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The Association of Adrenaline and Noradrenaline with Blood Platelets

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It is becoming apparent that blood platelets, in addition to their classical role in blood coagulation, have other important functions. Among these their function as carriers of pharmacologically active amines is of particular interest. Weil-Malherbe & Bone (1954*a*) have briefly reported that platelets contain adrenaline and noradrenaline, and more extensive data will now be presented. Experiments on the nature of this association will also be described.

The results were obtained with a fluorimetric method which, though not strictly specific for adrenaline and noradrenaline, is believed to be specific for the catechol grouping. Of other biologically occurring catechol compounds those possessing a carboxylic or ketonic group in the side chain give rise to much smaller fluorescence intensities than adrenaline and noradrenaline and would interfere only if they were present in great excess. Experiments with ion-exchange resins, paper chromatography and paper electrophoresis have shown that interfering substances do not significantly contribute to the fluorescence obtained when the method is applied to plasma (Weil-Malherbe & Bone, 1957*b*). Although we have so far been unable to demonstrate the presence of adrenaline by bioassay in plasma of resting human subjects, Born & Hornykiewicz (1957) were successful in demonstrating the presence of adrenaline, though not noradrenaline, by bioassay in pig platelets.

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METHODS

Collection of blood

When human blood was used, the donors were hospital patients suffering from mental disorders. Blood was collected from an antecubital vein with a transfusion needle (16 s.w.g.) mounted on a short length of polyvinyl chloride tubing and was run into a measuring cylinder containing anticoagulant solution. Unless stated otherwise, a solution containing 1% (w/v) of neutralized sodium ethylenediaminetetra-acetate (EDTA) and 2% (w/v) of $Na_2S_2O_3,5H_2O$ (Renton & Weil-Malherbe, 1956) was used in the proportion of 1 vol to 3 vol. of blood. This anticoagulant is referred to below as 'EDTA–thio solution'. Solid heparin, containing 107 units/mg. (Evans Medical Supplies Ltd.), was dissolved in a minimum volume of 0·9% NaCl and

used in the proportion of 200 units/10 ml. of blood. The composition of other anticoagulants is indicated in the tables.

Preparation of platelets

Blood was first centrifuged at about 600 *g* for 20 min. at room temperature; the supernatant ('platelet-rich plasma') was centrifuged a second time in the angle head, no. 6886, of the MSE refrigerator centrifuge (Measuring and Scientific Equipment Ltd., London) at 4000 rev./min. (about 2500 *g*) for 20 min. at 10°. The plasma ('platelet-poor') was thoroughly drained off and the deposit of platelets redistributed in a mixture of 3 vol. of 0·9% NaCl and 1 vol. of EDTA–thio, equal in volume to the original volume of plasma. The platelets were washed once or twice by centrifuging and suspension in the same solution; washing was sometimes omitted, especially in many of the estimations included in Table 2.

Estimation of adrenaline and noradrenaline

The platelet suspension, prepared from 15–20 ml. of blood, was adjusted to pH 8·5 with 0·5N-Na_2CO_3 solution and passed, under pressure (40 mm. Hg), through a column of 0·7 g. of alumina (5 mm. × 45 mm.) previously washed with 5 ml. of 0·2M-sodium acetate. Details of the technique have been described by Weil-Malherbe & Bone (1952). Filtration usually proceeded smoothly and the platelet suspension emerged apparently unchanged, macro- and micro-scopically, from the column, although comparative platelet numbers were not determined. Plasma, whether platelet-rich or platelet-poor, was treated as described by Weil-Malherbe & Bone (1952), i.e. it was diluted with an equal volume of sodium acetate buffer before filtration through alumina. The adsorbed catecholamines were eluted from the column and estimated by the differential fluorimetric method (Weil-Malherbe & Bone, 1952, 1953).

Concentrations of catecholamines in platelet-poor plasma and in platelets were calculated relative to the volume of platelet-rich plasma from which these fractions were prepared.

Platelet counts

For counts on whole blood the method of Brecher & Cronkite (1950) was used. Counts on platelet-rich plasma were carried out after 100-fold dilution with the following solution: 1 g. of EDTA (disodium salt), 0·7 g. of NaCl, 0·1 g. of Brilliant Cresyl Blue, 3 ml. of N-NaOH, in 100 ml. Platelets in 1 $mm.^2$ (400 small squares) of the improved Neubauer chamber were counted in each determination.

RESULTS

Distribution of adrenaline and noradrenaline between plasma and platelets

When 'platelet-rich' plasma was centrifuged, analysis showed the presence of catecholamines both in the 'platelet-poor' supernatant and in the deposit of platelets. As shown in Table 1, the sums of the catecholamine concentrations in the two fractions account, within the limits of error, for the concentrations in the unfractionated sample.

The data shown in Table 2 represent two series of estimations. The first of these was carried out between June 1954 and July 1955, the second between November 1956 and May 1957; in the interval, modifications of the fluorimeter were introduced (cf. Weil-Malherbe & Bone, 1957*a*), which may be responsible for the fact that the absolute concentrations were lower by about one-third in the second than in the first series, the differences being significant. Although there was a considerable scatter in individual samples, adrenaline and noradrenaline were both about evenly distributed between plasma and platelets in both series of estimations on the average. The distribution of adrenaline between plasma and platelets correlated with that of noradrenaline; the

Table 1. *Distribution of catecholamines in human plasma and platelets*

Details are given under Methods. The values represent means ± S.E.M. of six different samples.

	Adrenaline	Noradrenaline
	(μg./l. of plasma)	
(*a*) Plasma, platelet-rich	2·27 ± 0·27	8·39 ± 1·58
(*b*) Plasma, platelet-poor	0·88 ± 0·16	3·70 ± 0·23
(*c*) Platelets	1·18 ± 0·17	3·71 ± 0·33
Sum (*b* + *c*)	2·06 ± 0·31	7·41 ± 0·43

Table 2. *Concentrations of adrenaline and noradrenaline in plasma and platelets*

Values are means ± S.E.M. (with range). For details see text.

Series	No. of observations		Plasma (μg./l.)	Platelets (μg./l. of platelet-rich plasma)	Percentage in platelets of total adrenaline or noradrenaline
I	85	Adrenaline	1·11 ± 0·056 (0·42–2·80)	1·18 ± 0·047 (0·27–2·73)	51·95 ± 1·06 (21·6–70·3)
		Noradrenaline	3·76 ± 0·115 (1·74–6·36)	4·28 ± 0·158 (1·63–8·25)	52·66 ± 0·76 (35·1–70·8)
II	31	Adrenaline	0·74 ± 0·068 (0·39–2·41)	0·82 ± 0·058 (0·30–1·59)	52·6 ± 1·96 (28·5–73·6)
		Noradrenaline	2·60 ± 0·261 (1·17–9·40)	2·72 ± 0·236 (1·20–5·92)	50·9 ± 2·17 (24·2–72·6)

coefficient of correlation, calculated on the combined total of 116 samples, was 0·602.

Platelet counts on platelet-rich plasma were available for many of the samples in the first series and all those in the second series of thirty-one estimations. To facilitate comparison with the results of other authors who determined the concentrations of histamine (Graham, Lowry, Wheelwright, Lenz & Parish, 1955; Humphrey & Jaques, 1954) and serotonin (Humphrey & Jaques, 1954; Humphrey & Toh, 1954; Zucker & Borrelli, 1955, 1956) concentrations were expressed in μmg./10^9 platelets. The mean contents of adrenaline and noradrenaline/10^9 platelets (± S.E.M.) were 1·92 ± 0·13 μmg. (range 1·07–3·82 μmg.) and 6·01 ± 0·48 μmg. (range 2·81–14·6 μmg.) respectively. The concentration of catecholamines in platelets is thus about one-hundredth of that of serotonin (Zucker & Borrelli, 1955). From data collected by Maupin (1954) it appears that, for a count of 500 000/μl. of plasma, the platelet volume accounts for 0·85% of the volume of plasma. Assuming an equal distribution of catecholamines between platelets and plasma and allowing for dry matter [12·5 and 7% (w/w) respectively] it may be calculated that the concentration of catecholamines in platelets is about 125 times higher than in plasma.

Effects of collection procedures

The vulnerability of platelets is well known; contact with glass seems to be particularly injurious to them. It is therefore common practice to use silicone-coated glassware in experiments with platelets. However, as shown in Table 3, the use of untreated glassware did not appreciably affect the distribution of catecholamines between plasma and platelets. To exaggerate the effect of glass surfaces a pinch of powdered glass was thoroughly mixed with blood before centrifuging in one experiment, but not even this procedure led to a marked release of platelet-bound catecholamines. In most of the later experiments therefore untreated glassware was used.

The choice of anticoagulant solutions did not significantly alter the catecholamine content of plasma, but it had a marked effect on that of the platelet fraction (Table 4). The data for the platelet counts given in Table 4 show that this difference was due to the effect of the anticoagulant on the number of platelets in plasma. The amount of catecholamines per unit number of platelets was hardly affected by a change of anticoagulant.

Platelet yields were highest with EDTA as anticoagulant, whereas they were low with oxalate or fluoride, clumping being observed microscopic-

Table 3. *Effect of glass on distribution of catecholamines between plasma and platelets*

In Expt. 1 about 0·5 g. of powdered glass was added to blood collected in uncoated glassware and the mixture was gently inverted several times before being centrifuged. Standard anticoagulant.

		Plasma		Platelets	
		Adrenaline	Noradrenaline	Adrenaline	Noradrenaline
Expt. no.	Treatment of glassware	(μl./l. of plasma)			
1	Uncoated	0·58	2·94	0·62	3·70
	Silicone-coated	0·43	2·78	0·81	3·81
2	Uncoated	0·82	4·80	1·07	6·90
	Silicone-coated	0·91	4·10	1·44	7·00

Table 4. *Effect of different anticoagulants on platelet count and concentration of catecholamines*

P represents vol. of anticoagulant solution mixed with 10 vol. of blood. A = adrenaline, N = noradrenaline.

				Plasma		Platelets			
				A	N	A	N	A	N
Expt. no.	Anticoagulant	*P*	No. of platelets/ μl. of platelet-rich plasma	(μg./l. of plasma)				(μmg./10^9 platelets)	
1	EDTA–thio	3·3	503 000	0·63	1·51	0·82	1·65	1·63	3·28
	Heparin	—	265 000	0·66	1·20	0·59	0·90	2·22	3·40
2	Sodium EDTA, 2·5% (w/v)	2·0	614 000	0·63	1·90	0·79	2·37	1·29	3·85
	Potassium oxalate, 1·8% (w/v)	1·5	355 000	1·06	2·70	0·42	1·43	1·18	4·03
3	Sodium EDTA, 2·5% (w/v)	1·75	540 000	0·56	1·56	0·67	2·11	1·24	3·92
	Sodium citrate, 3·8% (w/v)	1·75	420 000	0·45	1·56	0·45	1·50	1·07	3·58
	Sodium oxalate, 1·3% (w/v)	1·75	280 000	0·67	1·50	0·33	1·11	1·18	3·96

Table 5. *Retention of catecholamines by platelets during blood clotting*

Anticoagulant was added to a portion of a blood sample and the other portion was allowed to clot. Catecholamines were then determined in the serum and in the plasma after removal of platelets by centrifuging.

Expt. no.	Anticoagulant	Fraction	Adrenaline (μg./l.)	Noradrenaline (μg./l.)
1	Heparin	Plasma	0·50	4·08
	None	Serum	0·45	3·52
2	EDTA–thio	Plasma	0·80	3·32
	None	Serum	0·73	3·50
3	EDTA–thio	Plasma	0·45	2·20
	None	Serum	0·36	1·60
	EDTA–thio	Serum*	0·51	2·28

* Clotting was induced by addition of 50 units of thrombin to 40 ml. of platelet-rich plasma.

Table 6. *Release of adrenaline (A) and noradrenaline (N) from platelets by lytic procedures*

Platelet samples used in any one experiment were prepared from the same sample of platelet-rich plasma; each platelet sample corresponded to 20 ml. of blood.

Expt. no.		μg./l. of plasma A	μg./l. of plasma N	% of content of untreated platelets A	% of content of untreated platelets N
1 (means of three experiments)	Platelets, untreated	0·84	3·77	—	—
	After freezing:				
	Insol. residue	0·37	1·40	44·0	37·2
	Supernatant	0·40	1·62	47·6	43·0
2	Platelets, untreated	0·67	2·60	—	—
	After suspension in 0·2% cetyltrimethylammonium bromide:				
	Supernatant	0·25	1·03	37·3	39·6
3	Platelets, untreated	1·07	6·90	—	—
	After extraction with butanol:				
	In extract	0·93	7·10	87	103

ally. Heparin and citrate gave intermediate yields. Silicone-coated glassware was used in these experiments, but in spite of this clumps of platelets were found to adhere to the walls of centrifuge tubes when oxalate or fluoride was used. When heparinized blood was cooled in ice clumping was very marked and, on centrifuging, a practically platelet-free plasma containing less than 1% of the original number of platelets was obtained. A similar observation relating to the effect of oxalate and citrate on platelet counts in plasma has been reported by Barkhan (1957).

Effect of clotting

In marked contrast with serotonin, which is partly released from platelets during the clotting process (Zucker & Borrelli, 1955; Hardisty & Stacey, 1955), catecholamines are not liberated, as shown by the fact that their concentration is the same in centrifuged plasma as in serum, whether formed by spontaneous or by thrombin-induced coagulation (Table 5).

Extraction of platelet-bound catecholamines

As described under Methods the catecholamine content of the platelet suspension was estimated by passing it through a column of alumina at pH 8·5. Catecholamines are quantitatively extracted by this procedure. This was shown in two ways: (1) after the platelet suspension had been passed a second time through a column of alumina, no further fluorescence-producing material could be eluted from the column; (2) when the platelet suspension, after filtration through the column, was centrifuged and the precipitate extracted with acid methanol (Montagu, 1956) no catecholamines were found.

The question arises whether adrenaline and noradrenaline are held within the platelets by a membrane-like structure or whether they are combined with insoluble constituents. If they are held by a membrane, lytic procedures would release them. Saline suspensions of platelets were therefore frozen at −40° for 20 min. and thawed at 37°.

After centrifuging, the residue and supernatant were analysed separately. The initial catecholamine content of the platelets was determined on an untreated portion of the suspension. In another experiment cetyltrimethylammonium bromide, a surface-active agent, was added to a platelet suspension to give a final concentration of 0·2%. The mixture was left at room temperature for 20 min. and centrifuged. Only the supernatant was analysed, since the residue could not be redispersed sufficiently to be passed through an alumina column. Finally a platelet suspension was extracted three times with 2 vol. of butanol and the extract was analysed as described by Weil-Malherbe & Bone (1954*b*).

The results are shown in Table 6. Less than half of the catecholamines in platelets are released by lysis, whether brought about by freezing and thawing or by surface activity. Extraction with butanol, on the other hand, was practically quantitative.

Stability of platelet-bound catecholamines in vitro

When platelet-rich plasma was incubated at 37° for 2 hr. in CO_2+O_2 (5:95, v/v), a considerable disappearance of catecholamines was often noticed. The loss was always larger in platelets than in plasma and greatly depended on the anticoagulant used. The different effects of anticoagulants are shown in Table 7; platelet-bound catecholamines were stable in heparinized or oxalated plasma, whereas there was some loss in citrated plasma. Losses were greatest with EDTA, although the effect was not strictly reproducible. Complete or nearly complete disappearance was found in five out of twenty-one observations, but little or no loss occurred in another six; in the remaining experiments losses varied between 30 and 70%. EDTA sometimes caused losses in the plasma fraction too. Its effects on both fractions were lessened by the exclusion of oxygen [in N_2+CO_2 (95:5, v/v)], but not by the addition of heparin.

With other chelating agents, namely sodium azide (0·005M), potassium cyanide (0·005M), sodium diethyldithiocarbamate (0·001M) and phenylthiourea (0·001M), added either to heparinized or to EDTA plasma (platelet-rich), no clear-cut effects on the stability of catecholamines were observed. On the other hand, the addition of small amounts of adrenaline (2·5 μg./l.) seemed to enhance the disappearance of platelet-bound catecholamines in EDTA plasma, though not in heparinized plasma.

The addition of EDTA decreased the stability of catecholamines in platelets to the same extent, whether the platelets were suspended in plasma, in serum, in serum inactivated at 56° (the latter two fractions were prepared from the same sample of blood as the platelets), in horse serum (Burroughs Wellcome and Co. London), in Ringer solution or in saline. The effect therefore does not depend on an interaction of plasma and platelet factors.

When EDTA was added to a suspension of platelets in Ringer solution or in normal saline, no catecholamines were found in the supernatant after an incubation of 2 hr. Since catecholamines added to saline are stable under these conditions it is unlikely that the losses caused by EDTA are due to leakage. In another experiment platelets which had been incubated in plasma containing EDTA were extracted with acid methanol after they had been passed through alumina. If the incubation had resulted, for instance, in a change of platelet permeability, the removal of catecholamines by the alumina column might have been incomplete and an additional fraction might have been extracted by the more drastic treatment with acid methanol, but, in fact, the extract contained no catecholamines.

Uptake of adrenaline by platelets in vitro

Since it is known that platelets are capable of accumulating serotonin against a concentration gradient (Humphrey & Toh, 1954; Hardisty & Stacey, 1955; Zucker & Borrelli, 1956) their ability to take up adrenaline was investigated. No

Table 7. *Stability of catecholamines in plasma and platelets under various conditions*

Platelet-rich plasma was incubated for 2 hr. at 37° in O_2 or N_2, each containing 5% (v/v) of CO_2. *P* represents volume of anticoagulant solution mixed with 10 vol. of blood.

Expt. no.	Anticoagulant	*P*	Gas	Plasma		Platelets	
				Adrenaline	Noradrenaline	Adrenaline	Noradrenaline
				(% of initial content)			
1	EDTA–thio	3·3	O_2	111	103	0	0
	Heparin	—	O_2	100	87	109	101
2	Heparin	—	O_2	90	103	100	108
	Heparin in EDTA–thio	2·5	O_2	48	50	30	46
	Heparin in EDTA–thio	2·5	N_2	70	70	59	77
3	Sodium EDTA, 2·5% (w/v)	1·7	O_2	24	53	0	0
	EDTA, 2·5% (w/v)	1·7	N_2	92	91	67	69
	Sodium oxalate, 1·3% (w/v)	1·7	O_2	82	92	90	78
	Sodium citrate, 3·8% (w/v)	1·7	O_2	87	100	49	56

Table 8. *Uptake of adrenaline by platelets* in vitro

Blood (8 vol.) was collected in 3·8% (w/v) sodium citrate (1 vol.). Samples (25 ml.) of platelet-rich plasma were incubated at 37° in $N_2 + CO_2$ (95:5, v/v). After 2 hr. the plasma was centrifuged and the residue of platelets washed twice with 10 ml. of 0·9% NaCl. 'Initial' samples were treated immediately. For further details see text.

Expt. no.	Adrenaline added (μg.)	Sample	Platelet-bound fraction: Adrenaline (μg./l. of plasma)	Platelet-bound fraction: Noradrenaline (μg./l. of plasma)	Platelet-bound fraction: Adrenaline (μmg./10^9 platelets)	Platelet-bound fraction: Noradrenaline (μmg./10^9 platelets)
1	0	Initial	0·88	2·38	3·06	8·25
	0	Final	0·78	2·28	2·71	7·93
	2	Final	2·64	2·80	9·2	9·7
	4	Final	2·95	2·80	10·3	9·7
2	0	Initial	0·56	1·13	1·35	2·71
		Final	0·39	0·74	0·94	1·77
	5	Initial*	0·43	0·61	—	—
		Final	1·44	1·04	3·44	2·50

* This sample was cooled in ice before the addition of adrenaline, which caused losses of platelets through clumping.

definite uptake was observed after incubation for 1 hr. at 37° in $O_2 + CO_2$ (95:5, v/v), when the concentration of adrenaline in heparinized platelet-rich plasma was increased about tenfold. In similar experiments in which EDTA was used as anticoagulant the addition of adrenaline even potentiated the disappearance of platelet-bound catecholamines. However, when citrate was used as anticoagulant and the concentration of adrenaline was raised by a factor 100–200, unequivocal evidence of adrenaline uptake was obtained (Table 8). In these experiments platelet-rich plasma was incubated in $N_2 + CO_2$ (95:5, v/v) for 2 hr. at 37° and the platelets were washed twice in 0·9% saline before analysis. The efficiency of the washing process was controlled in Expt. 2 of Table 8 by determining the initial value of the platelet-bound fraction after the addition of adrenaline. To minimize the interaction between platelets and adrenaline before analysis the sample of platelet-rich plasma was cooled in ice before the addition of adrenaline; this led to some clumping of platelets and therefore to lower values for platelet-bound catecholamines. The efficiency of the washing procedure, however, has been clearly demonstrated.

The uptake of adrenaline by platelets, though small in relation to the amount of adrenaline added, increased the concentration of platelet-bound adrenaline about threefold. The effect is thus comparable with that observed by Hardisty & Stacey (1955) for serotonin. Born & Hornykiewicz (1957), using still higher concentrations of adrenaline (1 μg./ml.), observed uptake of adrenaline by pig platelets, proportional to the amount of adenosine triphosphate in the platelets.

It has been pointed out above that the concentration gradient [adrenaline in platelets]/[adrenaline in plasma] is about 125. Since [adrenaline in platelets] was increased about threefold, [adrenaline in plasma] would have to be increased by a factor about 400 if the uptake had been due only to diffusion. The concentrations actually used were well below this margin.

Uptake of adrenaline by platelets in vivo

Concentrations of adrenaline such as those found to lead to adrenaline uptake *in vitro* might be reached or exceeded in blood of the suprarenal vein *in vivo*. A procedure which was previously found to lead to an increase of adrenaline in platelet-rich plasma, i.e. convulsion treatment (Weil-Malherbe, 1955), was chosen to study the possible changes of the platelet-bound fraction. Blood samples were obtained from patients undergoing convulsion treatment in a mental hospital. Convulsions were produced by the intravenous injection of Leptazol (5-pentamethylenetetrazol; about 15 mg./kg. body wt.) in four cases and by an electric discharge across the cranium in six cases. One blood sample was withdrawn before the administration of the convulsant agent, a second one immediately after the convulsion and a third one 15 min. later. The results are shown in Table 9. Immediately after the convulsion the adrenaline concentration in platelet-poor plasma rose in all cases and the mean increase was significant. Although there was an increase of the noradrenaline concentration in four cases, the mean increase of noradrenaline was not significant. The adrenaline and noradrenaline contents of the platelets, on the other hand, were significantly decreased in half of the cases, and the mean changes were also significant. This decrease was probably due to an increased adhesiveness of the platelets, since after the convulsion the platelet concentration in plasma was usually decreased, whereas there was

no corresponding change of the platelet count in blood. In these tests relative platelet concentrations were determined in samples of platelet-rich plasma by a turbidity method (Cruz, 1954) and figures for the catecholamine content of absolute numbers of platelets are not available, but this aspect will be investigated further.

Fifteen minutes after the convulsion the 'plasma' fraction of adrenaline was still significantly increased. Other increases were not statistically significant. There was thus no evidence for an uptake of catecholamines *in vivo* by platelets under these conditions, and it appears that the increase of adrenaline concentration previously observed is confined to the fraction present in platelet-poor plasma.

The concentration of catecholamines in bovine plasma obtained from the slaughterhouse is higher than in human plasma; this has been attributed to a discharge of catecholamines at the time of blood collection (Weil-Malherbe & Bone, 1957*b*). The proportion of platelet-bound catecholamines in bovine plasma was therefore studied in a small series of experiments (Table 10). The sum of free plus platelet-bound fractions is almost identical with the mean concentrations of adrenaline and noradrenaline previously found in unfractionated ox plasma (Weil-Malherbe & Bone, 1957*b*). The platelet-bound proportion is a little lower than in human plasma, but the difference is not significant. On the other hand, the content per unit number of platelets is about five times higher than in human plasma. The adrenaline content of pig platelets is higher still, according to Born & Hornykiewicz (1957). Whether this difference between human platelets on the one hand and bovine or pig platelets on the other is due to uptake of pre-mortally discharged catecholamines by the animal platelets or to species differences, remains to be investigated. Considerable species differences are known to exist in histamine and serotonin contents of platelets (Humphrey & Jaques, 1954).

DISCUSSION

The partial association of circulating catecholamines with blood platelets brings to mind the fact that tissue catecholamines are also partly bound to particles. This has been demonstrated for the adrenal medulla (Hillarp & Nilson, 1954; Blaschko, Hagen & Welch, 1955), phaeochromocytoma (Burger & Langemann, 1956), spleen and sympathetic nerves (Euler & Hillarp, 1956) and brain (Weil-Malherbe & Bone, 1957*c*). It seems natural to assume that there is an inherent functional purpose in this arrangement which may serve to prevent the reaction of catecholamines with receptor groups and inactivating enzymes. The particle-bound fraction may thus be conceived as an operational reserve from which active amines are released as required. Although this concept is well founded for the particles of adrenal medulla,

Table 9. *Effect of convulsion treatment on the concentration of catecholamines in plasma and platelets*

Sample 1: blood was withdrawn before treatment; sample 2: blood was withdrawn immediately after convulsion; sample 3: blood was withdrawn 15 min. after convulsion. Heparin was used as anticoagulant. Values are means ± S.E.M. from ten cases. For details see text. A = Adrenaline; N = noradrenaline.

Sample no.	Plasma A (μg./l. of plasma)	Plasma N (μg./l. of plasma)	Platelets A (μg./l. of plasma)	Platelets N (μg./l. of plasma)	10^{-3} × Platelet count/μl. of whole blood	$E_{620m\mu}$ of platelet-rich plasma (1:20)
1	1·13 ± 0·15	3·79 ± 0·36	1·32 ± 0·16	4·53 ± 0·47	227 ± 29	0·176 ± 0·028
2	2·15 ± 0·41	4·98 ± 0·99	1·08 ± 0·15	3·93 ± 0·54	250 ± 32	0·116 ± 0·025
3	1·55 ± 0·25	4·19 ± 0·53	1·43 ± 0·14	4·74 ± 0·45	279 ± 36	0·173 ± 0·025
	Percentage change after convulsion (value before convulsion = 100)					
2	191·2 ± 28·9*	131·8 ± 29·9	82·0 ± 5·5*	86·8 ± 5·4†	110·1 ± 9·3	66·0 ± 12·2†
3	136·9 ± 15·2†	110·5 ± 15·1	108·4 ± 8·9	104·6 ± 11·8	122·7 ± 6·0*	98·4 ± 8·6

* Significantly different from 100 ($P \leqq 0·01$). † Significantly different from 100 ($P < 0·05 > 0·01$).

Table 10. *Catecholamine distribution in bovine plasma*

Blood was collected at the slaughterhouse with heparin as anticoagulant. Values are means of four experiments ± S.E.M.

	Plasma (μg./l.)	Platelets (μg./l. of platelet-rich plasma)	μmg./10^9 platelets*	Percentage in platelets of total adrenaline and noradrenaline
Adrenaline	4·0 ± 0·72	1·65 ± 0·27	9·8	30·8 ± 6·9
Noradrenaline	8·62 ± 2·19	6·15 ± 1·51	33·5	43·1 ± 11·9

* Means of two experiments.

its applicability to blood platelets remains to be established.

It seems that catecholamines are less easily detached from platelets than is serotonin, as they do not pass into solution during clotting and do so only partly after lysis by freezing or exposure to a surface-active agent. Also, any changes in the platelets which may be produced by contact with glass do not substantially affect the retention of catecholamines; this, at least, is found when the platelets are suspended in a medium containing EDTA.

The unfavourable effect of EDTA on the stability of platelet-bound catecholamines *in vitro* requires some comment. The underlying reaction is probably an oxidation, since the destruction is smaller in nitrogen. It is well known that EDTA activates iron-catalysed autoxidations. Green, Mazur & Shorr (1956) have shown that EDTA accelerates about tenfold the oxidation of adrenaline by ferritin or Fe^{3+} ions at pH 7. Citrate has no effect on this reaction and oxalate inhibits it. The same order of activity was observed in our experiments on the effects of these anticoagulants on the stability of platelet-bound catecholamines. It seems likely therefore that EDTA acts by activating an iron-catalysed oxidation. Whether the catalyst is ferritin is doubtful since ferritin has not yet been demonstrated in blood except in certain pathological conditions (Granick, 1951). The catalyst probably occurs at a higher concentration in platelets where the effect is stronger than in plasma.

It might be concluded that the use of EDTA as anticoagulant in adrenaline estimations is inadvisable and that it ought to be replaced by heparin, citrate or oxalate. However, none of these equals EDTA in its ability to prevent adhesion and clumping of platelets (Dillard, Brecher & Cronkite, 1951). Moreover, the instability of catecholamines in platelets suspended in EDTA (Table 4) was observed in experiments at 37° and lasting for 2 hr. The yield of platelets is therefore highest with EDTA; slight losses of platelet catecholamines during processing cannot be ruled out, but they can be kept to a minimum by avoiding delay and by cooling of the sample.

Leach & Heath (1956) suggested that adrenaline is oxidized in plasma by coeruloplasmin, a copper-containing protein. In their experiments adrenaline was added to plasma *in vitro* at a final concentration about 10^6 times the physiological concentration. We have added diethyldithiocarbamate or phenylthiourea, both powerful inhibitors of catalysis by copper, to heparinized platelet-rich plasma and found that they did not affect the stability of preformed catecholamines in either platelets or plasma. We conclude therefore that there is no significant copper-catalysed oxidation under these conditions. It is probable that normal protective mechanisms are swamped when unphysiologically high concentrations are used and the physiological significance of the observations of Leach & Heath is therefore doubtful.

SUMMARY

1. The distribution of adrenaline and noradrenaline between plasma and blood platelets has been studied by a fluorimetric method.

2. In 116 samples of human plasma the platelets contained, on the average, a little over 50 % of the total catecholamines present in platelet-rich plasma. The platelet-bound proportion of adrenaline correlated with the platelet-bound proportion of noradrenaline. The mean amounts contained in 10^9 platelets were 2 μmg. of adrenaline and 7 μmg. of noradrenaline. It was calculated that the concentration of catecholamines is about 125 times higher in platelets than in plasma.

3. The distribution of catecholamines between platelets and plasma was unaffected by contact with uncoated glass surfaces. Some anticoagulants decreased the yield of platelets in platelet-rich plasma by causing clumping and adhesion. Highest platelet yields were obtained with ethylenediaminetetra-acetate.

4. No catecholamines passed into serum from platelets during clotting, whether spontaneous or induced. Lysis of platelets by freezing and thawing or by treatment with a surface-active agent resulted in a partial release. Extraction with butanol was practically quantitative.

5. Ethylenediaminetetra-acetate increased the disappearance in oxygen of platelet-bound catecholamines *in vitro*.

6. No uptake of adrenaline by platelets was observed in heparinized platelet-rich plasma at an adrenaline concentration of about 10 μg./l. An uptake resulting in a final concentration of platelet-bound adrenaline of about three times the initial concentration was found in citrated platelet-rich plasma at an adrenaline concentration of 80–200 μg./l.

7. In confirmation of earlier results an increase of adrenaline was found in platelet-rich plasma after convulsion treatment. This increase was confined to the 'free' fraction.

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Spectral Methods of Characterizing Anthocyanins

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Because of the close relationship that exists between anthocyanins and genetic factors controlling flower colour in higher plants (Lawrence, 1950), considerable efforts have been made to devise rapid and simple methods of characterizing this group of glycosidic pigments. Existing methods, e.g. the well-known colour and distribution tests (Robinson & Robinson, 1932), although of value, provide only limited information about the glycosidic nature of the anthocyanins. More recently paper-partition chromatography has been widely used as an alternative to the older tests (Bate-Smith, 1948, 1949; Hayashi & Abe, 1956), and it provides an excellent method of obtaining pure pigments on a small scale. A method of identifying the sugars of anthocyanins with this technique has recently been described (Harborne & Sherratt, 1957*a*, *b*). In a study of the genetic control of anthocyanin production in a number of different plants (Harborne, 1958*a*), the absorption spectra of a large number of chromatographically pure pigments were measured. As a result, new ways in which spectral measurement can be used for characterizing anthocyanins became apparent.

Although the spectra of many anthocyanins have been examined in the past (Schou, 1927; Hayashi, 1936; Robinson, 1954), little attempt was made to apply these data to the characterization of these pigments. More recently, spectral measurements have been used for identifying anthocyanidins, mainly in connexion with studies of plant leucoanthocyanins (Bate-Smith, 1954; Roux, 1957*a*, *b*). In addition, the effect of aluminium chloride on the spectra of anthocyanins has been described as distinguishing pigments containing *o*-dihydroxyl groups (Geissman, Jorgensen & Harborne, 1953; Geissman & Jurd, 1955).

In the present work complete spectral data are given for all those anthocyanidins which occur naturally as glycosides, as distinct from those which are obtained by treatment of leucoanthocyanins with acid. A method of distinguishing the two main classes of anthocyanin, the 3- and the 3:5-glycosides, is described. Some anthocyanins occur naturally in association with hydroxy aromatic acids in acylated form and the nature and amount of acyl components have now been determined by spectral means.

MATERIALS AND METHODS

Sources of pigments

Anthocyanidins. Small crystalline samples of authentic delphinidin, cyanidin, petunidin, malvidin and hirsutidin were available. Larger amounts of these pigments were prepared from suitable plant material. Pelargonidin was