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# THE ELIMINATION OF SALICYLIC ACID IN MAN: SERUM CONCENTRATIONS AND URINARY EXCRETION RATES

BY

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Previous communications from this laboratory (Cummings, 1963; Martin, 1964; Cummings & Martin, 1964; Cummings, Martin & Park, 1964) have referred to the observation that there is a limiting value for the rate of formation of salicyluric acid, the major metabolite of salicylic acid in man. Evidence has been subsequently presented by Bedford, Cummings & Martin (1965) indicating that the rate of elimination of salicylic acid can be described as a first order process only when the amount present in the body is less than 0.3 g. At higher levels elimination is not first order and this was considered to be a consequence of the formation of salicyluric acid by an apparent zero order process. This evidence was obtained from urinary excretion studies, and similar results have recently been reported by Levy (1965b). Corresponding features should, however, also be apparent in the decline of the plasma salicylate concentration.

The decline of the plasma salicylate concentration in various short-term studies has frequently been interpreted at being log-linear, that is first order (Swintosky, 1956; Brodie, Burns & Weiner, 1959; Bayles, 1963; Done, 1963; Levy & Hollister, 1964). An invariable first order rate of decline is not consistent with the urinary excretion results or with the widely different values reported for the half-life of salicylic acid at high and low plasma salicylate concentrations. Values of about 19 hr were calculated by Swintosky (1956) when the plasma salicylate concentration was high, whereas a range of 4 to 9 hr was observed by Brodie *et al.* (1959) when the levels were low.

It was considered that a long-term study of the decline of the salicylate concentration in serum from moderately high values would resolve these discrepancies. The present report describes an investigation of the rate of decline of the serum salicylate concentration in man over a period of 2 days and a study of the rate of excretion of salicyluric acid in urine. A new specific method has been used for the determination of salicyluric acid.

## METHODS

### *Drugs*

The salicylate was administered as acetylsalicylic acid (0.324 g) tablets B.P.

### *Experimental design*

Five healthy men of 20 to 30 years of age took part in the study; no restriction was placed on their diet or movements. Each received six doses of 0.972 g of acetylsalicylic acid at approximately

6 hr intervals. Blood samples were collected into plain tubes at 3, 8, 14, 20, 26, 33, 38, 45 and 50 hr after the final dose. A complete urine collection was made at 1.5 hr intervals between 8 a.m. and 8 p.m. on the 2 days following the final dose.

*Chemical methods*

*Serum salicylate.* A modification of the method of Brodie, Udenfriend & Coburn (1944) was used for the determination of the serum salicylate concentration. The serum (2 ml., or 1 ml. with 1 ml. of water) and 2% aqueous malonic acid (2 ml.) were extracted with chloroform (25 ml.). The filtered chloroform extract (20 ml.) was shaken with 0.05% ferric nitrate solution (4 ml.), and the extinction of the aqueous phase was measured at 530 mμ. Appropriate standards were treated similarly.

*Unconjugated salicyluric acid in the urine.* The 1.5 hr urine specimens were diluted to 200 ml. and were stored frozen solid until analysed. It has been observed that salicyluric acid in urine is stable for at least 10 days under these conditions, but that appreciable loss by hydrolysis to salicylic acid can occur if the urine becomes contaminated with micro-organisms. The salicyluric acid was isolated by thin-layer chromatography on silica gel (Merck GF 254), using the solvent system benzene:ether:acetic acid:methanol (120:60:18:1, v/v), which has previously been shown to separate salicylic acid and its metabolites (Cummings & King, 1966). The urine (0.3 ml.) was applied as a thin band along the width of a 20 × 20 cm plate and the chromatogram was developed until the solvent front had moved 12 cm. The salicyluric acid band, which was visualized in ultraviolet light, was quantitatively transferred into methanol (1 ml.) and well mixed. Water (4 ml.) was added and after shaking the silica gel was removed by centrifugation. The extinction of the supernatant fluid was measured at 295 mμ against a reagent blank. Salicyluric acid standard solutions were treated similarly and a plot of extinction against concentration was linear over the range used.

## RESULTS

The present investigations were designed to establish the general pattern of decline over a wide concentration range rather than to provide detailed information over a narrow range. The decline of the serum salicylate concentration has been determined in five men for a period of 2 days after dosage with acetylsalicylic acid. The results obtained in this study are given as plots of log concentration against time (Fig. 1) and concentration against time (Fig. 2). The logarithmic plots (Fig. 1) are curves which appear to approach linearity only when the serum salicylate level has fallen to below 0.3 mM (4 mg/100 ml.), and the curvature of these plots indicates that the rate of elimination of salicylate becomes relatively more rapid as the concentration falls. The plots in Fig. 2 appear to be almost linear for about 20 hr after the final dose.

The rates of excretion of salicyluric acid in the urine of three of the five men who took part in the study have been determined between the 5th to 16th hour and the 26th to 35th hour after the final dose of acetylsalicylic acid. The plots of log rate of excretion (mmole/1.5 hr) against time (Fig. 3) are in good agreement with results previously reported (Bedford *et al.*, 1965). Although the rate of excretion of salicyluric acid (Fig. 3) is almost constant in the first period, a distinct, though slight, decline in the rate is apparent which shows every indication of continuing until about the 20th hour. Thereafter, the decline of the rate is much more rapid and the plots show a marked change of slope. The maximum rates of excretion of salicyluric acid ranged between 0.6 to 0.92 mmole/1.5 hr.

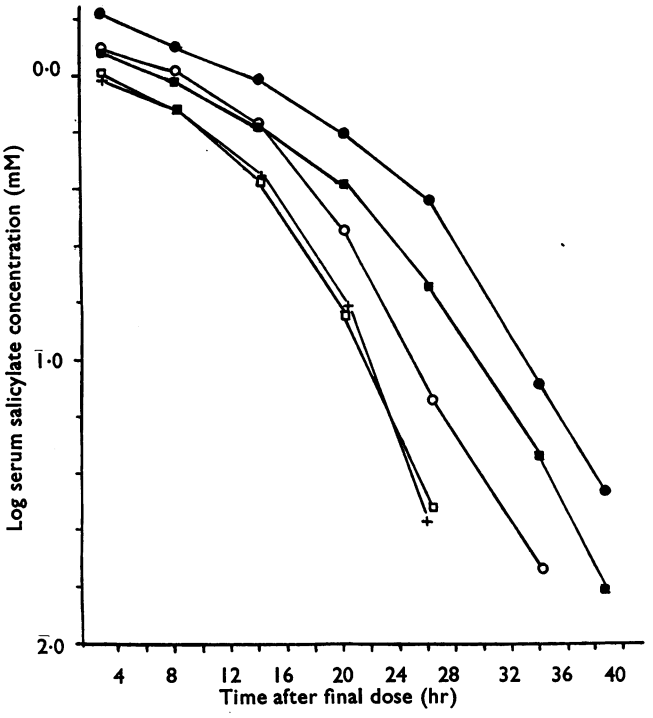


Fig. 1. The decline of the log of the serum salicylate concentration in five men after each had received six doses of 0.972 g of acetylsalicylic acid at approximately 6 hr intervals.

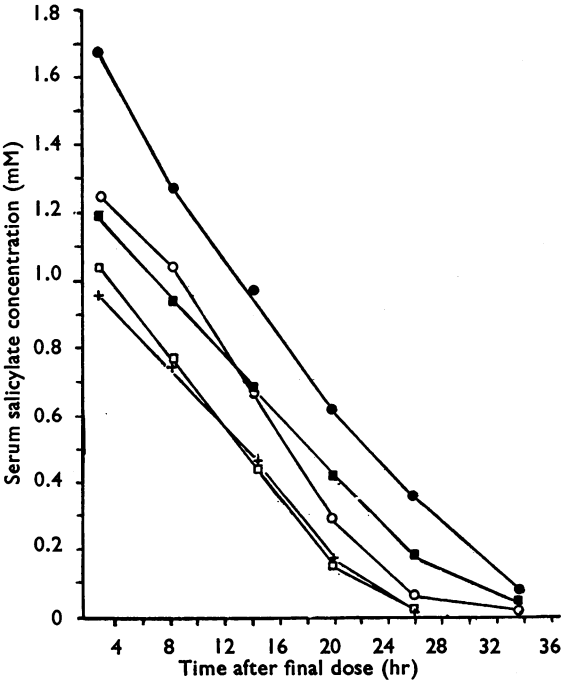


Fig. 2. The decline of the serum salicylate concentration in five men after each had received six doses of 0.972 g of acetylsalicylic acid at approximately 6 hr intervals.

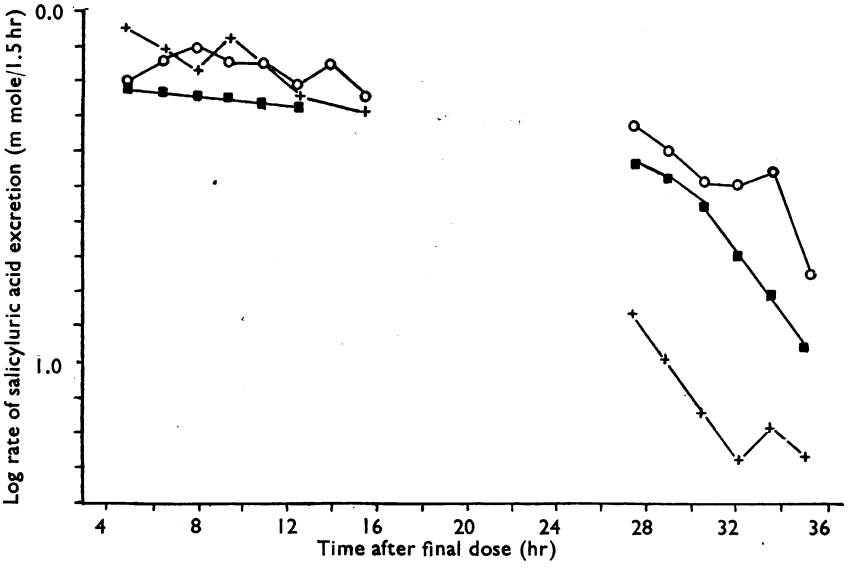


Fig. 3. The decline of the rate of excretion of salicyluric acid in three men after each had received six doses of 0.972 g of acetylsalicylic acid at approximately 6 hr intervals.

## DISCUSSION

The results of the present studies modify the commonly accepted concept that the decline of the log of serum salicylate concentration is linear over a wide range of concentrations. The elimination of salicylic acid in man has frequently been interpreted as log-linear by workers in this field, but the serum results on which these interpretations were based were usually obtained in studies restricted to relatively short periods of time. Nevertheless, Salassa, Bollman & Dry (1948) had reported results of a detailed study of the decline of the plasma salicylate concentration in one man over 32 hr which showed that the decline was not log-linear. The kinetic significance of their findings do not, however, appear to have been generally appreciated.

When the serum salicylate is within the range 1.5 to 0.3 mM (21 to 4 mg/100 ml.), the salicylate concentration closely approaches a simple linear rate of decline, which suggests that during this time the elimination of salicylic acid is predominantly zero order. The results of Salassa *et al.* (1948) can be similarly interpreted. This is in good agreement with the urinary excretion values of both the former study (Bedford *et al.*, 1965) and the present investigation. The rate of excretion of salicyluric acid shows only a slight decrease during this period and, as it constitutes the major route of elimination, indicates that the elimination of salicylic acid is largely zero order in character. When the serum salicylate concentration is below 0.3 mM, the elimination of salicylic acid may well be first order.

It has been previously shown that the excretion rate constant for salicyluric acid is considerably greater than the elimination rate constant for salicylic acid (Bedford *et al.*, 1965) and considerations of metabolic accrual indicate that if salicyluric acid were

formed by a zero order process its rate of excretion in the urine would increase to approach a limiting value equal to its rate of formation. It follows that the slight decline in the rate of excretion of salicyluric acid which is observed in the first period studied (Fig. 3) is not fully consistent with a zero order rate of formation. Therefore, the rate of formation of salicyluric acid progressively approaches a limiting vaue at high salicylate concentrations, but cannot be precisely described as zero order.

The velocity of an enzymic reaction is directly proportional to the substrate concentration only when the enzyme is present in considerable excess. At higher substrate concentrations the velocity becomes relatively slower and at very high concentrations, when the enzyme approaches saturation with substrate, the velocity approaches a limiting or maximum value. The changes observed in the rate of excretion of salicyluric acid appear to be of this pattern and to be explicable in terms of Michaelis-Menten kinetics (Levy, 1965a).

When the serum salicylate concentration exceeds 0.3 mM, one of the processes in the synthesis of salicyluric acid approaches a maximum velocity. This could be the result of the progressive saturation of an enzyme involved in the synthesis and, on this basis, the rate of formation of salicyluric acid could be limited by the amount of enzyme available at the metabolic site. An alternative explanation must also be considered, for transport systems which involve the concept of a "carrier" are also subject to saturation at high substrate concentrations and Michaelis-Menten kinetics are also applicable. Consequently the saturation of any carrier involved in the transport of an essential substrate to the site of salicyluric acid synthesis could provide an excretion pattern similar to that observed in the present study. On this basis, the rate of formation of salicyluric acid could be limited by the capacity of a carrier involved in the transport mechanism.

The possibility that the limiting factor may be the rate of transport of glycine to the site of synthesis has been considered (Bedford *et al.*, 1965). Calculations from available values (Quick, 1931) reveal that in the synthesis of hippuric acid glycine can be utilized at a rate which is about ten times greater than that required for the maximum observed rate of salicyluric acid synthesis. If the site and mode of synthesis of these metabolites are similar, these considerations would imply that the transport of glycine is suppressed by the simultaneous presence of salicylic acid. However, when *p*-aminobenzoic acid was administered after a loading dose of acetylsalicylic acid, the rate of excretion of *p*-aminohippuric acid was not obviously decreased (Cummings & Martin, unpublished), which suggests that salicylate has no effect upon the availability of glycine.

There is the alternative possibility of a limitation in the rate at which salicylic acid reaches the metabolic site. The absorption of salicylic acid from gastric fluid (*p*H 2) may be explained on the basis of passive diffusion of the undissociated molecule (Schanker, Shore, Brodie & Hogben, 1957), but transport of salicylic acid within the body presents a different situation. At *p*H 7.0 the ratio of salicylate ions to undissociated salicylic acid molecules is about 10,000 to 1 and, even if the specific rate constant for the transport of salicylate ion were very small, the transport of ion could under these conditions contribute appreciably to the total rate of transport. Current concepts imply that the transport of salicylate ion would require a carrier mechanism and this could

be subject to saturation at high salicylate concentrations and to competitive inhibition by other ions using the same transport system.

At the concentrations applying in the present studies, the major route for the elimination of salicylic acid was by metabolism to salicyluric acid and, therefore, the rate of elimination has closely corresponded to the rate of formation of salicyluric acid. However, due to the limitation on the rate of formation of salicyluric acid, a diminishing proportion of the drug will be eliminated by this route at very high salicylate concentrations and a correspondingly greater proportion will be eliminated as free drug and other metabolites, for example, glucuronides and gentisic acid, if these other metabolites are formed by first order processes. Then after very large or toxic doses, the elimination of salicylic acid could approach a first order rate. The results of Salassa *et al.* (1948) suggest that when the formation of salicyluric acid is suppressed by the simultaneous administration of *p*-aminobenzoic acid, the elimination of salicylic acid is first order. Also, Cumming, Duke & Widdowson (1964) have reported that, under conditions of alkaline diuresis, the decline of the serum salicylate concentration appeared to be log-linear within the concentration range 100 to 30 mg/100 ml.

## SUMMARY

1. The serum salicylate concentration closely approached a simple linear rate of decline when the concentration exceeded 0.3 mM.

2. Serum salicylate and salicyluric acid excretion values have indicated that the rate of elimination of salicylic acid is predominantly zero order when the rate of formation of salicyluric acid approaches a maximum value.

3. The limitation of the rate of formation of salicyluric acid has been considered in terms of the saturation of an enzyme or of a transport system.

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