3. The pseudo-cholinesterase concentrations have been expressed on the basis of enzyme activity under standard conditions. The concentration of pseudo-cholinesterase in the particular samples of full serum used were as follows: horse serum 8.8×10^{-8} M; human serum, 7.9×10^{-8} M; female mouse serum, 5.8×10^{-8} M; dog serum, 4.6×10^{-8} M; female rat serum, 1.7×10^{-8} M.

4. The pseudo-cholinesterases from mouse, rat, horse, human and dog serum form a series in which the ratio of pseudo-cholinesterase activity towards 0.06 M-acetylcholine over that towards 0.006 M-benzoylcholine diminishes progressively from 8.2 to 1.6. In this same series, the absolute activity of the pseudo-cholinesterases towards acetylcholine becomes progressively smaller. Other characteristics

of the pseudo-cholinesterases also vary considerably from one type of pseudo-cholinesterase to another, but do not show any correlation to the above series.

5. The significance of these results has been discussed with respect to the concept of the pseudo-cholinesterases as a special group of closely related enzymes.

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The Chemical Estimation of Adrenaline-like Substances in Blood

By H. WEIL-MALHERBE AND A. D. BONE
Research Department, Runwell Hospital, Wickford, Essex

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Of the numerous chemical methods which have been devised for the estimation of adrenaline the fluorimetric techniques seem most likely to reach a degree of sensitivity sufficient for the measurement of concentrations as low as those occurring in peripheral venous blood. Adrenaline, as is well known, forms an unstable fluorescent oxidation product in alkaline solution. Lund (1949*b*) recently described a method in which the fluorescent derivative is stabilized by the addition of ascorbic acid and which is capable of estimating concentrations of not less than $10 \mu\text{g./l.}$ Recovery of added adrenaline amounted to 80% and a factor was employed to correct for the loss. Lund did not find measurable

amounts of adrenaline in venous blood and concluded that adrenaline, if present at all, occurred in concentrations of less than $10 \mu\text{g./l.}$

Although the sensitivity of Lund's method could perhaps be increased by suitable modifications, the procedure seems to have other disadvantages. It is stated that adrenaline is quantitatively oxidized to adrenochrome by manganese dioxide without adrenochrome itself being further oxidized. The need for a rapid and complete removal of manganese dioxide is, however, emphasized and variations of such factors as temperature or the activity of the manganese dioxide preparation might, unless carefully controlled, entail the risk of a loss of adreno-

chrome. Secondly, as pointed out by Fischer, Derouaux, Lambot & Lecomte (1950), adrenochrome is liable to be reduced in the presence of ascorbic acid and may thus partly escape transformation to the fluorescent compound in alkaline solution. Whether these side reactions can seriously affect the practical value of the method has not been studied by us.

In the following, a fluorimetric method of adrenaline estimation will be presented which is not, like previous fluorimetric methods, based on the formation of adrenolutine (Lund, 1949*a*), but on a condensation with ethylenediamine. The reaction was discovered by Natelson, Lugovoy & Pincus (1949), but, as far as we are aware, it has not been further studied or applied to blood. The condensation takes place in alkaline solution in which adrenaline is readily autoxidizable. No reaction occurs if oxygen is excluded. If adrenaline is replaced by adrenochrome, a fluorescence intensity curve is obtained, both in presence and in absence of oxygen, which is identical with that produced by equimolar amounts of adrenaline in presence of oxygen. It may therefore be concluded that the condensation is preceded by an oxidation of adrenaline (I) to adrenochrome (II), and the reaction may be formulated as follows:

(I) Adrenaline. (II) Adrenochrome. (III) Ethylene diamine. (IV) Condensation product (hypothetical).

The constitution of the condensation product (IV) must at present be regarded as hypothetical.

The fluorescence of the final extract remains constant for at least 24 hr. The fact that the unstable, highly reactive adrenochrome is trapped in the nascent state and quantitatively converted into a completely stable condensation product is the most notable advantage of the method. In addition, the sensitivity is such that adrenaline concentrations of $1 \mu\text{g./l.}$ can be measured with accuracy; the recovery is quantitative and the use of a correction factor is therefore unnecessary; and finally, strictly additive results are obtained with mixtures of adrenaline and noradrenaline, whereas the production and the decay of fluorescence observed in an alkaline

solution of adrenaline is disturbed by noradrenaline (Heller, Setlow & Mylon, 1950).

The procedure of Natelson *et al.* (1949) has been modified: the condensation is carried out by heating the solution at 50° for 20 min. with a mixture of ethylenediamine and an aqueous solution of ethylenediamine dihydrochloride. The free base is added to produce the required degree of alkalinity (pH of the mixture = 10.4) and may be replaced by ammonia. Since the presence of ethylenediamine in the ionized form is essential, it is obviously the reactive agent. For the extraction of the fluorescent condensation product isobutanol was used in preference to amyl alcohol; a quantitative extraction was achieved by saturating the aqueous phase with sodium chloride.

Attempts to replace ethylenediamine by *o*-phenylenediamine were abandoned owing to the formation of highly fluorescent oxidation products in the reagent blank.

The initial separation of adrenaline by chromatography introduced by Lund (1949*b*) has been adopted, with some modifications designed to reduce the autoxidation of adrenaline before and during the adsorption. Acid-washed alumina is used and its quantity is reduced. Another point

which was found to be particularly important is the removal of traces of heavy metal from the sodium acetate solution that is added to plasma prior to adsorption. This is done by passing the solution through a column of cation-exchange resin. Finally, the precise adjustment of the diluted plasma to pH 8.4 is controlled by a glass electrode.

The blood is drawn directly into a solution of fluoride and thiosulphate which combines anti-oxidant with anticoagulant properties. The apparent adrenaline content remains constant for at least 24 hr. at refrigerator temperature. In a few instances red blood cells were washed and cytolysed. Their apparent adrenaline content was about equal to that of plasma in confirmation of Lund's results,

but the alumina columns were liable to become clogged when haemolysates were used, and for this reason most estimations were carried out with separated plasma.

METHOD

Reagents

Sodium fluoride-sodium thiosulphate solution. NaF (2 g.) and $\text{Na}_2\text{S}_2\text{O}_3$ (3 g.), are dissolved in glass-distilled water to 100 ml. The solution is autoclaved and transferred to sterile ampoules, each containing about 6 ml.

Sodium carbonate. A 0.5N solution.

Sodium acetate buffered at pH 8.4. A 0.2M solution of sodium acetate is passed over a column of Zeo-Karb 215 (Permutit Co. Ltd.) and adjusted to pH 8.4 by the addition of 0.5N Na_2CO_3 (about 5 ml./l.) with the aid of a glass electrode. The Zeo-Karb column is prepared by repeated washings with 2N-HCl, water and 4% NaCl, the last being continued until the pH of the filtrate rises to about 6. Excess NaCl is flushed out with glass-distilled water.

Alumina, acid-washed. Al_2O_3 (100 g.; British Drug Houses Ltd., 'for chromatographic adsorption analysis') is stirred with 500 ml. boiling 2N-HCl for 20 min., filtered and washed on the funnel with 500 ml. of hot 2N-HCl. It is then repeatedly washed by decantation with distilled water and dried at 300° for 3 hr.

Acetic acid. A 0.2N solution.

Ethylenediamine dihydrochloride, 2M solution. The salt is prepared from freshly redistilled ethylenediamine by the slow addition of the calculated amount of 6N-HCl (A.R.). Crystals are filtered by suction, dried on a water bath and finally over H_2SO_4 .

Ethylenediamine, or ethylenediamine hydrate. Redistilled.

isoButanol. A laboratory chemical grade is satisfactory.

Procedure

Fluoride-thiosulphate solution (5 ml.) is drawn up into a 20 ml. syringe and about 15 ml. blood collected from the cubital vein. The syringe is emptied into a 25 ml. measuring cylinder and the volume is noted (v_1 ml.). The plasma is separated by centrifugation (v_2 ml.) and an equal volume of sodium acetate buffer added. The mixture is brought to pH 8.40 by the addition of 3-5 drops of Na_2CO_3 solution, precise adjustment being controlled by a glass electrode.

Thistle funnels with a 50 ml. bulb and a 5 mm.-bore stem are used for the chromatographic separation. At a distance of about 18 cm. from the bulb, the stem has a constriction on which is placed a plug of glass wool. The funnel is mounted on a filter flask. The column is prepared by pouring in 0.7 g. of dry acid-washed alumina followed by 5 ml. acetate buffer. With the aid of a drawn-out glass rod the alumina is stirred up until all trapped air bubbles have escaped. After allowing it to settle, mild suction is applied which is so regulated that the rate of filtration does not exceed 20-30 drops/min. The plasma-acetate mixture is added next, followed by 5 ml. acetate buffer and 5 ml. glass-distilled water. The filtrates are rejected. The adsorbed adrenaline is eluted by passing 5 ml. 0.2N-acetic acid, followed by 5 ml. water, through the column.

After the addition of 0.5 ml. ethylenediamine dihydrochloride solution and 0.7 ml. ethylenediamine (or 1 ml. ethylenediamine hydrate) the eluate is heated at 50° for

20 min., cooled to room temperature and saturated with solid NaCl (about 4 g.). The solution is extracted with 6 ml. isobutanol in a mechanical shaker for 4 min. and lightly centrifuged to break up the emulsion. The isobutanol layer is removed completely and 5 ml. are measured into the fluorimeter tube. To avoid unnecessary transfer the eluate is collected in a glass-stoppered centrifuge tube in which the condensation and extraction procedures are carried out.

A standard containing 0.2 μg . adrenaline in 10 ml. and a reagent blank are carried through the condensation and extraction procedures together with the plasma eluates.

Measurement of fluorescence

A 125 W. high-pressure mercury vapour lamp with stabilized arc (Type MBL/D, British Thomson-Houston Co. Ltd.), operated in conjunction with choke and voltage stabilizer (Type MT140A, Advance Components Ltd.) serves as light source. The exciting light is passed through a blue filter (Chance OB10, 2 mm. thick) and focused by a thin-walled, flat-bottomed round flask of 250 ml. capacity, filled with water, on to a thin-walled test tube of non-fluorescent glass (11 mm. internal diameter). The test tube is held inside a light-tight box to which is attached, at right angles to the beam of the exciting light, the photocell compartment of a Unicam S.P. 500 spectrophotometer. The fluorescent light is filtered through a yellow filter (Chance OY4, 2 mm. thick) before entering the photocell. The photocell current is measured with the aid of the electrical controls provided by the Unicam spectrophotometer with slight modifications. It was necessary to change the bias on the first amplifier valve which controls the range of illumination at which the photocell works. This involved fixing a 100 000 Ω variable potentiometer between two taps on battery no. 3 of the instrument, and this control was fitted with a built-in switch to return the instrument to its normal operating state when used as a spectrophotometer.

Readings are taken 45 min. after switching on the mercury lamp in order to eliminate drift during the warming-up period. The reagent blank is used to establish the zero position of the 'Transmission' control: by means of the new control for rough adjustment and the 'Dark Current' control for fine adjustment the potential of the photocell at this illumination level is backed-off until balance is obtained with the 'Transmission' dial set at zero. The standard is now introduced into the fluorimeter box, and with the 'Transmission' dial at 100, balance is obtained by operating the 'Sensitivity' control. Next the unknown sample is inserted and balance is established by turning the 'Transmission' control. The position of the dial shows the intensity of the unknown fluorescence as a percentage of the standard fluorescence. When a reading is taken, the shutter which normally protects the photocell is opened and replaced as soon as the necessary adjustments have been made so as to avoid undue fatigue. In each series of readings the position of the zero and the 100 setting is checked at intervals by re-introducing the reagent blank and the standard, respectively. When a series has been completed, the readings are repeated in the same order. Duplicate readings obtained in this way may differ somewhat owing to instabilities in the amplifier circuit or to fluctuations of the mercury lamp output. The mean of the duplicate readings is used to calculate the result.

Calculation

If the fluorimetric measurement indicates the presence of $a \mu\text{g.}$ adrenaline, the amount, per litre of plasma, is

$$\frac{a \times v_1 \times 1000}{v_2(v_1 - 5)} \mu\text{g.}$$

RESULTS AND DISCUSSION

Choice of standard

When a standard of $0.2 \mu\text{g.}$ adrenaline is used to adjust the sensitivity setting the calibration curve obtained is linear (Fig. 1), indicating proportionality between fluorescence intensity and photocell current.

Fig. 1. Calibration curve of fluorimetric estimation of adrenaline.

This setting was optimal for the equipment available. Attempts to increase the sensitivity to the point where a dial reading of 100 was obtained with a standard of $0.1 \mu\text{g.}$ resulted in a calibration curve which was no longer linear; in addition, there was a disproportionate loss of discrimination. The sensitivity setting obtained with a standard of $0.2 \mu\text{g.}$ proved satisfactory for our purpose, since with most blood samples the scale readings were between 15 and 20 on a scale of 100 divisions. If it should be required, the sensitivity could no doubt be increased, either by changing the type of photocell, or by intensifying its illumination.

Error of fluorimeter readings

On the scale of 100 divisions, 1 division corresponds to $0.002 \mu\text{g.}$ adrenaline, given the usual sensitivity setting. Readings were taken to 0.1 of a division, the last figure being obtained by approximate estimation. Owing to slight instabilities in the set-up, duplicate readings differed to some extent. The standard deviation of single readings was calculated

from the sum of the squares of the differences (Δ) between n pairs of duplicate readings according to the formula $s = \sqrt{\frac{\sum(\Delta^2)}{2n}}$. The results show that the error does not appreciably vary in different ranges of dial readings, the standard deviation being close to 0.5 division throughout (Table 1). This indicates that single readings are accurate to approximately ± 1 division and the mean of duplicate readings to approximately $\pm 1/\sqrt{2} = 0.7$ division, or $0.0014 \mu\text{g.}$ adrenaline.

Table 1. Error of fluorimeter readings

No. of samples	Range of dial readings (divisions)	Standard deviation of single reading (divisions)
31	5-10	0.406
67	10-15	0.437
45	15-20	0.388
27	20-40	0.601
15	40-70	0.482

Recovery

Both adrenaline and noradrenaline were quantitatively recovered when pure solutions were carried through the chromatographic adsorption process. The recovery from plasma was studied by adding varying amounts of adrenaline to 10 ml. samples of sterile horse serum. The results of ten such experiments showed that recovery was quantitative with a standard deviation of about 5% over the entire range investigated (Table 2). To be significant at a probability level of 0.01, the difference between two results must exceed 15% or, at a probability level of 0.05, 11%.

Table 2. Recovery of adrenaline added to horse serum (Ten experiments for each quantity of adrenaline added.)

Adrenaline added ($\mu\text{g./10 ml.}$)	Mean recovery (%)	Standard deviation of recovery
0.02	100.55	6.34
0.03	100.01	4.70
0.05	97.89	4.90
0.10	97.34	4.47

Equally good recoveries, obtained by different methods, have been reported by many other authors. Nevertheless, the estimates of the normal level of blood adrenaline differ widely according to the method used. Obviously, a satisfactory recovery of added adrenaline excludes an underestimate, but not an overestimate. This can only be done by obtaining further evidence.

Specificity

Fluorescence of variable intensity is produced by all catechol derivatives tested, with the exception of dihydroxyphenylalanine (Table 3). This presumably forms a derivative which is not extracted

by *isobutanol* from the alkaline solution, or only to a slight extent. None of the non-catechol substances tested produced any significant fluorescence after their solutions had been carried through the chromatographic adsorption process.

The reaction is thus less specific than that resulting in the formation of adrenolutine or similar com-

diamine was omitted and plasma eluates were directly extracted with *isobutanol*, the fluorescence of the extract was smaller than that of the usual reagent blank. This indicates that none of the fluorescence measured in the usual test is due to preformed plasma components, but is produced entirely during the condensation reaction.

Table 3. *Specificity of method*

Catechol derivatives	Formula	Relative fluorescence
L-Adrenaline	(3) $\text{HO}-\text{C}_6\text{H}_3\cdot\text{CHOH}\cdot\text{CH}_2\cdot\text{NHCH}_3$ (1)	100
Adrenochrome		100
L-Noradrenaline	(3) $\text{HO}-\text{C}_6\text{H}_3\cdot\text{CHOH}\cdot\text{CH}_2\cdot\text{NH}_2$ (1)	20
Epinephrine	(3) $\text{HO}-\text{C}_6\text{H}_3\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{NHCH}_3$	48
Isoprenaline	(3) $\text{HO}-\text{C}_6\text{H}_3\cdot\text{CHOH}\cdot\text{CH}_2\cdot\text{NHCH}(\text{CH}_3)_2$	56
Catechol	(1) $\text{HO}-\text{C}_6\text{H}_4$	96
L-Dihydroxyphenylalanine	(3) $\text{HO}-\text{C}_6\text{H}_3\cdot\text{CH}_2\cdot\text{CHNH}_2\cdot\text{CO}_2\text{H}$	0.2
Other compounds		
Ephedrine	$\text{C}_6\text{H}_5\cdot\text{CHOH}\cdot\text{CH}(\text{NH}_2)\text{CH}_3$	2×10^{-4}
Amphetamine	$\text{C}_6\text{H}_5\cdot\text{CH}_2\cdot\text{CH}(\text{NH}_2)\text{CH}_3$	1×10^{-6}
Tyramine	(p) $\text{HO}\cdot\text{C}_6\text{H}_4\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{NH}_2$	3×10^{-3}
Resorcinol	(1) $\text{HO}-\text{C}_6\text{H}_4$	~ 0
Quinol	(1) $\text{HO}-\text{C}_6\text{H}_4$	~ 0
Nicotinamide	$\text{C}_5\text{H}_4\text{N}\cdot\text{CONH}_2$	~ 0

pounds, for which indole ring closure is required. But this is of no practical consequence since physiological catechol derivatives which are not capable of indole ring closure, such as dihydroxyphenylalanine and, possibly, dihydroxyphenylserine, are largely eliminated by the extraction procedure.

When the condensation reaction with ethylene-

The fluorescence intensity produced by nor-adrenaline is one-fifth that produced by an equimolar amount of adrenaline. The fluorescence produced by a mixture of both substances is equal to the sum of the fluorescence produced by the separate components. There is thus no mutual interference like that observed under different conditions by Heller *et al.* (1950).

Rate of autoxidation

When plasma was diluted with an equal amount of sodium acetate buffer, adjusted to pH 8.4, and shaken in air, added adrenaline was found to disappear at about the same rate as the preformed material (Table 4). To facilitate autoxidation, sodium thiosulphate was omitted from the anti-coagulant solution and the sodium acetate solution used in the experiment was not previously purified by cation exchange.

The experiment shows that in its autoxidizability the preformed substance behaves like adrenaline.

Table 4. *Autoxidation of preformed and added adrenaline in human plasma*

(Blood (150 ml.) collected in 50 ml. 2% NaF (no $\text{Na}_2\text{S}_2\text{O}_3$.)

Adrenaline ($\mu\text{g./l.}$)			
Found			Loss (%)
Added	Initial	After 30 min. shaking in air (37°, pH 8.4)	
0	3.1	1.6	48.5
20	22.9	9.8	57

Action of amine oxidase

It could further be shown that an extract of rabbit liver removed preformed material and added adrenaline at an equal rate from plasma (Table 5). The extract was inactivated by boiling and inhibited by ephedrine, a specific inhibitor of amine oxidase (Blaschko, Richter & Schlossmann, 1937). The inhibition by ephedrine was the same whether the enzyme acted on the preformed substance or on added adrenaline. It may therefore be concluded that the action of the liver extract was due to its content of

amine oxidase, and that the substance preformed in plasma is an amine derived from catechol with affinities for amine oxidase similar to those of adrenaline.

It remained to be shown that the entire fluorescence measured in the test was due to this substance and was not the resultant of several components. It was found that amine oxidase

Fig. 2. Action of amine oxidase on preformed reacting material in human plasma. Enzyme prepared as in experiment of Table 5. Plasma (15 ml.) incubated with 3 ml. liver extract at 37°. Results were corrected by subtracting the fluorescence obtained in controls containing 15 ml. water + 3 ml. liver extract.

quantitatively removes the preformed material from plasma (Fig. 2). The plot is that of a first-order reaction, i.e. a straight line is obtained on a semi-logarithmic scale. The bulk of the reacting material is therefore either a single substance or a mixture of substances with similar affinities for the enzyme.

Table 5. *Action of amine oxidase on preformed and added adrenaline in plasma*

(Enzyme: rabbit liver homogenized with 1 part water and centrifuged. Residue washed with 1 part water. Combined supernatants dialysed against running distilled water at 0° for 16 hr. Blood (150 ml.) collected in 50 ml. NaF- $\text{Na}_2\text{S}_2\text{O}_3$ solution. Samples contained 15 ml. plasma, with or without 3 ml. liver extract. Incubation at 37° for 2 hr.)

		Adrenaline found ($\mu\text{g./l.}$)	
		Preformed	After adding 10 $\mu\text{g./l.}$
Sample no.	Addition to plasma		
S1	0	3.7	11.2
S2	0.01 M-Ephedrine	4.0	11.7
S3	Liver extract, boiled	4.3	13.0
S4	Liver extract + 0.01 M-ephedrine	3.4	10.1
S5	Liver extract	1.4	4.1
Disappearance (S3 - S5)		2.9	8.9
Percentage disappearance $\left(\frac{(S3 - S5)}{S3} \times 100 \right)$		67.5	68.5
Percentage inhibition by ephedrine $\left(\frac{(S4 - S5)}{(S3 - S5)} \times 100 \right)$		69	67.5

*The identity of the adrenaline-like substances
in blood*

It is now generally accepted that a mixture of adrenaline and noradrenaline, in varying proportions, occurs not only in the adrenal medulla, but in all tissues containing functional post-ganglionic sympathetic fibres, and that it is discharged into the blood stream when these fibres are stimulated (cf. references in a review by von Euler, 1950). The sympathomimetic amine occurring in human and cattle blood is, according to von Euler & Schmiterlw (1947), largely L-noradrenaline with an admixture of about 4 % adrenaline; rabbit blood, on the other hand, contains mainly adrenaline, according to West (1947). A third sympathomimetic amine, hydroxytyramine, may be the physiological precursor of noradrenaline (cf. Blaschko, 1950). Traces have been found in the adrenals (Goodall, 1950) and in urine (Holtz & Credner, 1942; von Euler & Hellner, 1951), but its concentration in blood is probably negligible.

Chemical, biological and chromatographic methods are available to distinguish between these amines, but these methods are generally not sensitive enough for application to the low concentrations occurring in blood. Thus the measurable limits which can be attained in the set of five pharmacological tests devised by Gaddum, Peart & Vogt (1949) are given as 10 $\mu\text{g./l.}$ for adrenaline and 100 $\mu\text{g./l.}$ for noradrenaline.

Nevertheless, at our request, Dr T. B. B. Crawford of the Department of Pharmacology, University of Edinburgh, applied the rat-uterus method to a plasma eluate which we had sent. Its apparent adrenaline content had previously been estimated by our method and amounted to 3.0 $\mu\text{g./l.}$ In the rat-uterus test adrenaline causes a depression of the contraction elicited by carbachol. The plasma eluate had a small effect in the wrong direction, since the response to carbachol was slightly increased. The addition of adrenaline to the eluate in a concentration of 2.5 $\mu\text{g./l.}$ was more than enough to antagonize the slight stimulant action. A reagent blank did not affect the definite response of the rat uterus to a solution containing 2.5 $\mu\text{g.}$ of adrenaline/l.

In our opinion, this result does not yet definitely exclude the identity of the plasma substance with adrenaline. As pointed out by Gaddum *et al.* (1949), blood contains interfering substances whose effect on the rat uterus is antagonistic to that of adrenaline. The eluate tested was not free of this interference and it is conceivable that the amount of preformed adrenaline was just adequate to counterbalance most of the antagonistic effect. It is, however, far more probable, especially in view of the results of von Euler & Schmiterlw (1947), that the bulk of the plasma substance is identical with noradrenaline rather than adrenaline. Work is in progress

to decide this problem by paper-chromatographic methods.

For many questions of clinical interest relative figures for the combined level of sympathomimetic amines in blood would be of value, provided there are no drastic changes in the proportion of the components. Such figures can be obtained in the clinical laboratory by the method described. Pending a final identification of the reacting material the results have been expressed in terms of adrenaline. To convert them to noradrenaline concentrations the figures have to be multiplied by 5.

*The concentration of adrenaline-like substances
in human blood*

Fifty-four samples of blood were collected from the staff and patients of Runwell Hospital under ordinary conditions. The mean amine concentration corresponded to $3.39 \pm 0.043 \mu\text{g.}$ adrenaline/l. plasma. Variations from the mean were comparatively slight, the range being from 2.9 to 4.3 $\mu\text{g./l.}$

The values for the normal adrenaline content of blood reported by previous authors are notoriously divergent (for reviews of the literature see Lehmann & Michaelis, 1942; Jrgensen, 1945; Blaschko, 1950). Most of them are much higher than those found by our method, a clear indication that the methods used were lacking in specificity. There are, however, some results, such as those of Bloor & Bullen (1941) and especially those recently obtained by Anrep, Barsoum & Gabrawy (1950), which broadly agree with ours, or such as those of Lund (1949*b*) which, at least, do not contradict them. Expressed as L-noradrenaline our results are in remarkably good agreement with the value of 10–20 $\mu\text{g./l.}$ found by von Euler & Schmiterlw (1947).

Some authors (Stewart & Rogoff, 1917; Trendelenburg, 1923; Guyton & Gillespie, 1951) tried to calculate the circulating concentration of adrenaline from a consideration of the rates of secretion and elimination or the amounts required to maintain blood pressure after adrenalectomy. They all arrived at figures in the neighbourhood of 1 $\mu\text{g./l.}$ It seems that, in order of magnitude, these estimates which neglected extra-adrenal sources of sympathomimetic amines are confirmed by our observations.

The extremely high values obtained by Lehmann & Michaelis (1942, 1949) and by Annersten, Grnwall & Kiw (1950) in non-deproteinized plasma are attributed by these authors to a protein-bound, biologically inactive form of adrenaline. For this there is at present no experimental support. As pointed out by Lund (1949*b*), it is unlikely that the adrenaline-protein complex would remain undissociated when plasma is passed through an alumina column. There is certainly no retention of adrenaline when it is added to plasma.

*Changes of plasma adrenaline concentration
under various conditions*

Electrical stimulation of the brain by external electrodes may increase the apparent adrenaline level in plasma by as much as 100%, whether convulsions occur or not. In general anaesthesia, on the other hand, the adrenaline content may fall to about 40% of the initial value.

Contrary to a widely held opinion a highly significant fall of the adrenaline level in blood is invariably observed during insulin hypoglycaemia. This phenomenon is fully developed within 30 min. after the intramuscular injection of insulin, long before consciousness becomes clouded. The effect is not due to an exhaustion of the adrenergic system, since an intravenous injection of glutamic acid, or the conduction of electric current through the brain, during hypoglycaemic coma, causes a pronounced rise of blood adrenaline.

These effects will be fully reported in a later publication.

SUMMARY

1. A fluorimetric method for the estimation of adrenaline-like substances in blood is described. It consists of the following steps: (a) filtration of plasma-buffer mixture (pH 8.4) through a column of acid-washed alumina and elution of the adsorbed amines by dilute acetic acid; (b) heating of the eluate at 50° with a mixture of ethylenediamine and ethylenediamine dihydrochloride; (c) extraction of a stable fluorescent condensation product with

isobutanol; (d) measurement of fluorescence. The method has the advantage, compared with previous methods, that adrenochrome, a labile oxidation product of adrenaline formed as an intermediary, is trapped in the nascent state and quantitatively converted into a stable condensation product.

2. A series of recovery experiments showed that added adrenaline was quantitatively recovered with a standard deviation of about 5%. Concentrations $\geq 1 \mu\text{g./l.}$ may be determined by the method.

3. A study of the specificity of the method and of the action of amine oxidase led to the conclusion that the reactive material in blood consists entirely of amines derived from catechol. Whether this is adrenaline, noradrenaline or a mixture of both, remains to be further investigated.

4. Expressed in terms of adrenaline the mean concentration observed in human venous blood under normal conditions is about $3 \mu\text{g./l.}$ Since the fluorescence formed from noradrenaline is one-fifth of that produced by adrenaline, figures of adrenaline concentrations have to be multiplied by 5 to convert them to noradrenaline concentrations.

5. Some further results obtained with the method are briefly reported.

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