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Letter to the Editor

A problem with quantitation of mephenytoin enantiomers due to chiral interference from its N-demethylated metabolite

Sir,

In an earlier publication [1], a chiral capillary gas chromatography assay was reported for the enantiomeric resolution of mephenytoin and its N-demethylated metabolite phenylethylhydantoin (PEH) in plasma and blood. The key to simultaneously quantitating the isomers of mephenytoin and PEH by this assay lies in selectively propylating PEH at the 3-position on the hydantoin ring with 1-iodopropane. Recently, this method was applied to the analysis of over 100 blood samples from rats infused with racemic mephenytoin in our laboratory. Upon analyzing the data, it was discovered that the concentration of each isomer of mephenytoin was strongly correlated with the corresponding isomer of PEH (Fig. 1). The possibility this relationship was an artifact arose when it was found that the concentration of mephenytoin was less and its isomeric composition different when PEH was not derivatized in duplicate blood samples. This indicated some PEH might be converted to mephenytoin during the derivatization process by methylation rather than propylation of the hydantoin ring. To test this suspicion, 10 μg of PEH, the PEH internal standard (5-phenyl-5-propylhydantoin) and the internal standard for mephenytoin (5-phenyl-5-isopropyl-3-methylhydantoin) were carried through the derivatization process described by Wedlund et al. [1]. Following this derivatization, measurable levels of mephenytoin were found. Since the 1-iodopropane (Aldrich, Milwaukee, WI, U.S.A.) was >99% pure and no methyliodide could be detected by NMR, the methanol solvent used to solubilize PEH was suspected of having a role in methylating PEH. When the methanol solvent was replaced by propanol, it was found that a reduced but significant amount of mephenytoin was still formed from PEH. The reduced quantity of mephenytoin formed from PEH in the presence of propanol was found to result from incomplete derivatization of PEH in propanol rather than prevention of the methylation process.

One report has suggested the need to redistill 1-iodopropane prior to its use to remove trace amounts of iodomethane which might be present [2]. The redistillation of iodoalkanes prior to PEH derivatization, however, has not been reported elsewhere [3—6], and no data have been provided on the

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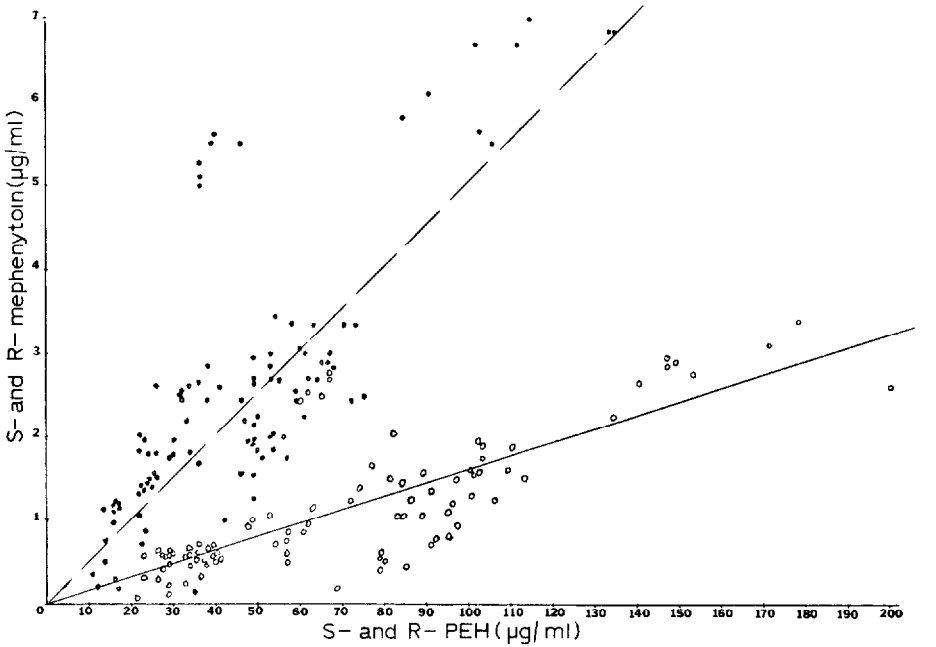

Fig. 1. Relationships between *R*-mephenytoin and *R*-PEH (○) and *S*-mephenytoin and *S*-PEH (●) concentrations in blood samples from rats infused with racemic mephenytoin.

extent to which trace impurities in commercial 1-iodopropane affect quantitation of mephenytoin concentrations. To determine whether the distillation of 1-iodopropane could prevent the methylation of PEH, 40 ml of 1-iodopropane were redistilled in an all-glass distillation assembly. The distillate was collected into two fractions boiling at 45—95°C (15 ml) and from 96—102°C (25 ml). The first fraction was discarded and the second fraction used for the subsequent derivatization of PEH as previously described [1]. The formation of mephenytoin from PEH was found to be markedly reduced by using redistilled 1-iodopropane, although not entirely eliminated. Based on standard curves for mephenytoin, approximately 0.7% of the total PEH is converted to mephenytoin when undistilled 1-iodopropane of 99% purity is used for derivatization. This conversion was decreased to only 0.1% when the 1-iodopropane was redistilled.

In most instances, this minor degree of interference from PEH is of little consequence. However, it can become a major concern when PEH levels are greater than 5 μg/ml and exceed mephenytoin levels by 50—100 fold, as they do in rats receiving mephenytoin chronically. It is hoped this cautionary notice on the relative importance of trace contamination of 1-iodopropane will help to prevent similar problems for other investigators.

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