effective than imipramine or fenfluramine and as active as Lilly 110140. These data are in close agreement with those recently presented by Tuomisto (1974) and add further support to the usefulness of platelets as a model for the study of serotoninergic nerve endings.

Possibly, the methodology for measuring 5-HT uptake by platelets and its pharmacological inhibition can be further improved (e.g. by shortening the incubation period even more and/or reducing the platelet number and/or stopping the reaction by means more effective than lowering the temperature).

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A new g.c. procedure, based on nitrosation, for the simultaneous determination of propoxyphene and norpropoxyphene in biological material

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Propoxyphene is a widely used oral analgesic which is metabolized in man mainly to the secondary amine norpropoxyphene (Lee, Scott & Pohland, 1959) and to a lesser extent to the primary amine dinorpropoxyphene (McMahon, Sullivan & others, 1973).

We present a method in which norpropoxyphene is converted into *N*-nitrosonorpropoxyphene by reaction at low temperature with sodium nitrite. Under these conditions propoxyphene does not react appreciably; norpropoxyphene is converted almost quantitatively into *N*-nitrosonorpropoxyphene thus permitting the extraction of both compounds from an acidic medium into small volumes of chloroform. At the same time the primary amine dinorpropoxyphene may be expected to be converted into an alcohol with different chromatographic properties. This selective concentration thus permits and eliminates most other basic contaminants which contribute to the background on the gas chromatograms.

Dextropropoxyphene hydrochloride and (+)-norpropoxyphene hemicitrate (Eli Lilly Inc.) were used. The impurities normally present in samples of norpropoxyphene (Verebely & Inturissi, 1973) were shown to be carried through the extraction with norpropoxyphene itself but were satisfactorily separated at the chromatographic stage. Solvents were acid and alkali washed and distilled in an all-glass apparatus. All glassware was silylized in the usual manner.

Standard solutions were prepared by adding 0–5 μg of propoxyphene and 0–20 μg of norpropoxyphene to 2 ml of urine and 0–5 μg each of both drugs to 10 ml of serum. Calibration curves were constructed using cocaine (10 mg per 100 ml ethanol) as an internal standard. 70 μl of this solution were added to urine samples and 50 μl to serum samples at the beginning of the analysis.

Blood samples were centrifuged within 1–2 h of collection and the serum stored at —15° until analysed. Urine samples were similarly stored at —15°.

A Packard Model 824 gas chromatograph fitted with dual F.I.D. and silylized 0·5 m glass columns (3 mm internal diameter) packed with 1% SE 30 on

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Chromosorb 750 (100–120 mesh) was used. The instrument settings were as follows:

Flow rates: hydrogen 15 ml min^{-1}; nitrogen 40 ml min^{-1}; air 350 ml min^{-1}. Attenuation 3×10^{-11}. Injection block temperature 270°; detector temperature 280°. Samples were injected at a column temperature of 180° which was maintained until the appearance of the propoxyphene peak (8 min). The temperature was then rapidly increased at 32° min^{-1} to 210° when the internal standard (11 min) and *N*-nitrosonorpropoxyphene (21 min) were eluted.

To 5–10 ml of serum were added 1·0 ml of carbonate buffer (pH 9·8; 1M) and 1 drop of octanol. The mixture was saturated with respect to NaCl and extracted on the shaker with butyl chloride (7 ml, 6 ml per 10 min). After centrifugation, 10·0 ml of the combined butyl chloride phase were extracted with 0·01N H_2SO_4 (3 ml, 2 ml per 10 min). The combined acid extract was washed once with n-hexane (5 ml per 3 min) and the organic phase discarded. The aqueous acid phase was then cooled in ice, 100 μl of sodium nitrite solution added (29 mg of $NaNO_2$ ml^{-1} distilled water), and after mixing, the solution was left for 15 min in ice. Thereafter, 1·0 ml 1M sodium acetate buffer pH 4·0 was added and the solution saturated with respect to NaCl. A 5·0 ml portion was transferred to a 'nipple tube' (ground glass-stoppered tube with drawn-out bottom portion) and extracted with chloroform (2 ml, 1 ml per 5 min). After centrifugation the combined chloroform phase was transferred to a clean nipple tube, 0·2 ml of 2,2-dimethoxypropane added and the mixture concentrated (nitrogen stream in a water bath at 50°) to approximately 20–40 μl final volume of which 2 μl were injected into the chromatograph. For urine an identical procedure was used starting with 2 ml of urine and 1·0 ml of carbonate buffer.

Nitrosation proceeds virtually to completion when 4 ml of a 0·01N H_2SO_4 solution containing up to 20 μg of norpropoxyphene was treated at low temperature (4°) with 100 μl of a sodium nitrite solution containing 29 mg of $NaNO_2$ ml^{-1} for 15 min. Propoxyphene is not appreciably effected under these conditions. After nitrosation and buffering the acid solution at pH 4·0 with 1 M acetate buffer, both propoxyphene and *N*-nitrosonorpropoxyphene could be satisfactorily extracted into chloroform. In the analysis of patients' samples no evidence of the presence of dinorpropoxyphene (which would be converted into the corresponding alcohol) was obtained.

By analysing the same urine and serum samples (which contained 4 μg of propoxyphene and 13·4 μg of norpropoxyphene per 2 ml of urine and 3 μg each of the two drugs per 10 ml serum) ten times, the relative standard deviations obtained were propoxyphene 7·5%; norpropoxyphene 7·0% (for urine) and propoxyphene 8·1% and norpropoxyphene 7·2% (for serum).

Accuracy was assessed by recovery experiments in which known amounts of propoxyphene and norpropoxyphene had been added to serum and urine samples. All values were automatically corrected for the non-quantitative extraction of both drugs by the fact that standard curves prepared in urine and serum samples were used to evaluate the results. Average results (10 experiments) were as follows: Urine (2 μg ml^{-1} added of each drug) propoxyphene 101·3%; norpropoxyphene 104·4%. Serum (1 μg ml^{-1} added of each drug) propoxyphene 96·0%; norpropoxyphene 98·4%.

Phenobarbitone, diphenylhydantoin, acetylsalicylic acid and diazepam or their metabolites in man do not interfere in this assay.

The choice of cocaine as internal standard was based upon its retention time, the fact that it is extracted—like propoxyphene—by the organic solvents used from both alkaline (pH 9·8) and acid (pH 4·0) aqueous solutions and the fact that it does not react with nitrite under the present conditions. An important advantage is that cocaine may be added to the serum or urine samples right at the beginning of the analyses; in this respect it has decided advantages over androsterone (Evenson, M. A. and Koellner, S.) and SKF 525-A (Verebely & Inturissi, 1973) previously used as internal standards. Presumably other compounds with similar solubility characteristics and suitable chromatographic properties such as certain phenothiazines, pethidine, methadone etc. could be used instead of cocaine.

The facile conversion of a drug secondary amine (norpropoxyphene) into a potentially carcinogenic nitrosamine reported herein raises the question of the nitrosatability *in vivo* of secondary amine drugs in general and the possible carcinogenic effects of the nitrosamines formed in this manner. These aspects as well as the preparation and chemical characterization of the nitroso compounds concerned have been dealt with elsewhere (Serfontein & de Villiers, 1975 a, b).

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