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# A KINETIC STUDY OF THE ELIMINATION OF SALICYLATE IN MAN

BY

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The elimination of salicylate in man is a slow process, after a single 1 g dose of aspirin the average half-life of salicylate is reported to be 6 hr (Brodie, Burns & Weiner, 1959), while after very high doses a value of 19 hr has been obtained by Swintosky (1956). It can be inferred that the half-life of salicylate varies with dosage, that is with body salicylate concentration, and therefore that elimination cannot be invariably described by first-order kinetics and the ratio in which the metabolites are formed may change with dosage and time after dosage.

Only a small proportion of the dose is normally excreted directly as unconjugated salicylate and the removal of salicylate from the body is almost entirely dependent upon the formation of the metabolites salicyluric acid and the two glucuronides of salicylic acid. Salicyluric acid is the major metabolite and may account for more than half the total metabolites of salicylic acid excreted in the urine (Baldoni, 1915; Smith, Gleason, Stoll & Ogorzalek, 1946; Salassa, Bollman & Dry, 1948; Schachter & Manis, 1958). It has been previously suggested that the rate of formation of salicyluric acid is limited when the plasma salicylate concentration exceeds some low level (Cummings, 1963).

The present paper describes a study of the rate of " total salicylate " and salicyluric acid excretion in the urine after various doses of aspirin. In this context, " total salicylate " denotes those metabolites which, when hydrolysed by the specified process, may be estimated as salicylic acid. Unconjugated salicylate, salicylic acid glucuronides and salicylurate are considered to be included in this term. Also, for the purpose of this investigation, it has been convenient to divide the total salicylate into the two fractions, salicyluric acid and the other derivatives of salicylic acid. The latter fraction is considered to include unconjugated salicylate and the two salicylic acid glucuronides.

## METHODS

Seven healthy men between 20 and 40 years of age took part in these studies. They pursued their normal occupation during the experiments and no restriction was placed upon their diet or fluid intake.

*Drugs.* The salicylate was administered as aspirin powder B.P. in hard gelatin capsules or as aspirin tablets B.P.

Salicyluric acid (melting point, 170 to 171° C) was prepared by the method of Buzas & Dufour (1959), and was administered as a powder in hard gelatin capsules.

*Chemical methods*

A modification of the method of Brodie, Udenfriend & Coburn (1944) was used for the determination of salicylic acid and salicyluric acid in urine.

" *Total salicylate* " *in urine.* The urine was hydrolysed by heating 4 ml. with 2 ml. of 12 N-sulphuric acid in an autoclave at 115° C for 3 hr. The hydrolysate was diluted to 25 ml. with water and 4 ml. of this solution was extracted with 25 ml. of chloroform; 20 ml. of the chloroform were filtered and shaken with successive 5-ml. volumes of ferric nitrate solution (75 mg/100 ml.) until the colour of the ferric nitrate solution remained unchanged. The ferric nitrate solutions were combined, the total volume was noted and the extinction was determined at 530 mμ.

Appropriate standard solutions of salicylic acid were treated similarly.

*Salicylic acid and salicyluric acid in urine.* The urine was hydrolysed by heating 2 ml. with 1 ml. of 8 N-sulphuric acid in a boiling-water bath for 1 hr. This procedure hydrolyses salicylic and salicyluric acid glucuronides to their respective acids without appreciably hydrolysing salicyluric acid to salicylic acid. The hydrolysate was successively extracted first with 25 ml. of carbon tetrachloride and secondly with 25 ml. of chloroform, for the determination of salicylic and salicyluric acids respectively. The carbon tetrachloride and chloroform extracts were then shaken with the ferric nitrate solution as above.

The extinctions obtained in repeated determinations when a standard solution, containing 10 mg of salicylic acid and 50 mg of salicyluric acid per 100 ml., was treated in the same manner as urine were within 5% of the mean value.

Various amounts of salicylic and salicyluric acids were added together to normal urines. Table 1 gives the amounts recovered using the above method.

TABLE 1

RECOVERIES OF SALICYLIC AND SALICYLURIC ACIDS ADDED TO NORMAL URINE

| Amount added | | Recovery | |
|---|---|---|---|
| Salicylic acid (mg/100 ml.) | Salicyluric acid (mg/100 ml.) | Salicylic acid (%) | Salicyluric acid (%) |
| 5 | 25 | 101·5 | 108·5 |
| 10 | 50 | 90·0 | 92·0 |
| 15 | 100 | 109·0 | 95·0 |
| 20 | 150 | 109·0 | 92·0 |
| 30 | 200 | 106·0 | 90·0 |

*Experimental designs*

*The excretion of total salicylate after* 0.32 *and* 0.96 *g of aspirin.* On separate occasions two men each took one tablet and three tablets of aspirin. A complete collection of urine was made at intervals over the following 24 to 30 hr. The " total salicylate " concentration was determined.

*The rate of excretion of salicyluric acid.* Salicyluric acid (0.5 g) was administered orally to two men. Urine was collected at short intervals for 6 hr and the salicyluric acid concentration was determined after hydrolysis.

*The effect of dosage upon the rate of total salicylate excretion.* A group of five men each received on separate occasions 0, 0.16, 0.24, 0.32, 0.64 and 0.96 g of aspirin together with 100 ml. of water at 9.30 a.m. A complete collection of urine was made at 1.5-hr intervals for the following 7.5 hr, the urines being pooled at each collection. The " total salicylate " concentration of the urines was determined.

The experiment with 0.64 g of aspirin was repeated twice with the following modifications:

(1) 4 g of glycine were administered with the aspirin at 9.30 a.m. and a further 2 g of glycine were given at each 1.5-hr period except the last.

(2) The 0.64 g of aspirin was dissolved in 100 ml. of warm water immediately before administration.

*The effect of various doses of aspirin on salicyluric acid excretion.* Another group of five men, including three who took part in the previous experiment, each received 0, 2, 4, 8 and 12 mg of aspirin per kg body

weight. Urine collections were made as before. The salicylic acid and salicyluric acid concentrations were determined. In a second study they each received 5 mg/kg of salicyluric acid and on subsequent occasions the same dose of salicyluric acid together with 2, 4 and 8 mg/kg of aspirin. The urine salicylic acid and salicyluric acid concentrations were determined.

*Multiple-dose studies.* In the following experiments two men received 1 g doses of aspirin at approximately 6-hr intervals:

(1) Total salicylate excretion. In this instance seven doses were taken in all, the final dose being taken at 12.30 p.m. Complete urine collections were made at 3 and 6 hr after the final dose and at every 1.5 hr between 6.30 and 11 p.m. on the first day and 8 a.m. and 11 p.m. on the second day. The "total salicylate" concentration was determined.

(2) Salicyluric acid excretion. The above study was repeated, except that six doses of aspirin were taken. In order to study the excretion pattern over a period of more than 30 hr, but to avoid the necessity of collecting urine throughout the night, the study was again repeated with a different time schedule. Accordingly, the final dose was taken at 10.30 a.m. and urine collections were made every 1.5 hr from 6.30 a.m. to 11 p.m. on the following day. The concentrations of salicyluric acid and other salicylic acid derivatives were determined.

## RESULTS

The time course of excretion of total salicylate in the urine of two men who each received 0.32 and 0.96 g doses of aspirin is shown in Fig. 1, with the logarithm of the rate of excretion plotted against time after dosage.

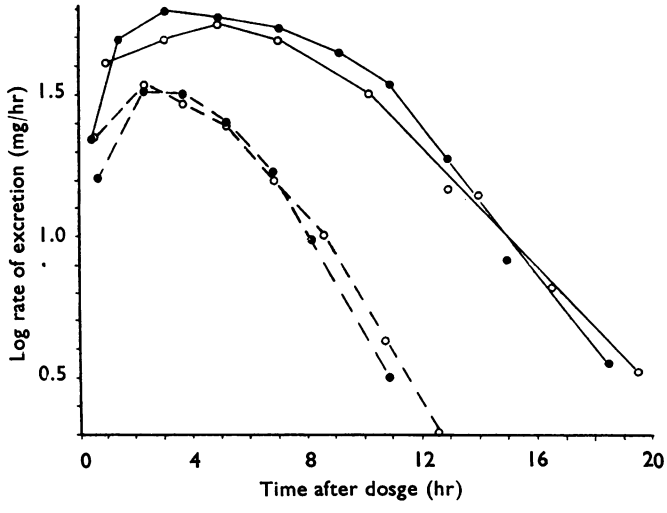


Fig. 1. The rate of excretion of total salicylate in two men (●, ○) after 0.32-g (— —) and 1-g (—) doses of aspirin.

A log-linear rate of decline is not obtained until about 8 hr after dosage. After 0.96 g of aspirin, the rate of total salicylate excretion is almost constant between the 2nd and the 8th hour after dosage and has an apparent first-order rate of decline from about the 12th hour. It may be noted that in these instances approximately 70% of the dose had been excreted before the rate of excretion was apparently first order.

The excretion of total salicylate by the group of five men who received graded doses of aspirin was studied over the first 7.5 hr after dosage and the results obtained are given in

Table 2 and Fig. 2. These results are remarkable in that after the three lowest doses the amount of total salicylate excreted is directly proportional to the dose given, whereas after the higher doses there is a deviation from this pattern and the rate at which total salicylate is excreted does not increase proportionately.

TABLE 2

THE COMBINED EXCRETION OF TOTAL SALICYLATE IN THE URINE OF FIVE MEN

The men each received oral doses of 0·16, 0·24, 0·32, 0·64 and 0·96 g of aspirin. * 4 g of glycine was given at 0 hr, and 2 g at 1·5, 3, 4·5 and 6 hr. †Aspirin was dissolved in 100 ml. of water

| Time of urine collection after dosage (hr) | Total salicylate excretion (mg) for dose of aspirin (g) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0·16 | 0·24 | 0·32 | 0·64 | 0·64* | 0·64† | 0·96 |
| 1·5 | 77·0 | 103 | 141 | 165 | 239 | 259 | 259 |
| 3 | 104·0 | 177 | 212 | 283 | 305 | 240 | 419 |
| 4·5 | 112·5 | 158 | 225 | 323 | 313 | 323 | 416 |
| 6 | 88·0 | 129 | 195 | 358 | 293 | 318 | 453 |
| 7·5 | 51·0 | 79 | 114 | 241 | 265 | 263 | 395 |
| % of dose excreted in 7·5 hr | 70·5 | 70·3 | 71·4 | 55·1 | 57·5 | 60·4 | 52·1 |

The effect of giving glycine or of administering the aspirin in solution is most marked in the first 1.5-hr period, which suggests that in both instances the initial rate of absorption of aspirin is increased slightly.

In the above experiments no allowance was made for differences in the body weight of the individuals taking part, and therefore the experiments were repeated with another group of five men who each received doses of 2, 4, 8 and 12 mg/kg of aspirin. The results

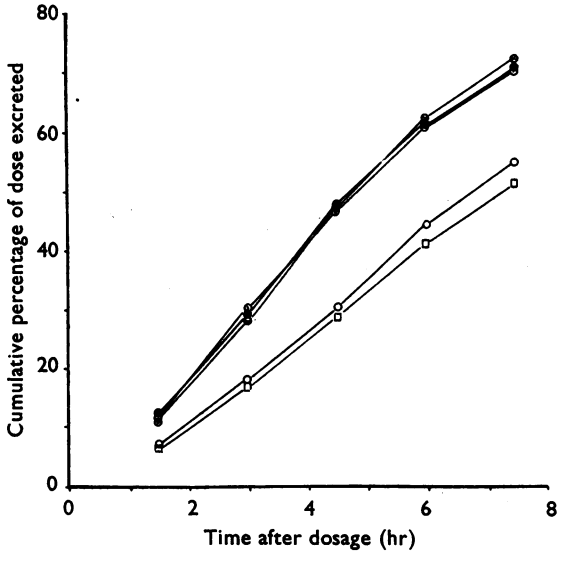


Fig. 2. The cumulative percentage of dose excreted as total salicylate in the urine of five men who each received 0.16 g (●), 0.24 g (○), 0.32 g (⊗), 0.64 g (○) and 0.96 g (□) of aspirin.

of this study (Table 3 and Fig. 3) again show that, as the dose increases, a decreasing proportion of the dose is excreted in the period of the experiment. This is related to the amount of salicyluric acid excreted, which does not increase in proportion to the dose. On the other hand, the amounts of the other salicylic acid derivatives excreted appear to increase in direct proportion to the dose.

TABLE 3

THE COMBINED EXCRETION OF SALICYLURIC ACID (SUA) AND OF OTHER METABOLITES OF SALICYLIC ACID (SA) BY FIVE MEN

On separate occasions each man received oral doses of 2, 4, 8 and 12 mg of aspirin per kg of body-weight. Amounts of salicyluric acid are expressed as equivalent of salicylic acid

| Time of urine collection after dosage (hr) | Excretion of salicyluric and salicylic acids (mg) per dose of aspirin (mg/kg) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | | | 4 | | | 8 | | | 12 | | |
| | SUA | SA | SUA / SA | SUA | SA | SUA / SA | SUA | SA | SUA / SA | SUA | SA | SUA / SA |
| 1·5 | 61·5 | 19 | 3·3 | 75 | 29 | 2·6 | 134 | 54 | 2·5 | 111 | 67 | 1·6 |
| 3 | 105·5 | 22 | 4·8 | 180 | 44 | 4·1 | 261 | 75 | 3·5 | 284 | 107 | 2·6 |
| 4·5 | 89·0 | 17 | 5·3 | 186 | 37 | 5·0 | 280 | 77 | 3·6 | 334 | 107 | 3·1 |
| 6 | 73·0 | 15 | 4·9 | 146 | 33 | 4·4 | 262 | 72 | 3·7 | 345 | 101 | 3·4 |
| 7·5 | 53·0 | 10 | 5·2 | 127 | 23 | 5·5 | 214 | 50 | 4·3 | 350 | 93 | 3·8 |
| Total excreted | 382·0 | 83 | 4·6 | 714 | 166 | 4·3 | 1,151 | 328 | 3·5 | 1,424 | 475 | 3·0 |
| % of dose excreted in 7·5 hr | 83·1 | | | 77·6 | | | 65·0 | | | 56·0 | | |

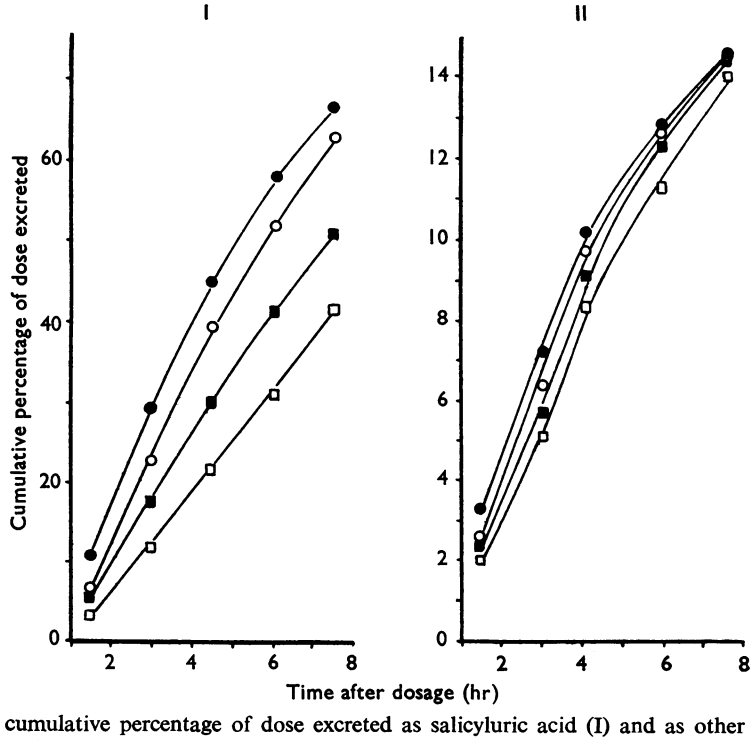


Fig. 3. The cumulative percentage of dose excreted as salicyluric acid (I) and as other metabolites of salicylic acid (II) by five men who each received 2 mg (●), 4 mg (○), 8 mg (■) and 12 mg (□) of aspirin per kg body weight.

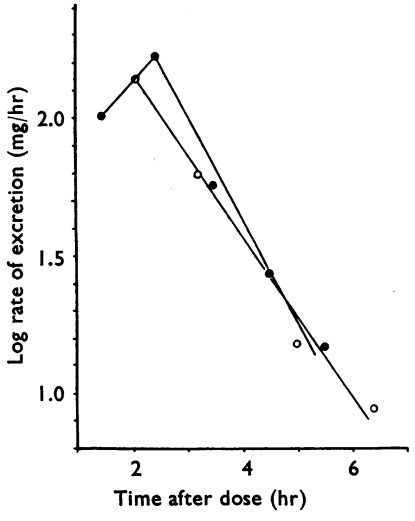


Fig. 4. The rate of excretion of salicyluric acid in the urine by two men (●, ○) after an oral dose of 0.5 g of salicyluric acid. Two results typical of those obtained in a number of repeated experiments are shown.

TABLE 4

THE EXCRETION OF TOTAL SALICYLATE (TSA), SALICYLURIC ACID (SUA) AND OTHER SALICYLIC ACID METABOLITES (SA) BY TWO MEN

TSA excretion was determined after seven doses, and SUA and SA after six doses each of 1 g of aspirin, taken at approximately 6-hr intervals. Salicyluric acid is expressed as equivalent of salicylic acid. Pairs of columns for SUA and SA refer to separate experiments

| Time of urine collection after final dose (hr) | Excretion of salicylate and salicyluric acid (mg) for Subject A | | | | | Subject B | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | TSA | SUA | | SA | | TSA | SUA | | SA | |
| 6 | 147 | 122 | — | 47·0 | — | 160 | 130 | — | 36·5 | — |
| 7·5 | 136 | — | — | 59·0 | — | 156 | 120 | — | 38·0 | — |
| 9 | — | 138 | 130·5 | 57·5 | 36·4 | 159 | 125 | 130 | 49·0 | 36·0 |
| 10·5 | 140 | 111 | 110·5 | 50·0 | 36·0 | 157 | 127 | 130 | 53·5 | 30·0 |
| 12 | — | — | 94·5 | — | 28·0 | 140 | — | 111 | — | 24·0 |
| 13·5 | — | — | 89·0 | — | 25·0 | — | — | 107 | — | 21·5 |
| 15 | — | — | 94·0 | — | 25·5 | — | — | 100 | — | 17·6 |
| 16·5 | — | — | 84·0 | — | 22·5 | — | — | 98·5 | — | 15·0 |
| 18 | — | — | 86·5 | — | 20·0 | — | — | 69 | — | 13·2 |
| 19·5 | — | — | 79·5 | — | 18·0 | — | 52·0 | 55·5 | 11·0 | 11·0 |
| 21 | 102 | 60·5 | 70·0 | 12·0 | 14·0 | 79 | 32·0 | 38·0 | 5·5 | 7·5 |
| 22·5 | 97 | 52·0 | 64·0 | 11·0 | 10·5 | 72 | 23·0 | 28·0 | 4·2 | 6·5 |
| 24 | 93 | 37·5 | 37·0 | 7·5 | 7·0 | 56 | 17·5 | 14·5 | 3·3 | 4·7 |
| 25·5 | 89 | 24·5 | — | 4·8 | — | 41 | 12·0 | — | 2·1 | — |
| 27 | 77 | 18·0 | — | 3·5 | — | 30 | 9·0 | — | 1·6 | — |
| 28·5 | 63 | 12·0 | — | 2·7 | — | 22 | 7·0 | — | 1·3 | — |
| 30 | 48 | 6·0 | — | 1·5 | — | 16 | 4·5 | — | 0·9 | — |
| 31·5 | 38 | 4·0 | — | — | — | 12 | 2·5 | — | — | — |
| 33 | 25 | 2·3 | — | — | — | 9 | 1·8 | — | — | — |
| 34·5 | 17 | 1·7 | — | — | — | — | — | — | — | — |

The effect of salicylate upon the excretion of salicyluric acid was investigated in the group of five men who took part in the above study. Aspirin did not appear to affect the rate of excretion of salicyluric acid and, conversely, salicyluric acid did not appear to affect the rate of excretion of the metabolites directly derived from the salicylic acid. A value of about 50 min was obtained for the half-life of salicyluric acid which was calculated from the plot of log rate of excretion against time (Fig. 4). Salicyluric acid was excreted mostly unconjugated and in these experiments about 90% of the dose was recovered.

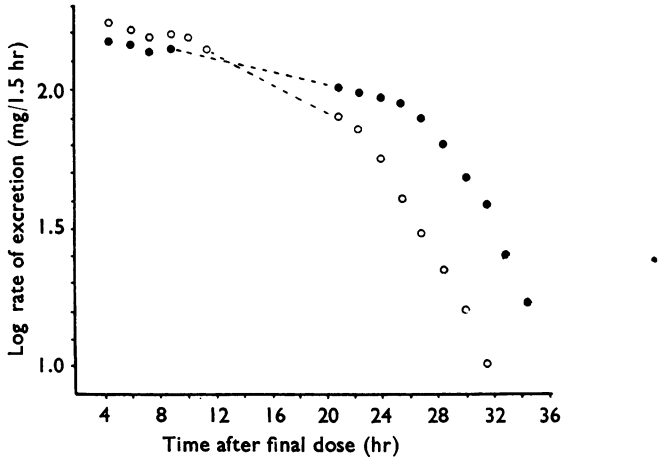


Fig. 5. The rate of excretion of total salicylate in two men (●, ○) after seven doses of aspirin (1 g) taken at approximately 6-hr intervals.

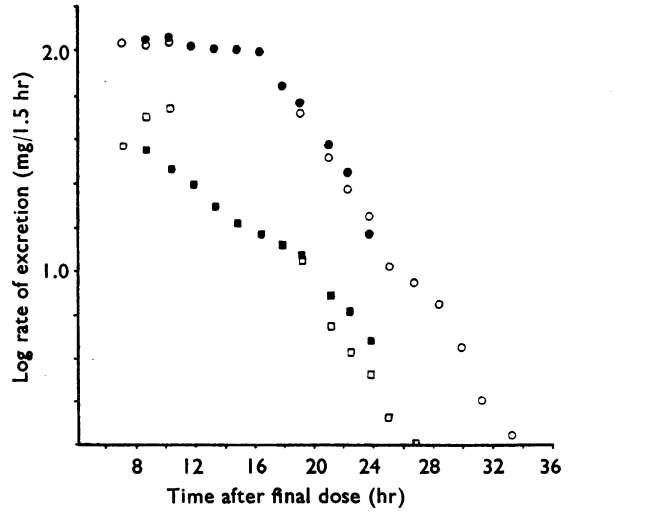


Fig. 6. The rate of excretion of salicyluric acid (○, ●) and other salicylic acid metabolites (□, ■) after six doses of aspirin (1 g) taken at approximately 6-hr intervals. The results of two separate experiments in one man are shown.

In the above studies no allowance has been made for variation in the rates of absorption and distribution which may occur with the different doses of aspirin and to obviate these factors the multiple dose studies were undertaken. The excretion of total salicylate and of salicyluric acid was determined in the urine of two men after administration of six or seven 1 g doses of aspirin (Table 4 and Figs. 5 and 6). The plots of log excretion rate of total salicylate and salicyluric acid against time are given from the 8th hr after the final dose until the excretion was almost complete; in these instances this was about the 34th hr.

From the results, it is seen that the plots of the log rate of excretion (mg/1.5 hr) of both total salicylate (Fig. 5) and salicyluric acid (Fig. 6) show a marked change of slope about 20 hr after the final dose. This change of slope is also discernible about 12 hr after a single 0.96 g dose of aspirin (Fig. 1). Also, the ratio of salicyluric acid to the other salicylic acid derivatives in the urine increases up to about the 20th hr but thereafter remains constant, indicating that the metabolites are then formed in a constant ratio.

## DISCUSSION

The multiple dose studies were undertaken to obtain moderately high levels of salicylate in the body and this method of drug administration was considered preferable to a single large dose. The higher the body salicylate level, the longer will be the period available for the study of excretion after the effects associated with drug absorption and metabolite accrual become negligible. In the present studies these effects are thought to be apparent for about 8 hr after the final dose and the results obtained during this initial period are for the moment excluded, but receive consideration later. After this initial period, there is an intermediate period from about the 8th to the 20th hr, during which the rate of excretion of salicyluric acid is virtually constant (Fig. 5) and a plot of the log rate of excretion of salicyluric acid against time is an almost horizontal straight line. At the end of this period there is an abrupt change in slope and there follows a final period which extends to about the 34th hr when excretion is virtually complete.

It appears, therefore, that when the amount of salicylate in the body exceeds a certain level there is a limit on the rate at which salicyluric acid appears in the urine. This may be attributed to a limit in its rate of formation, to a limit in its rate of renal clearance, or to a change in its renal clearance causing increased accumulation of salicyluric acid which then inhibits its further formation.

The available evidence indicates that the above limitation does not include a renal effect. Experiments involving the administration of salicyluric acid indicate that this substance is rapidly excreted with a half-life of 50 min, and its excretion has been observed to be first order over a range which appreciably exceeds the limited rate of excretion observed after the administration of aspirin. There is, therefore, no simple limit in the excretion of salicyluric acid when present alone. The administration of various doses of aspirin with salicyluric acid have failed to demonstrate that the simultaneous presence of aspirin and its metabolites with salicyluric acid appreciably modifies the total rate of excretion. Furthermore, if a renal effect were involved, an appreciable accumulation of salicyluric in the plasma would be expected, but this was not apparent after the administration of large doses of sodium salicylate in the studies of Schachter & Manis (1958). If the limited rate of excretion of salicyluric acid after aspirin were due solely to a renal effect, the rate of formation of

salicyluric acid and hence the rate of elimination of salicylate would not be affected. Results reported in the literature (Swintosky, 1956; Brodie *et al.*, 1959; Cummings & Martin, 1964) imply that the decline of the plasma salicylate may be log-linear only over a limited range of concentrations, and more recent studies by Cummings & Martin (unpublished) have confirmed this. Therefore, it is considered that the limited rate of excretion of salicyluric acid after the administration of aspirin is due to a limit in its rate of formation. Accordingly, the present findings are interpreted on the basis that, up to about the 20th hr, salicyluric acid is formed by an apparent zero-order process and that after this time it is formed by an apparent first-order process.

The plot of the log rate of excretion of total salicylate (Fig. 5) as from the 8th hr may again be considered in terms of an intermediate and final period and to consist of two linear sections, one of relatively shallow slope, followed by a second of much steeper slope. The change in slope which occurs at about the 20th hr is relatively sharp and the two sections of different slope coincide in time with the intermediate and the final phases encountered in the excretion pattern of salicyluric acid. It was expected that the log rate of excretion of total salicylate might be linear in the final period, but the observation that it was also linear in the intermediate period when salicyluric acid was formed by an apparent zero-order process was not expected.

Cummings, Martin & Park (1964) have recently considered an idealized model system in which a drug is eliminated by simultaneous first-order and zero-order processes and their results provide a theoretical basis for the present observation. They demonstrated that if a drug D is eliminated as two metabolites, M′ and M″, which are formed respectively by zero-order and first-order processes with specific rate constants $k'_{of}$ and $k''_f$, then:

$$\ln \frac{dM}{dt} = \ln (k'_{of} + k''_f D_o) - k''_f t \quad \ldots\ldots\ldots\ldots \quad (1)$$

where $M = M'_f + M''_f$, $M'_f$ and $M''_f$ represent respectively the amount of the metabolites formed in time t, and $D_o$ is the amount of drug present at t=o. Equation (1) indicates that a plot of the log rate of formation of total metabolites against time is a straight line having the same slope as if metabolite $M''_f$ were formed alone.

It has been suggested that when a metabolite is formed by a first-order reaction its rate of excretion cannot be equated to its rate of formation (Cummings & Martin, 1963), but if its specific excretion rate constant is appreciably larger than the specific rate constant governing its formation, then, after a certain time, the log rate of excretion of the metabolite will decline in parallel with its log rate of formation. Under the same conditions the rate of excretion of a metabolite formed in a zero-order process asymptotically approaches its rate of formation and there will, in this instance, be a period during which the two rates may be considered to be equal. After some time, t, when both the above conditions apply, changes in the rate of excretion of total metabolites will reflect changes in their rate of formation. The greater the first-order rate constants for the excretion of the metabolites, the shorter will be the time t. Then the rate of excretion of total metabolites ($dM_e/dt$) will be described by an equation of the same form as (1), that is:

$$\ln \left(\frac{dM_e}{dt}\right) = \text{Constant} - k''_f t \quad \ldots\ldots\ldots\ldots \quad (2)$$

Thus, equations (1) and (2) indicate that plots of the log rate of formation and log rate of excretion of total metabolites against time are parallel straight lines having a slope equal to $k''_t$.

When equation (2) is applied to the excretion of salicylic acid metabolites, M corresponds to the total salicylate, M′ to salicyluric acid and M″ to the group of metabolites which together with unchanged drug are estimated as salicylic acid. There is, therefore, theoretical support for the observation that the plot of the log rate of excretion of total salicylate is linear during the intermediate period when salicyluric acid is formed by an apparent zero-order process.

Although the excretion of total salicylate has a log-linear rate of decline, the elimination of salicylate is not first order during this intermediate period. The half-life of salicylate in the body under these conditions depends on the amount of salicylate present and decreases as elimination proceeds, for, when a drug is eliminated in the manner described above, its half-life ($t_{0\cdot5}$) is given by:

$$t_{0\cdot5} = \frac{1}{k''_t}\left[\ln 2 + \ln\left(\frac{k'_{ot} + k''_t D_o}{2k'_{ot} + k''_t D_o}\right)\right] \quad \ldots\ldots\ldots\ldots \quad (3)$$

This expression is derived from equation (2) in the paper by Cummings *et al.* (1964).

The ratio in which the metabolites occur in the urine during the intermediate period (Table 4) shows a progressive increase in the percentage of total metabolites excreted as salicyluric acid. This is consistent with the theoretical considerations. The rate of formation of salicyluric acid is $k'_{ot}$, whilst the rate of formation of the other metabolites at time t will be equal to $k''_t D_t$ which therefore diminishes as the amount of drug in the body, $D_t$, becomes smaller; consequently the ratio $k'_{ot} : k''_t D_t$ increases during this intermediate period.

During the final period the plot of the log rate of excretion of salicyluric acid is apparently linear and parallel to the plot of the log rate of total salicylate excretion and it is considered that all metabolites are now formed in constant ratio by first-order processes.

A number of rate constants may be calculated from data such as those obtained in the present studies. For example, the slope of the log rate of excretion of total salicylate during the final period (Fig. 5) provides the overall rate constant ($K = -2.303 \times$ slope). This constant will apply to the elimination of small doses of aspirin (0.32 g) but only to the initial and final phases of elimination after large doses. The slope of the first linear section of the same plot provides the sum of the individual first-order rate constants for the formation of all metabolites except salicyluric acid. The difference between the two rate constants, calculated as above, represents the first-order rate constant for the formation of salicyluric acid which is applicable at low plasma salicylate levels. At higher plasma levels, the rate of formation of salicyluric acid is zero order and the rate constant approximates to the observed amount of salicyluric acid excreted per hour. However, critical comment must be made concerning the calculation of the slope of the log rate of excretion of total salicylate and of salicyluric acid during the final period. The accrual of a metabolite formed in a zero-order reaction will continue until the rate limitation ends and there will then be a further interval before the plot of its log rate of excretion becomes linear and parallel to the log of its first-order rate of formation. The accrual of all other metabolites will be complete within the initial period. This, together with experimental errors which assume an increasing

importance with the decline of the rate of excretion to very low levels, makes it difficult to establish when these plots become truly linear.

For practical purposes it is convenient to refer to a particular dose of aspirin, or to a particular plasma salicylate level, above which the rate of elimination of salicylate can no longer be described by first-order kinetics. The limitations of doing so must be realized, for more accurately the rate of formation of salicyluric acid will approach a limiting value when the salicylate concentration at the site of the synthesis exceeds a certain critical concentration. The dose of aspirin, or the plasma salicylate level required for this critical concentration to be exceeded, will depend upon a number of personal factors in an individual, which include body weight, the binding capacity of the plasma proteins and, in particular, the rate of absorption of aspirin from the gastrointestinal tract. In this last respect a given dose of aspirin, if rapidly absorbed, could provide a salicylate level in excess of the critical concentration, whereas the same dose slowly absorbed might provide a level which would remain below the critical concentration. An explanation based on this fact was advanced by Cummings & Martin (1964) to explain the different excretion patterns observed when aspirin is rapidly absorbed and when it is more slowly absorbed from aloxiprin. An approximate value of the critical dosage was first obtained by studying the excretion of total salicylate in the pooled urine of five men after various small doses of aspirin (Tables 2 and 3 and Figs. 2 and 3). In these studies, the rate of total salicylate excretion was observed to be proportional to the dose of aspirin administered when this did not exceed 0.32 g of aspirin. When the dose exceeded 0.32 g the observed rate of total salicylate excretion was no longer proportional to the dose. This could be attributed to the fact that the amount of salicyluric acid excreted does not increase in proportion to the dose.

It is of interest to seek a correlation between the body salicylate level or dose at which the elimination of salicylate ceases to be first order in the multiple-dose studies and the single-dose studies. From a knowledge of the total salicylate excreted during the final log-linear phase of the multiple-dose studies, and assuming the recovery of 80% of a dose, it can be calculated that 150 to 300 mg of salicylic acid were present in the body at the beginning of this period. These figures are in reasonable agreement with the findings of the single-dose study, namely that the rate of excretion of total salicylate was not proportional to the dose when two tablets of aspirin were administered, that is 500 mg of salicylic acid.

Such calculations are, however, based on the assumption that no metabolite of salicylic acid is present in the body and, consequently, overestimate the amount of drug actually present. An alternative method of calculation may be based on the relationship $k'_{ot}=k''_{t}D_{crit}$, where $D_{crit}$ is the critical amount of the drug.

The excretion of total salicylate during the first 8 hr after aspirin administration (Fig. 1) presents a changing pattern which may be attributable to a number of factors. It is considered that a 0.32 g dose of aspirin is rapidly absorbed from the gastrointestinal tract so that the plasma salicylate concentration and the rate of metabolite formation reach their respective maxima within 2 hr of dosing and thereafter decline in parallel with apparent first-order rate constants. The effects associated with the absorption, distribution and the hydrolysis of aspirin, will only appreciably affect the rate of excretion during the first 2 hr. Whilst a maximum rate of metabolite formation has probably been reached within 2 hr, the rate of total salicylate excretion does not become maximal for at least 4 hr and achieves an apparent first-order rate of decline only after 8 hr (Fig. 1). These results may be

explained by the concept of metabolite accrual which has been discussed in a previous paper (Cummings & Martin, 1963). After a 1 g dose of aspirin an additional factor becomes apparent, the limited rate of metabolite formation. The rate of excretion of total salicylate has the same final log-linear rate of decline from about 12 hr, but an approach to a log-linear decline of shallow slope is also discernible from the 8th to the 12th hr. That is, the excretion pattern obtained in this instance shows a resemblance to the pattern of total salicylate excretion observed after higher doses of aspirin.

The half-life of salicylate is constant only when the amount of salicylate present in the body is less than the critical level, when all the metabolites are formed by first-order processes. The critical dose has been shown to be about 300 mg of salicylic acid. Calculation of the half-life from the plot of the log rate of excretion of total salicylate during the final period gives values of 2.5 to 4 hr.

The most remarkable feature of this investigation is the observation that the rate of formation of salicyluric acid is limited at such an exceptionally low body salicylate level. It is well known that the velocity of an enzymic reaction reaches a limited value as the substrate concentration increases, and the concentration of the substrate then declines with a zero-order rate constant; also, the overall velocity of a series of enzymic reactions will be that of the slowest reaction.

The change in the rate of formation of salicyluric acid as the body salicylate concentration increases can be attributed to the saturation of an enzyme system resulting in a limited availability of an essential reactant, so that the velocity of the reaction becomes dependent on the concentration of this reactant and not upon the concentration of salicylate present. In this way the concentration of the reactant will reach a limiting value as the concentration of salicylate is increased and then salicyluric acid will be formed with an apparent zero-order rate constant. The limited rate of formation of salicyluric acid (*o*-hydroxyhippuric acid) may be compared with that of hippuric acid. It has long been known that the rate of formation of hippuric acid from benzoic acid is limited in man (Quick, 1931; Snapper, Greenspan & Saltzman, 1946), and a detailed kinetic study of this phenomenon in the rabbit was made by Bray, Thorpe & White (1951, 1952), who established that after large doses of benzoic acid the elimination proceeded by simultaneous zero-order and first-order processes.

The kinetics of salicyluric acid formation are not known in detail and the rate limiting step in the reaction must therefore be the subject of speculation. If it is assumed that salicyluric acid is formed *in vivo* by a process essentially the same as that which forms hippuric acid from benzoic acid (Schachter & Taggart, 1953), at least two processes may be effected by the presence of salicylate. Salicylate is known to suppress the transport of certain amino acids across cell membranes (Segal & Blair, 1963; Quastel, 1963) and in this instance may be limiting the access of glycine to the conjugating enzyme at the site of salicyluric acid synthesis. If this were so, the administration of glycine together with aspirin would not appreciably alter the rate of salicyluric acid synthesis; this was a finding of the present study.

Salicylate is also known to uncouple oxidation and phosphorylation and its presence could limit the amount of adenosine triphosphate available which may then govern the velocity of the reaction.

Despite many observations relating to the influence of salicylic acid on various enzyme systems and its action in uncoupling oxidation and phosphorylation, the consequence of these actions is not clearly or directly apparent after therapeutic or even after toxic doses of aspirin. The present observation of a limit in the rate of formation of one of the metabolites of salicylic acid in man, at a dosage level of one aspirin tablet, demonstrates a biochemical effect which operates at therapeutic levels. If this effect can be attributed to the saturation of a particular metabolic or transport system, it is pertinent to speculate concerning its effect on any other endogenous substance which uses the same system.

## SUMMARY

1. The rate of elimination of salicylate following the administration of single and multiple doses of aspirin has been investigated by determining the rate of excretion in the urine of " total salicylate " and salicyluric acid.

2. The excretion patterns are considered during: (i) an initial period when drug absorption and distribution and metabolite accrual affect the rate of excretion; (ii) an intermediate period when the rate of excretion indicates that drug elimination is by simultaneous apparent first-order and zero-order processes; and (iii) a final period when the log rates of excretion tend to decline in parallel, indicating that all metabolites are now formed by apparent first-order processes.

3. Plots of the log rates of excretion against time show a marked change of slope between the intermediate and final periods. The length of the intermediate period in particular depends upon the dose of aspirin; all three periods are discernible after 1 g and are readily apparent after higher doses.

4. Results indicate that the formation of salicyluric acid is rate limited at the body salicylate concentration obtained after a single dose of aspirin greater than 0.3 g.

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