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An analysis of the inter-subject variation in the metabolism of pentazocine

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Significant inter-subject variation in the cumulative urinary excretion of pentazocine, following its oral administration, was observed. Individual variations in the rate of metabolic oxidation of pentazocine are responsible for the variations in the urinary excretion of pentazocine (24 h cumulative). Smoking did not affect the metabolism of the drug. Pharmacokinetic analysis of the urinary excretion rate of pentazocine, using an open three compartment model, indicated that the fractional metabolic clearance is correlated with the cumulative urinary excretion (24 h) of the drug under conditions of acidic urinary pH. A g.l.c. method for the determination of pentazocine in urine is described.

The urinary excretion of pentazocine in man shows large inter-subject variations under uncontrolled (Berkowitz & Way, 1969) and controlled (Beckett, Kourounakis & others, 1970) conditions of urinary pH. We have, therefore, attempted to evaluate the inter-subject variation in the metabolism of pentazocine in smokers and non-smokers after oral administration of the drug by using an acidic urinary pH to prevent reabsorption of unchanged drug in the kidney tubules and we have analysed pharmacokinetically the rate of urinary excretion of pentazocine in some subjects.

The elimination of codeine was also examined in some subjects to investigate inter-subject variations in drug metabolism by glucuronidation.

The method of Beckett, Taylor & Kourounakis (1970) for the quantitative determination of pentazocine in biological fluids has been modified.

MATERIALS AND METHODS

Codeine assay

Codeine was extracted from urine and quantitatively analysed by the method of Vaughan & Beckett (1973a), using a Perkin-Elmer F11 gas chromatograph with a flame ionization detector.

Analysis of pentazocine

Apparatus. A Perkin-Elmer F11 chromatograph fitted with a flame ionization detector and coupled to a 0–2·5 mV Hitachi-Perkin-Elmer Model 159 recorder was used. The chromatographic column was glass tubing, $\frac{1}{4}''$ o.d., 2 m long, packed with 80–100 mesh chromosorb G, A.W.—DMCS, coated with 2·5% w/w SE 30. The column was conditioned for 24 h under the operating conditions: injection port temperature 230°, oven temperature 220°, nitrogen (carrier gas) flow rate 60 ml min^{-1}. The inlet pressures of the flame gases were 15 lb $inch^{-2}$ for hydrogen and 20 lb $inch^{-2}$ for air.

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Reagents and compounds. Ammonium hydroxide 2·0 N; acetic acid 2·0 N. Internal marker: the equivalent of 5 μg base ml^{-1} of dipipanone hydrochloride in distilled water. Diethylether (Analar) freshly distilled, n-butanol reagent grade. Pentazocine hydrochloride (Sterling Winthrop) codeine (British Drug Houses) and dipipanone hydrochloride (Burroughs, Wellcome & Co.) were authenticated by mass spectrometry and chemical assay before use.

Treatment of biological samples. Urine (5ml), combined with internal marker solution (1 ml), was adjusted to pH 9·0–9·02 with ammonium hydroxide and acetic acid using a Dynacap pH meter equipped with a Pye-Unicam combined glass and reference electrode, EO2. The resultant solution was extracted with ether (6 × 4 ml) and the extracts combined in a test-tube with a finely tapered base (Beckett, 1966). Samples whose pH had decreased by 0·1 units during extraction were re-analysed by the above procedure after acidification with acetic acid (0·2 ml) to increase the buffering capacity at pH 9·0.

Chromatographic analysis. The bulked ether extracts were concentrated to 5 ml by evaporation at 43° in a water bath, n-butanol (20 μl) was added and the evaporation continued to about 40 μl. 5 μl was injected on to the gas chromatographic column. The amount of pentazocine present in a sample was determined by measuring the peak height ratio of the drug to internal marker and relating it to a previously constructed calibration graph. Retention times relative to solvent front were pentazocine 9·5 min and dipipanone 17·5 min.

The specificity and reproducibility of the assay for pentazocine in urine and the recovery of the drugs by extraction. Urine samples from 20 subjects who had not received pentazocine were analysed and did not produce interfering peaks. Pentazocine was added to samples of pooled urine to give standard solutions of the drugs in urine (0·5 μg base ml^{-1}). Replicate analyses (20) were made on the standard solution and gave a s.d. of $\pm$ 2%. Calibration graphs constructed from data obtained by extracting urine containing various concentrations (0·1–30·0 μg base ml^{-1}) of pentazocine were linear (s.d. $\pm$ 4%). The recovery of pentazocine was 97% and of marker 98%.

Estimation of pentazocine glucuronide. Since pentazocine is unstable to heat in aqueous acidic media (Vaughan & Beckett, 1973b) the following method was used:— Urine (5 ml) was adjusted to pH 5·0 with acetic acid (0·1 N); Walpole's acetate buffer (1 ml double strength, Documenta Geigy) and 'Ketodase' (Warner-Chilcott; 2 ml, 5000 units ml^{-1}) were added. The resultant solution was incubated in a water bath (37°) for 24 h and then analysed for pentazocine as described previously. The amount of pentazocine glucuronide present was calculated from the concentrations of the drug in the sample before and after the hydrolysis procedure: determinations were duplicated. Blank urine samples after enzymatic hydrolysis on subsequent analysis, by g.l.c., did not produce chromatographic peaks at or close to the retention times of pentazocine or the internal marker. Pentazocine was stable under the conditions of the hydrolysis procedure.

The conditions used to hydrolyse pentazocine glucuronide in urine were in excess of the optimal since maximal increases in the amount of extractable pentazocine could be obtained with smaller amounts of ketodase (1·2 ml) and shorter incubation periods (14 h).

Pentazocine and codeine administration. Pentazocine was administered orally to seventeen healthy subjects (age 22–28), as an aqueous solution (100 ml containing 100 mg pentazocine HCl = 88·7 mg base). On a separate occasion an aqueous solution of codeine (100 mg base in 100 ml) was administered to four of the subjects and to a subject (subject 18) whose urinary excretion of pentazocine had been determined previously (Beckett & others, 1970).

Conditions of drug trials. The general procedures adopted for diet and collection of urine were similar to those described by Beckett & Rowland (1965a). Induction and maintenance of an acidic urinary pH (pH $\geqslant$ 5·0) was as described by Beckett & Brookes (1967). In two subjects, the cumulative 24 h urinary excretion of pentazocine was obtained when the urinary pH was uncontrolled, when it was kept acidic and using acid diuresis (Beckett & Hossie, 1969).

Pharmacokinetics

Apparatus. Electronics Associates Ltd. PACE TR—20R Analogue computer equipped with a X-Y recorder (Bryans Ltd.) and a digital volt meter (Roband Ltd.).

Method. The appropriate pharmacokinetic model to describe the absorption, metabolism, distribution and urinary excretion was programmed (see Appendix 1 for details). Experimental urinary excretion rate data (% dose h^{-1}) were plotted on the X-Y recorder and the settings of the rate potentiometers, other than the one corresponding to the urinary excretion rate constant (K_e) (see discussion) were systematically varied until the computer-generated curves corresponded to the experimental data points. When the best fit was obtained, the settings of the rate constant potentiometers were read from the digital voltmeter. Delay times were estimated by manually setting the abscissa zero of the X-Y recorder. The potentiometric setting for K_e was at 0·102 h^{-1} namely the mean figure calculated from data obtained after intravenous pentazocine (Beckett & others, 1970).

RESULTS

Urinary excretion of pentazocine and pentazocine glucuronide. Under uncontrolled conditions of urinary pH, the 24 h urinary recoveries of orally administered pentazocine, in two subjects (subjects 5 & 9) varied from 1–1·5 and 1·8–4% of the administered dose. However, under conditions of controlled acidic urinary pH (pH $\geqslant$ 5·0), in the same individuals, reproducible amounts of the drug were excreted in urine (2·9 $\pm$ 0·2% and 5·4 $\pm$ 0·15% respectively* mean of 4 trials each); acid diuresis did not increase these amounts.

Reproducible amounts of codeine (about 10% of the dose) are excreted in urine when the urine is acidic (Vaughan & Beckett, 1973a).

The 24 h urinary recoveries of unchanged pentazocine, expressed as a percentage of the administered dose and under conditions of acidic urinary pH, for all subjects are represented in Fig. 1 and the 24 h urinary excretions of the drug relative to the excretion of its glucuronide metabolite (% dose 24 h) are presented in Fig. 2.

Urinary excretion of pentazocine and codeine. The 24 h urinary excretions of pentazocine and codeine (% dose 24 h), under similar conditions of acidic urinary pH

* These percentages correspond to 'low excretors' reported previously (Beckett & others, 1970).

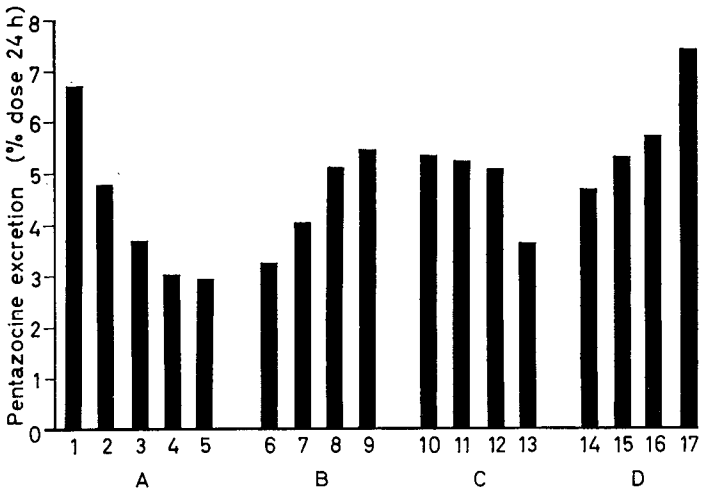


FIG. 1. 24 h urinary recoveries of pentazocine expressed as a percentage of the administered dose in seventeen subjects (acidic urinary pH) after oral administration ('solution' dose ≡ 88·7 mg base). A, Male non-smokers; B, Male smokers; C, Female non-smokers; D, Female smokers.

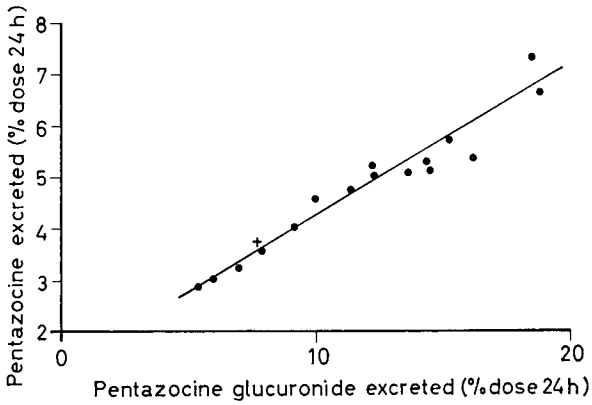


FIG. 2. 24 h urinary recovery of pentazocine (% dose) as a function of the 24 h urinary recovery of pentazocine glucuronide (% dose) in various individuals after the oral administration of pentazocine ('solution' dose ≡ 88·7 mg/base) and under conditions of an acidic urinary pH. +(Two data points represented).

(pH ≯ 5·0) in five subjects, after oral administration of the drugs, on separate occasions, are presented in Fig. 3.

Pharmacokinetic analysis. The computer generated urinary excretion rates of pentazocine (% dose h^{-1}) after oral administration obtained with the pharmacokinetic model (Fig. 4) and the experimental data points of urinary excretion rates, for two subjects, are shown in Fig. 5: agreement between the computer generated and measured urinary excretion rates was similarly found with *all* the data subjected to pharmacokinetic analysis. Table 1 gives the computed rate constants for the distribution and elimination of pentazocine and Fig. 6 shows the relation between the metabolic rate constant (K_m), the fractional metabolic clearance $[K_m/(K_m + K_{21})]$ and the cumulative urinary excretion of pentazocine (% dose in 24 h).

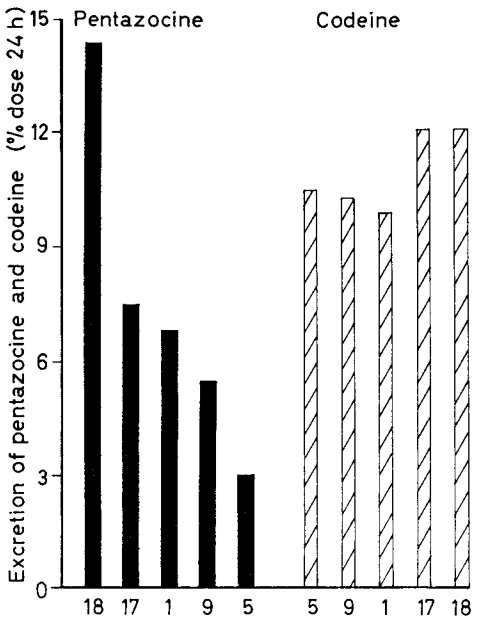


FIG. 3. 24 h urinary recoveries (% dose) of pentazocine (solid columns: 'solution' dose ≡ 88·7 mg base) and codeine (hatched columns: 'solution' dose ≡ 100 mg base), after oral administration of the drugs in five subjects under conditions of acidic urinary pH.

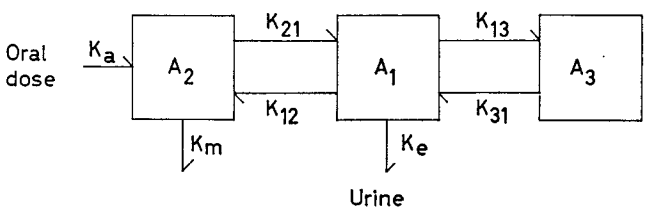


FIG. 4. The three compartment linear model used to describe the kinetics of absorption, metabolism and excretion of pentazocine in man after oral administration of a solution dose of the drug under controlled conditions of an acidic urinary pH.

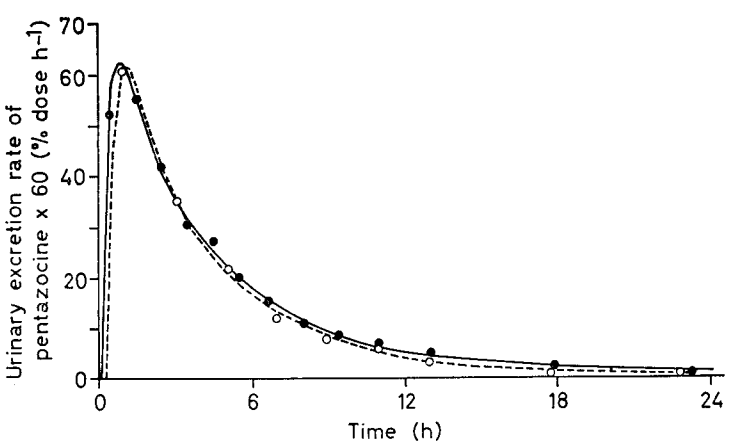


FIG. 5. Computer curves and experimental data points for the urinary excretion of pentazocine, after oral administration of 100 mg of pentazocine hydrochloride (≡ 88·7 mg base) in a solution dose form, in two subjects (subjects 9 and 12).

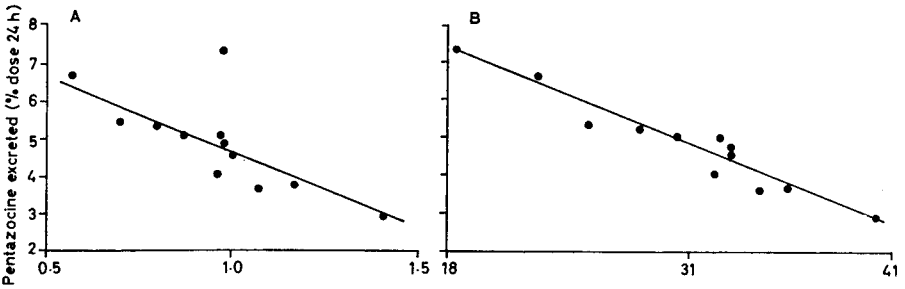


FIG. 6. 24 h urinary recovery (% dose) of pentazocine as a function of A, the metabolic rate constant (K_m) and B, the fractional metabolic clearance $\{K_m/(K_m + K_{21})\}$ in various individuals after the oral administration of pentazocine ('solution' dose ≡ 88·7 mg base) under conditions of an acidic urinary pH. Abscissa scale for A = K_m and for B = $K_m/(K_m + K_{21})\cdot 100$.

Table 1. *Kinetic parameters for the absorption metabolism and excretion of pentazocine after the oral administration of pentazocine (solution dose = 88·7 mg base).*

Subject	Delay time (min)	K_a (h^{-1})	K_m (h^{-1})	K_e (h^{-1})	K_{21} (h^{-1})	K_{12} (h^{-1})	K_{13} (h^{-1})	K_{31} (h^{-1})	$\frac{K_{13}}{K_{31}}$	$\frac{K_m}{(K_m + K_{31})}\cdot 100$
1	15	1·714	0·564	0·102	1·894	4·448	5·156	1·00	2·3	22·9
2	15	2·097	1·165	0·102	2·039	4·928	7·742	1·21	2·2	33·0
3	15	1·5	1·07	0·102	2·00	5·3	4·79	1·00	2·7	34·9
5	8	2.12	1·400	0·102	2·018	5·701	5·3	1.00	2·8	41·1
7	15	1·229	0·958	0·102	2·00	5·352	3·102	1·00	2·7	32·4
8	15	1·47	0·976	0·102	1·977	4·146	4·606	1·00	2·1	32·8
9	4	2·122	0·695	0·102	2·015	5·701	3·01	1·00	2·8	25·6
10	30	1·23	0·792	0·102	2·00	4·714	4·197	1·00	2·4	28·4
12	15	2·004	0·868	0·102	1·991	4·771	3·497	1·00	2·4	30·4
13	15	2·097	1·165	0·102	2·038	4·928	7·742	1·21	2·4	36·4
14	15	2·3	1·01	0·102	2·001	4·268	4·592	1·758	2·1	33·3
17	15	2·62	0·98	0·102	4·306	6·304	3·344	1·62	1·6	18·5

DISCUSSION

The replacement of benzene as a solvent for the extraction of pentazocine (Beckett & others, 1970) by ether avoids the extensive purification of the benzene that was required by the published method.

Unlike many other basic drugs, e.g. amphetamine (Beckett & Rowland, 1965b) and dihydrocodeine (Vaughan & Beckett, 1973a), the cumulative urinary excretion of pentazocine (% dose 24 h), under conditions of acidic urinary pH (pH $\not>$ 5·0), exhibited a 2·5 fold inter-subject variation (see Fig. 1).

Although only small amounts of drug were excreted unchanged, it is proposed to compare the relative amounts of unchanged pentazocine excreted in urine (% dose, 24 h) by each subject to assess the relative inter-subject variation in the metabolism of the drug. This is done because when the urine is acidic (a) only slight intra-subject variation in the cumulative urinary excretion of the drug occurs and acid diuresis does not increase the amount of drug excreted, (b) the excretion of pentazocine is completed in 24 h, (c) among subjects who excrete different amounts of pentazocine into the urine (% dose 24 h) the ratios of the areas under the pentazocine blood level time curves and the urinary excretion curves are similar and the ratios are independent of

the route of drug administration (Beckett & others, 1970); also the urinary rate of pentazocine is directly related to the blood concentrations of the drug. If clearance of pentazocine from blood by the kidney showed considerable inter-subject variation, the ratio of the areas under the pentazocine blood level-time curves and urinary excretion curves would not be similar in subjects who excreted widely different amounts of pentazocine. Thus inter-subject variation in the urinary excretion rate (K_e) of pentazocine must be small under conditions of an acidic urinary pH. (d) Following oral administration the differences in urinary excretion of pentazocine (% dose 24 h) cannot be accounted for by a failure to make the drug available for absorption since faecal recoveries of unchanged pentazocine are negligible and the inter-subject variation in the cumulative urinary excretion is also observed after intravenous or intramuscular administration of the drug (Beckett & others, 1970).

Fig. 1 shows that different amounts of pentazocine are required in different subjects to obtain similar cumulative urinary excretion profiles; since the differences in pentazocine recoveries (% dose 24 h) arise from differences in the rate of excretion (μg min^{-1}) at any given time, different doses would also be required to produce similar blood drug level profiles. There is a direct correlation between the plasma levels of pentazocine and the analgesia produced by the drug 30 min after intravenous administration (Berkowitz, Asling & others, 1969), thus the results of the present study (see Fig. 1) indicate that different doses of pentazocine are required in each subject to produce a similar analgesic effect.

Although no controlled assessment of the adverse effects produced by pentazocine was attempted, subjects 1, 9, 16, 17 who excreted the most unchanged drug were nauseated.

The major metabolic routes of pentazocine are glucuronidation and oxidation of the terminal carbon atoms, producing the corresponding alcoholic and carboxylic metabolites (Pittman, Resi & others, 1969). A variation in the rate of oxidative metabolism, rather than a variation in the rate of glucuronidation, is considered responsible for the inter-subject variations in the excretion of unchanged pentazocine (% dose 24 h see Fig. 1). The evidence is as follows: (a) the total amount of pentazocine glucuronide (% dose 24 h) excreted in the urine increases as the amount of unchanged drug excreted increases (2·8–7·4% of pentazocine and 5·4–18·8% of pentazocine glucuronide is excreted in urine, see Fig. 2). (b) the inter-subject variation in the excretion of pentazocine is not paralleled by a similar variation in the excretion of codeine after oral administration and under similar conditions of urinary pH (see Fig. 3). Codeine is extensively metabolized in man by glucuronidation and inter-subject variations in this metabolic process would produce wide variations in the excretion of unchanged codeine (% dose 24 h).

The distribution and elimination of pentazocine in man under conditions of acidic urinary pH, can be described by an open three compartment linear kinetic model (see Fig. 4); see later why a two compartment model is unsatisfactory (for other three-compartment models see Nagashima, Levy & O'Reilly, 1968).

Based on this model, the following rate equations may be written (all symbols are defined in Appendix 1).

Post-lag time:

$$\frac{dA_1}{dt} = K_{31} A_3 + K_{21} A_2 - (K_e + K_{21} + K_{13}) A_1$$

$$\frac{dA_2}{dt} = K_a G + K_{12} A_1 - (K_m + K_{21}) A_2$$

$$\frac{dA_3}{dt} = K_{13} A_1 - K_{31} A_3$$

$$\frac{dG}{dt} = -K_a G$$

$$\frac{dU}{dt} = K_e A_1$$

The analogue computer program for the solution of this model is given in Appendix 1.

The pharmacokinetic model (Fig. 4) accounts for the following: (a) under conditions of an acidic urinary pH the cumulative urinary excretion of pentazocine following oral administration, even after complete absorption is less than that obtained after intravenous administration to the same subject (Beckett & others, 1970): this is because following oral absorption all the drug traverses the liver and some is metabolized before the absorbed dose reaches the general circulation. After intravenous administration less than 30% of the dose of a drug traverses the liver in the first circulatory pass (Harris & Riegelman, 1969). Pentazocine is, therefore, considered to be directly absorbed into the metabolizing compartment following oral administration but directly into the central compartment following intravenous administration (b) after the initial absorption phase of orally administered pentazocine, the urinary excretion rate can be described by a biexponential equation

$$\frac{dU}{dt} = K_e (Be^{-\beta t} + Ce^{-\gamma t}),$$

where B, C, β and γ are hybrid constants. The terminal regression of the urinary excretion rate of pentazocine, where $dU/dt \rightarrow K_e Ce^{-\gamma t}$, was only observed 12 h after drug administration* thus indicating that a 'deep tissue' compartment is required to describe the kinetics of pentazocine's distribution. A two compartment model is inappropriate to describe the experimental data because such models are inadequate to deal with drugs that exhibit substantial metabolism during the first passage through the liver as occurs with pentazocine.

Since K_e was invarient in the kinetic analysis, the computed rate constants (Table 1) cannot be regarded as absolute values. However, the ratio of rate constants are meaningful since they can be used to estimate the sizes of tissue and metabolizing compartments relative to that of the central compartment. In the kinetic model (Fig. 4) the ratio of the volume of the metabolizing compartment to the volume of the central compartment is given by K_{12}/K_{21} and an increase in K_{12} relative to K_{21}, all other constants remaining unchanged, would result in a larger fraction of the dose being present in the metabolizing compartment at any one time with the consequence that a larger fraction of the dose would be eliminated from the body by metabolism; the converse results from a decrease of K_{12} relative to K_{21}. However K_{12}/K_{21} is not correlated with the cumulative urinary excretion of pentazocine (see Table 1). The metabolic rate constant is not directly correlated ($r^2 = 0{\cdot}475$) with the amount of un-

* Obtained by applying residual subtractions to the log of urinary excretion rate versus time plots.

changed pentazocine excreted in the urine (see Fig. 6). However, the urinary excretion of pentazocine (% dose in 24 h) is correlated ($r^2 = 0{\cdot}943$) with the fractional metabolic clearance of the drug $*K_m/(K_m + K_{21})$; see Fig. 6. Since K_m and K_{12} are computed from the urinary excretion data, the fractional metabolic clearance should directly correlate with the cumulative urinary excretion. The correlation obtained, therefore, demonstrates the fit of the experimental data to the three compartment model chosen.

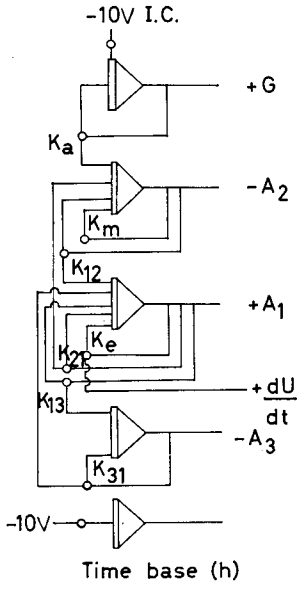
In conclusion the inter-subject variation in the amounts of pentazocine excreted in the urine (% dose 24 h) can be regarded as representing the variations in the individual metabolic clearances for the drug.

Up till now, when urinary pH is acidic, minimal inter-subject variation in the *N*-dealkylation or reduction of drugs in man has been observed, e.g. the keto reduction and *N*-de-ethylation of diethylpropion; *N*-dealkylation of *N*-methyl, *N*-ethyl and *N*-propylamphetamine (Testa & Beckett, 1973; Beckett & Shenoy, 1973). In contrast, large individual differences in the rates of elimination of drug that are predominantly metabolized by hydroxylation are known, e.g. phenylbutazone (Burns, Rose & others, 1953) and diphenylhydantoin (Kutt, Winters & others, 1964).

Pentazocine is not metabolized by *N*-dealkylation but by glucuronidation and hydroxylation of the terminal carbon atom. In common with other drugs that are predominantly metabolized by oxidation not at the basic centre and its α-carbon atoms, wide intersubject variations in the metabolism of pentazocine are observed.

Appendix 1

The analogue computer program for the solution of an open three compartmental model is given below.



* Fractional metabolic clearance of pentazocine = $\dfrac{\text{the amount of drug cleared from the metabolizing compartment by metabolism alone}}{\text{the amount of drug cleared from the metabolizing compartment by all processes}}$

Analogue computer program. The terms used in the equations are:
t, time in h after ingestion of the dose. Lagtime, the time interval between ingestion of the dose and the appearance of the drug in the central compartment. U, the amount of pentazocine in the urine. A_1 the amount of pentazocine in compartment 1 (the central compartment). A_2 the amount of pentazocine in compartment 2 (the metabolising compartment). A_3 the amount of pentazocine in compartment 3 (the deep tissue compartment). G the amount of pentazocine present in the gastrointestinal tract. K_a the first-order rate constant for the absorption of pentazocine from the gastrointestinal tract into compartment 2. K_m the first-order rate constant for the formation of all the metabolite of pentazocine. K_{21} & K_{12} the first-order rate constant for the transfer of pentazocine from compartment 2 to compartment 1 and from compartment 1 to compartment 2 respectively. K_{13} & K_{31} the first-order rate constant for the transfer of pentazocine from compartment 1 to compartment 3 and from compartment 3 to compartment 1 respectively. K_e the first-order rate constant for the excretion of pentazocine from compartment 1 into the urine.

REFERENCES

BECKETT. A. H. (1966). *Dansk. Tidsskr. Farm.*, **40**, 197–223.

BECKETT, A. H. & BROOKES, L. G. (1967). *J. Pharm. Pharmac.*, **19**, 425–495.

BECKETT, A. H. & SHENOY, E. V. B. (1973). *Ibid.*, **25**, 793–799.

BECKETT, A. H. & HOSSIE, R. D. (1969). *Ibid.*, **21**, 1575–1615.

BECKETT, A. H., KOUROUNAKIS, P., VAUGHAN, D. P. & MITCHARD, M. (1970). *Ibid.*, **22**, 16) – 1745.

BECKETT, A. H. & ROWLAND, M. (1965a). *Ibid.*, **17**, 59–60.

BECKETT, A. H. & ROWLAND, M. (1965b). *Ibid.*, **17**, 728–639.

BECKETT, A. H., TAYLOR, J. F. & KOUROUNAKIS, P. (1970). *Ibid.*, **22**, 123–128.

BERKOWITZ, B. A., ASLING, J. H., SCHNIDER, S. M. & WAY, E. L. (1969). *Clin. Pharmac. Ther.*, **10**, 320–328.

BERKOWITZ, B. A. & WAY, E. (1969). *Ibid.*, **10**, 681–689.

BURNS, J. J., ROSE, R. K., CHENKIN, T., GOLDMAN, A., SCHULERT, A. & BRODIE, B. B. (1953). *J. Pharmac. exp. Ther.*, **109**, 346–357.

HARRIS, P. A. & REIGELMAN, S. (1969). *J. pharm. Sci.*, **58**, 71–75.

KUTT, H., WINTERS, W., KOKENGE, R. & MCDOWELL, F. (1964). *Arch. Neurol.*, **11**, 642–648.

NAGASHIMA, R., LEVY, G. & O'REILLY, R. A. (1968). *J. pharm. Sci.*, **57**, 1888–1895.

PITTMAN, K. A., RESI, D., CHERNIAK, R., MEROLA, A. J. & CONWAY, W. D. (1969). *Biochem. Pharmac.*, **18**, 1673–1678.

TESTA, B. & BECKETT, A. H. (1973). *J. Pharm. Pharmac.*, **25**, 119–124.

VAUGHAN, D. P. & BECKETT, A. H. (1973a). *Ibid.*, **25**, *Suppl.* 104P–108P.

VAUGHAN, D. P. & BECKETT, A. H. (1973b). *Ibid.*, **25**, 993–995.