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# A KINETIC STUDY OF DRUG ELIMINATION: THE EXCRETION OF PARACETAMOL AND ITS METABOLITES IN MAN

BY

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In the preceding paper (Cummings, Martin & Park, 1967) theoretical considerations have been presented relating to a drug which is eliminated by apparent first order processes of urinary excretion and metabolism. These considerations have indicated that when the excretion rate constants of drug metabolites are greater than the elimination rate constant of the drug, plots of log rate of excretion of drug and of drug metabolite eventually become linear and parallel to each other. Experimental verification of this is now attempted with a study of the excretion of paracetamol and its metabolites in man.

When paracetamol is administered or is formed in the body from other drugs, such as acetanilide and phenacetin, it is eliminated by metabolism and to a small extent by excretion (Greenberg & Lester, 1946; Brodie & Axelrod, 1948, 1949). Although its metabolism in man does not appear to have been studied in detail, paracetamol is usually considered to be mainly metabolized to the sulphuric acid and glucuronic acid conjugates (Smith, 1958). It is also metabolized to a small extent to S-(1-acetamido-4-hydroxyphenyl) cysteine (Jagenburg & Toczko, 1964). Lester & Greenberg (1947) report that determinations of the increased excretion of ethereal sulphates and glucuronic acid in urine after the administration of acetanilide showed that approximately two-thirds of the *p*-aminophenol was excreted as sulphuric acid ester and one-third as glucuronide. The isolation of paracetamol sulphate from urine of patients who had received acetanilide was reported by Mörner (1889). There appears to be no report of the isolation of paracetamol glucuronide from human urine, although it has been isolated from the urine of rabbits dosed with paracetamol or with a drug which gives rise to paracetamol (Smith & Williams, 1949).

Studies of paracetamol elimination in man have been based on the determination of the free paracetamol and total paracetamol conjugates in urine. The half-life of paracetamol, calculated from the results of such studies, has been reported to be within the range 1.6 to 2.8 hr (Nelson & Morioka, 1963).

The present report gives details of the separate estimation of the rate of excretion of paracetamol, and of its glucuronide and sulphate in urine.

## METHODS

### *Chemical methods*

The following compounds were used as standards in the analytical procedures:

(i) Paracetamol B.P.

(ii) Paracetamol sulphate, potassium salt monohydrate. Potassium *p*-nitrophenylsulphate was prepared by the method of Burkhardt & Wood (1929) and reduced to the *p*-aminophenylsulphate by hydrogenation in aqueous solution using 5% palladium on charcoal at room temperature and atmospheric pressure. This was acetylated with acetic anhydride and recrystallized from aqueous ethanol to give *p*-acetamidophenylsulphate, which was isolated as the potassium salt. Analysis of the product gave: C, 33.0; H, 3.51; N, 4.91; $H_2O$, 6.27%. $CH_3CO$, 15.0%. (Calc. for $C_8H_8NOS$, K, $H_2O$: C, 33.4; H, 3.51; N, 4.88; $H_2O$, 6.35%. $CH_3CO$, 15.3%).

(iii) Paracetamol glucuronide, potassium salt, was isolated by the method of Kamil, Smith & Williams (1952) from the urine of rabbits which had been dosed with paracetamol. The product was dissolved in ethanol, 2 N-potassium hydroxide in methanol was added until the solution was slightly alkaline (pH 8). The precipitate was dissolved in a small volume of methanol, 0.5 vol. of ethanol was added and the precipitate collected. The product which was a white powder was dried and stored in a desiccator. Analysis gave: C, 45.6; H, 5.3; N, 3.8%. (Calc. for $C_{14}H_{17}NO_8$, K: C, 45.9; H, 4.7; N, 3.8%).

### *Separation of the standard compounds*

The separation of paracetamol, paracetamol sulphate and paracetamol glucuronide standards was effected by thin-layer chromatography on silica gel using the solvent system, ethyl acetate, methanol, water, acetic acid (60:30:9:1, v/v). With an ascending development, the $R_F$ values of these compounds approximated to 0.82, 0.63 and 0.25 respectively.

### *The determination of paracetamol and of two of its metabolites in urine after dosage with paracetamol*

Total urine collections were made at 1.5 hr intervals for 15 hr after the administration of paracetamol (12 mg/kg body-weight) to four men. Urine was also collected over four 1.5 hr periods on the day before the study, for the determination of an average "blank" value for the drug and the metabolites for each man.

Thin-layer chromatography of the urine collected after dosage with paracetamol revealed three substances with $R_F$ values respectively corresponding to those of paracetamol, paracetamol sulphate and paracetamol glucuronide. Further evidence for the identity of the substances isolated from the chromatograms, was obtained by comparison of their respective infra-red absorption spectra with those of the standard compounds.

Paracetamol, paracetamol sulphate and paracetamol glucuronide were determined in the 1.5 hr urine samples as follows. The urine (0.75% of volume) was applied to form a narrow 18 cm band near the bottom of a $20 \times 20$ cm thin-layer chromatography plate spread with a 0.3 mm layer of silica-gel (Merck, GF 254). The chromatograms were developed with the solvent described above until the front had travelled 12 cm, then paracetamol and the two metabolites were revealed as dark bands against a fluorescent background by examination under ultraviolet light. Horizontal lines were drawn across the plates to isolate three strips of silica gel each containing one of these compounds and for any one compound the width of the strip was kept constant; this procedure ensured a constant blank value. The whole of the silica gel within this strip was scraped off and quantitatively transferred into 5 ml. of water. Then, after shaking for 30 min the silica gel was removed by centrifuging and the extinction of the supernatant solution was measured at 240 mμ against a reagent blank. All three compounds exhibited maximum absorption at this wavelength.

Standard solutions of paracetamol, paracetamol sulphate and paracetamol glucuronide were treated in the same way as the urines. Plots of extinction against concentration for the three compounds were linear over the required range. The reagent blank depended upon the amount of silica gel removed from the chromatogram, the average values were equivalent to 22, 46 and 121

μ-mole/1.5 hr for paracetamol, its sulphate and its glucuronide respectively. The average urine blank values were zero for paracetamol and paracetamol sulphate and equivalent to 69 μ-mole/1.5 hr for paracetamol glucuronide.

*Recovery experiments*

Known amounts of paracetamol, paracetamol sulphate and paracetamol glucuronide were added to pooled urine from men who had received no recent medication and these solutions were analysed by the above procedure. The recovery of paracetamol and of each metabolite was 90–110% of the amount added.

## RESULTS

The amounts of paracetamol, paracetamol sulphate and paracetamol glucuronide excreted in the urine in consecutive 1.5 hr periods up to the fifteenth hour after dosage are given in Table 1. These results show the glucuronide to be the major metabolite accounting for an average of 49% of the dose administered, the corresponding values for the sulphate and the unchanged paracetamol were 26% and 4% respectively.

The results have been plotted at the mid-point of each time interval to give log "Rate" plots (Fig. 1 (a)) and were also treated by an alternative procedure in which the calculated total amount excreted minus the cumulative amount excreted at each time, was plotted on a logarithmic scale against time after dosage (Fig. 1 (b)), these are termed

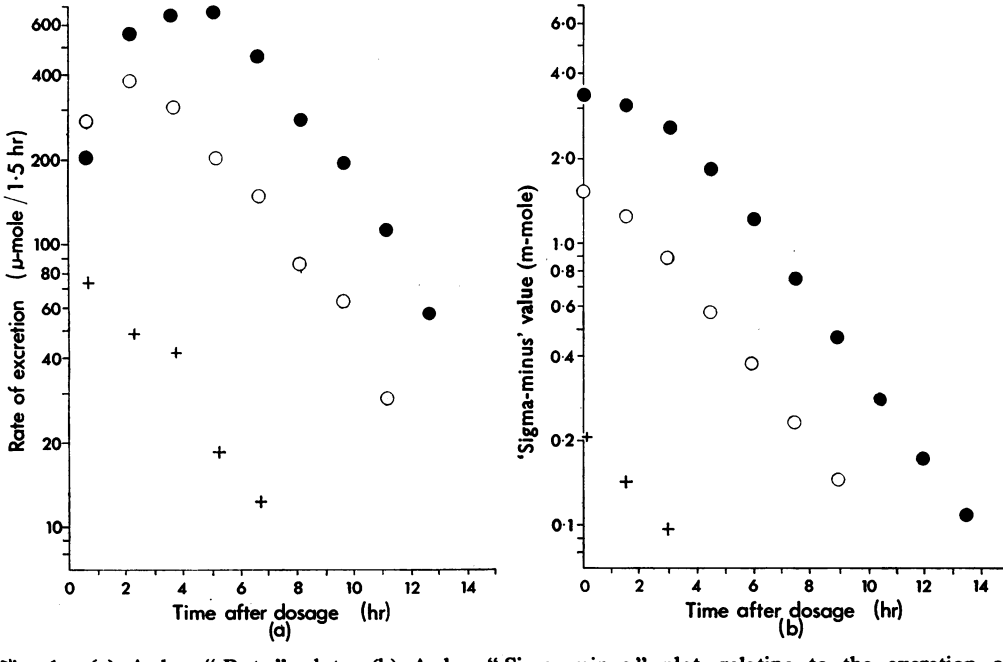


Fig. 1. (a) A log "Rate" plot. (b) A log "Sigma-minus" plot, relating to the excretion of paracetamol (+), paracetamol sulphate (○) and paracetamol glucuronide (●) in the urine of one man (D). The terminal sections of the three plots in Fig. 1 (a) closely correspond to the straight lines:

$$\log y = 2.05 - 0.15x$$
$$\log y = 3.14 - 0.15x$$
$$\log y = 3.66 - 0.15x$$

TABLE 1

THE AMOUNT OF PARACETAMOL (P), PARACETAMOL SULPHATE (PS) AND PARACETAMOL GLUCURONIDE (PG) EXCRETED IN THE URINE OF FOUR MEN (A, B, C, D) AFTER DOSAGE WITH PARACETAMOL (12 mg/kg BODY-WEIGHT)

| Subject and Dose (g) | | Time of urine collection (hr after dosage) | | | | | | | | | | % of [total excreted* | % of dose excreted* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1·5 | 3·0 | 4·5 | 6·0 | 7·5 | 9·0 | 10·5 | 12·0 | 13·5 | 15·0 | | |
| | | Amount excreted (μ-mole/1·5 hr) | | | | | | | | | | | |
| A | P | 29 | 51 | 45 | 25 | 18 | 11 | — | — | — | — | 4·6 | |
| 0·762 | PS | 170 | 320 | 220 | 190 | 130 | 100 | — | — | 29 | 20 | 28·7 | 89 |
| | PG | 170 | 370 | 450 | 510 | 450 | 360 | — | — | 110 | 63 | 66·7 | |
| B | P | 45 | 40 | 42 | 22 | 10 | — | — | — | — | — | 4·9 | |
| 0·684 | PS | 200 | 320 | 230 | 160 | 110 | 66 | 31 | 25 | 9 | — | 34·0 | 77 |
| | PG | 220 | 490 | 500 | 370 | 230 | 140 | 80 | — | 26 | — | 61·0 | |
| C | P | 31 | 49 | 31 | 23 | 11 | — | — | — | — | — | 4·4 | |
| 0·84 | PS | 107 | 360 | 290 | 220 | 123 | 69 | 53 | 35 | — | — | 34·5 | 69 |
| | PG | 120 | 380 | 550 | 440 | 290 | 223 | 127 | 71 | 33 | — | 61·0 | |
| D | P | 74 | 45 | 39 | 17 | 11 | — | — | — | — | — | 4·4 | |
| 0·993 | PS | 250 | 360 | 290 | 190 | 140 | 80 | 60 | 27 | — | — | 30·9 | 72 |
| | PG | 190 | 510 | 610 | 630 | 430 | 260 | 180 | 110 | 53 | — | 64·7 | |

* Calculated by extrapolation to negligible rate of excretion.

" Sigma-minus " plots (Martin, 1967). The total amount of paracetamol and of each of the metabolites which were excreted in the urine was calculated from the extrapolated rate plots. This procedure is not entirely free from criticism, for it involves the assumption that the excretion of these substances continues to be first order.

The results of the present investigation are considered to be explicable in terms of the theoretical considerations of the preceding paper (Cummings *et al.*, 1967). Typically, the maximum rate of excretion of paracetamol in urine was observed within a period of 3 hr after drug administration (Table 1). After a further short period of time its decline could be interpreted as log-linear, indicating that the process of drug absorption had then become negligible and that the process of elimination was first order. The rate of excretion of paracetamol glucuronide continued to increase for periods of 3 to 6 hr after drug administration and reflects the accrual of this metabolite in the body. In all four subjects the observed maximum rate of excretion of paracetamol glucuronide occurred later than that of paracetamol sulphate and in two subjects the maximum rate of excretion of paracetamol sulphate was delayed relative to the maximum rate of excretion of paracetamol (Table 1).

After attaining their respective maximum values the rate of excretion of both metabolites decreases and plots of the log rate of excretion eventually appear to become linear (Fig. 1 (a)). A close correlation then exists between the slope of the plots of the log rate of excretion of the metabolites and of the drug, and statistical analysis of the results indicates that the terminal sections of these plots can be interpreted as linear and parallel. It may therefore be deduced that both paracetamol sulphate and paracetamol glucuronide have excretion rate constants which are appreciably larger than the elimination rate constant for the drug.

*The evaluation of rate constants*

The elimination rate constant (K) for paracetamol was obtained by calculation of the slope of the linear parts of the plots of log rate of excretion of paracetamol or its metabolites (Fig. 1 (a)), or from the slope of the corresponding " Sigma-minus " plots (Fig. 1 (b)).

The elimination constant consists of the sum of the rate constants which respectively govern the excretion of unchanged drug and the formation of each metabolite, thus:

$$K = k_D + k'_F + k''_F \quad \ldots\ldots\ldots\ldots (1)$$

where $k_D$ and $k_F$ are the rate constants for drug excretion and metabolite formation respectively.

The individual rate constants may be calculated from the relationships:

$$\frac{k_F}{k_D} = \frac{M_{U\infty}}{D_{U\infty}} \quad \ldots\ldots\ldots\ldots (2)$$

$$k_D = \frac{KD_{U\infty}}{D_A} \quad \ldots\ldots\ldots\ldots (3)$$

where $D_{U\infty}$ and $M_{U\infty}$ respectively represent the total amount of drug and of metabolite ultimately excreted and $D_A$ represents the total amount of drug which participates in the

elimination process—that is, the amount of drug which is absorbed. The evaluation of $D_A$ presents no problem when it is possible to account for the whole dose in terms of the urinary recovery of drug metabolites. When this is not so, any attempt to calculate the individual rate constants must involve either or both of the following considerations, that the dose administered was not completely absorbed, and that appreciable drug elimination takes place by other additional routes. Then

$$k_D = K \frac{D_{U\infty}}{D_{U\infty} + M'_{U\infty} + M''_{U\infty} + D_X} \quad \ldots\ldots\ldots\ldots\ (4)$$

where $D_X$ represents the amount of drug and metabolite eliminated by routes other than urinary excretion and any metabolite present in urine but not determined by the method of analysis.

TABLE 2

THE RATE CONSTANTS CALCULATED FROM URINARY EXCRETION DATA OF FOUR MEN (A, B, C, D)

| | | A | B | C | D |
|---|---|---|---|---|---|
| | | | Elimination Rate Constant ($hr^{-1}$) | | |
| Paracetamol | | 0·35 | 0·27 | 0·30 | 0·34 |
| | | | Half-life (hr) | | |
| | | 2·0 | 2·6 | 2·3 | 2·0 |
| | | | Formation Rate Constant ($hr^{-1}$) | | |
| Paracetamol sulphate | | 0·09 | 0·07 | 0·07 | 0·07 |
| Paracetamol glucuronide | | 0·16 | 0·16 | 0·13 | 0·15 |
| | | | Excretion Rate Constant ($hr^{-1}$) | | |
| Paracetamol | | 0·014 | 0·011 | 0·010 | 0·010 |
| Paracetamol sulphate | *(i) | 1·51 | 1·41 | 1·27 | 0·80 |
| | *(ii) | 1·50 | 1·53 | 1·25 | 0·84 |
| Paracetamol glucuronide | *(i) | 1·01 | 0·53 | 0·47 | 0·48 |
| | *(ii) | 0·91 | 0·55 | 0·51 | 0·53 |

* Calculated by (i) "Rate *v* Amount" method, and (ii) "Terminal ratio" method respectively.

In the present study, an average of about 75% of the dose of paracetamol could be accounted for in the form of drug and the two metabolites estimated in urine. The rate constants for the excretion of paracetamol and for the formation of paracetamol sulphate and paracetamol glucuronide have been calculated (Table 2) on the basis that all the dose was absorbed and that the remainder was eliminated as other metabolites which have not been estimated. The rate constants calculated from the following equations therefore represent minimum values,

$$k_D = K \cdot \frac{D_{U\infty}}{D_0} \text{ and } k_F = K \cdot \frac{M_{U\infty}}{D_0}$$

where $D_0$ is the amount of drug administered.

The Rate *v* Amount method (Martin, 1967) and the Terminal Ratio method (Cummings *et al.*, 1967) for the evaluation of excretion rate constants have been applied to assess the excretion rate constants of paracetamol sulphate and paracetamol glucuronide (Table 2). Both methods depend essentially on the ability to establish the decline in

the rate of excretion of drug in the urine. The proportion of the dose of paracetamol which is excreted unchanged is small and this tends to diminish the accuracy of the results in this instance.

## DISCUSSION

The results of the study of the excretion of paracetamol, paracetamol sulphate and paracetamol glucuronide conform closely with the theoretical considerations previously advanced (Cummings *et al.*, 1967). The plots of log rate of excretion of the metabolites are at first curves but their terminal sections can be interpreted as linear and parallel to the plot for paracetamol and the elimination rate constant for paracetamol can in theory be calculated from the slope of any of these plots. However, relatively few experimental values are available for the urinary excretion of paracetamol and the slope of the plot of its log rate of excretion cannot be determined with great precision, the equations for the set of three parallel straight lines (Fig. 1 (a)) are therefore based largely upon the metabolite results. The question then arises whether the rate of excretion of the metabolites achieves a true log-linear decline within the period of the experiment. If this is not achieved, both the elimination rate constant of paracetamol (K) and the excretion rate constants of the metabolites ($k_U$) will be underestimated. The time required for the log "Rate" plots of the metabolites to become linear is related to the values of K and $k_U$, and in the present studies the smallest difference is observed between the value of K and the value of $k_U$ of paracetamol glucuronide. When theoretical log "Rate" plots are constructed from the urinary excretion results of a model drug and one of its metabolites, using the rate constants appropriate to paracetamol and paracetamol glucuronide, it is found that the slope of the metabolite plot is within 10% of its terminal value from about 9 hr after drug administration. This is in fair agreement with the experimental results of the present studies and indicates that the error in the calculation of K from the slope of the metabolite plots is likely to be less than 10%.

The corresponding "Sigma-minus" plots of the results become apparently linear from about 6 hr after dosage, and the elimination rate constant of paracetamol may also be calculated from their slope. This might seem to be the best method of calculating these rate constants, but it must be noted that in this instance, the calculated total amount of drug and total amount of metabolite ultimately excreted, on which the "Sigma-minus" plots are based, were dependent upon the extrapolation of the terminal linear parts of the log "Rate" plots.

The values of the elimination rate constant of paracetamol calculated from the results of the present studies are in good agreement with those calculated by Nelson & Morioka (1963) from the results of their study of the excretion of paracetamol and "total paracetamol metabolites" in urine.

Greenberg & Lester (1946) reported that paracetamol was the major metabolite of acetanilide in man and that this was excreted in the urine mainly as sulphate. In the present study, however, paracetamol was eliminated predominantly (49%) as its glucuronic acid conjugate.

## SUMMARY

1. Paracetamol, paracetamol sulphate and paracetamol glucuronide excreted in the urine after the administration of paracetamol, have been separated by thin-layer chromatography and estimated spectrophotometrically.

2. Plots of the log of the rate of excretion in urine of paracetamol, of paracetamol sulphate and of paracetamol glucuronide against time eventually become linear and parallel.

3. Two methods have been used to calculate the urinary excretion constants of paracetamol sulphate and paracetamol glucuronide.

4. The results are considered to be in accord with theory in respect of a drug having a first order elimination rate constant appreciably smaller than the first order excretion rate constants of its metabolites.

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## REFERENCES

BRODIE, B. B. & AXELROD, J. (1948). The fate of acetanilide in man. *J. Pharmac. exp. Ther.*, **94**, 29–38.

BRODIE, B. B. & AXELROD, J. (1949). The fate of acetophenetidin (*phenacetin*) in man, and methods for the estimation of acetophenetidin and its metabolites in biological material. *J. Pharmac. exp. Ther.*, **97**, 58–67.

BURKHARDT, G. N. & WOOD, H. (1929). Nitro-arylsulphuric acids and their reduction products. *J. Chem. Soc.*, 141–152.

CUMMINGS, A. J., MARTIN, B. K. & PARK, G. S. (1967). Kinetic considerations relating to the accrual and elimination of drug metabolites. *Br. J. Pharmac. Chemother.* **29**, 136–149.

GREENBERG, L. A. & LESTER, D. (1946). The metabolic fate of acetanilid and other aniline derivatives. I. Major metabolites of acetanilid appearing in the urine. *J. Pharmac. exp. Ther.*, **88**, 87–98.

JAGENBURG, O. R. & TOCZKO, K. (1964). The metabolism of acetophenetidine. Isolation and characterization of S-(1-acetamido-4-hydroxyphenyl)-cysteine, a metabolite of acetophenetidine. *Biochem. J.*, **92**, 639–643.

KAMIL, I. A., SMITH, J. N. & WILLIAMS, R. T. (1952). Studies in detoxication. 41. A study of the optical rotations of the amides and triacetyl methyl esters of some biosynthetic substituted phenylglucuronides. *Biochem. J.*, **50**, 235–240.

LESTER, D. & GREENBERG, L. A. (1947). The metabolic fate of acetanilid and other aniline derivatives. II. Major metabolites of acetanilid appearing in the blood. *J. Pharmac. exp. Ther.*, **90**, 68–75.

MARTIN, B. K. (1967). Drug urinary excretion studies—a new method of treating the data. *Nature (Lond.)* In press.

MÖRNER, K. A. H. (1889). Stoffwechselproducte des Acetanilids im menschlichen Körper. *Hoppe-Seyler's Z. physiol. Chem*, **13**, 12–25.

NELSON, E. & MORIOKA, T. (1963). Kinetics of the metabolism of acetaminophen by humans. *J. pharm. Sci.*, **52**, 864–868.

SMITH, J. N. & WILLIAMS, R. T. (1949). Studies in detoxication. 22. Metabolism of phenacetin (p-ethoxyacetanilide) in the rabbit and a further observation on acetanilide metabolism. *Biochem. J.*, **44**, 239–242.

SMITH, P. K. (1958). Metabolism and intermediary products. In *Acetophenetidin*, 15–19. Interscience, London.