

## Research Articles

# Pharmacokinetics of Salicylate Elimination in Man

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Salicylate elimination kinetics was studied over a dose range of from 0.25 to 2.0 Gm. of aspirin. It was found that when the amount of salicylate in the body of normal adult test subjects exceeded approximately 360 mg. aspirin equivalent, conjugation of salicylic acid with glycine reached a maximum rate and thus proceeded by zero-order kinetics. The over-all elimination of salicylate was found to proceed by first-order kinetics at very small doses, and by parallel zero and first-order processes at higher doses. A kinetic model was developed, and values for appropriate rate constants were determined which make it possible to reconcile apparent half-lives for salicylate elimination ranging from about 3 hr. to over 20 hr. which have been reported in the literature. Unusual patterns in salicylate elimination kinetics observed by others, such as a change from zero to first-order kinetics upon concomitant administration of *p*-aminobenzoic acid, and zero-order elimination in a newborn infant, have been rationalized and interpreted by means of the kinetic model evolved from the present study. The pharmacokinetics of salicylate elimination were found to be unusual both qualitatively and quantitatively, and the results of the present study have potentially important therapeutic, toxicologic, and pharmacogenetic implications.

RELATIVELY little is known about the kinetics of salicylate elimination in man. This is not only unusual in view of the long and extensive use of salicylates, but also unfortunate since such information is necessary for establishing rational dosage regimens (1). For example, intensive salicylate therapy, as in the treatment of arthritis or rheumatic fever, frequently requires maintenance of plasma salicylate levels in a high but narrow range, and this can be accomplished only by means of a dosage schedule based on the elimination rate of the drug (2). Salicylate intoxication, particularly in children, is sometimes due to accumulation of the drug during therapy (rather than due to accidental ingestion) if the rate of salicylate administration exceeds the rate of its elimination from the body (3). Treatment of salicylate intoxication is directed mainly toward facilitating the elimination of the drug since all toxic manifestations are readily reversible with removal of salicylate from the body (4).

A review of the literature reveals that the apparent half-life of salicylate elimination increases with dose (Table I). The reported half-lives

range from 2.4 to 19 hr. in adults. The half-life of salicylate elimination in children intoxicated with this drug was found to range from 15 to 29 hr., with a mean value of 20 hr. (12). Similar results are evident from reported studies of urinary excretion of total salicylates (salicylate and its metabolites) as a function of dose (Table II), which show that the per cent of the dose excreted at a given time (prior to the completion of excretion) decreases with increasing dose. While an increase in half-life with increasing dose is not unknown (13), it is a rarely observed phenomenon since the elimination of most drugs is describable by first-order kinetics with half-lives independent of dose.

Elimination of salicylate occurs mainly by the following parallel processes: renal excretion of unchanged salicylic acid, formation of salicyluric acid by conjugation with glycine, and formation

TABLE I.—MEAN HALF-LIFE OF SALICYLATE ELIMINATION IN ADULT HUMANS AS REPORTED IN THE LITERATURE

Drug	Dose Gm.	Dose mmoles	Route	Half- Life, hr.	Ref.
Sodium salicylate	0.25	1.6	i.v.	2.4	(5)
Aspirin	1.0	5.6	Oral	5.0	(2)
Aspirin	1.3	7.2	Oral	6.1	(6)
Salicylic acid	1.3	9.4	i.v.	6.1	(7)
Sodium salicylate	10-20	62.5-125	i.v.	19.0	(8)

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TABLE II—URINARY EXCRETION OF TOTAL SALICYLATE BY ADULT HUMANS AS REPORTED IN THE LITERATURE

Dose, Gm. <sup>a</sup>	% of Dose Recovered	Collection Period, hr.	Ref.
Sodium Salicylate			
1.0	78.6	24	(9)
2.1	54.5	24	
3.1	49.4	24	
4.2	50.8	24	
Sodium Salicylate			
0.5	82.7	24	(10)
1.0	78.6	24	
2.0	70.8	24	
Aspirin			
0.16	70.0	7.5	(11)
0.24	70.4	7.5	
0.32	71.5	7.5	
0.64	55.3	7.5	
0.97	52.1	7.5	

<sup>a</sup> Oral route.

of salicyl ester and ether glucuronides by conjugation with glucuronic acid (14). The purpose of the study to be reported here was to determine which of these processes was responsible for the observed decrease in the relative elimination rate of salicylate with increasing dose. In the course of this investigation it was found that the pharmacokinetics of salicylate elimination are most unusual and that their elucidation made it possible to rationalize and correlate numerous previously reported and often apparently contradictory findings.

#### METHODS AND EXPERIMENTAL PROCEDURES

The indicated amounts of aspirin or salicylic acid were administered orally in solution (dissolved in 200 ml. water by addition of equimolar amounts of sodium bicarbonate) to healthy male subjects who had fasted overnight. No food or beverages were ingested by the subjects until at least 2 hr. after drug administration. Total urine collections were made at appropriate times. Blank urine samples were obtained immediately before drug administration and for an extended period of time after termination of drug excretion. (In most experiments, urine collections were carried out for 70 hr. or more.) The test subjects drank water at a frequency and in amounts sufficient to result in adequate urine output (usually 50–100 ml./hr.).

Quantitative determinations of salicylic acid, salicyluric acid, salicyl glucuronides, and total salicylate in the urine were carried out by the

method of Smith *et al.* (15) as modified by Levy (16). Urine pH was determined immediately after voiding (except in the case of urines collected at night which were stored in the refrigerator and pH determined the following morning). Analyses were carried out within 1 to 2 days after collection of urines. Up to that time, all urines were stored in the refrigerator.

#### RESULTS

The effect of dose on the metabolic fate of aspirin is shown in Table III. Almost all of the administered drug was recovered in the urine as salicylic acid or its metabolites. Urinary pH's, though variable, did not change with dose. The fraction eliminated as salicyluric acid decreased with increasing dose, while the fraction eliminated as unchanged salicylic acid and salicyl glucuronides, being due to parallel and competing processes, increased accordingly.

The time course of elimination of salicylate as a function of dose is shown for two representative subjects in Figs. 1 and 2, respectively. The first experimental point after zero time in each of these figures is affected by the absorption phase and is therefore not representative of excretion alone. All subsequent points represent postabsorption data since aspirin is rapidly absorbed when given in solution (17–19). Salicylate elimination did not follow first-order kinetics until the amount remaining in the body was decreased to about 400 mg. aspirin equivalent. Prior to that time (*i.e.*, in the case of the 1.0, 1.5, and 2.0-Gm. doses), elimination was not an exponential process (nor was it a zero-order process). The relative rate of salicylate elimination decreased with increasing dose, as is evident from the times necessary to eliminate half the administered dose. These times, indicated in the figures by vertical arrows, are somewhat affected by the absorption phase which tends to diminish the real differences. A better relative measure is obtained by subtracting about 0.5 hr. from each indicated time in order to account for the absorption phase. This will show that the time required to eliminate 50% of the administered dose increased from about 3 hr. with a 0.25-Gm. dose to about 9 hr. with a 2.0-Gm. dose. The first-order elimination rate constant describing the exponential phase of drug elimination was essentially the same regardless of the amount of drug administered.

The time course of salicyluric acid excretion after administration of 1.5 and 2.0 Gm. aspirin, respectively, is shown in Figs. 3 and 4. These data were obtained from the same experiments as the data represented by the top curves in Figs. 1 and 2. Elimination of salicyluric acid proceeded at an essentially constant rate for over 12 hr., and thus was a zero-order process during this time.

TABLE III.—EFFECT OF DOSE ON METABOLIC FATE OF SALICYLATE IN HEALTHY ADULT HUMANS

Subject (Sex, Age) →	A (M, 36)			B (M, 22)			C (M, 23)		D (M, 23)	
Dose, mg. <sup>a</sup>	250	1000	2000	250	1000	1500	250	1000	250	1000
Amount excreted, mg. <sup>b</sup>	232	928	1958	234	934	1490	236	935	215	928
Excreted as salicyluric acid, %	79.6	67.5	59.2	78.1	77.0	74.0	83.5	59.9	91.4	76.9
Excreted as salicylic acid, %	2.5	7.3	17.1	5.1	3.6	10.3	2.0	6.7	3.9	3.6
Excreted as salicyl glucuronides, %	17.9	25.2	23.7	16.8	19.4	15.7	14.5	33.4	4.7	19.4

<sup>a</sup> Aspirin taken orally. <sup>b</sup> Total salicylates as aspirin equivalent.

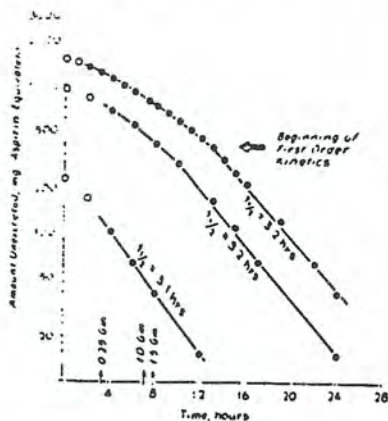


Fig. 1.—Elimination of salicylate by subject *B* as a function of dose. Doses taken were 0.25, 1.0, and 1.5 Gm. aspirin, respectively. Vertical arrows on the time axis indicate the time necessary to eliminate 50% of the dose.

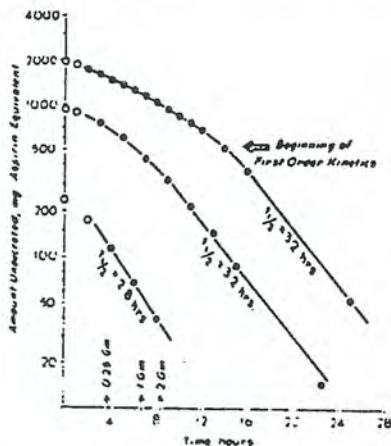


Fig. 2.—Elimination of salicylate by subject *A* as a function of dose. Doses taken were 0.25, 1.0, and 2.0 Gm. aspirin, respectively. Vertical arrows on the time axis indicate the time necessary to eliminate 50% of the dose.

A detailed tabulation of the data collected after administration of 1.5 and 2.0 Gm., respectively, of aspirin is presented as Tables IV and V. Not included in these tables are blank values obtained from the time of termination of drug elimination to about 70 hr. after drug administration. From the tables it can be seen that the average salicylic acid output was about 78 mg. or 400  $\mu$ m./hr. from the second to the eighth hour and 60 to 70 mg./hr. thereafter until elimination changed from zero to first-order kinetics. Salicylic acid excretion was essentially an exponential process, but exhibited the well-known sensitivity to urinary pH (20). This can be seen in Table IV at 3 and 5 hr., when urinary pH and salicylate excretion rate were relatively high, and at 7 $\frac{1}{4}$ , 8, and 10 hr., when urinary pH and salicylate excretion rate were relatively low. Similar effects are noticeable in Table V—namely, at 5, 9, and 16 hr. (high pH) and at 4, 7, and 12 hr. (low

pH). Salicyl glucuronide elimination as listed represents both ester and other glucuronides and, since the maximum excretion of the former occurs considerably earlier than that of the latter (21), there is no clearly recognizable pattern of excretion rate versus time. In addition, the excretion rate of salicyl glucuronides is affected by the variable excretion rate of unchanged salicylic acid, since salicylate excretion and glucuronide synthesis are parallel and therefore competing processes.

First-order rate constants for over-all salicylate elimination as well as for the individual elimination processes can be obtained from experimental data collected after administration of 0.25 Gm. aspirin.

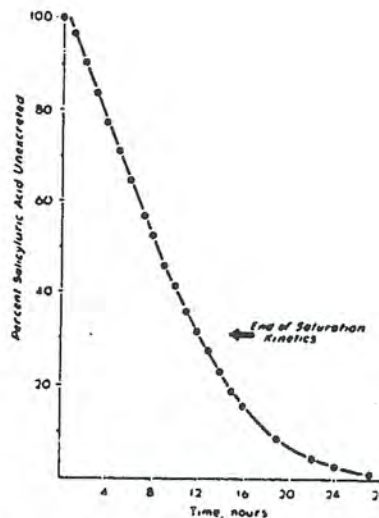


Fig. 3.—Excretion of salicylic acid as a function of time after oral administration of 1.5 Gm. aspirin to subject *B*.

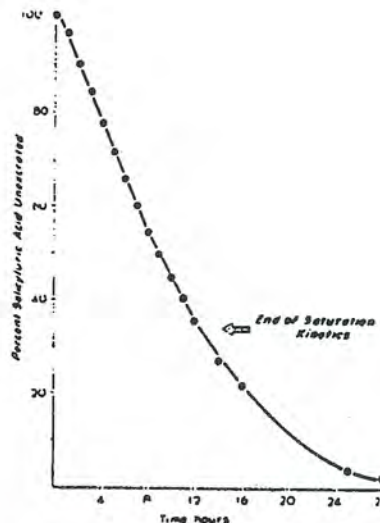


Fig. 4.—Excretion of salicylic acid as a function of time after oral administration of 2.0 Gm. aspirin to subject *A*.

TABLE IV.—URINARY EXCRETION OF SALICYLATE AND ITS MAJOR METABOLITES AFTER ORAL ADMINISTRATION OF 1.5 GM. ASPIRIN (SUBJECT B)

Hr. After Drug Administration	pH	Salicylic Acid		Salicyluric Acid		Salicyl Glucuronides <sup>a</sup>		Cumulative Amt. of Total Salicylates Excreted, mg. Aspirin Equivalent
		Amt. Excreted, mg.	Excretion Rate, mg./hr.	Amt. Excreted, mg.	Excretion Rate, mg./hr.	Amt. Excreted, mg.	Excretion Rate, mg./hr.	
1	6.3	15.1	15.1	39.7	39.7	14.9	14.9	76
2	6.6	13.6	13.6	78.2	78.2	8.60	8.60	177
3	7.1	17.3	17.3	81.8	81.8	5.37	5.37	282
4	6.6	14.3	14.3	78.5	78.5	11.4	11.4	388
5	6.95	15.7	15.7	74.8	74.8	5.45	5.45	485
6	6.7	12.4	12.4	80.8	80.8	7.88	7.88	586
7 <sup>1/4</sup>	5.7	2.47	1.98	96.8	77.4	12.9	10.3	695
8	5.9	2.38	3.17	55.0	73.3	6.58	8.77	758
9	6.1	4.71	4.71	69.9	69.9	8.82	8.82	840
10	5.5	0.99	0.99	64.4	64.4	7.04	7.04	910
11	5.65	1.29	1.29	69.2	69.2	8.21	8.21	986
12	5.8	1.36	1.36	53.3	53.3	6.18	6.18	1045
13	6.4	2.76	2.76	49.8	49.8	7.05	7.05	1104
14	6.9	5.75	5.75	55.1	55.1	5.05	5.05	1169
15	7.1	4.0	4.0	50.5	50.5	5.24	5.24	1227
16	5.6	0.45	0.45	37.5	37.5	5.15	5.15	1269
19	5.9	1.20	0.40	88.2	29.4	10.2	3.40	1366
22	5.5	0.55	0.18	49.8	16.6	10.2	3.40	1425
24	5.45	0.27	0.13	19.4	9.7	4.67	2.33	1450
27	6.6	0.83	0.28	17.4	5.8	5.25	1.75	1474
30	6.45	0.59	0.20	5.60	1.87	1.57	0.52	1482
32	5.3	0.13	0.06	2.25	1.12	0.87	0.43	1485
36	5.8	0.23	0.06	2.39	0.60	0.03	0.007	1488
39	6.5	0.29	0.10	0.59	0.20	0.82	0.27	1490

<sup>a</sup> Expressed in terms of salicylic acid.

TABLE V.—URINARY EXCRETION OF SALICYLATE AND ITS MAJOR METABOLITES AFTER ORAL ADMINISTRATION OF 2 GM. ASPIRIN (SUBJECT A)

Hr. After Drug Administration	pH	Salicylic Acid		Salicyluric Acid		Salicyl Glucuronides <sup>a</sup>		Cumulative Amt. of Total Salicylates Excreted, mg. Aspirin Equivalent
		Amt. Excreted, mg.	Excretion Rate, mg./hr.	Amt. Excreted, mg.	Excretion Rate, mg./hr.	Amt. Excreted, mg.	Excretion Rate, mg./hr.	
1	7.0	39.6	39.6	45.8	45.8	18.9	18.9	118
2	7.05	30.9	30.9	81.5	81.5	8.85	8.85	245
3	6.6	17.7	17.7	74.8	74.8	15.3	15.3	358
4	6.25	9.93	9.93	81.3	81.3	19.5	19.5	471
5	6.9	23.7	23.7	77.3	77.3	15.7	15.7	594
6	6.6	17.3	17.3	74.5	74.5	15.6	15.6	705
7	6.25	10.1	10.1	74.1	74.1	19.5	19.5	812
8	6.5	15.4	15.4	72.2	72.2	20.1	20.1	925
9	7.13	21.6	21.6	60.6	60.6	14.6	14.6	1029
10	7.0	13.9	13.9	63.7	63.7	14.4	14.4	1124
11	6.6	6.45	6.45	59.3	59.3	14.6	14.6	1206
12	5.5	1.07	1.07	61.8	61.8	17.1	17.1	1287
14	6.7	15.6	7.80	107.7	53.8	26.5	13.2	1442
16	7.35	21.8	10.9	64.2	32.1	48.1	24.0	1592
25	6.35	14.0	1.55	225.9	25.1	67.2	7.47	1906
28	6.6	1.13	0.38	20.2	6.73	7.55	2.52	1936
31	6.35	0.86	0.29	8.2	2.73	2.40	0.80	1948
34	5.8	0.44	0.15	3.82	1.27	1.18	0.39	1954
37	5.8	0.36	0.12	1.85	0.62	0.49	0.16	1957
39	7.0	0.35	0.17	0.77	0.38	0.10	0.05	1958

<sup>a</sup> Expressed in terms of salicylic acid.

since elimination at this dose level is an exponential process. Thus,

$$\log A = \log A_0 - \frac{k}{2.3} t \quad (\text{Eq. 1})$$

where  $A$  is the amount of drug remaining unexcreted at time  $t$ ,  $A_0$  is the total amount of drug eventually excreted, and  $k$  is the first-order elimination rate constant, expressed in reciprocal hours.

$k$  can be determined from the slope of the straight line obtained when  $\log A$  is plotted as a function of time. Half-life ( $t_{1/2}$ ) values can be calculated on the basis of the relationship

$$t_{1/2} = \frac{0.693}{k} \quad (\text{Eq. 2})$$

The first-order rate constants for component processes can be calculated from  $k$  values and the frac-

TABLE VI.—KINETIC PARAMETERS FOR SALICYLATE ELIMINATION DETERMINED AFTER ADMINISTRATION OF 0.25 Gm. ASPIRIN TO FOUR HUMANS

Subject <sup>a</sup>	<i>t</i> <sub>1/2</sub> , hr.	<i>k</i> , hr. <sup>-1</sup>	<i>k</i> <sub>ST</sub> , hr. <sup>-1</sup>	<i>k</i> <sub>SA+SG</sub> , hr. <sup>-1</sup>
B	3.1	0.224	0.164	0.046
A	2.8	0.248	0.183	0.047
C	2.7	0.257	0.203	0.040
D	3.0	0.231	0.182	0.017
Mean <sup>b</sup>	2.9	0.239	0.182	0.032

<sup>a</sup> Ages and amounts excreted are listed in Table III. <sup>b</sup> *t*<sub>1/2</sub> expressed as arithmetic mean; rate constants as harmonic means.

tion of the total excreted dose which is eliminated as the particular metabolite, provided that rate of formation is considerably lower than rate of excretion (22). This is true for salicyl glucuronides and for salicyluric acid, as will be shown subsequently. Thus

$$k_{SU} = \frac{SU}{ST} \cdot k \quad (\text{Eq. 3a})$$

where *k*<sub>SU</sub> is the first-order rate constant for salicyluric acid synthesis, SU is the total amount (in moles) of salicylate which is eliminated as salicyluric acid, ST is the total amount (in moles) of salicylate eliminated in all forms, and *k* is the first-order rate constant for over-all salicylate elimination. Similarly,

$$k_{SA+SG} = \frac{SA + SG}{ST} \cdot k \quad (\text{Eq. 3b})$$

where SA and SG are the total excreted amounts of salicylic acid and salicyl glucuronides, respectively. Separate estimation of *k*<sub>SA</sub> and *k*<sub>SG</sub> values is possible also (though not necessary for the purpose of the present study), but experimentally determined *k*<sub>SG</sub> values tend to be too low and *k*<sub>SA</sub> values too high since a considerable part of salicyl glucuronides may be hydrolyzed prior to urine collection (9). On the other hand, *k*<sub>SA+SG</sub> will not be affected by partial hydrolysis of salicyl glucuronides. Values for *k*, *t*<sub>1/2</sub>, *k*<sub>ST</sub>, and *k*<sub>SA+SG</sub> for four subjects are listed in Table VI. The mean half-life of salicylate elimination was found to be 2.9 hr., and the major process responsible for salicylate elimination at this low dose was the conjugation of salicylic acid with glycine to form salicyluric acid.

The renal excretion kinetics of salicyluric acid were determined by oral administration of this metabolite of salicylic acid. Salicyluric acid was well absorbed and rapidly excreted as shown in Fig. 5. Renal excretion was describable by first-order kinetics, showing no saturation effects in the dose range studied and no decrease in rate constant with increasing dose. The half-life for excretion was about 37 min., which is equivalent to a rate constant of about 1.1 hr.<sup>-1</sup>. The maximum observed average excretion rate was 337 mg./hr., which is equivalent to 1730 μm., and thus is far in excess of the maximum excretion rate of salicyluric acid observed after salicylate administration (400 μm./hr.).

DISCUSSION

**Kinetic Model for Salicylate Elimination.**—The elimination of salicylate administered as aspirin can be depicted by the kinetic model in Scheme I.

In Scheme I, ASA is aspirin, SA is salicylic acid, SU is salicyluric acid, SG represents salicyl glucuronides, and the subscripts *b* and *u* refer to drug in the body and in the urine, respectively. *k*'s are first-order rate constants for rate-limiting processes, while *k*<sup>\*</sup> designates first-order rate constants for processes that are not rate-limiting since they are preceded by processes with smaller rate constants (i.e., *k*<sub>SU</sub> < *k*<sup>\*</sup><sub>ST</sub>, and *k*<sub>SG</sub> < *k*<sup>\*</sup><sub>SG</sub>). The formation of minor metabolites (such as gentisic acid) and excretion by extra-renal routes can be neglected in the following discussion, since these pathways are quantitatively insignificant.

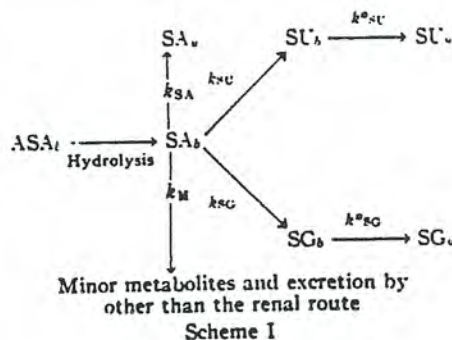
When 0.25-Gm. doses of aspirin are given, over-all salicylate elimination and therefore each of the participating processes proceeds by first-order kinetics. Thus

$$k = k_{SA} + k_{SU} + k_{SG} \quad (\text{Eq. 4})$$

where *k* is the first-order rate constant for over-all salicylate elimination. The *in vivo* hydrolysis of aspirin to salicylic acid is extremely rapid (23) as compared to salicylic acid elimination and thus need not be considered.

When higher doses of aspirin, such as 1 Gm. or more, are given, the formation of salicyluric acid reaches a maximum rate, and this process follows apparent zero-order kinetics until the amount of salicylate in the body has declined sufficiently for the formation rate to be lower than the maximum rate. The over-all elimination consists therefore of parallel zero- and first-order processes. This possibility has been suspected only recently (24, 25).

In the present investigation, the maximum salicyluric acid formation rate was found to be about 330 to 400 μm./hr. It is of interest that similar values can be found by examination of data previously reported by others. Schachter's data (9) indicate a maximal salicyluric acid excretion rate of 408 μm./hr. after administration of 4.2 Gm. sodium salicylate. Schachter and Manis found a maximal salicyluric acid excretion rate of 312 ± 66 μm./hr. in 19 subjects given 0.4 μm./Kg. of sodium salicylate (21). The data of Salassa and co-workers (26) indicate a maximum excretion rate of approximately 320 μm./hr. after administration of 3 Gm. sodium salicylate. Quick's maximum values, reported in 1933 (27), are somewhat lower (about 250 μm./hr. after ingestion of 1.0-3.5 Gm. salicylic acid), but this may be due to the less accurate analytical methods available at that time, or it may be a characteristic of the test subject used in his study. Clearly then, there has been indirect evidence in the



literature for some time which should have suggested that salicyluric acid is formed at a constant low rate from salicylate even when the latter is administered in doses well within the therapeutic range. It is interesting that this has been overlooked for many years by workers in the field, including the author.

The limiting value for the excretion rate of salicyluric acid is not due to a renal tubular transport maximum [although salicyluric acid is known to be excreted by both glomerular filtration and active tubular secretion (28)]. This is evident from the exponential excretion of orally administered salicyluric acid even when excretion rate was far in excess of the maximum value observed after salicylate administration (Fig. 5).

It is known that the formation rate of hippuric acid by conjugation of benzoic acid with glycine also reaches a limiting value, but this maximum formation rate is considerably higher than the maximum rate of salicyluric acid synthesis. For instance, Quick (29) has reported maximum hippuric acid formation rates in the range of from 7300 to 9300  $\mu\text{m.}/\text{hr.}$  for an adult. Freiberg and West (30) have studied hippuric acid formation in children and found maximum rates about 50% higher (on a body weight basis) than those reported by Quick. Schachter (9) has reported that maximum hippuric acid formation rates in adults were around 7800  $\mu\text{m.}/\text{hr.}$ , which is in agreement with the values established by Quick. Thus, it is evident that the maximum rate of conjugation of benzoic acid with glycine is about 20 times higher than the maximum rate of conjugation of salicylic acid with glycine. It will be shown in a subsequent report that this is due to the fact that the rate-limiting process in hippuric acid formation is not the same as the rate-limiting process in salicyluric acid formation (31).

**Relationship Between Dose and Relative Rate of Elimination.**—The kinetic model for salicylate elimination presented above and the kinetic parameters listed in Table VI make it possible to reconcile the wide differences in reported half-lives of salicylate elimination which are summarized in Table I. According to the findings of the present study, the half-life of salicylate elimination is about 2.9 hr. when the administered dose is sufficiently small so that all elimination processes proceed by first-order kinetics. This is in good agreement with the shortest reported half-life of salicylate elimination (Table I). When the administered dose is very high, the contribution of the zero-order process to salicylate elimination is negligible until most of the drug is eliminated. At these high doses, elimination occurs mainly by salicylate excretion and salicyl glucuronide formation. The rate constant for these combined processes was found in the present study to have a mean value of 0.032  $\text{hr.}^{-1}$  (Table VI), which is equivalent to a half-life of about 22 hr. This is similar to the maximum value reported by Done (12) in the case of salicylate poisoning, and it agrees also with the longest average half-life reported for salicylate elimination under therapeutic conditions, as tabulated in Table I. Thus, the relationship between dose and the time required for 50% elimination is as follows. (a) When the dose is sufficiently small so that all elimination processes proceed by first-order kinetics, the half-life is about 2.9 hr. (b) When the dose is so large that the con-

tribution of the salicyluric acid formation process to the elimination of salicylate is negligible, the half-life increases to about 22 hr. Elimination appears to be a first-order process (until most of the drug is eliminated) since the contribution of the zero-order process is too small to be noticeable except during the terminal phase of salicylate elimination. (c) When the administered dose is intermediate in size, the zero-order formation of salicyluric acid can be a significant contributory factor to the total elimination of salicylate. Apparent half-lives will increase continuously with dose in the range between the minimum and maximum values reported above. However, salicylate elimination definitely does not follow first-order kinetics in this range, although this can be overlooked easily. A graph of the logarithm of plasma salicylate concentration versus time (following administration of 1 Gm. or more of aspirin) usually looks linear for a period of time equal to more than one apparent half-life even though elimination proceeds by parallel zero- and first-order processes. The same is true for urinary excretion curves (see Figs. 1 and 2). Interpretation of such graphs is complicated further by the initial absorption phase (after oral administration) or distribution phase (after intravenous administration). A downward bend in such curves is usually only apparent if elimination rate is followed for very long times (*i.e.*, over three orders of magnitude of uneliminated drug). In the case of plasma salicylate determinations, this would require intensive blood sampling on an around-the-clock basis. For practical purposes (such as for calculating appropriate dosage regimens), apparent half-life values obtained by drawing a best-fitting line through the initial part of the semilogarithmic plot (*i.e.*, to the point of 50% drug elimination) can be used. However, rate constants derived from such values are best referred to as functional rate constants to distinguish them from "true" first-order rate constants. The true first-order rate constant for salicylate elimination cannot be used for calculating salicylate dosage regimens in the usual therapeutic dose range.

Some comments are appropriate concerning the maximum half-life for salicylate elimination as estimated in the preceding paragraph. This value was derived from  $k_{\text{BA}+\text{BG}}$  values determined in the present study.  $k_{\text{SA}}$  is extremely sensitive to urinary pH and can increase more than tenfold when urinary pH is raised from below 6.0 to above 7.0 (20). Thus, it is reasonable to expect considerable inter- and intrasubject variation in  $k_{\text{SA}+\text{BG}}$  and therefore in maximum half-life of salicylate elimination. In the present study,  $k_{\text{SA}+\text{BG}}$  values ranged from 0.017 to 0.047  $\text{hr.}^{-1}$ , which is equivalent to half-lives of 15 to 41 hr. Indeed, half-lives as long as 30 hr. for salicylate elimination after ingestion of large doses have been reported (4). On the other hand, elimination will be considerably more rapid, due mainly to an increase in  $k_{\text{BA}}$ , when urine pH is increased by administration of alkalinizing agents such as sodium bicarbonate or Tris.

**Estimation of Threshold for Apparent Saturation of the Salicyluric Acid Formation Process.**—Since the maximum rate of salicyluric acid synthesis in healthy adults is about 400  $\mu\text{m.}/\text{hr.}$ , it is possible to calculate the amount of salicylate in the body which must be exceeded before salicyluric acid formation changes from first-order to zero-order kinetics. At

below threshold levels

$$\frac{dSU}{dt} = k_{st} \cdot SA_b \quad (\text{Eq. 5})$$

where  $dSU/dt$  is the rate of formation of salicylic acid,  $k_{st}$  is the first-order formation rate constant, and  $SA_b$  is the amount of salicylic acid in the body. Rearranging the equation to solve for  $SA_b$ , and introducing the values  $400 \mu\text{m.}/\text{hr.}$  for  $dSU/dt$  and  $0.198 \text{ hr.}^{-1}$  for  $k_{st}$  (from Table VI) yields

$$SA_b = \frac{400 \mu\text{m.}/\text{hr.}}{0.198 \text{ hr.}^{-1}} = 2000 \mu\text{m.} \text{ or } 2 \text{ mmoles}$$

This is equivalent to 276 mg. of salicylic acid or 360 mg. of aspirin. Reference to Figs. 1 and 2 shows that this was indeed the amount of aspirin equivalent in the body at which the kinetics changed. Additional evidence in support of these estimations is available from the urinary excretion data reported by Cummings and Martin (11), which are summarized in Table II of the present paper. The percent of a dose of aspirin excreted in 7.5 hr. was the same with doses of 0.16, 0.24, and 0.32 Gm., but decreased successively with doses of 0.64 and 0.97 Gm. The dose of aspirin necessary to obtain a body content equal to or exceeding 2 mmoles of salicylate depends in part on the rate of absorption of the drug. With conventional tablets, this dose should be between 400 and 500 mg. aspirin. In other words, the body content of salicylate at which salicylic acid formation rate reaches a maximum in human adults is exceeded ordinarily when only two aspirin tablets are taken! Similar apparent saturation effects are known for elimination of only a few other drugs (notably, the elimination of ethanol and glycine conjugation of benzoic and *p*-aminobenzoic acids), and such saturation is encountered only at considerably higher doses.

**The "Constant Infusion" Case—Independent Verification of the Kinetic Model.**—Schachter and Manis (21) have determined plasma salicylate and salicyl conjugate levels as a function of time, after administration of 0.4 mmole/Kg. sodium salicylate to adult humans. They found that plasma salicylate levels declined from an average of 1782  $\mu\text{moles}/\text{L.}$  at 2 hr. to 467  $\mu\text{moles}/\text{L.}$  at 24 hr. after drug administration. On the other hand, plasma salicylate levels remained constant during the entire period—namely, 9.8, 10.1, 9.5, and 10.5  $\mu\text{moles}/\text{L.}$  at 2, 4, 8, and 24 hr., respectively. Such plateau effects are obtained typically when a drug is infused at a constant rate and is eliminated by first-order kinetics. Schachter and Manis used a dose of salicylate which was large enough (according to the calculations presented in the preceding paragraph) to result in salicylic acid formation at a constant rate for a considerable time (apparently more than 24 hr.). This is equivalent to a constant infusion case and permits the application of an equation (32) which relates infusion rate ( $R_0$ ), apparent volume of distribution ( $V_d$ ), and the first-order elimination rate constant ( $k$ ) to the plateau plasma level ( $C_p$ ) eventually obtained:

$$\frac{R_0}{V_d k} = C_p \quad (\text{Eq. 6})$$

Equation 7 may be solved for  $V_d$  by introducing a value of  $10 \mu\text{moles}/\text{L.}$  salicylic acid for  $C_p$  (from

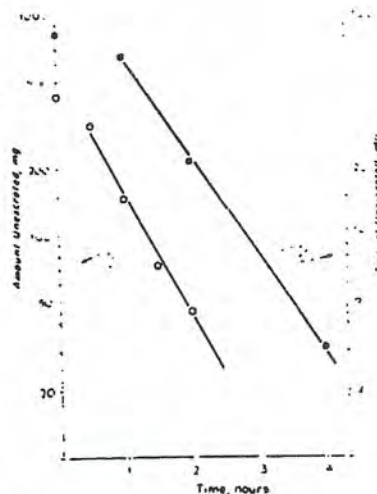


Fig. 5.—Excretion of salicylic acid as a function of time after oral administration of 0.1 Gm (●) and 0.5 Gm. (○), respectively, of salicylic acid (subject A).

Schachter and Manis' data),  $1.1 \text{ hr.}^{-1}$  for  $k$  (as determined in the present study from the experimental data shown in Fig. 5),<sup>1</sup> and  $312 \mu\text{moles}/\text{hr.}$  for  $R$  (the value for the maximum rate of salicylic acid formation, as found by Schachter and Manis) Thus

$$V_d = \frac{312 \mu\text{moles}/\text{hr.}}{(10 \mu\text{moles}/\text{L.})(1.1 \text{ hr.}^{-1})} = 28.4 \text{ L.}$$

The renal clearance of a drug can be calculated from  $k$  and  $V_d$  since

$$\text{renal clearance} = \frac{k C V_d}{C} = k V_d \quad (\text{Eq. 7})$$

where  $C$  is the plasma level of the drug. The renal clearance of salicylic acid, as determined by Eq. 7 from a  $V_d$  value of 28.4 L. and  $k$  value of  $1.1 \text{ hr.}^{-1}$  is  $31.2 \text{ L.}/\text{hr.}$  or  $520 \text{ ml.}/\text{min.}$  The renal clearance of salicylic acid obtained experimentally by Schachter and Manis (21) was  $444 \pm 112 \text{ ml.}/\text{min.}$  The good agreement of these values constitutes an independent verification of the kinetic model for salicylate elimination developed in this paper and supports also the value for the first-order elimination rate constant for salicylic acid obtained in the present study. The apparent volume of distribution of salicylic acid determined above is two to three times greater than the volume of distribution of salicylic acid reported recently by Levy and Hollister (33), although exact comparison is not possible due to the concentration dependence of these values. However, the difference in volumes of distribution is consistent with the much greater plasma protein binding of salicylic acid, as compared to salicylic acid (21).

**Change in Salicylate Elimination Kinetics Due to Concomitant Administration of a Drug Which Competes for Glycine Conjugation.**—Following an initial observation by Dry *et al.* (34) that plasma salicylate levels increased when *p*-aminobenzoic

<sup>1</sup> In the context of the present discussion,  $k$  equals  $k_{st}$  as previously defined.

acid was co-administered. Salassa *et al.* (26) studied the effect of *p*-aminobenzoic acid on the metabolism and excretion of salicylate. Their data (as depicted in Figs. 1 and 2 of their paper) show that elimination of salicylate (given as 3 Gm. sodium salicylate) proceeded at a practically constant rate (*i.e.*, by zero-order kinetics) and that most of the drug was eliminated as salicylic acid. When sodium salicylate was given together with repeated doses of *p*-aminobenzoic acid (3 Gm. every 3 hr.), salicylate elimination occurred by first-order kinetics and there was practically no salicylic acid formed. The half-life for salicylate elimination was about 26 hr. These observations can be rationalized and explained readily by means of the kinetic model and rate constants evolved in the present study. Salicylate elimination in the first experiment (without PAB) occurred primarily by salicylurate formation at a constant rate. The data show that this rate was about 320  $\mu$ moles/hr. which is in good agreement with the maximum rates observed in the present study. The over-all elimination process was describable by zero-order kinetics since the contribution of the first-order processes (salicylic acid excretion and salicyl glucuronide formation) was relatively small. When sodium salicylate was administered together with PAB, the latter competed so effectively with the former in the glycine conjugation process that there was almost no salicylic acid formed. Under these circumstances, salicylate elimination was due almost solely to salicylic acid excretion and salicyl glucuronide formation. Since both of these are first-order processes, the over-all salicylate elimination proceeded by essentially first-order kinetics. The applicable rate constant for elimination under these conditions is  $k_{SA+SG}$  and the corresponding mean half-life determined in the present study is 22 hr. (range 15 to 41 hr.). This agrees well with the 26-hr. half-life apparent from the data of Salassa and co-workers (26).

**Salicylate Elimination Kinetics in the Newborn.**—Earle (35) recently reported a case of congenital salicylate intoxication in an infant whose mother attempted suicide by taking 15–18 Gm. aspirin 27 hr. before the baby was born. The infant was treated by exchange transfusion, and plasma salicylate levels were determined subsequently for a total of 6 days. A plot of these values on a linear scale (rather than on a logarithmic scale as was done in the original paper) against time yields a straight line: *i.e.*, salicylate elimination apparently proceeded by zero-order kinetics.<sup>2</sup>

Glucuronide conjugation capacity is deficient in the newborn (36). Blood level data obtained by Vest and Rossier (37) after administration of single doses of *N*-acetyl-*p*-aminophenol (APAP) to newborn infants (1 to 6 days old) show a constant level of APAP-glucuronide with time while APAP levels decreased. This is indicative of zero-order (saturation) kinetics with respect to phenolic glucuronide conjugation. Similar saturation conditions are probably operative in ester glucuronide formation. It is also known that renal function, particularly tubular function, is very poorly developed in newborn infants (38). Thus, salicylate elimination in new-

born given appreciable amounts of the drug may be expected to involve mainly two parallel zero-order processes (salicylic acid and salicyl glucuronide formation), with only a minor contribution from salicylic acid excretion processes. One of the latter (tubular secretion) is probably also saturated, while the other (glomerular filtration) may be expected to follow first-order kinetics. Under these conditions, over-all elimination will proceed at a very slow and constant (*i.e.*, zero-order) rate. This is evident from the data reported by Earle (35); plasma salicylate levels of the intoxicated infant declined at a constant rate and required over 70 hr. to decrease by 50%.

**Additional Considerations.**—Limitations in conjugating capacity, such as described with respect to the conjugation of salicylate with glycine, possibly may occur also with other drugs. For example, examination of recently reported blood levels of *p*-aminosalicylate obtained after administration of large single doses (39) suggests that zero-order kinetics may be operative in one or more of the elimination processes involved. A component zero-order process may be difficult to recognize on the basis of plasma level data in the presence of a parallel first-order process (or processes) of appreciable magnitude, but the occurrence of such kinetics should be considered whenever there is an apparent increase in half-life of elimination with increasing dose.<sup>3</sup>

Individual differences in apparent half-life of salicylate elimination, as reported by Levy and Hollister (2), probably reflect differences in maximum salicylic acid formation capacity. Any search for genetic differences in salicylate elimination by salicylic acid formation must be directed not only toward the determination of individual first-order rate constants for this process (which requires that the administered doses be small), but must also include the determination of individual maximum salicylurate formation rate capacities. Either one or the other (or both) could show genetically determined differences, if existent. An apparently genetically determined difference in glucuronide conjugation of salicylate and other drugs has already been observed (10).

When the observations of Alpen *et al.* (40), who studied the metabolism of salicylic acid in cancer patients, are interpreted against the background of information obtained or reviewed in the present study, the extent of salicylic acid formation in these patients appears unusually limited. This raises the possibility that malignancy may decrease salicylic acid forming capacity. It would be desirable to determine salicylic acid forming capacity in children as a function of age, and to establish if this capacity is altered in arthritics and in other individuals taking large doses of salicylate for some time. There is presently no published information suggesting enzyme induction effects in the metabolism of salicylates. A recently reported study on dogs (41), which showed no change in salicylate elimination kinetics after repeated dosings,

<sup>2</sup> This report was brought to the author's attention by Professor Eino Nelson, State University of New York at Buffalo, who also pointed out that the kinetics were apparently zero-order.

<sup>3</sup> It has been reported recently (44) that the extent of acetylation of *p*-aminobenzoic acid (PABA) decreases with increasing dose, and increases when absorption rate is decreased. These observations suggest that acetylation of PABA occurs by apparent zero-order kinetics when a certain body level of PABA is exceeded.



has only limited predictive value with respect to humans. This becomes apparent upon comparison of the human and dog data obtained by Salassa and co-workers (26).

#### ADDENDUM

Most biotransformation processes treated pharmacokinetically as apparent first-order reactions probably follow Michaelis-Menten kinetics (42), *viz.*,

$$\frac{1}{v} = \frac{K_m}{V_{\max} C_s} + \frac{1}{V_{\max}} \quad (\text{Eq. 1A})$$

where  $v$  is the initial velocity of the reaction,  $K_m$  is the Michaelis constant (which is numerically equal to the concentration of substrate necessary to attain half-maximum initial velocity),  $V_{\max}$  is the maximum initial velocity (which is attained when the enzyme is "saturated"), and  $C_s$  is the concentration of substrate (*i.e.*, the drug). Equation 1A can be rearranged to

$$\frac{1}{v} = \frac{1}{V_{\max}} \left( \frac{K_m}{C_s} + 1 \right) \quad (\text{Eq. 2A})$$

to show that, when  $K_m \gg C_s$ , the expression reduces to

$$\frac{1}{v} = \frac{K_m}{V_{\max} C_s} \quad (\text{Eq. 3A})$$

Equation 3A, when inverted, yields the first-order rate equation

$$v = K C_s \quad (\text{Eq. 4A})$$

where  $K = V_{\max}/K_m$ .

The maximum amount of a drug which can be administered safely to humans usually yields  $C_s$  levels which are very much smaller than  $K_m$  values. Therefore, the biotransformation process can be described adequately in these instances on the basis of first-order kinetics. While experimental difficulties (for example, control of the over-all process by another rate-limiting process) and toxicologic considerations have usually restricted the characterization of Michaelis-Menten kinetics of enzymic processes to isolated enzyme systems, cell fractions, or tissue slices, we have recently been able to demonstrate in intact man that salicylurate formation follows Michaelis-Menten kinetics (43).

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