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The distribution of methadone in man

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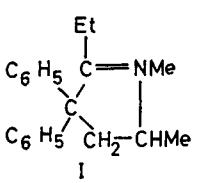
The distribution of methadone and its metabolite, 1,5-dimethyl-3,3-diphenyl-2-ethylidene pyrrolidine, in man, postmortem, is presented. Quantitative data for methadone and the metabolite in blood, bile, urine, liver, kidney, spleen, lung and brain samples show that methadone blood concentrations range from 0·22–3·04 μg/ml and are less than in bile and urine. The metabolite is found particularly in bile and urine. The liver and kidney concentrations are approximately equivalent unless the survival time is reduced by the presence of another CNS-depressant drug. Lung tissue is the richest source of methadone and brain the poorest. Chromatographic data for three other methadone metabolites are included.

Early urinary excretion studies of methadone in man (Scott & Chen, 1947; Cronheim & Ware, 1948; Way, Sung & McKelway, 1949) suffered from the analytical limitations of the solvent-soluble base-dye complex methods while the distribution studies of Alha & Ohela (1956) relied upon a non-specific colorimetric test together with a positive Straub reaction for measurement and characterization of the drug. In 1957, Vidic found methadone-derived primary and secondary amines in human urine after administration of the drug. An attempt by Pohland, Sullivan & Lee (1959) to synthesize *N*-desmethylmethadone resulted in the formation of 1,5-dimethyl-3,3-diphenyl-2-ethylidene pyrrolidine (I), which was isolated and identified by Beckett, Taylor & others (1968) as the major urinary excretion product of methadone in man.



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In this paper the distribution of methadone and its major metabolite (referred to as metabolite 1) in man postmortem in relation to the cause of death, is described.

MATERIAL AND METHODS

Reagent grade chemicals were used. Methadone hydrochloride, benzhexol hydrochloride and lignocaine hydrochloride were of B.P. quality. A sample of metabolite 1 was made available by Dr. A. F. Casy. All tissues were deep frozen and all body fluids were refrigerated as soon as possible after collection.

The nature of the drugs present was established in each case by appropriate preliminary qualitative tests (see for example Clarke, 1969).

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Extraction procedure for methadone

Urine. To urine (10 ml) were added benzhexol hydrochloride solution (120 μg in 0·2 ml as internal standard) and 2N sodium hydroxide (1 ml). The mixture was then extracted with ether (2 × 20 ml) and the ether extracts evaporated in a tapered tube at 50° to 100 μl. Portions (3 μl) were used for g.l.c.

Blood. To blood (10 ml) were added benzhexol hydrochloride solution (30 μg in 0·05 ml as internal standard) and 2N sodium hydroxide (1 ml). The mixture was shaken with ether (2 × 20 ml). The ether solution was extracted with N sulphuric acid (10 ml) which was then made alkaline with 40% w/v sodium hydroxide solution (1·5 ml) and extracted further with ether (15 ml). The final ether extract was evaporated in a tapered tube at 50° to 100 μl and portions (3 μl) used for g.l.c.

Bile To bile (10 ml) were added benzhexol hydrochloride (120 μg in 0·2 ml) and 2N sodium hydroxide (1 ml). The mixture was shaken with ether (2 × 20 ml). The ether extracts were extracted as for blood. Portions (3 μl) from the final ether extract were used for g.l.c.

Tissues. Defrosted tissue (50 g) was homogenized without added fluid. Benzhexol hydrochloride solution (120 μg in 0·2 ml), 2N sodium hydroxide (10 ml) and distilled water (10 ml) were added to the homogenate and the mixture was extracted with ether (3 × 75 ml). The ether extracts were reduced to 100 ml by evaporation and then extracted with N sulphuric acid (40 ml). Sodium hydroxide (5 ml 40% w/v) solution was added to the acid and the whole was extracted with further ether (50 ml). This ether extract was evaporated in a tapered tube at 50° to 100 μl. Portions (3 μl) were used for g.l.c.

Gas-liquid chromatography (g.l.c.)

An F & M Biomedical 400 Gas Chromatograph fitted with a flame ionization detector was used.

Column 1. 3·8% W98 on Diatoport S 80–100 mesh packed in a four foot glass column of 3 mm i.d. Conditions: oven temperature 175°, flash heater temperature 255°, detector temperature 185°; nitrogen (carrier gas) pressure 40 lb/inch², flow rate 80 ml/min; air pressure 30 lb/inch²; hydrogen pressure 25 lb/inch². The retention time for methadone was 5·4 min, for benzhexol 7·4 min and for metabolite 1, 3·7 min.

Column 2. 1% cyclohexane dimethanol succinate on Diatomite CQ, 100–120 mesh, packed in a 6 foot glass column of 3 mm i.d. Conditions: oven temperature 185°; flash heater temperature 290°; detector temperature 220°; nitrogen (carrier gas) pressure 40 lb/inch², flow rate 80 ml/min; air pressure 30 lb/inch²; hydrogen pressure 25 lb/inch². The retention times were as follows: methadone, 4·6 min; metabolite 1, 6·9 min; lignocaine, 2·6 min; methaqualone, 13·3 min.

Quantitative analysis for methadone

Calibration curves using column 1 were prepared for the assay of blood samples using 30 μg of benzhexol hydrochloride for up to 50 μg methadone, and, for the assay of bile, urine and tissue samples, 1200 μg of the internal standard for up to 250 μg methadone. Linear relations were observed for these ranges and standards were included in every set of samples for assay.

Comparison of the small slightly impure sample of metabolite 1 with methadone showed equivalent response of the flame ionization detector for the two substances on a molar basis. Hence, calibration curves for methadone were applicable also to metabolite 1.

Column 2 was used when the preliminary tests indicated the presence of methaqualone. Lignocaine was a suitable internal standard: 30·5 μg of the hydrochloride was used for the blood samples and 102 μg for urine, bile and tissue homogenates, the range of methadone levels being as before.

Recoveries of methadone and metabolite 1 added to blank tissues and fluids were 87 and 88% respectively and of benzhexol 80% and lignocaine 89%.

Thin-layer chromatography (t.l.c.)

Silica gel G (E. Merck & Co.) was spread on glass plates in 0·25 mm layers which were dried at 110° for 1 h before use. Two solvents systems were used: (1) methanol–ammonia (s.g. 0·88) (100 : 1·5, by volume) and (2) benzene–dioxan–ethanol–ammonia (s.g. 0·88) (50 : 40 : 5 : 5, by volume). Solvent development was allowed to proceed for 10–12 cm from the point of sample application. After drying, the plates were examined under ultraviolet light (264 nm) before spraying for basic substances with Dragendorff reagent and then over-spraying with iodoplatinate solution.

RESULTS AND DISCUSSION

Qualitative data

Methadone and metabolite 1 were detected chromatographically in all extracts of urine. Both substances reacted with Dragendorff and iodoplatinate reagent on thin-layer chromatograms prepared using solvent 1; nicotine (derived from tobacco smoking) was usually present and the other unidentified bases thought to be derived from methadone administration, were also found in subjects 1, 2, 3, 4, 5, 10 and 11 (Table 1). The reactions of the Dragendorff-positive substances on the chromatograms to other reagents are shown in Table 1, which also includes the retention times

Table 1. *Chromatographic characteristics of methadone, metabolite* 1 *and other *basic substances in urine extracts.*

Substance	Relative R_F value System 1	Relative R_F value System 2	Cis-aconitic anhydride	*p*-Nitroaniline/ Alkali	Ninhydrin	Relative retention time g.l.c. Column 1†
Methadone	1·00	1·00	Pink	Pink	Grey	1·00
Metabolite 1	0·35	0·95	—	Purple	Pink/mauve	0·69
Nicotine	1·54	0·66	Yellow	Pink	Pink	
Nicotine metabolite	0·50	0·55				
Metabolite 2	1·07	0·78	Pink	Red-black	—	1·2
Metabolite 3	0·80	0·10	—	Pink	Mauve	0·58
Metabolite 4	0·17	0·46	—	—	Mauve	

* i.e. Dragendorff-positive reacting substances, probably derived from methadone, referred to as metabolites 2, 3 and 4; no reactions were observed with Folin-Ciocalteau reagent, sodium nitroprusside, or reduced sodium nitroprusside (Ziegler & Pettit, 1964).

† Additional peaks present in extracts of blank urine specimens from smokers and non-smokers had relative retention times of 0·32, 0·38, 0·45 and 0·90.

of the substances after elution with ether from alkaline suspension of the silica gel "spot", concentration and chromatography on column 1, relative to methadone. From these data, it appears that metabolite 2 may contain a tertiary amino-group

because of its reaction with *cis*-aconitic anhydride, while metabolites 3 and 4 may each contain a primary amino-group (see Table 1). Vidic (1957) previously detected a primary amine in urine extracts after administration of methadone. Further identification of these metabolites, which appeared to be present in minor proportions to metabolite 1, was not attempted.

Quantitative data

The quantitative distribution of methadone and metabolite 1 in postmortem tissues from eleven male subjects is given in Table 2.

From Table 2 it may be seen that, except for subject 7, methadone was present in the blood; the absence of the metabolite from the blood samples may be a consequence of the assay method (limit of detection in a 10 ml sample is 5 μg). Methadone blood concentrations were always low compared with other specimens which may be an indication of protein binding in the tissues as demonstrated by Sung, Way & Scott (1953) in the rat.

The presence of methadone in the stomach content of subjects 2 and 4 is notable since recent injection sites were evident and oral administration of the drug was unlikely. Secretion of methadone in the gastric juice may account for these observations which are consistent with our previously unpublished finding of nicotine in the gastric content of smokers. This is not an unexpected observation in man in view of the work of Shore, Brodie & Hogben (1957) in animals.

Both the unchanged drug and metabolite 1 were found in specimens of bile and urine, the amount of the metabolite in the bile usually exceeding that of methadone whereas the reverse was found in the urine. Beckett (1969) reported the urinary excretion of 9·6% of an oral dose of methadone unchanged and 17·7% as metabolite 1 in 24 h, the proportion of methadone excreted in the urine ranging from 1–20% according to the controlled urinary pH value. It would appear from the results that the extent of biliary secretion at least of the metabolite 1 may indicate faecal excretion of a significant proportion of the drug in this form.

In the tissues both methadone and metabolite 1 are usually present with the exception of those subjects where a suspected acute overdose of methadone in the absence of tolerance is probable (subjects 9 and 10). Even so, the survival time might have been sufficient to allow formation and retention of the metabolite in some of the tissues.

The concentrations of methadone found in the tissues vary from one subject to another, probably reflecting individual tolerance and drug-taking habits. It is perhaps more informative to consider the relative tissue concentrations for each subject and these are shown in Table 3, the results being derived from Table 2 by relating each concentration to that of the liver taken as 1. This Table shows that liver and kidney levels are approximately equal except in subjects 9 and 10 in whom lack of tolerance or at least a reduced tolerance to the drug was probable. Also, the time interval between administration of methadone and death of subject 11 was shorter than cases in which methadone alone was the significant drug, i.e. the presence of a barbiturate or morphine as well as methadone decreased the survival time compared with an acute fatal overdose of methadone.

Methadone, as may be expected of a CNS-active drug, was found in the brain although in lesser amounts than in the other organs analysed. In three subjects (2, 4, and 8), metabolite 1 was also present.

Table 2. *Methadone and metabolite* 1 *concentrations*† *in body fluids and tissues from* 11 *male subjects.*

Subject No.	Age		Blood	Bile µg/ml	Urine	Liver	Kidney	Spleen µg/g	Lung	Brain	Stomach content mg	Other drugs	Circumstances
1	23	Methadone	2·13	8·9	19·3	19·2			24·0	2·2	7·0	Methylamphetamine (Tincture of Cannabis)	Probable acute oral overdose ? Loss of tolerance. About 100–200 mg 9 h before death.
		Metabolite 1	—	14·6	5·3	1·3			0·15	—			
2	22	Methadone	0·3	1·56	5·2	0·92	0·89	0·89	3·0	0·19	1·5	*Barbiturate (short-acting) morphine Amphetamine	? Overdose. Daily dose 10 mg.
		Metabolite 1	—	7·9	0·72	0·3	0·42	0·22	0·64	0·03	—		
3	22	Methadone	0·95	40·6	132	2·5	2·9	4·1	7·2	—	—	None	Survived 9 h after last dose.
		Metabolite 1	—	+	46·2	0·25	1·9	0·15	0·32	—	—		
4	19	Methadone	2·3	7·1	17·4	3·8	3·2	2·7	3·5	0·6	2·5	None	Probable acute overdose; possibly tolerant. Sur. 6 h.
		Metabolite 1	—	110	13·4	+	+	+	+	+	—		
5	27	Methadone	1·6	15·0	18·0	4·0	4·3	1·5	8·5	—	36·6	None	Took about 200 mg 16 h before death.
		Metabolite 1	—	102	9·2	0·41	0·16	0·04	0·98	—			
6	21	Methadone	1·2	10·2	5·0	3·7	3·32	3·8	8·7	2·0	—	Morphine	Had been taking 30 mg/day but not during last 3 days in hospital. Supplied by visitors?
		Metabolite 1	—	6·1	6·4	0·33	0·7	1·56	0·29	—	—	Methaqualone	
7	34	Methadone	—	—	0·52	0·58	0·67	1·56	—	—	—	Morphine	None given during 4 days in hospital before death.
		Metabolite 1	—	0·67	—	0·14	1·13	1·0	0·11	—	—		
8	28	Methadone	1·43	42·5	8·8	0·4	0·48	0·2	0·8	0·05	—	*Methaqualone	Oral methadone available; methaqualone prescribed.
		Metabolite 1	—	9·8	3·5	+	+	+	+	+	—	Alcohol	
9	32	Methadone	3·04	11·9	76·2	49·5	18·3	20·9	110	—	—	Methaqualone	Probable acute overdose; considered 'off drugs'.
		Metabolite 1	—	9·85	1·6	—	2·7	—	—	—	—		
10	19	Methadone	0·54	9·6	34·6	3·3	2·03	2·32	11·1	0·23	43	Amphetamine	Considered to have been 'off drugs'.
		Metabolite 1	—	0·2	4·5	0·8	0·63	—	—	—	—	Iprindole	
11	28	Methadone	0·22	1·9	5·5	0·25	0·14	0·18	1·35	—	—	*Morphine	Took 30 mg per day probably with other drugs; survived about 5 h after barbiturate injection.
		Metabolite 1	—	17·7	1·65	0·13	0·17	0·40	0·07	—	—	*Barbiturate (Intermediate-acting) Lymecycline	

* Significant in respect of the cause of death.
† Since the internal standard was added to the specimens before extraction no correction factors have been applied to the results.

Table 3. *Ratios of tissue concentrations of methadone to the concentrations found in the liver, calculated on a wet weight basis.*

Subject No.	Liver concn μg/g	Liver	Ratio of concentrations to that of liver			
			Kidney	Spleen	Lung	Brain
1	19·2	1·0			1·25	0·12
2	0·92	1·0	0·97	0·97	3·26	0·21
3	2·5	1·0	1·16	1·64	2·88	
4	3·8	1·0	0·84	0·71	0·92	0·16
5	4·0	1·0	1·01	0·375	2·12	
6	3·7	1·0	0·89	1·025	2·35	0·54
7	0·52	1·0	1·12	1·29	3·0	
8	0·4	1·0	1·2	0·5	2·0	0·13
9	49·5	1·0	0·37	0·42	2·24	
10	3·3	1·0	0·61	0·70	3·36	0·07
11	0·25	1·0	0·56	0·71	5·4	

Lung tissue concentrations usually exceeded that of the liver (except subject 4). The finding of comparatively large amounts of methadone in the lungs compared with other organs correlates with animal work (Rickards, Boxer & Smith, 1950) and unpublished data (in the Department) for some other CNS-active drugs including tricyclic anti-depressants and phenothiazine derivatives. Although many organic bases may accumulate in the lungs of dosed animals, we have not always found a parallel in man. In toxicological analyses, when only qualitative results are needed, there are advantages in testing lung tissue in preference to liver especially since the extracts present fewer experimental difficulties.

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