A METHOD FOR THE ESTIMATION OF ADRENALINE AND NORADRENALINE IN URINE

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A method for the estimation of adrenaline and noradrenaline in urine is described. The catecholamines are adsorbed on aluminium oxide and eluted with acid. The adrenaline and noradrenaline are estimated separately after oxidation with manganese dioxide at different pH values and conversion to the trihydroxyindole derivative for fluorimetric estimation. A mean recovery of 82 per cent (±5 per cent S.D.) was obtained for total amines estimated as noradrenaline, adrenaline recovery was 83 per cent (±6 per cent S.D.) and noradrenaline recovery was 80 per cent (±6 per cent S.D.). The results were in good agreement with biological assay results.

MODERN methods of estimation of adrenaline and noradrenaline in urine involve adsorption of the amines on aluminium oxide (Pitkanen, 1956; Lund, 1952) or on an ion-exchange resin (Weil-Malherbe and Bone, 1957; Crawford and Law, 1958; Wright, 1958). The amines are then eluted and estimated biologically (Euler and Hellner, 1951; Burn, 1953) or fluorimetrically (Lund, 1952; Crawford and Law, 1958; Weil-Malherbe and Bone, 1952; Euler and Floding, 1955).

Attempts to use existing fluorimetric methods of assay have not been entirely satisfactory in our laboratories, results frequently differing from concurrent biological assays, inconsistent recoveries and anomalous high results occasionally being obtained.

This paper describes a modification of Lund's (1952) fluorimetric method for the estimation of adrenaline and noradrenaline.

EXPERIMENTAL AND RESULTS

Reagents

Aluminium oxide (Chromatographic B.D.H.); adrenaline solution, 1 in 1,000 (Burroughs Wellcome); Ascorbic acid, B.P., which was used to prepare a 1 per cent aqueous solution and was not kept longer than 5 days at 0°; Manganese dioxide (B.D.H.), technical grade, pretreated as described by Crawford and Law (1958); noradrenaline solution, 1 in 1,000 (Levophed, Bayer); buffer solution, pH 3·5–50 ml. 0·2M potassium hydrogen phthalate plus 7·9 ml. 0·2N hydrochloric acid: buffer solution, pH 6·5–0·1M disodium hydrogen phosphate; freshly distilled water. All other reagents were of Analar quality.

Extraction

Urine (100 ml.), ethylenediaminetetra-acetate (10 to 20 mg.) and aluminium oxide (4 g.) were stirred magnetically. Sodium hydroxide was added over 2 min. to give a blue colour to thymol blue paper (about

pH 8·5). Stirring was continued for a further 6 min. and the mixture was then centrifuged at 2,000 r.p.m. for 2 min.

The precipitate was washed with two quantities of 50 ml. of distilled water. Elution was with two portions, each of 10 ml. of N sulphuric acid and separated by centrifugation. Elution was repeated with a further three quantities each of 10 ml. of distilled water. All eluates were then pooled.

Estimation of Total Amines as Noradrenaline

Five ml. of the pooled eluates was mixed with 25 ml. of 0·1M disodium hydrogen phosphate solution. The precipitate formed adsorbed catecholamines, but these are still susceptible to oxidation and the resultant adrenochrome is not adsorbed by the precipitate.

This solution, at pH 6·5, was divided into three quantities of 10 ml. in three 50 ml. centrifuge tubes, designated standard, test and blank. Hydrochloric acid (1 ml., 0·01N) was added to the test and blank tubes and the same quantity containing 0·1 μg. of noradrenaline was added to to the standard tube. Manganese dioxide (100 mg.) was added to each tube which was then shaken continuously by hand for exactly 45 sec. and centrifuged for 2 min.

Nine ml. of clear supernatant from each tube was transferred to 15 ml. centrifuge tubes designated standard, test and blank. To the test and standard tubes, ascorbic acid solution (0·15 ml., 1 per cent) was added. Sodium hydroxide solution (0·85 ml., 5N) was added to all three tubes which were closed by rubber stoppers and inverted three times to mix. After exactly 4 min. ascorbic acid solution (0·15 ml., 1 per cent) was added to the blank and, after mixing by inversion, all three tubes were centrifuged for 6 min. to remove the traces of brown mucilaginous precipitate which appeared. The supernatant was pipetted off. The fluorescence of test, blank and standard solutions was compared in a direct reading E.I.L. fluorimeter using a Chance OB 10 primary filter and a Chance OY 2 secondary filter.

Estimation of Adrenaline

To a 5 ml. portion of the pooled eluate was added buffer solution (25 ml., pH 3·5). The solution was then divided into three 9 ml. portions designated standard, test and blank. Adrenaline (0·1 μg. in 1·0 ml. of 0·01N hydrochloric acid) was added to the standard and hydrochloric acid (1·0 ml., 0·01N) was added to the test and blank solutions. The solutions were then inverted to mix and the standard and test solutions were oxidised with 100 mg. of manganese dioxide for 45 sec. After centrifuging all three tubes for 2 min., the fluorescent derivative was prepared by the addition of ascorbic acid solution (0·15 ml., 1 per cent) followed by 0·85 ml. of 5N sodium hydroxide solution. The fluorescence was then examined as before.

After determination of the relative fluorescence of adrenaline to noradrenaline at pH 6·5, the noradrenaline content was calculated.

Demonstration of linear relationship of concentration of adrenaline and noradrenaline to fluorescence. A series of concentrations of adrenaline and noradrenaline in separate solutions, pH 6·5, were estimated fluorimetrically. A linear relationship between concentration and fluorescence was demonstrated for adrenaline and noradrenaline at concentrations between 0·025 and 0·20 μg./ml.

Estimation of adrenaline and noradrenaline in pure solution. In a series of ten estimations, the mean recovery for total amines as noradrenaline was 81·7 per cent (S.D. ± 0·08 per cent), noradrenaline 80·3 per cent (S.D. ± 0·18 per cent) and adrenaline 83·1 per cent (S.D. ± 0·10 per cent).

Recovery of adrenaline and noradrenaline added to urine. Adrenaline (5 μg.) and noradrenaline (50 μg.) were added to 1,000 ml. of urine of known adrenaline and noradrenaline content. After estimating the adrenaline and noradrenaline content the percentage recovery was determined.

In a series of ten estimations the mean recovery for total amines as noradrenaline was 81·7 per cent (S.D. ± 4·8 per cent), noradrenaline 80·3 per cent (S.D. ± 6·3 per cent) and adrenaline 83·1 per cent (S.D. ± 6·3 per cent).

Comparison of results obtained with results of bioassay on the same sample of urine. Concurrent fluorimetric and biological estimations were carried out on urine samples from 25 hypertensive patients. Total amines were determined biologically as noradrenaline using a cat blood-pressure method (Willoughby, 1958). The mean fluorimetric results were 99·3 per cent of the mean biological results (S.D. ± 13·4 per cent).

Determination of the specificity of the method. A solution was prepared containing adrenaline (1 μg.), noradrenaline (5 μg.), DOPA (1 μg.) and dopamine (50 μg.) in 50 ml. of 1·0N hydrochloric acid. A 10 ml. sample was extracted and estimated and found to contain 0·8 μg. adrenaline and 4·3 μg. noradrenaline in 50 ml. of the original solution. This suggests that DOPA and dopamine do not interfere in this fluorimetric estimation.

DISCUSSION

Lund's original method has been modified in an attempt to develop a method which gave the same results as those obtained by biological assay.

Recovery has been improved by the addition of EDTA, slow addition of alkali and rapid magnetic stirring. Under these conditions, breakdown of adrenaline and noradrenaline is minimal and constant.

Substances which caused quenching or potentiation of fluorescence were a source of error in initial experiments. The use of internal standards of adrenaline at pH 3·0 and noradrenaline at pH 6·5 has removed this source of error.

Non-specific fluorescence, extracted from the urine with the catecholamines, interfered with the preparation of the blanks as it faded in a similar manner to adrenolutin and noradrenolutin. The blank at pH 3·0

was therefore prepared by omitting the oxidation. At pH 6·5, fading of the blank was prevented, after a 4 min. interval, by the addition of ascorbic acid solution.

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