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Methadone in Autopsy Cases

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Summary. In the last year 5 cases of fatal methadone intoxications have been investigated. The mass spectrum of methadone and the results of the gas chromatographic determinations are presented. The variations in plasma half-life of methadone are discussed. Blood and tissue levels in fatal methadone intoxications are reported and discussed.

Zusammenfassung. Im letzten Jahr wurden insgesamt 5 Methadonvergiftungen untersucht. Das Massenspektrum von Methadon und die Ergebnisse von gaschromatographischen Untersuchungen werden angegeben. Die Variationen in Plasmahalbwertszeit sowie die Konzentration von Methadon im Blut und Gewebe bei tödlichen Vergiftungen werden angegeben und diskutiert.

Key word: Methadone intoxications, autopsy values.

Methadone was synthesized in Germany in 1941. It has found great application in the treatment of opiate addicts (Dole and Nyswander, 1965). During recent years, however, methadone taken parenterally has become a popular form of drug abuse (Fraser, 1971). Although fatal methadone intoxications are not rare (Bieter and Hirsh, 1948; Naeve and Körner, 1954; Alha and Ohela, 1955; Peterson, 1955; Schmidt, 1955; Wagner, 1958; Wilson and Fisher, 1969; Garner, 1970a, b; Dole *et al.*, 1971), few toxicological data are published in the literature (Worm and Schou, 1970; Robinson and Williams, 1971). The metabolism, excretion and disposition of methadone have been extensively studied (Becket *et al.*, 1968; Baselt and Casarett, 1972; Inturrisi and Verebely, 1972a) and metabolites have been identified and synthesized (Beckett *et al.*, 1971; Pohland *et al.*, 1971). The main metabolite is 2-ethylidine-1.5-dimethyl-3.3-diphenylpyrrolidine. Baselt and Casarett (1972) have shown that the concentration of unchanged drug and this metabolite in random urine samples are dose dependent and that the urinary excretion increased with increasing dose. In subjects on a methadone maintenance program receiving a single daily oral dose of 80 mg, about 70% of this dose was excreted in the urine in 24 hrs. At a dose of 150 mg more than 90% of the dose was excreted in the urine in 24 hrs. In thise cases the urine pH ranged from 5.7 to 5.9 and more than 60% of the dose was excreted in the urine as unchanged methadone. The urinary excretion may, however, be affected by changes in urine pH. Neither methadone nor its metabolite were present in the urine as the glucuronide conjugate (Inturrisi and Verebely, 1972c). The plasma level of methadone has been determined after terapeutic dosage of 10 mg (Inturrisi and Verebely, 1972b), and in addicts receiving a daily oral dose of 100 or 120 mg (Inturrisi and Verebely, 1972c). The amount of methadone bound to human plasma albumin is

relatively independent of the concentration of methadone, but more dependent on the concentration of albumin (Olsen, 1972).

During 1972, 5 cases of fatal methadone intoxications have been investigated in our institute and the results of the toxicological examinations are presented in this paper. Since only a few other cases of fatal intoxications in drug addicts have been investigated in the same period of time, the number of methadone intoxications are surprisingly high.

Case Reports[1]

Case 1. A 21-year-old man died after a party where drugs were taken intravenously. He was earlier known to be a drug addict, but was now probably off drugs. Autopsy showed fresh needle marks on the left arm. Trachea and bronchi contained frothy fluid, and the lungs were large and edematous.

Case 2. A 23-year-old drug addict was found dead in his apartment. It is likely that during the last days before death he had taken large amounts of narcotics intravenously. Autopsy showed fresh needle marks on both arms. Aspirated stomack contents were found in the air passages. The lungs weighted 1450 g and were edematous.

Case 3. A 22-year-old addict injected himself with methadone before taking off on a travel. He seemed all right during the same night, but the next morning he had breathing difficulties and was heard gargling. Later in the day he was found dead. Autopsy revealed fresh needle marks on both arms. The lungs showed bronchopneumonia, the lymph nodes of the liver hilus were enlarged and the liver showed signs of infection.

Case 4. A 23-year-old drug addict come home one night, when he was under the influence of narcotics. Just after arrival he injected himself. After a while he felt unwell and went to bed. The next morning he was found dead. Autopsy showed fresh needle marks on both arms. Trachea and bronchi contained frothy fluid and the lungs were edematous. Large lymph nodes were seen in the liver hilus. Signs of chronic and acute liver infections were seen.

Case 5. A 42-year-old alcoholic and drug addict was brought to an institution at about 1 p.m. He was treated with methadone tablets 4×25 mg and chlorpromazine 2×50 mg. In the evening he seemed all right, but the next morning he was found dead. Autopsy showed frothy fluid in the air passage and heavy and edematous lungs, and a fatty liver.

Material and Methods

Samples of blood, brain, liver, kidney and urine were collected at autopsy and delivered for toxicological examination. The samples were stored at —20° C. Methadone is a weak base and may be isolated by extraction from alkaline solution. Tissue samples were prior to extraction homogenized in 70% ethanol according to Frøslie and Schubert (1970).

10—20 ml of blood was diluted with water to about 50 ml, made alkaline with concentrated ammonia and extracted three times with 100 ml ether. Tissue samples (30 g) were homogenized with 20 g of water and thoroughly mixed with 100 g of ethanol. The sherry was refrigerated overnight and then centrifugated and filtrated. 50 g of the ethanol extract, corresponding to 10 g of tissue, was evaporated to a small volume on a boiling water bath under a stream of air. The residue was diluted with 40 ml water and made alkaline with concentrated ammonia. After being transferred to a separating funnel the solution was extracted three times with 100 ml ether. 5—10 ml of urine was diluted to 40 ml, made alkaline and extracted three times with 100 ml ether.

All the ether extracts were stored overnight in the dark. The next day the ether was washed with a small amount of water; the washings being discarted, and reextracted twice with 40 ml 0.1 N sulfuric acid. The acid was poured directly into a new separating funnel, made alkaline with concentrated ammonia, and extracted twice with 150 ml chloroform. The

1 The autopsies were performed by Drs. J. Hognestad, J. Lundevall and B. Olaisen.

final extracts were stored overnight in the dark, evaporated to a small volume, transferred to centrifuge tubes with ground glass stoppers and carefully evaporated to dryness under a stream of air. The residue was dissolved in at least 100 µl of methanol and 2 µl injected into the gas chromatograph.

Phenmetrazine was isolated by the same extraction procedure as described above. A few drops of methanolic hydrochloric acid were added to the chloroform extract prior to the evaporation in order to avoid loss of phenmetrazine. Chlorpromazine was also isolated by same extraction procedure, but the samples were hydrolyzed with potassium hydroxide on a boiling water bath prior to extraction with ether. Both substances were determined on the gas chromatograph.

Gas Chromatography

The samples were analysed on a Varian Aerograph Series 2700 gas chromatograph equipped with flame ionization detectors. The columns were 5 ft. × 1/8"I.D. stainless steel filled with 3% SE-30 on Varaport-30 100—120 mesh support and 6 ft. × 2 mm I.D. glass coil filled with 3% OV-17 on Chromosorb G 80—100 mesh support. The nitrogen flow was 50 ml/min, the oven was operated at 210°C for the SE-30 column and 250°C for the OV-17 column. The injector and detector temperatures were 30 and 40°C higher, respectively.

Mass Spectrometry

The mass spectra were run on a Varian MAT CH7 mass spectrometer connected with a Varian Aerograph Series 1400 gas chromatograph using a 10% OV-17 glass column. The instrument was operated at 70 eV and the ionization temperature was 190°C. The gas chromatograph was programed from 180 to 300°C at 10°C/min. The helium gas flow was 30 ml/min and 4 µl of the urine extracts were injected into the instrument.

Results and Discussion

The extraction procedure described above gives a very pure extract and a high recovery. On an average 87% of methadone added to different samples were recovered. The results of the gas chromatographic determinations are presented in Table 1. Alcohol was not detected in any of the cases and only in case 4 and 5 small amounts of other drugs were present. The most intense peak in the mass spectrum is m/e 72 corresponding to the fragment $C_4H_{10}N$. The relative intensity of all other peaks are less than 10%. The mass spectrum shown in Fig. 1 is almost identical with the spectrum from pure methadone.

The diagnosis of intravenous narcotism was stated in case 3 and 4, and in case 2 aspirated material was seen in the lungs, a not uncommon autopsy finding in drug addicts (Helpern and Rho, 1966; Siegel *et al.*, 1966; Johnston *et al.*, 1969; Siegel and Bloustein, 1970). In case 3 bronchopneumonia was stated and this may have contributed to the death.

The plasma half-life of methadone has been determined in three normal volunteers receiving 10 mg of methadone (Inturrisi and Verebely, 1972b) and in 5 methadone maintenance subjects receiving a single daily oral dose of 100 or 120 mg (Inturrisi and Verebely, 1972c). In the first group the mean half-life was 7.3 hrs (7.6, 6.7, 7.5). In the second group the mean apparent half-life was 25 hrs (29, 47, 31, 21, 13, 17) and the peak plasma level occurred after 4 hrs. These results indicate that a longer half-life may be expected in drug addicts using methadone than in normal subject. In the present cases it is not known if

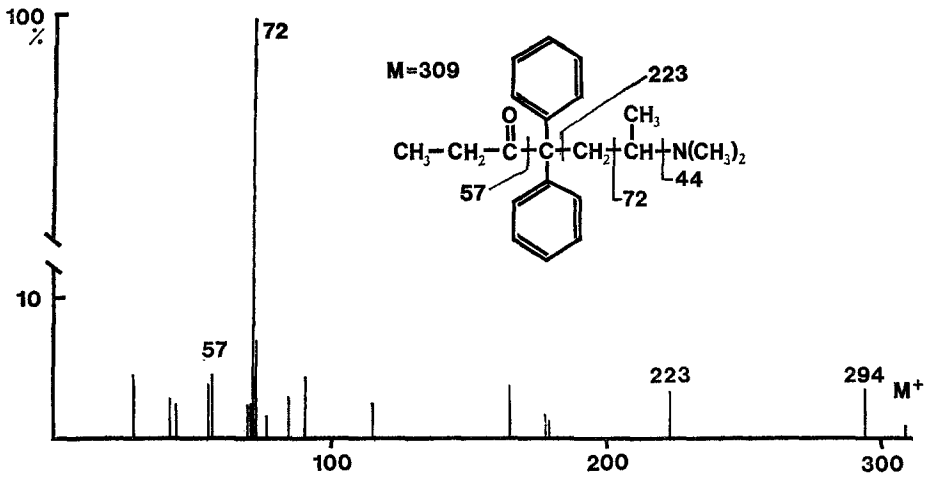

Fig. 1. Mass spectrum of methadone extracted from urine (case 1). The spectrum of pure methadone base is almost identical with the spectrum above

Table 1. The concentration of methadone base in different samples in 5 autopsy cases. The concentrations are calculated as mg/100 ml(g)

	Blood	Brain	Liver	Kidney	Urine	Remarks
Case 1	0.043	0.045	0.28		1.80	
2			0.22		0.74	Aspirated material in the lungs
3	0.051		0.12	0.14	1.60	Intravenous narcotism Bronchopneumonia
4	0.10		0.31		0.87	Intravenous narcotism 0.3 mg phenmetrazine/100 ml urine
5	0.036		0.13		0.32	0.01 mg chlorpromazine/100 ml blood 0.03 mg chlopromazine/100 g liver 0.04 mg chlorpromazine/100 ml urine

methadone had been used for a longer period or not, although is it reason to believe that tolerance had been developed in cases 2, 3 and 4.

The plasma level of methadone in the five methadone maintenance subjects mentioned above varied from 0.020 to 0.108 mg/100 ml over a 24-hour period after a single oral dose (Inturrisi and Verebely, 1972c). In the present cases concentrations of the same order of magnitude were found. These doses, except in case 5, were taken intravenously giving a peak plasma level short after injection, and the determinations were performed on postmortem blood. Together this indicates that the doses were considerable.

Only in case 5 the amount of drug taken prior to death is known. Although chlorpromazine may have potentiated the effect of mathadone, this example does emphasize the high toxicity of methadone.

Robinson and Williams (1971) have determined the methadone concentration in 11 autopsy cases. Only in 3 of these cases no other drugs were found and the methadone concentrations in liver in these cases were 0.25, 0.38 and 0.40 mg/100 g, respectively. This is in good agreement with the results presented here. However,

in 2 of the 11 cases mentioned above a considerable higher concentration of methadone was found.

The present investigation confirms the high toxicity of methadone. It also indicates the concentration level of methadone in blood and tissue that may be expected in fatal intoxications.

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