ESTIMATION OF EPINEPHRINE AND NOREPINEPHRINE CONCENTRATIONS IN HUMAN PLASMA BY THE TRIHYDROXYINDOLE METHOD[1]

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We have applied a variant of the trihydroxyindole fluorimetric method of Lund (4) to the detection of epinephrine and norepinephrine in human plasma. Our interest lay in improving both the sensitivity and specificity of the original method of analysis.

The technique developed by us is similar to the original but differs from it in relying upon excitation of solutions of adrenolutine and noradrenolutine with light of two wavelengths, rather than upon oxidation at two pH levels for the separation of a mixture into its constituents. Sensitivity is thereby increased, and amounts of epinephrine as small as 1 mμg can be detected (6).

Unlike the ethylenediamine condensation methods (5, 7), which detect catechols in general, the trihydroxyindole reaction occurs theoretically only when a side chain of unique configuration is attached to the catechol nucleus. Apparently the side chain must contain two carbon atoms, must terminate in an amino nitrogen, and the β-carbon must be hydroxylated in order for fluorescence to occur (3). If this configuration alone produces fluorescence by the trihydroxyindole method, then the method should be highly specific for the naturally occurring catecholamines epinephrine, norepinephrine, or isopropylnorepinephrine. We have examined its specificity by subjecting a number of closely related substances to our analytical procedure.

Our present technique utilizes light of 400 and 436 mμ to excite the fluorescent solutions, while the emission is measured at 500 mμ. The emission detected at 500 mμ when the fluorescent products of various substances were excited at 400 and 436 mμ and at pH 5 or pH 12 has been summarized in Table 1. In addition to exciting the substances with light of two wavelengths, we have read the fluorescence when the reaction products were maintained at two pH levels (5 and 12–13). The fluorescence of epinephrine measured at pH 5 has arbitrarily been assigned the value 100, and the fluorescence of the other substances is compared to it. All results are in terms of the base or acid. It can be seen that, at pH 12, only norepinephrine, isopropylnorepinephrine, and dopa produced fluorescence greater than 1% of that produced by epinephrine. The fluorescence of dopa was reduced to 1% of that at pH 12 by acidifying the solution before exciting it. In general, acidifying the solution increased the fluorescence of the catecholamines, while reducing that of dopa and 5-hydroxytryptamine. At pH 5 no compound

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TABLE 1

*Fluorescence of hydroxyphenyl compounds**

Substance	Ring†	Side Chain	Filter	Relative Fluorescence (%)	
			mμ	*pH5*	*pH13*
l-Epinephrine	R_1–	$CHOHCH_2NH$	400	100.	85.
		CH_3	436	100.	76.
DL-Isopropylnorepinephrine	R_1–	$CHOHCH_2NH$	400	72.	—
		$CH(CH_3)_2$	436	75.	—
l-Norepinephrine	R_1	$CHOHCH_2 NH_2$	400	85.	71.
			436	37.	27.
Threo-DL-3,4-dihydroxyphenylserine	R_1	$CHOHCHNH_2$	400	0.19	0.08
		COOH	436	0.08	0.08
Erythro-DL-3,4-dihydroxyphenylserine	R_1–	$CHOHCHNH_2$	400	0.03	0.01
		COOH	436	0.01	0.001
DL-Dopa	R_1–	CH_2CHNH_2	400	0.051	5.0
		COOH	436	0.002	0.05
Dopamine	R_1–	$CH_2CH_2NH_2$	400	0.13	0.14
			436	0.04	0.04
3,4-Dihydroxyphenylacetic acid	R_1–	CH_2COOH	400	0.004	0.014
			436	0.002	(−)0.002
3,4-Dihydroxymandelic acid	R_1–	CHOHCOOH	400	(−)0.001	—
			436	(−)0.001	—
5-Hydroxytryptamine (serotonin)	R_2–	3-$CH_2CH_2NH_2$	400	0.022	0.11
			436	0.027	0.26

* This table is reprinted, by permission, from the Journal of Laboratory and Clinical Medicine, 50: 771, 1957.

† R_1 = 3,4-dihydroxyphenyl; R_2 = 5-hydroxyindole.

other than the β-OH catecholamines produced fluorescence greater than 0.2% of that produced by epinephrine. Epinephrine and isopropylnorepinephrine produced nearly equal fluorescence when excited at either wavelength and cannot therefore be separated by the present method; however, norepinephrine can be differentiated. This method is thus reasonably specific.

Plasma derived from blood drawn from 40 normal subjects was analyzed and found to contain 0.00 to 0.25 μg/l of epinephrine and 0.1 to 0.6 μg/l norepinephrine. In general, the concentrations of epinephrine in arterial (brachial) plasma were greater than in venous (antecubital), while the opposite was true of norepinephrine. This suggests that norepinephrine was liberated from the forearm tissues, while epinephrine was taken up by them. The average concentration of epinephrine in venous plasma was nearly zero (less than 0.1 μg/l), while that of norepinephrine was 0.3 μg/l. The fact that nearly identical levels of epinephrine and norepinephrine were estimated when the eluates derived from human plasma

were analyzed at pH 5 (5) and pH 12 to 13 (1) indicates that the eluates could not have contained significant amounts of substances the fluorescence of which is drastically altered by pH changes.

The plasma concentrations estimated by the trihydroxyindole method are close to those determined biologically (2) but are much less than those estimated by the ethylenediamine method. The explanation for this is unknown but lack of specificity of the ethylenediamine method for biologically active catecholamines could explain the discrepancy.

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