LETTER TO THE EDITOR

ON THE PHENYLHYDRAZINE REACTION FOR CORTICOSTEROIDS

Sir,

The standard technique of estimation of plasma corticosteroids (principally Cortisol) by phenylhydrazine reaction (2,3) involves treatment of extracted steroid in dichloromethane with sulphuric acid - ethanol reagent containing phenylhydrazine (SER-PH). The absorbancy of faint yellow coloured hydrazones formed is finally measured spectrophotometrically at 410 nm. This is true if the reagents used are in pure form. However, carbonyl impurities (CI) if present even in traces in Absolute ethanol reagent (AE) used, react with phenylhdrazine (PH) in SER-PH to produce brown colouration and render the estimation impossible at 410 nm.

There are basically two methods employed for removing CI from absolute ethanol. The first one suggests treating AE with ammoniacal silver nitrate overnight, followed by distillation of decanted supernate in Vigreaux column and collecting intermediate fraction of distilled AE (2). This method mostly oxidises aldehydes present in AE and keto impurities still remain. In the second technique, oxidation of mainly keto group is carried out by chromic acid and potassium permanganate (1). All these methods are time consuming and there is need for much simpler and effective technique of removing both aldehydes and keto impurities from AE at the same time in single sitting. The authors have come out with such a method.

The knowledge that 2,4-dinitrophenyl hydrazine (2,4-DNPH) reacts promptly with both aldehydes and ketones and form stable hydrazones in slightly acidic medium, is used in the present technique. The advantage of 2,4-DNPH over PH is that the hydrazones formed with former are not converted to azines.

Previously Siggia (4) employed 2,4-DNPH for removal of CI from Methanol reagent during colorimetric determination of carbonyl group. We used identical proportions of AE and 2,4-DNPH in this method. A litre of AE was added to 10 *gms* of 2,4-DNPH in presence of few drops of concentrated HCl in a Pyrex flask and mixture was boiled for 30 min under reflux arrangement following which AE was distilled twice at 78°C. So prepared AE was incorporated in preparation of SER and treated with PH. No colour developed even on keeping it for 72 hrs suggesting complete removal of CI from absolute

ethanol. The relative comparison of present technique with other methods is mentioned in Table I.

TABLE I : Comparative evaluation of purification methods for AE.

Methods for AE purification	*Reaction with PH* (within 24 hrs)*	*Effectiveness of procedure*
Ammoniacal silver nitrate	+++	—
Potassium permanganate	++	—
Chromic acid	++	—
2,4-DNPH	—	+++

*relative degree of colour formation due to CI : subjective comparison.

Since ethanol of analytical grade is not available, the present method may successfully be used for preparing AE suitable for corticosteroid estimation by Spectrophotometric procedure.

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