## The Use of β-Thiopropionic Acid for Stabilising the Fluorescence of Adrenolutine and Noradrenolutine

SIR,—The determination of small amounts of adrenaline and noradrenaline by the fluorometric trihydroxyindole method using two sets of filters for 405 and 436 mμ (e.g. Euler and Lishajko, 1959) is made difficult by the increasing fluorescence of the final solution with time—particularly at 436 mμ. Firstly, ascorbic acid breaks down in strong alkali with the formation of a pink coloured compound. The rate of breakdown is influenced by the strength of ferricyanide used for oxidation, the temperature and the presence of substances from biological fluids which have come through the alumina separation procedure. Fluorescence may also depend on the presence or absence of adrenolutine or noradrenolutine; blank solutions often increase in fluorescence more rapidly than those containing these lutines. The increase in blank fluorescence is particularly troublesome at low concentrations of the amines where the blank or control sample may often fluoresce more than the standard after a few min. Another factor which may contribute to the rising fluorescence is the presence of small quantities of plasma proteins (or related compounds) or other unknown substances which come through the alumina separation procedure. Precipitation of plasma proteins (Vendsalu, 1960) may reduce this effect but may also introduce variable losses because preliminary experiments with Sephadex G50 (Medium) have demonstrated the affinity of the plasma proteins for adrenaline and noradrenaline.

Euler and Lisajko (1961) have recommended the use of ethylenediamine for stabilising the fluorescence of the final alkali-ascorbic mixture in the analysis of adrenaline and noradrenaline in urine. At high concentrations ($> 0{\cdot}2$ μg./ml. of alumina eluate), ethylenediamine improves the stability of fluorescence particularly at 436 mμ. At concentrations of the amines of 5 ng. and below there are still difficulties, particularly when assaying plasma.

With ethylenediamine the fluorescence increases unsteadily to a plateau and may then decrease after a variable time. The rate of increase to this plateau depends critically upon the amount of ethylenediamine added, its purity and upon other unidentified factors which are related to the efficacy of washing the alumina in the separation procedure. The ratio of adrenolutine to noradrenolutine may also vary continuously. Increasing the concentrations of ethylenediamine above the authors' recommended figure (0·2 ml./10 ml. alkali-ascorbic mixture) produces a slight improvement in stability—though at the expense of reduced readings (particularly of adrenolutine) and also in an overall increase in background fluorescence.

To overcome the difficulties of stabilising the fluorescence many substances alone and in combination with ascorbic acid have been tried. β-Thiopropionic acid (TPA) was found to be easily the best and it effectively reduced the increase in fluorescence of both the alkali-ascorbic and plasma-alkali mixtures.

The alkali-ascorbic-TPA mixture is prepared as follows: TPA (0·2 ml.) is placed in a sample tube (3 ml.); and approximately 15 sec. before use ascorbic acid (0·1 ml. 5 per cent) followed by sodium hydroxide (1·9 ml., 5N) are added to this sample tube from a Seligson automatic pipette. This mixture is then added to a graduated flask (5 ml.) containing the pH-adjusted alumina eluate, previously oxidised by ferricyanide (0·25 per cent) for 2½ min. This method of preparation is simple and reliable—the 5N sodium hydroxide is stored in a plastic container (with $CO_2$ absorbent air intake) attached to the pipette, and the 5 per cent ascorbic acid is kept in a plastic bottle for up to a week in a refrigerator. The ferricyanide is conveniently stored and dispensed (0·1 ml.) from

a plastic ultra-micro-pipette. The glass containers were rinsed with normal hydrochloric acid, tap water and double distilled water between use, and a particular glass cuvette was used for a batch of analysis.

Results using β-thiopropionic acid show an easily obtained reproducibility of ± 100 pg. for adrenaline and noradrenaline in the hands of relatively unskilled laboratory personnel.

The improvement in the stability of the fluorescence is most useful at 436 mμ though the parallel improvement at 405 mμ makes this latter wavelength most suitable for estimations of small amounts of total adrenaline + noradrenaline. It is probably that a suitable secondary filter could be found which would equalise the fluorescence of adrenolutine and noradrenolutine (with added TPA) at 405 mμ. Without the addition of TPA the fluorescence of the two lutines was exactly equal at 405 mμ.

The ratio of adrenaline to noradrenaline at 436 mμ, using TPA was 2·6 (± 0·1) : 1 using 2A and 47B as primary filters* and 2A-15 as a secondary filter*). The ratio of adrenaline to noradrenaline at 405 mμ was 0·75 (± 0·05) : 1 (using a 405* and an Ilford Bright 623 as a primary and secondary filter respectively). The fluorometer used was a Turner model III.

The use of this thioacid opens up possibilities of reliably and routinely assaying adrenaline and noradrenaline in picrogram quantities.

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