

## **The kinetics of elimination of salicylic acid and the formation of gentisic acid**

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1. A method for the estimation of gentisic acid in urine has been devised which is based on thin-layer chromatography and fluorimetry.
  2. In man the urinary excretion of gentisic acid accounted for 0.6% of a 0.32 g dose of aspirin and 1.1% of a 1.28 g dose. The increase in the percentage of the dose excreted as gentisic acid provides further evidence that the elimination of salicylic acid cannot be entirely described by first order kinetics.
  3. Equations are presented which describe the amount of gentisic acid formed from various doses of salicylic acid in a model system, when elimination proceeds partly by simultaneous first order and zero order kinetics. The close agreement of the experimental and theoretical results indicates that the model provides an acceptable interpretation of salicylic acid elimination in man.
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The elimination of salicylic acid in man is complex. After a small dose, elimination closely corresponds to a first order process, salicyluric acid being the major metabolite. After a large dose, elimination cannot be described by first order kinetics during the entire period of elimination and a smaller proportion of the dose is excreted as salicyluric acid (Cummings & Martin, 1964; Cummings, Martin & Renton, 1966). There appears to be a limit to the rate at which the body can form salicyluric acid and this limit is approached when the amount of salicylic acid in the body exceeds a value of about 0.3 g (Bedford, Cummings & Martin, 1965; Levy, 1965). At higher concentrations the elimination of salicylic acid can be more accurately described by simultaneous zero order and first order kinetics (Cummings, Martin & Park, 1964; Cummings & Martin, 1968). Theoretical considerations indicate that the other metabolites of salicylic acid, unless similarly limited in their rate of formation, would then account for a large fraction of the dose.

One of the minor metabolites of salicylic acid in man is gentisic acid (2:5-dihydroxybenzoic acid). It has been reported that in man this accounts for the excretion of 4–8% of doses of salicylic acid (Kapp & Coburn, 1942; Roseman & Dorfman, 1951; Batterman & Sommer, 1953; Arata & Mangiola, 1962). After a single 1 g dose of aspirin, gentisic acid accounted for 1% of the total (Alpen, Mandel, Rodwell & Smith, 1951).

The present work describes the excretion of gentisic acid in the urine after single doses of aspirin, particular attention being given to the effect of the size of the dose

on the proportion eliminated as gentisic acid. Equations are presented which describe the amount of gentisic acid formed from various doses of salicylic acid in a model system, when elimination proceeds partly by simultaneous first order and zero order kinetics. Close agreement of the experimental and theoretical results would support the present interpretation of salicylic acid elimination in man.

## Methods

The total amount of gentisic acid excreted in the urine was measured after a low dose (0.32 g) and after a high dose (1.28 g) of acetylsalicylic acid. The dose of 0.32 g was selected on the basis that the elimination of salicylic acid resulting from this dose could be interpreted as proceeding entirely by first order processes. Aspirin tablets (0.32 g) were administered as a conventional precursor of salicylic acid.

Six healthy men between 20 and 40 years of age took part in this study; no restriction was placed on their diet or physical activity. Control urine samples were collected on the days before drug administration. Each subject then received a single oral dose of acetylsalicylic acid (0.32 g) and a complete urine collection was made for 33 hr after dosing. A second dose of acetylsalicylic acid (1.28 g) was administered 48 hr after the first dose and urine was again collected for 33 hr.

### *Chemical methods*

Preliminary studies confirmed the findings of Batterman & Sommer (1953) and Arata & Mangiola (1962) that after the administration of gentisic acid or aspirin some of the gentisic acid in the urine was present in a conjugated form. In order to estimate the total gentisic acid formed in the body from salicylic acid, the urine was heated with acid for 30 min to hydrolyse the conjugate. This treatment did not decompose the gentisic acid, and increasing the period of hydrolysis to 1 hr did not further increase the amount of free gentisic acid.

Urine samples were made up to 3,000 ml. with water. An aliquot (50 ml.) was heated on a steam bath for 0.5 hr with concentrated HCl (8 ml.), cooled, and continuously extracted with ether for 2 hr. The ether was evaporated, the residue dissolved in ethanol and made up to 5 ml.

The gentisic acid was isolated by thin-layer chromatography on silica gel (Macherey, Nagel G-HR/UV 254) using the solvent system, benzene:ether:acetic acid:methanol (120:60:8:2 v/v). Aliquots (100  $\mu$ l.) of the ethanol solutions were applied as bands to thin-layer plates (20 cm  $\times$  20 cm) divided into 4 cm strips. An aliquot of the corresponding blank sample was applied to an adjacent strip.

After development, the solvents were allowed to evaporate and the gentisic acid visualized in ultraviolet light. An area of silica gel containing the gentisic acid was quantitatively transferred to a filter tube and eluted with water (4 ml.). Acetate buffer solution (5 ml., 0.1 M, pH 6) was added and the sample made up to 10 ml. with water. A corresponding area from the blank sample was treated in an identical manner. The relative fluorescent intensities of the samples were recorded on an Aminco-Bowman spectrophotofluorimeter (activating wavelength 325 m $\mu$ , fluorescent wavelength 445 m $\mu$ , uncorrected; No. 3 slit arrangement).

Known amounts of gentisic acid were added to samples of control urine and estimated using the above procedure to provide a calibration curve. The graph of relative fluorescent intensity against concentration was linear over the range used.

## Results

The results obtained in this study are shown in Table 1. All subjects excreted a higher proportion of the dose as gentisic acid after the large dose (1.28 g) of aspirin than after the small dose (0.32 g). The average percentage of the dose excreted as gentisic acid was 0.6% and 1.1% respectively for the low and high doses and there was no overlap in the range of the individual values (0.3–0.8% and 0.9–1.3%).

## Discussion

The percentage of the dose eliminated as gentisic acid after the 1.28 g dose of aspirin is on average about double the percentage excreted after the 0.32 g dose. This provides further support for the view that the elimination of salicylic acid cannot be described by first order kinetics over this dosage range.

It is desirable to consider whether the increase in the proportion excreted as gentisic acid corresponds in magnitude with that which might be predicted if drug elimination proceeds partly by simultaneous first order and zero order processes. The model used by Cummings *et al.* (1964) and Cummings & Martin (1968) can be extended and applied to this consideration.

The following model portrays the elimination of a drug by urinary excretion and by the formation of four metabolites:

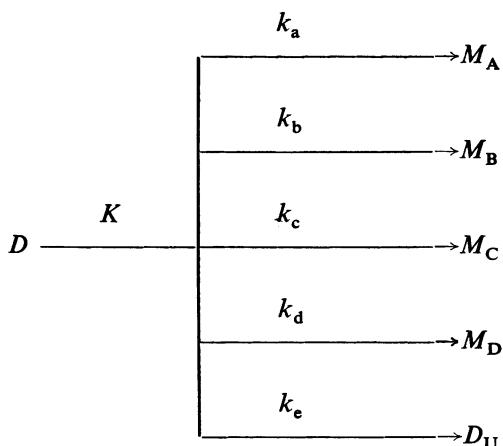


TABLE 1. Amount of gentisic acid excreted by six subjects after the administration of 0.32 g and 1.28 g of acetylsalicylic acid.

Subject	Gentisic acid excreted after aspirin				
	Dose 0.32 g		Dose 1.28 g		% of 1.28 g dose
	mg	% of dose	mg	% of dose	% of 0.32 g dose
A	1.2	0.4	13.3	1.2	3.0
B	1.3	0.5	14.5	1.3	2.6
C	2.1	0.8	11.1	1.0	1.3
D	0.8	0.3	11.0	1.0	3.3
E	2.3	0.8	11.3	1.0	1.3
F	1.3	0.5	9.8	0.9	1.8
	Average 0.6		Average 1.1		Average 2.2

Duplicate analyses were performed in many instances and did not differ by more than 6%.

where  $D$  is the amount of drug in the body at time  $t$ ,  $D_U$  is the amount of drug in the urine at time  $t$  and  $M_A$ ,  $M_B$ ,  $M_C$  and  $M_D$  are the amounts of the respective metabolites formed at time  $t$ .

When the amount of drug in the body does not exceed a certain critical level,  $D_A$ , drug elimination in the model proceeds entirely by first order processes and  $k_a$ ,  $k_b$ ,  $k_c$ ,  $k_d$  and  $k_e$  represent the relative first order rate constants and

$$K = k_a + k_b + k_c + k_d + k_e \dots\dots\dots(1)$$

When the amount of drug in the body exceeds the critical value  $D_A$ , the rate of formation of one of the metabolites,  $M_A$ , reaches a limiting value and it is then formed by a zero order process. The change in the kinetics of formation of  $M_A$  from first order to zero order is considered to occur abruptly when the drug level exceeds  $D_A$  and the zero order rate constant for its formation can therefore be equated to  $k_a D_A$ . The urinary excretion of drug and the formation of the other metabolites remain first order processes. When  $D$  is greater than  $D_A$ , then

$$-\frac{dD}{dt} = (k_b + k_c + k_d + k_e)D + k_a D_A \\ = (K - k_a)D + k_a D_A.$$

Integrating, with  $D = D_o$  at  $t = 0$ , gives

$$t = \frac{1}{K - k_a} \ln \left[ \frac{k_a D_A + (K - k_a) D_o}{k_a D_A + (K - k_a) D} \right] \dots\dots\dots(2)$$

Putting  $D = D_A$  in equation (2) therefore gives the time  $t_A$  that must elapse for  $D_o$  to decline to  $D_A$  and for all processes involved in drug elimination to become first order. Rearranging the equation,

$$t_A = \left[ \ln \left( 1 + \frac{(D_o - D_A)(K - k_a)}{D_A K} \right) \right] / (K - k_a) \dots\dots\dots(3)$$

If  $(M_A)t_A$  represents the amount of metabolite  $M_A$  formed in time  $t_A$ , then

$$(M_A)t_A = k_a D_A t_A \dots\dots\dots(4)$$

The amount of drug excreted in the urine and the amounts of the other metabolites formed (B, C and D) when the drug level declines from  $D_o$  to  $D_A$  can now be calculated, for together they account for an amount of drug equal to  $(D_o - D_A) - k_a D_A t_A$ . Thus, for metabolite B

$$(M_B)t_A = \frac{k_b}{(K - k_a)} [(D_o - D_A) - k_a D_A t_A] \dots\dots\dots(5)$$

Corresponding equations apply to the other metabolites.

The subsequent elimination of the remaining amount of drug  $D_A$  will proceed entirely by first order processes.

$$\text{So the amount of } M_A \text{ formed when } D_A \text{ is eliminated} = \frac{k_a}{K} D_A \dots\dots\dots(6)$$

$$\text{and the amount of } M_B \text{ formed when } D_A \text{ is eliminated} = \frac{k_b}{K} D_A \dots\dots\dots(7)$$

$M_{A\infty}$ , the total amount of  $M_A$  formed in the elimination of  $D_o$  is the sum of (4) and (6), so

$$(M_{A\infty}) = k_a D_A t_A + \frac{k_a}{K} D_A \dots\dots\dots(8)$$

Similarly, ( $M_{B\infty}$ ) is the sum of (5) and (7), so

$$(M_{B\infty}) = \frac{k_b}{(K - k_a)} [(D_o - D_A) - k_a D_A t_A] + \frac{k_b}{K} D_A \dots\dots\dots (9)$$

Subject to certain limitations this model can be applied to the elimination of salicylic acid; salicyluric acid assumes the character of metabolite A and gentisic acid, the metabolite selected for study, corresponds to metabolite B. The equations can therefore be used to calculate the amount of gentisic acid formed from various doses of salicylic acid in the model system. Equation (7) is used when the dose of salicylic acid is less than the critical amount ( $D_o < D_A$ ), for elimination will proceed entirely as a first order process. This equation can also be applied to a slightly larger dose when the absorption rate is such that this does not give rise to a drug level in excess of  $D_A$ . Equations (3) and (9) are used when the dose is in excess of critical amount ( $D_o > D_A$ ); equation (3) permits the evaluation of  $t_A$  which is then substituted in equation (9).

The results obtained by using a range of values of  $K$ ,  $k_a$  and  $D_A$  in the model system are shown in Table 2. The calculations relate to doses of 0.25 g and 1.0 g of salicylic acid, so as to correspond closely to the doses of aspirin used in the experimental study. The results are expressed as the ratio ( $R$ ) of the percentage of the dose excreted as gentisic acid after the high dose to the percentage excreted after the low dose.

The values of the ratio calculated in the model (range 1.5–3.0) are comparable with those observed experimentally (range 1.3–3.3). The close similarity of these results provides further support for the view that above a certain level the elimination of salicylic acid can be more accurately described by simultaneous first order and zero order kinetics.

The values of  $K$ ,  $k_a$  and  $D_A$  used in the calculations are those quoted in the literature. The first order rate constants,  $K$  and  $k_a$ , can be evaluated from experimental data collected after the administration of 0.32 g of aspirin. Calculations based on the data of Bedford *et al.* (1965) and Levy (1965), together with other results obtained in this laboratory, indicate that the first order elimination rate constant for salicylic acid ( $K$ ) is within the range 0.2–0.3 hr<sup>-1</sup>. The urinary recovery of salicyluric acid accounts

TABLE 2. *Excretion of gentisic acid in the theoretical model after the administration of salicylic acid at two different dosage levels.*

$D_A$ (g)	$R = \frac{\text{Gentisic acid (\% of dose) after 1.00 g dose}}{\text{Gentisic acid (\% of dose) after 0.25 g dose}}$			$R$
	$K$ (hr <sup>-1</sup> )	$k_a$ (hr <sup>-1</sup> )	$k_b$ (hr <sup>-1</sup> )	
0.15	0.200	0.150	0.0010	2.0
0.15	0.200	0.190	0.0010	3.0
0.20	0.200	0.150	0.0010	1.7
0.20	0.200	0.190	0.0010	2.3
0.25	0.300	0.225	0.0015	1.6
0.25	0.300	0.285	0.0015	2.0
0.25	0.200	0.150	0.0010	1.6
0.25	0.200	0.190	0.0010	2.0
0.30	0.200	0.150	0.0010	1.5
0.30	0.200	0.190	0.0010	1.7

for 70–92% of this dose, so that  $k_a = 0.70 K$  to  $0.92 K$ . In the present study, gentisic acid accounted on average for 0.6% of the 0.32 g dose of aspirin, so that  $k_p = 0.006 K$ .

It is difficult to assign a precise value to the critical level  $D_A$ . Bedford *et al.* (1965) observed that salicylic acid elimination closely conformed to a first order process after all doses of aspirin which did not exceed 0.32 g, but that a major departure from first order kinetics occurred after a dose of 0.64 g. On this evidence, the critical amount of salicylic acid is not greatly in excess of 0.24 g. Levy (1965) reported a figure of 0.28 g. Other studies based on the decline of the plasma salicylic acid concentration indicated that elimination could not be interpreted as first order until the salicylic acid in the body had decreased to 0.15–0.30 g (Bedford *et al.*, 1965). Whereas it is convenient to refer to a critical salicylic acid level in terms of the amount of drug in the body, Bedford *et al.* (1965) emphasize that this would be more accurately expressed in terms of the drug concentration at the metabolic site. Considerations of body weight imply that the amount of drug necessary to produce comparable drug concentrations may extend over a wide range. Accordingly, values of the critical amount which range from 150–300 mg have been used in the model.

There are certain inherent limitations in seeking to apply the theoretical model to the elimination of salicylic acid. Whereas the change in the kinetics of formation of salicyluric acid appears in practice to approach completion within a relatively short time, the change cannot be considered to be abrupt as in the model. A further limitation occurs in the attempt to relate  $D_0$  to the dose of salicylic acid (or equivalent dose of aspirin) which is administered orally. Absorption of drug is not instantaneous and after the oral administration of any large dose there will be a short period when the amount of drug in the body is below the critical level. Drug elimination will therefore proceed for a short time as a first order process. Furthermore, the maximum drug level attained in the body will consequently also be slightly less than  $D_0$ . The rate of drug absorption therefore introduces a further variable factor in respect of any dose which is greater than the critical amount  $D_A$ . Slow absorption of the drug will tend to reduce the effect associated with the critical level.

The present studies are in agreement with the report of Alpen *et al.* (1951) that the urinary excretion of gentisic acid in man accounts for about 1% of a 1 g dose of aspirin. They also establish that the proportion of the dose excreted as gentisic acid is dose dependent and offer an explanation of the higher values reported by other workers. In subsequent studies involving the administration of 4 g of aspirin daily, it was observed that gentisic acid constituted 1.3–3.3% of the dose (Boreham, Cummings & Martin, unpublished).

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