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A note on the effects of 2, 4-diamino-5-phenylthiazole and 1, 2, 3, 4-tetrahydro-9-aminoacridine on morphine metabolism

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Chromatographs of extracts of urine from patients receiving mixtures of morphine plus amiphenazole or morphine plus 1,2,3,4-tetrahydro-9-aminoacridine (THA) have a spot which is due to a substance with properties similar to morphine-*N*-oxide. This spot was not present in urine of patients given morphine, amiphenazole or THA alone. Morphine-*N*-oxide may be a metabolite of morphine, and amiphenazole and THA may inhibit the further metabolism of morphine-*N*-oxide.

THE use of combinations of 2,4-diamino-5-phenylthiazole (amiphenazole) and morphine, or 1,2,3,4-tetrahydro-9-aminoacridine (THA) and morphine for the relief of chronic pain has been reported to have advantages over treatment with morphine alone. In patients with intractable pain from terminal carcinoma given morphine and amiphenazole there was no narcosis, and the respiratory depressant action of morphine was absent (Shaw & Shulman, 1955, 1955a). McKeogh & Shaw (1956) confirmed these observations and showed that, with this drug combination, analgesia was not impaired. Marked tolerance to morphine was not observed, and when this drug regimen was withdrawn there was no evidence of an abstinence syndrome. Similar effects were reported when THA was administered concurrently with morphine (Shaw, 1960; Stone, Moon & Shaw, 1961). These advantages were attributed by Gershon, Bruce & others (1958) to the *in vivo* formation of a "complex" between morphine and amiphenazole. The present work deals with an attempt to detect and identify such a substance in the urine of cancer patients given morphine plus amiphenazole, or morphine plus THA, or amiphenazole, THA or morphine alone.

Experimental

Examination of urine samples. Urine samples were collected over a 24 hr period from cancer patients (Austin Hospital, Heidelberg, Victoria) receiving morphine, THA or amiphenazole, or mixtures of amiphenazole and morphine or of THA and morphine; other drugs were given in addition to the above as demanded by the needs of the patients.

The urine sample (250 ml) was adjusted to pH 9·0 with 50% NaOH and shaken with an equal volume of an isopropanol–chloroform (1 : 3) mixture for 10 min. Two layers slowly formed and the separated organic phase was centrifuged at 2,800 rev/min for 10 min. This phase was reduced under low pressure to <2 ml, and then made up to 2 ml with the isopropanol–chloroform mixture.

Paper chromatography. The concentrated organic extract (30 μl) was spotted on Whatman No. 1 paper and thoroughly dried. Solutions containing 25 μg each of amiphenazole, THA, morphine, amiphenazole plus morphine, and THA plus morphine were used as markers. Ascending

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chromatography was carried out at 20°. The solvent systems were n-butanol–ammonia (sp. gr. = 0·88)–water (4 : 1 : 3, v/v), n-butanol–pyridine–water (6 : 4 : 3), n-butanol–methyl isobutyl ketone–33% ethylamine (2 : 2 : 1) and n-butanol–isobutyric acid–water (7 : 2 : 3). The detecting agents were diazotized *p*-aminoacetophenone (Lundgren, 1956), iodoplatinate and bromcresol green (Smith, 1960).

Synthesis of a substance formed by the interaction of morphine and amiphenazole. This was as reported by Woo (1965) and involves mixing solutions of morphine and amiphenazole in methanol, adding concentrated ammonia and allowing the mixture to react for 15 hr at room temperature (20°). Unreacted morphine and amiphenazole are then removed from the mixture after drying by extraction with ethyl acetate under alkaline conditions. The product is purified using column chromatography and by recrystallization.

Spectroscopy. Infrared spectra of solid samples were measured, using paraffin oil mulls, with a Perkin-Elmer infrared spectrophotometer (model 137). Ultraviolet spectra were measured with a Beckman spectrophotometer DU (model G2400).

Elemental analysis for C, H, N and O were performed by the Australian Micro-analytical Service (C.S.I.R.O.).

Morphine-*N*-oxide (m.p. 272–273°) was synthesized using the method of Freund & Speyer (1910, m.p. 273°).

Results

Chromatograms of extracts of the urine of 5–10 patients receiving either morphine and amiphenazole or morphine and THA showed a spot not found in the urine of patients receiving morphine, amiphenazole or THA alone nor was it present in urine from patients who were not receiving drugs. The Rf values of the unknown substance and its colour reactions with various detecting agents were identical to those of authentic morphine *N*-oxide (Table 1). But we have not, as yet, isolated sufficient to make a positive identification.

TABLE 1. Rf VALUES AND COLOUR REACTIONS WITH DETECTING AGENTS

Solvent system	Morphine	Amiphenazole	THA	Substance in urine	Substance synthesized	Morphine *N*-oxide
			Rf values			
n-Butanol–ammonia–water	0·70	0·88	0·90	0·15	0·15	0·14
n-Butanol–pyridine–water	0·81	0·87	0·55	0·55	0·55	0·53
n-Butanol–methylisobutyl ketone–ethylamine ..	0·45	—	0·85	0·00	0·00	0·00
n-Butanol–isobutyric acid–water	0·48	0·56	0·73	0·62	0·62	0·64
Reagent			**Colour of chromatograph spots**			
Diazotized *p*-amino acetophenone	Purple	Yellow-orange	Nil	Deep-purple	Deep-purple	Deep-purple
Iodoplatinate	Purple-blue-black	Nil	Violet	Violet-purple	Violet-purple	Violet-purple
Bromcresol green ..	Blue	Nil	Powder-blue	Purple-blue	Purple-blue	Purple-blue

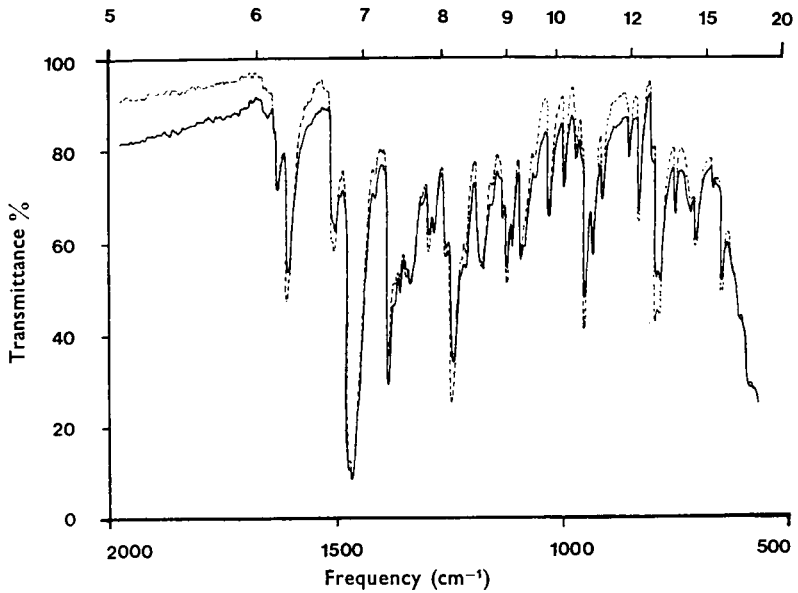

FIG. 1. Infrared spectra of morphine-*N*-oxide (broken line) and the substance synthesized by mixing morphine and amiphenzaole (solid line).

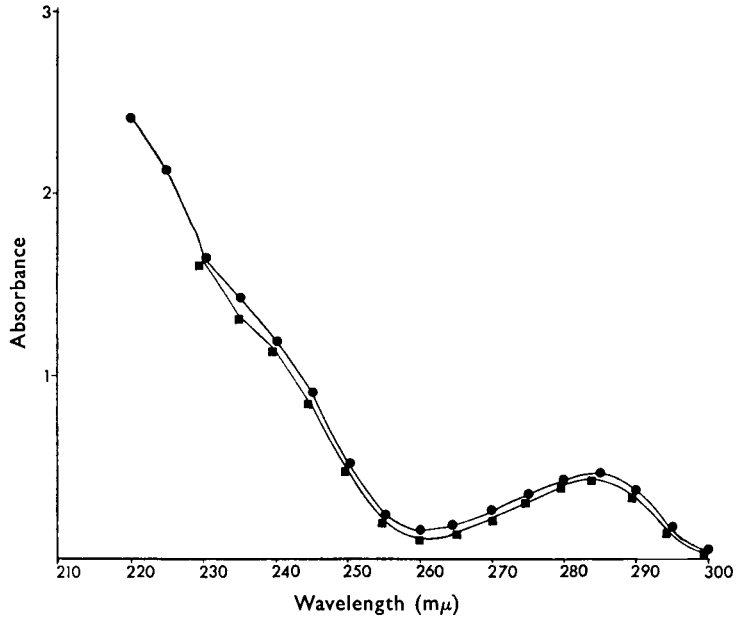

FIG. 2. Ultraviolet spectra of aqueous solutions of morphine-*N*-oxide (●——●) and the substance synthesized by mixing morphine and amiphenazole (■——■).

The substance synthesized from the mixture of morphine and amiphenazole was a white powder, m.p. 251–256°. Elemental analysis of three samples (mean found: C, 66·7; H, 6·3; N, 4·7%) showed that the empirical formula ($C_{16·5}$,$H_{18·6}$,N, O_4) corresponded closely to that of morphine *N*-oxide (C_{17},H_{19},N, O_4) suggesting that the substance synthesized was morphine *N*-oxide, and this was further confirmed by the similarity of the colour reactions and chromatographic properties (Table 1) in comparison with the authentic morphine *N*-oxide, and of the close similarity of their infrared (Fig. 1) and ultraviolet spectra (Fig. 2).

Discussion

The substance synthesized by reacting together morphine and amiphenazole would seem to be morphine *N*-oxide on the evidence of the infrared and ultraviolet spectra, elemental composition and paper chromatography. Because of the similarity in chromatographic properties of the synthetic substance and the substance detected in urine, it is possible that morphine *N*-oxide is a metabolite of morphine. Several drugs are already known to be excreted to some extent by man, as *N*-oxides, notably chlorpromazine (Fishman, Heaton & Goldenberg, 1962), imipramine (Fishman & Goldenberg, 1962) and chlorcyclizine (Kuntzman, Phillips & others, 1967). Also, studies with animals have shown that other drugs undergo *N*-oxidation and that NADPH- and oxygen-dependent liver microsomal enzymes are capable of forming *N*-oxides *in vitro* (McMahon, 1966).

A generally recognized metabolic fate of morphine is demethylation. The evidence pointing to the metabolic formation of morphine *N*-oxide from morphine is of interest because of the hypothesis originally suggested by Fish, Johnson & others (1955) that *N*-oxides are intermediates in the oxidative demethylation of drugs.

The substance excreted in the urine was detected only when either THA or amiphenazole was administered in conjunction with morphine but not when morphine, THA or amiphenazole were given alone. This suggests that THA and amiphenazole cause excretion of morphine *N*-oxide either by inhibiting its degradation to other metabolites or by inhibiting an alternative pathway for the metabolism of morphine. Less likely is the possibility that THA and amiphenazole stimulate the formation of morphine *N*-oxide directly. As the *N*-oxide was not found in the urine of patients treated with morphine alone it seems unlikely that its formation could be due to an artefact arising from the extraction procedure or by bacterial action.

The hypothesis of Gershon & others (1958) that combined administration of morphine with amiphenazole or THA leads to formation of a complex between morphine and those drugs would not appear to be substantiated.

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