any, metabolism of the drug. About 90% was eliminated unchanged over 8 days in a normal volunteer given 1.5 g by mouth, with half appearing in the urine and half in the faeces (Figure 2). Plasma, whole blood and salivary drug concentrations are also indicated in Figure 2, as is the urinary excretion rate.

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References

- ALKALAY, D., KHEMANI, L., WAGNER, W.E. & BARTLETT, M.F. (1975). Pharmacokinetics of phenformin in man. *J. clin. Pharmac.*, **15**, 446–448.
- ASSAN, R., HEUCLIN, Ch., GANEVAL, D., BISMUTH, Ch., GEORGE, J. & GIRARD, J.R. (1977). Metformin-induced lactic acidosis in the presence of acute renal failure. *Diabetologia*, **13**, 211–217.
- BRITISH MEDICAL JOURNAL (1977). Biguanides and lactic acidosis in diabetes. *Br. med. J.*, **2**, 1436.
- GARRETT, E.R., TSAU, J. & HINDERLING, P.H. (1972). Application of ion-pair methods to drug extraction from biological fluids. II: Quantitative determination of biguanides in biological fluids and comparison of protein binding estimates. *J. pharm. Sci.*, **61**, 1411–1418.
- MATIN, S.K., KARAM, J.H. & FORSHAM, P.H. (1975). Simple electron capture gas chromatographic method for the determination of oral hypoglycemic biguanides in biological fluids. *Analyt. Chem.*, **47**, 545–548.

THE URINARY EXCRETION OF PHENELZINE

The substituted hydrazine drug, phenelzine, prescribed under the name Nardil, has been used for some years in the treatment of neurotic depression (Medical Research Council, 1965). While there is some evidence that clinical efficacy and side effects of the drug may be related to acetylator status, it is only since analytical techniques have become available (Caddy, Tilstone & Johnstone, 1976; Caddy & Stead, 1977) which enable levels of this drug in body fluids to be determined, that its excretion can be examined in more detail. The present paper describes the application of an iodate oxidation procedure to the analysis of phenelzine levels in the urine of five patients.

Chemicals, apparatus and analytical procedure

The assay of phenelzine in urine by its oxidation to β -phenylethanol with acidic potassium iodate has been adequately discussed in an earlier publication (Caddy & Stead, 1977).

Drug regimen and acetylator status

Following the oral administration of phenelzine (30 mg three times daily) at regular intervals, urine samples for assay of the drug were collected from five hospitalized patients on days 1 and 13 of a continuous course of treatment. The time of sampling and the volume of urine excreted are given in Table 1.

Acetylator status was determined by the method of Evans (1969).

The half-life of the drug was calculated from the gradient (G) obtained from a plot of $\log_e$ (excretion rate) against time, using the equation:

$$T_{\frac{1}{2}} = \frac{0.693}{G}$$

The times of sampling (1 and 13 days) were chosen because it was necessary to compare analytical results obtained before with those obtained during treatment. It was also assumed that 13 days was sufficient time for a steady state pharmacokinetic equilibrium to have been established.

The results of analyses of the urine samples obtained from the five patients are summarized in Table 1. The pH of all such urine samples lay in the range 6 to 7. Note that the large urine outputs are symptomatic of the high fluid intake resulting from the dry mouth experienced with phenelzine chemotherapy.

The drug excretion profiles in these patients show a marked change in pharmacokinetic behaviour during the course of treatment. The time of maximum excretion rate, the amount excreted over the 8 h period between dosing and the acetylator phenotypes of each patient are recorded in Table 2.

The pharmacokinetic data are consistent with the expected changes resulting from a successful treatment of depression with phenelzine. Since it can be postulated that, during the 13 days of drug administration, monoamine oxidase activity has been reduced by irreversible inhibition of the enzyme, it is to be expected that at the beginning of the course of treatment the half-lives would be less. Examination of the data shows this to be true; the averaged half-lives of the five patients studied at the beginning of treatment was calculated to be 0.87 ± 0.47 h, which is significantly different ($P < 0.05$ Mann-Whitney U Test) from the half-lives at the end of the course of treatment.

There is little variation of the times of maximum excretion rate amongst depressed patients for days 1 and 13, these values being 1.70 ± 0.37 h and 2.06 ± 0.55 h respectively.

Table 1 The urinary excretion of phenelzine†

<i>Patient 1</i>		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>Urine sample number</i>																	
Time of day when sample collected	A	*	10.00	10.30	11.00	11.30	12.00	12.30	*	13.30	14.00	14.30	15.00	15.30	16.00		
	B	9.00	10.00	*	11.00	11.30	12.00	*	*	13.30	14.00	14.30	15.00	15.30	16.00		
Collection time (h)		1	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8		
Volume of urine collected (ml)	A	*	600	770	210	350	460	400	*	200	300	250	325	400	320		
	B	50	100	*	190	260	250	*	*	250	230	250	300	450	350		
Phenelzine sulphate concentration (µg/ml)	A	*	1.04	0.13	0.08	**	**	—	—	—	—	—	—	—	—		
	B	7.00	10.33	*	2.80	0.85	0.58	*	*	—	0.29	**	—	—	—		
Phenelzine sulphate excretion (µg/h)	A	*	311	203	32	**	**	—	*	—	—	—	—	—	—		
	B	350	1033	*	531	440	289	*	*	—	132	*	—	—	—		
<i>Patient 2</i>																	
Time of day when sample collected	A	9.00	9.30	10.00	10.30	11.00	11.30	12.00	12.30	13.00	13.30	14.00	14.30	15.00	15.30	16.00	
	B	9.00	9.30	10.00	10.30	11.00	11.30	12.00	12.30	13.00	13.30	14.00	14.30	15.00	15.30	16.00	
Collection time (h)		1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	
Volume of urine collected (ml)	A	70	110	435	575	553	531	541	537	553	247	455	509	280	231	130	
	B	451	**	480	570	520	505	422	500	450	413	460	490	510	325	452	
Phenelzine sulphate concentration (µg/ml)	A	0.93	0.97	0.08	0.05	0.03	0.11	0.05	0.00	*	0.02	0.00	0.00	*	*	*	
	B	0.75	**	0.65	0.49	0.29	0.20	0.22	0.19	0.14	0.10	0.13	0.09	0.10	0.20	0.11	
Phenelzine sulphate excretion (µg/h)	A	65	212	70	52	32	111	105	26	*	8	2	2	*	*	*	
	B	340	**	620	559	298	206	181	191	122	81	124	88	106	127	102	
<i>Patient 3</i>																	
Time of day when sample collected	A	8.00	9.10	9.40	10.05	10.30	11.10	11.45	12.10	12.45	—	13.20	14.00	14.30	15.00	15.30	16.00
	B	8.00	9.00	9.30	10.00	10.30	11.00	11.30	12.00	12.30	13.00	13.30	14.00	14.30	15.00	15.30	16.00
Collection time (h)		—	0.5	1.25	1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75	6.25	6.75	7.25	7.75
Volume of urine collected (ml)	A	—	49	18	112	180	280	180	180	180	—	250	180	250	310	250	310
	B	—	20	14	60	210	240	275	200	225	275	300	260	250	300	300	275
Phenelzine sulphate concentration (µg/ml)	A	—	1.97	3.82	0.70	0.51	0.33	0.12	0.09	0.12	—	0.11	0.13	0.09	0.07	0.08	0.07
	B	—	3.65	3.76	1.23	0.51	0.43	0.31	0.39	0.36	0.28	0.24	0.22	0.29	0.21	0.20	0.21
Phenelzine sulphate excretion (µg/h)	A	—	49	137	187	220	138	41	39	37	—	46	36	47	42	39	41
	B	—	73	105	148	215	206	172	157	161	151	145	134	141	125	120	116
<i>Patient 4</i>																	
Time of day when sample collected	A	9.00	9.30	10.00	10.30	11.00	11.30	12.00	12.30	13.00	13.30	14.00	14.30	15.00	15.30	16.00	
	B	9.00	*	*	10.30	11.00	11.30	12.00	12.30	*	13.30	14.00	14.30	15.00	15.30	16.00	
Collection time (h)		1.00	1.50	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50		

Volume of urine collected (ml)	A	168	176	420	10.30	11.00	11.30	12.00	12.30	13.00	13.30	14.00	14.30	15.00	15.30	16.00
	B	200	*	350	190	250	260	300	*	490	190	300	225	250		
Phenelzine sulphate concentration (µg/ml)	A	0.38	0.24	0.44	0.26	0.19	0.14	0.13	—	0.07	*	*	*	*	*	*
	B	1.62	—	—	1.65	1.64	1.19	1.10	0.95	*	1.02	1.25	0.64	0.62	0.40	
Phenelzine sulphate excretion (µg/h)	A	63	84	369	252	187	130	121	—	44	*	*	*	*	*	*
	B	325	—	—	385	623	593	573	571	—	499	476	386	281	201	
Patient 5																
Time of day when sample collected	A	9.30	10.00	10.30	11.00	11.30	12.00	12.30	13.00	13.30	14.00	14.30	15.00	15.30	16.00	
Collection time (h)		1.50	2.00	2.50	3.00	3.50	4.00	4.30	5.00	5.50	6.00	6.50	7.00	7.50	8.00	
Volume of urine collected (ml)	A	25	290	336	340	370	134	30	187	45	184	300	430	50	30	
Phenelzine sulphate concentration (µg/ml)	A	*	0.62	0.27	0.21	0.15	0.25	1.09	0.18	0.63	0.14	0.07	*	T	*	
Phenelzine sulphate excretion (µg/h)	A	—	359	180	177	110	67	65	66	57	52	40	*	T	*	
A, B Samples of urine collected on days 1 and 13 respectively of a continuous treatment with the drug.																
* No sample available.																
** No drug detected.																
— Not analyzed.																
T Trace.																
† Phenelzine sulphate measured as the pure drug and/or metabolite retaining the hydrazine moiety.

Figure 1 Metabolites of phenelzine which would be indistinguishable from phenelzine in the assay procedure. I N-hydroxyphenelzine; II phenelzine hydroxydiazine.

Assuming that the urine excretion efficiency does not vary, the estimated amount of phenelzine excreted over the 8 h period between dosage (Table 1) measured as a total of both unchanged drug and phenelzine metabolite retaining the hydrazine linkage, follows the same pattern as did the half-lives calculated from the data of depressed patients on days 1 and 13. Since the monoamine oxidase activity has been reduced by the enzyme's inhibition, a greater percentage of the drug is likely to be eliminated unmetabolized by this enzyme. The amount of drug excreted at day 13 ($4.67 \pm 1.22\%$) expressed as a percentage of the ingested dose is significantly greater than that eliminated on day 1 ($1.14 \pm 0.26\%$ [$P < 0.05$ Mann-Whitney U Test]).

Because the number of patients used in this pilot study are too few, clinical ratings cannot be given. However, since it is normal to expect a clinical response by the 13th day of treatment (Johnstone & Tilstone, 1976) one may wish to speculate on the success of such treatment by reference to the analytical data. Thus, for example, the ratio of $T_{1/2(13)}$ (half-life of phenelzine on day 13) to $T_{1/2(1)}$ and that of $Q_{(13)}$ (the amount of phenelzine excreted over 8 h on day 13) to $Q_{(1)}$ may be an indication of the patient's improvement.

Since the assay procedure used will not distinguish between phenelzine and/or some of its metabolites and/or metabonates (Beckett, Coutts & Ogunbona, 1973) which retain the hydrazine function, it is always possible that some metabolites and metabonates produced neither by the catabolism of phenelzine by monoamine oxidase nor by its acetylation are present in the urine, which are indistinguishable from the parent drug. A metabolic pathway which involves hydroxylation in either the aromatic ring or the aliphatic side chain would be readily detected by the analytical procedure used in the present work, but N-hydroxylation to give Compound I or its related oxidation product, hydroxydiazine II (Figure 1), would produce a metabolite which would be indistinguishable from phenelzine. While there is no proof that N-hydroxylation of phenelzine occurs, Nelson, Mitchell, Timbrell, Snodgrass & Corcoran (1976) have produced some evidence that this process arises with isopropylhydrazine, a metabolite of iproniazid.

Table 2 Pharmacokinetic data for the urinary excretion of phenelzine

Patient	Acetylator status	Day 1			Day 13		
		$T_{\frac{1}{2}}(h)$	Q (%)	T(h)	$T_{\frac{1}{2}}$	Q(%)	T (h)
1	Slow	0.40	1.43	1.50	1.40	4.66	1.50
2	Slow	0.58	0.73	1.25	1.25	3.37	1.75
3	Slow	0.72	1.13	2.25	6.60	4.34	2.25
4	Fast	1.03	1.27	1.75	3.25	6.30	2.75
5	Fast	1.61	1.14	1.75	**	**	**
Mean of individual patients		0.87 ± 0.47	1.14 ± 0.26	1.70 ± 0.37	3.11 ± 2.49	4.67 ± 1.22	2.06 ± 0.55

Q the approximate amount of the drug excreted, measured as phenelzine sulphate and/or metabolite retaining the hydrazine moiety during the 8 h period of sampling expressed as a percentage of the ingested dose.

T the time of maximum excretion rate.

** no data available.

A more specific but less sensitive assay for phenelzine (Caddy *et al.*, 1976) based on the reaction of acetone with the free hydrazine group of the drug to give the acetonide, will theoretically discriminate between the parent compound and any N-substituted metabolite and/or metabonate.

However, since Beckett *et al.* (1973) have reported that N-hydroxy compounds will revert to the amine on gas chromatographic analysis, by analogy, any N-hydroxy phenelzine might revert to phenelzine which, in the presence of acetone residues from the extraction procedure, would be converted to the acetonide. Consequently, N-hydroxy phenelzine would not be distinguished from the parent drug using this more specific procedure. While the presence of N-hydroxy phenelzine in the urine of depressed patients is only speculative, should it be there, its urinary level may play an important role in any interpretation of excretion data.

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This study was carried out under the auspices of the Ethical Committee of Northwick Park Hospital and was conducted in accordance with its rules.

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References

- BECKETT, A.H., COUTTS, R.T. & OGUNBONA, F.A. (1973). Metabolism of amphetamines: Identification of N-oxygenated products by gas chromatography and mass spectrometry. *J. Pharm. Pharmac.*, **25**, 708–717.
- CADDY, B., TILSTONE, W.J. & JOHNSTONE, E.C. (1976). Phenelzine in urine: Assay and relation to acetylator status. *Br. J. clin. Pharmac.*, **3**, 633–637.
- CADDY, B. & STEAD, A.H. (1977). Indirect determination of phenelzine in urine. *Analyst*, **102**, 42–49.
- EVANS, D.A.P. (1969). An improved and simplified method of determination of acetylator phenotype. *J. med. Genet.*, **6**, 405–407.
- GELBICOVA-RUZICKOVA, J., NOVAK, J. & CHUNDELA, B. (1971). Determination of β -phenethylhydrazine in pharmaceuticals and urine by gas chromatography. *Biochem. Med.*, **5**, 537.
- JOHNSTONE, E.C. & TILSTONE, W.J. (1976). The relevance of acetylator status for response to phenelzine. *Br. J. clin. Pharmac.*, **3**, 355P–356P.
- Medical Research Council (1965). Clinical trial of the treatment of depressive illness. Report to the Medical Research Council. *Br. med. J.*, **1**, 881.
- NELSON, S.D., MITCHELL, J.R., TIMBRELL, J.A., SNODGRASS, W.R. & CORCORAN III, G.B. (1976). Isoniazid and Iproniazid: Activation of metabolites to toxic intermediates in man and rat. *Science*, **193**, 901–903.
- SIEGEL, S. (1956). *Non-parametric statistics for the behavioural sciences*, p. 116, New York: McGraw Hill.