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Catecholamines in blood

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There can be little doubt that the catecholamines, adrenaline (AD), noradrenaline (NA) and dopamine (DA) can be found in the circulating blood of many animals. From the early years of this century there has been a great deal of pressure to develop reliable and accurate assay methods for catecholamines in a variety of body fluids. A natural consequence has been the publication of many assay methods of widely varying reliability and complexity, and, of course, reviews attempting to find some order in the apparent chaos.

Why another review? This question could be asked with some justification.

However, from the earliest days at the Pharmacological Department of The Square, catecholamines and their assay were of major concern. Later, Monica Mann made the detection of catecholamine secreting tumours by the bioassay of urine samples into a cottage industry, and provided hospitals with a valuable diagnostic service.

The most successful bioassay for AD and NA in the blood is the rat blood pressure method. This assay must be preceded by rigorous extraction and purification of the amines. The sensitivity of the method is enhanced by the use of drugs that inhibit the uptake of the injected amines into the adrenergic nerves and their effects on the vascular β-adrenoceptors (Vanov & Vogt, 1963; Haefely, Hürlimann & Thoenen, 1965). Catecholamines can also be assayed by superfusion of blood, immediately after its removal from the experimental animal, over a variety of isolated tissues. This approach has been greatly exploited by Vane (1969).

The evolution into chemical methods of assay of the catecholamines in tissues and body fluids came about when Rosemary Cass joined the Department. She developed the butanol extraction method of Shore & Olin (1958) into probably the most reliable assay method then available (Cass & Spriggs, 1961). After extraction the amines were assayed fluorimetrically.

Until recently, of all the chemical methods available, only fluorimetry could provide any real estimate of the normal amounts of catecholamines to be found in the blood. The most reliable fluorimetric methods were those in which the amines were converted to their highly fluorescent lutine derivatives by controlled oxidation and rearrangement in alkali—the trihydroxyindole (THI) method, (for reviews see Callingham, 1967, 1975). The amines were extracted from the blood plasma, before assay, by precipitation of the plasma proteins with perchloric acid followed by ion-exchange chromatography. The sensitivity of the method was such that about 10–20 ml of blood was required for a successful assay. In consequence, most observations have been made on man. The need for large volumes of blood made repeated sampling difficult and precluded studies on babies and young children.

The collection of the blood sample is not without stress which can in itself greatly modify the circulating amounts of the catecholamines. In general, venepuncture will give rise to higher blood concentrations of AD and NA in the sample than is found in samples obtained from indwelling catheters. Observations on small animals are doubly difficult since if an anaesthetic is used, this may also change the circulating concentrations of the amines (see Callingham, 1975).

Nowadays, fluorimetric and bioassay methods are rivalled by techniques involving the conversion of the catecholamines to labelled products either enzymically or chemically, which can then be counted by liquid scintillation. The most popular are those developments of the 'double isotope' method described by Engelman, Portnoy & Lovenberg (1968) and Engelman & Portnoy (1970). The assay is based on the conversion of the amines to their corresponding 3-*O*-methylated derivatives by allowing them to react with the methyl donor ([^{3}H] or [^{14}C] methyl labelled *S*-adenosyl methionine) and a partially purified sample of catechol-*O*-methyl transferase. Internal standards of the amines labelled with the appropriate isotope can then be added as a check on the efficiency of the method.

There are many variations on this basic theme which overcome the problem that the original method does not distinguish between the different amines. Separation of the methylated products by electrophoresis on thin-layer cellulose (Siggers, Salter & Toseland, 1970) and by thin-layer chromatography (Engelman & Portnoy, 1970; Christensen,

1973a) are but two examples. In its developed form the method can detect AD, NA and DA in normal human plasma with a good degree of reliability. However at present, the highest sensitivity for NA in plasma is given by methods in which the amine is converted to labelled adrenaline by incubation with labelled *S*-adenosyl methionine and the enzyme phenylethanolamine-*N*-methyl transferase (Henry, Starman & others, 1975).

The method currently in use in our laboratory is based upon the COMT method. A partially purified preparation of pig liver COMT is made by ammonium sulphate precipitation (Nikodejevic, Senoh & others, 1970) and aliquots are deep frozen until required. One ml samples of blood plasma obtained by venepuncture are incubated with the enzyme and [^{3}H]*S*-adenosyl methionine in tris buffer at pH 9·1, together with pargyline to inhibit any monoamine oxidase that might be present. After incubation for 50 min at 37° the reaction is stopped by placing the tubes in ice followed by the addition of borate buffer at pH 11. Metanephrine, normetanephrine and methoxytyramine are added as carriers. The methylated amines are extracted with a mixture of toluene and amyl alcohol and then back extracted into 0·1 N HCl. The amines are now acetylated by the addition of acetic anhydride and sodium hydrogen carbonate in a similar manner to that described by Laverty & Sharman (1965). The acetylated methylated amines are separated by descending paper chromatography in a solvent system consisting of the organic phase from a mixture of toluene–methanol–water–ethyl acetate (10:5:5:4), on a paper cut as described by Laverty & Sharman (1965). After equilibration and running for 2–2·5 h the papers are dried in air and the bands corresponding to the three derivatives of AD, NA and DA are made visible by spraying with ammonia and Folin and Ciocalteau reagent. The stained bands are then cut out and counted for radioactivity by liquid scintillation. The limiting sensitivities at present are AD 25–50, NA 100–200, and DA 25–50 pg.

Whatever method or modification is used, none is simple and rapid. They are as tedious and time-consuming as the fluorimetric methods they sought to displace. But they do have greater sensitivity, enabling reliable assays to be done on 5 ml of blood or even less (Passon & Peuler, 1973; Cryer, Santiago & Shah, 1974). Reasonable confidence can be placed in the current methods and useful comparisons made between the results they produce and those from the best of the fluorimetric and biological methods.

After the validity of the assay has been established, it must be remembered that the circulating concentrations of the catecholamines represent what remains after the interaction of a complex set of processes, and may therefore not give such an intimate picture of the situation as was at first confidently expected. The NA in the venous outflow from an organ is only that which has escaped the avid uptake processes of uptake$_1$ and uptake$_2$. Even when the amine is in the blood vessel it is exposed to uptake into the blood vessel wall (Gillespie, 1974). Passage through the liver, for example, will greatly reduce its concentration (see Vane, 1969; Sharman, 1975). It is little wonder that AD and NA have a half-life in the circulation of less than 20 s (Ferreira & Vane, 1967).

The catecholamine content of the blood may be much changed if the activities of the uptake systems are altered by drugs or by other factors. The amounts of the amines in the blood are known to rise in stress. This rise could be augmented by a prolonged stressful situation that caused an increase in the circulating glucocorticoids. Salt (1972), using the perfused rat heart, has shown that the normal blood concentrations of steroids, with the exception of cholesterol, do not significantly affect uptake$_2$. However, the amounts that can occur in stress lead to a great reduction in the activity of this uptake process. This would direct more of the NA towards uptake$_1$ and towards escape from the synaptic gap.

There are many reports concerning the dispositon of the catecholamines into and between the various blood elements that could well have a bearing on the validity of assays of blood catecholamines. Often, reports are conflicting, but, in general, it seems that the amines are relatively more stable in plasma than in saline. The amines may be taken up into red cells by a rapid saturating process together with a very slow diffusional component, which would imply that it is desirable to remove the red cells as soon as possible after collection of the sample. Platelets will also take up catecholamines concentrating AD more readily than NA (see Callingham, 1967; Sharman, 1975).

There is dispute concerning the importance and implications of the binding of the amines to the plasma proteins. Some while ago Antoniades, Goldfien & others (1958) showed that AD bound to human plasma albumin in preference to NA, the side chain being important in this interaction (Zia, Cox & Luzzi, 1971). Danon & Sapira (1972) found that there were at least two components involved in the binding of AD and NA to plasma proteins.

The characteristics of the binding of NA appear to be similar in the plasma of rabbit, rat, ox and man, the binding being best at 37° with a Q_{10} of 2 to 3 (Cohen & Rodriguez Farre, 1975). In their discussion of the significance of protein binding of the catecholamines, these authors point out this binding could be involved in the regulation of the action of NA in the circulation. Although the time to maximum binding in their experiments was long (greater than 19 h), significant binding took place almost immediately. Osswald & Branco (1973) infused NA into the arterial supply to the dog hind limb at a rate of 1 μg kg^{-1} min^{-1}. They could only find about 42 μg litre^{-1} in the venous effluent instead of an expected 80–100 μg litre^{-1}. They suspected that protein binding may have been implicated in this discrepancy. Subsequently, Branco, Fleming Torrinha & Osswald (1974) showed that around 40% of ^{3}H-NA, when incubated with dog plasma, became bound to protein and could not be assayed by fluorimetry after precipitation of the proteins with perchloric acid, or after alumina extraction of the plasma itself. But they also observed that the protein binding did not interfere with the bioassay on the pithed rat blood pressure. The binding in these experiments was rapid and did not increase with time or with raising of the temperature. Their results may have been influenced to some degree by the fact that they used concentrations of NA in the range of 50 to 1000 ng ml^{-1}. They were unable to displace the bound amine with oxytetracycline, although Powis (1974) had been more successful with lower concentrations of the amine. They presumed that the bulk of the NA was bound to sites that were different from those binding the oxytetracycline. All the protein fractions examined bound the amines, the highest affinity residing with the globulins although more bound to the albumin since it was present in the greatest proportion in the plasma. Powis (1975) found similar results with human plasma. NA bound more readily than AD and was highly resistant to removal from the protein by procedures such as equilibrium dialysis. Again, more bound to the albumin fraction. He suggested that the binding of the NA to the high molecular weight protein IgM and to very low density lipoproteins may be evidence for a transport function of these compounds. Diseases that modify the amount and proportions of the plasma proteins may have an influence on the binding of the catecholamines in the blood. Powis (1975) quotes a case of Crohn's disease in which the plasma binding capacity was increased almost 20 fold. This change could not, however, be reconciled with the change in proteins, which might indicate the presence of yet another factor affecting the binding of the amines.

It is at present difficult to decide just how protein binding can affect the validity of any subsequent assay. If all the bound material can readily take part in the physiological processes in the blood, an assay of all the blood catecholamine content would be necessary. If this is not the case, an assay of the readily available amine could be the more useful. The fact that Branco & others (1974) found that the binding did not interfere with bioassay gives some hope for the enzyme assays done on the native plasma. By this approach it may be possible to assay bound and free amine but not the conjugated amine that is also present. Conjugated NA has been reported to comprise about 2 to 3 times the concentration of free NA (Häggendal, 1963).

In spite of all the limitations and as yet unsolved problems, blood catecholamine assays provide a useful experimental and diagnostic tool when adequate controls are available. Comparisons of results obtained by widely different methods is decidedly hazardous. However, surprisingly, over the years there has emerged a picture of the blood catecholamines correlated with a large number of factors including age, pulse rate, postural change, exercise and stress both mental and physical.

Table 1 summarizes some selected values of plasma catecholamines obtained using the enzymic methylation method both by ourselves and by others. Our results were derived from volunteers, none of whom fasted, the blood samples being taken during a brief interuption of their normal work (for values obtained by fluorimetry, see for example, Callingham, 1967; 1975). The results in Table 1 bear out the earlier

Table 1. *Catecholamine contents of venous plasma samples from normal human subjects*

No. and sex (if given) of subjects		Plasma content (ng ml^{-1} ± s.e.m.) AD	NA	DA	Ref.
12	m†	0·06 ± 0·01	0·20 ± 0·02	—	1
10	f†	0·04 ± 0·01	0·21 ± 0·03	—	1
4	—	0·09 ± 0·05	0·42 ± 0·01	—	2
16	m + f†	0·05 ± 0·01	0·22 ± 0·02	—	3
7	—*	—	0·25 ± 0·02	—	4
6	—	—	—	0·20 ± 0·02	5
18	m + f**†	0·04 ± 0·01	0·22 ± 0·02	—	6
10	m**	0·17 ± 0·02	0·29 ± 0·05	0·20 ± 0·07	7
6	f**	0·13 ± 0·01	0·41 ± 0·05	0·19 ± 0·04	7

Values obtained by the enzymic methylation method of assay using COMT except (*) in which phenylethanolamine-*N*-methyl transferase was used. ** Assay performed directly in plasma sample. † Fasting, rested supine subjects.

1. Engelman & Portnoy (1970). 2. Siggers, Salter & Toseland (1970). 3. Christensen (1973a). 4. Henry, Starman & others (1975). 5. Christensen (1973b). 6. Cryer, Santiago & Shah (1974). 7. Callingham & Barrand (unpublished results).

observations that there is no significant difference between the catecholamine contents of blood from males and females. In our estimations there is a close correlation of pulse rate and amine content. In a series of 33 subjects of either sex with pulse rates varying from 60 to 140 beats min^{-1}, correlation coefficients of 0·92, 0·73, 0·91 and 0·38 were found for total amines, AD, NA and DA respectively. It is to be expected that, of the three catecholamines, it is NA that correlates best with pulse rate, since it should be the amine most closely associated with sympathetic nervous activity. Christensen & Brandsborg (1973) showed that the rise in NA was less on light exercise than on simple standing. No rise in NA was seen until the increase in pulse rate exceeded 30 beats min^{-1}. They suggested that the initial increase in pulse rate was due to removal of parasympathetic control. On standing the increase in vasoconstrictor tone would be greater than that during moderate exercise. In our experiments where 3 subjects first exercised gently and then to near exhaustion, the plasma NA rose by 74 and 250%. There was no change in either AD or DA on gentle exercise but they rose by 80 and 33% respectively when the subjects were nearly exhausted. Exercising volunteers at a standardized work load sufficient to double their pulse rates (3 min at 20 km h^{-1} at a force of 20 Newtons) caused both the AD and NA to rise but with only little effect on the DA content. Both the resting and exercise concentrations of NA were higher within an hour of a meal. Pavlik & Frenkl (1975) have reported that during the digestive phase following a meal, the pressor responses to NA in volunteers are smaller than before the meal. It would seem that a greater sympathetic drive and consequent overflow of transmitter into the blood is needed to maintain and to double the post prandial pulse rate.

The relation between posture, exercise and the plasma DA concentrations is at present unresolved. Cuche, Kuchel & others (1972) have shown that on changing from the supine to the upright posture there is a fall in the excretion of DA in the urine together with a rise in NA excretion. These changes are accompanied by a decrease in renal plasma flow, glomerular filtration and sodium excretion. An increase in plasma DA leads to an increase in renin secretion which is unaffected by β-blocking agents but sensitive to haloperidol (Imbs, Schmidt & Schwartz, 1975). At present no clear story has emerged that reveals the physiological significance of the DA in human blood.

It is apparent that the sophistication of the assay techniques has vastly increased over the years. But it is still difficult to apportion the blame for the large variation between results from supposedly normal people that occurs from time to time. Clearly animal variation is a vital factor. What is normal for one could be abnormal for another. However, the fact that the assay methods currently available are forced to operate at the limit of their sensitivity introduces an error that is difficult to assess. It is to be hoped that the sensitivity of the enzyme method will soon be increased to overcome this problem, but there is a long way to go before a simple method appears. It would be pleasant to review this development for the Centennial.

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