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Interference by endogenous *p*-hydroxyphenylacetic acid with estimation of *N*-acetyl-*p*-aminophenol in urine by gas chromatography

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Prescott (1971) described a gas liquid chromatographic method for the assay of phenacetin and its metabolite *N*-acetyl *p*-aminophenol (acetaminophen or paracetamol) in biological fluids after conversion to trimethylsilyl (TMS) derivatives using *N*-trimethylsilyl-imidazole (TMSI) or *N*, *O*-bis(trimethylsilyl) acetamide (BSA). This method was modified by Thomas & Coldwell (1972) by substituting the more powerful silylating reagent bis(trimethylsilyl) trifluoroacetamide (Regisil) and a more selective extractant (diethyl ether). Using this modified method we observed that extracts of normal human urine regularly show a peak with an identical retention time to *N*-acetyl-*p*-aminophenol (NAPA). The peak was considerably accentuated after ingestion of ethanol.

A Hewlett-Packard Model 5750 gas chromatograph with flame ionization detector was used with helium as the carrier gas at a flow rate of 50 ml min^{-1}. Hydrogen and air flow rates were 30 and 300 ml min^{-1} respectively. The glass column 6 ft × 6 mm was packed with 3% OV-1 on 80/100 mesh chromosorb W H.P. and run at 160°. The injector and detector temperatures were 180° and 210° respectively.

Samples of urine and serum were collected before and 6 to 8 h after ethanol ingestion by healthy volunteers. Over 1 h, nine volunteers received 1 litre of beer each, and three received 50 ml ethanol in orange juice. These volunteers had no previous history of ingesting phenacetin, NAPA or any other drug for at least two weeks. Urine or plasma samples were extracted by the method of Thomas & Coldwell (1972), except that extraction was performed twice with diethyl ether. The two extracted ether layers were combined and dried with nitrogen. The residue was dissolved in 50 μl of Regisil (Regis Chemical Co., Chicago, Illinois) and kept at 50° for 30 min. After cooling, 1 μl of the sample was injected for chromatography. The retention times for *p*-bromoacetanilide, phenacetin and NAPA were 3·1, 3·5 and 4·1 min respectively.

Fig. 1 shows a typical gas chromatogram of urine extracts before and after ingestion of ethanol. Peak III with a retention time identical to NAPA, was found in each case when extracts of urine collected before ingestion of ethanol were chromatographed. Analysis of the extracts of urine collected before and after ethanol ingestion by coupled gas-liquid chromatography mass spectrometry (g.l.c.-ms) revealed this peak to be the TMS derivative of *p*-hydroxyphenylacetic acid (*p*-HP AA). Reanalysis of the derivatized extracts after spiking

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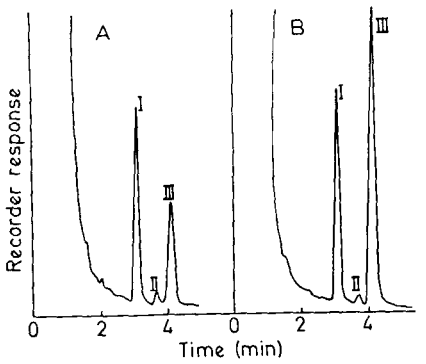

FIG. 1. A gas chromatogram of urine extract obtained from a healthy subject, (A) before, and (B) after ingestion of ethanol. The internal standard in all chromatograms was *p*-bromoacetanilide (Peak I). Peak II with a retention time 3.7 min remains unidentified. Peak III with a retention time of 4.1 min, identical to *N*-acetyl *p*-aminophenol, is *p*-hydroxyphenylacetic acid.

them with small amounts of NAPA yielded a larger peak III having a mass spectrum clearly that of a mixture of NAPA and *p*-HPAA TMS derivatives. The chromatographic conditions of g.l.c.-ms analysis (LKB-9000) were the same as above, and in addition, spectra were measured at 70 eV, 60 μA ionizing energy, with an ion source temperature of 290°.

The average concentration corresponding to peak III was 2·63 μg ml^{-1} or 1·10 μg mg^{-1} creatinine (ranges 0·49–4·5 μg ml^{-1} and 0·75–1·80 μg mg^{-1} creatinine) with reference to *p*-HPAA as standard and 2.57 μg ml^{-1} or 1.07 μg mg^{-1} creatinine (ranges 0.49-4.4 μg ml^{-1} and 0.73-1.75 μg mg^{-1} creatinine) with reference to NAPA as standard. No such peak was observed in the plasma samples.

We have confirmed the report of Witten, Levine & others (1973) that the amount of *p*-HPAA excreted in urine after ingestion of ethanol is increased, with concentrations increasing by as much as a factor of 4 in this study to 9·33 μg ml^{-1} with *p*-HPAA as standard and 9·10 μg ml^{-1} with NAPA as the standard.

An additional minor unidentified peak was regularly found in urine extracts with a retention time of 3·7 min compared with 3·5 min for phenacetin. It was always < 1 μg ml^{-1} with phenacetin as standard and appeared as a shoulder on the phenacetin peak which could be resolved. There was no significant change in its magnitude after ingestion of ethanol ($P > 0{\cdot}1$).

When accurate measurement of NAPA in urine is required at low concentrations, as in pharmacokinetic and other studies of the metabolism of phenacetin or NAPA, the errors due to the *p*-HPAA peak could be serious, particularly if alcohol were taken during the study.

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