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Molecular complexation of morphine and indol-3-yl sulphuric acid in the dog

During the course of investigation on morphine conjugates in the dog (Misra, Yeh & Woods, 1970) it was found that the methanolic eluate from Amberlite XAD-2 resin column on which urinary metabolites of morphine-*N*-methyl[^{14}C] had been adsorbed, on descending paper chromatography with the solvent systems n-butanol–acetic acid–water (I, 4:1:5 v/v organic phase; II, 35:3:10 v/v; III 100:4:24 v/v) consistently showed the presence of another iodoplatinate-positive radioactive spot having an Rf higher than that of free morphine and morphine conjugates. The Rf values of free morphine and this unknown radioactive spot in systems I-III were 0·53, 0·63; 0·43, 0·52; 0·38, 0·55 respectively. Paper and thin-layer chromatography using n-butanol-mineral acid or ammonia systems however showed the morphine spot only. Washing the methanolic residue of unknown product from Whatman 3 MM paper chromatograms with 4% K_2HPO_4 solution, saturated bicarbonate or ammonia solutions, and subsequent extraction with ethylene dichloride containing 30% n-amyl alcohol, gave an extract which showed the presence of morphine only. Similarly autoclaving the residue of unknown product with 2·4 N hydrochloric acid at 15 p.s.i. for 1 h, basification to pH 9, and solvent extraction showed a light indigo blue colour in the organic phase which on evaporation and paper chromatography showed the presence of morphine only.

A positive colour test for indol-3-yl sulphate with Ehrlich reagent (Decker, 1955; Rodnight, 1956) and morphine on acid or alkaline treatment suggested that the unknown radioactive product was a molecular complex of morphine and indol-3-yl sulphuric acid. Co-chromatography of the eluted unknown product with a synthetic morphine-indol-3-yl sulphuric acid complex prepared as described below substantiated this point. A single spot (Rf 0·52), positive to iodoplatinate and Ehrlich reagents, with a single peak of radioactivity coincidental to this spot was obtained using system III (Rf non-labelled morphine, 0·36). The complex isolated from Whatman 3 MM paper chromatograms with methanol and purified on neutral alumina column was a brownish hygroscopic powder of ill-defined melting point softening at 155–160° and melting at 175–178° (decomp.).

Molecular complexes of morphine, nalorphine, normorphine and tryptamine bases 1:1 with indol-3-yl potassium sulphate (indican) were prepared by the method of Boyland, Sims & Williams, 1956. Morphine complex: turns blue at 150°, m.p. 167–170° (decomp.), $C_{25}H_{26}N_2O_7S$, $\frac{1}{2}H_2O$, yield 55%. Nalorphine complex: m.p. 186–187° (decomp.), $C_{27}H_{28}N_2O_7S$, $\frac{1}{2}H_2O$, yield 80%. Normorphine complex: sinters 167°,

m.p. 173–175° (decomp.), $C_{24}H_{24}N_2O_7S$, yield 58%. Tryptamine complex: m.p. 160–161° (decomp.), $C_{18}H_{19}N_3SO_4$, yield 50%. Analyses for C, H and N were within the usual limits. The melting points of the morphine and normorphine complexes were ill-defined.

The 5-hydroxytryptamine complex, 5-hydroxytryptamine, indol-3-yl sulphate had Rf 0·55, 0·44, 0·45 respectively in system I.

Indol-3-yl potassium sulphate is a normal constituent of blood serum (Townsend, 1938) (26–85 μg %) and cerebrospinal fluid (Tinelli, 1945) (23·7 μg %) and its range of excretion (Bryan, 1965) in man on a normal balanced diet has been reported as 83 ± 36 mg/24 h. Its content and retention in blood and cerebrospinal fluid however markedly increased in various nervous pathological conditions (Mitolo, 1955) (67·5 μg %), morphine poisoning (Brocher, 1931), renal insufficiency (147–180 μg %) and intestinal obstruction (Haas, 1916). It was reportedly not destroyed or retained by human organs and excreted unchanged (Schlierbach, 1937) in man within 48 h of intravenous injection of a 100 mg dose. It was the major product of indole metabolism (King, Parke & Williams, 1966) in various animal species and it has been suggested (Posner, Mitoma & Udenfriend, 1961) that it is formed by liver microsomal hydroxylation of indole rather than by bacterial fermentation in the gut.

Synthetic morphine: indol-3-yl sulphuric acid complex (13·1 mg/kg) injected subcutaneously in male Sprague-Dawley rats showed analgesia comparable to morphine sulphate (10 mg/kg) by the hot plate technique. The demonstration of molecular complexation between morphine and indol-3-yl sulphuric acid does not necessarily imply that such a complex forms *in vivo*. However the possibility of such complexation as an artifact should not be overlooked in experiments on identification of narcotic analgesics (Fujimoto and Wang, 1970) in similar excretion studies.

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