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Letter to the Editor

Catecholamine determination by gas–liquid chromatography

Sir,

Whilst there is a need for development of relatively simple and rapid techniques for analysis of small concentrations of catecholamines that occur in biological fluids and tissues, it is essential that new methods reported claiming to fulfil these criteria should be reproducible and adaptable in different laboratories. Therefore it is surprising that the report by Lovelady and Foster[1] describing the measurement of adrenaline and noradrenaline in plasma, red blood cells and urine with detection sensitivity in the sub-picogram range has to date produced no comment in this journal with respect to several factors relating to the viability of the assay procedure.

Firstly, apart from the well established radioenzymatic method, the analysis of picogram ($10^{-12} - 10^{-14}$ moles) quantities of catechol- and related biogenic amines by gas chromatographic techniques has been accomplished by means of the highly sensitive mass spectrometry and electron capture detection[2]. In contrast the report by Lovelady and Foster[1] states that quantitative detection of similar amounts of catecholamines can be accomplished by gas–liquid chromatography using a dual hydrogen flame ionization detector with relatively little clean up of the biological extracts. In practice the detection limits of gas chromatography using a flame ionization detector for the determination of compounds extracted from biological fluids does not generally extend below the low nanogram range and it is of interest to note that Lovelady and Foster[1] present data showing linear calibration for adrenaline and noradrenaline from less than 0.2 pg to approximately 2 pg with a minimum detection of "approximately 0.1 pg". This stated detection limit is even numerically below the minimum detectability that is specified for flame ionization detectors on most commercially available gas chromatographs (in the order of 10^{-11} g/sec for alkanes[3]).

Secondly, we have shown elsewhere[4] that the extraction technique used by Lovelady and Foster[1] and also that described in a separate report by Lovelady[5] for extraction of catecholamines from red blood cells is unlikely to give significant recovery of these compounds. That is, the extraction of a protein free extract of red blood cells with water–4-methyl-2-pentanone–*n*-hexane (1:1:1) mixture as described by the authors[1,5] will not yield significant partition of the free catecholamines into the top organic phase, the bulk of catecholamines will remain in the aqueous phase. This is in contrast with the results of Lovelady and Foster[1] and Lovelady[5] that greater than 90% recovery of free catecholamines from red blood cells is achieved following extraction with this solvent system.

Thirdly, Lovelady and Foster[1] show that tripalmitin, used as an internal standard, has a retention time of approximately 11 min on a 6 ft. × ⅛ in. O.D. steel column packed with 7% DC-11 on Gas-Chrom P operating isothermally at 115° with carrier flow-rate of 20 ml/min. Yet it is well established that gas chromatography of glycerol esters of long chain fatty acids require considerably higher temperatures

higher carrier flow-rates, shorter column length and less percentage liquid phase for their elution from the column. For example triglycerides with carbon number 30–40 (the carbon number of tripalmitin is 48) are consecutively eluted at 250° on a 40-cm column of 2.25% SE-30 on Chromosorb W with carrier flow-rate of 75 ml/min[6]. Furthermore, if tripalmitin is used as an internal standard as stated by the authors[1] in nanogram amounts then it is unlikely to be able to differentiate the added tripalmitin from the endogenous tripalmitin together with the other closely related triglycerides which occur in human plasma at considerably higher concentration[7].

Fourthly, the catecholamine derivatives were prepared by simultaneous reaction with a mixture of bis-(trimethylsilyl)acetamide (BSA)–trifluoroacetic anhydride (TFA) (1:1) in tetrahydrofuran[1,5] and were simply referred to as the "TFA–BSA derivatives"[5]. No further information as to the nature of these compounds have to date been given. Presumably the derivatisation was an attempt to prepare the N-acyl, O-trimethylsilyl ether derivatives originally described by Horning *et al.*[8] where catechol- and related amines were reacted in a two-step sequential reaction with a trimethylsilyl donor then the acyl anhydride in anhydrous conditions. Similar derivatives, again using a two-step procedure have been prepared by other workers[9,10]. The method of Lovelady and Foster[1] also introduces the presence of water following extraction of the "TFA–BSA products" with water–organic solvent pair and we consider this likely to lead to hydrolysis of the formed derivatives. We have attempted to prepare the same derivatives of adrenaline, noradrenaline and dopamine by the method described by Lovelady and Foster[1] and found no formed products which eluted under the chromatographic conditions described in their report.

We are therefore led to conclude that the method of Lovelady and Foster[1] is of doubtful value for the measurement of catecholamines in blood or plasma, especially in the concentrations in which these compounds normally occur.

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