

## CONTENT AND SUBCELLULAR LOCALIZATION OF CATECHOLAMINES AND 5-HYDROXYTRYPTAMINE IN HUMAN AND ANIMAL BLOOD PLATELETS: MONOAMINE DISTRIBUTION BETWEEN PLATELETS AND PLASMA

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1 The content of adrenaline (Ad), noradrenaline (NA) and dopamine was measured in human, guinea-pig, cat, rabbit and rat blood platelets by a highly sensitive and specific radioenzymatic method.

2 In all platelet specimens analyzed, the content of the three catecholamines (CA) was several thousand times lower than that of 5-hydroxytryptamine (5-HT).

3 In basal conditions, the NA concentration in platelets and plasma always exceeded that of Ad and dopamine.

4 In rat and rabbit platelets, Ad, NA and dopamine were present only in the free (unconjugated) form.

5 Platelets of rats with storage pool deficiency (Fawn-hooded) contained much less 5-HT and CA than normal rat platelets.

6 Following restraint stress, platelets of Fawn-hooded rats, in contrast to normal rat platelets, did not accumulate CA in spite of a dramatic rise in plasma CA.

7 Reserpine, a monoamine depletor, released CA as well as 5-HT from rabbit platelets *in vivo*.

8 Subcellular fractionation experiments with rabbit platelets indicate that both CA and 5-HT are most concentrated in the fraction consisting of pure 5-HT organelles.

9 Both in humans and rabbits the concentration gradient between platelets and plasma was much lower for CA than for 5-HT, indicating that a high affinity transport mechanism operates *in vivo* for 5-HT but not for CA.

10 In conclusion, the present data show that both human and animal platelets contain Ad, NA and dopamine. The bulk of the CA seems to be stored as unconjugated amines together with 5-HT, histamine and *p*-octopamine in a multitransmitter storage site, namely the 5-HT organelle.

### Introduction

Blood 5-hydroxytryptamine (5-HT, serotonin) and catecholamines (CA) are more concentrated in blood platelets than in other circulating blood cells. Biochemical and ultrastructural studies of platelets of rabbit (Tranzer, Da Prada & Pletscher, 1966; Da Prada, Pletscher, Tranzer & Knuchel, 1967), guinea-pig (Da Prada, Pletscher & Tranzer, 1971), rat and man (Da Prada, Tranzer & Pletscher, 1972) have revealed that platelets possess specific subcellular storage sites for 5-HT and histamine, the 5-HT organelles (Da Prada & Pletscher, 1968; Pletscher,

Da Prada, Berneis & Tranzer, 1971). Indirect experimental evidence indicates that 5-HT organelles might also be the storage sites for platelet CA (Born, Hornykiewicz & Stafford, 1958; Weissbach, Bogdanski & Udenfriend, 1958; Weil-Malherbe & Bone, 1958; Hughes & Brodie, 1959; Born & Smith, 1970; Boullin & O'Brien, 1970). However, the very low amounts of CA present in the platelets and the consequent methodological difficulties of their measurement account for the lack of data regarding content and localization of platelet adrenaline (Ad), noradrenaline (NA) and dopamine. In the present study we took advantage of the high sensitivity and specificity of a recently developed radioenzymatic method (Da Prada

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& Zürcher, 1976) for measuring the content of Ad, NA and dopamine in platelets and plasma of humans and different animal species including Fawn-hooded rats, rodents with a storage pool deficiency (SPD) (Tschopp & Weiss, 1975). In addition in all these platelet and plasma specimens, parallel measurements of the 5-HT have been carried out.

The content and subcellular distribution of endogenous Ad, NA, dopamine and 5-HT was also determined in different subcellular organelles isolated from rabbit platelet homogenates submitted to density gradient centrifugation.

## Methods

### *Blood collection and isolation of platelets*

Human blood samples were obtained from healthy volunteers (aged 20 to 35 years) by cubital venipuncture. Animals (rabbits, cats, guinea-pigs) were exsanguinated under light ether anaesthesia, and blood collected through a polyethylene cannula inserted in a carotid artery. In some experiments rabbits were injected with reserpine (5 mg/kg i.p.) 16 h before exsanguination. Blood was also drawn from the abdominal aorta of normal (Wistar) and SPD (Fawn-hooded) rats, exposed in some experiments to restraint stress (Bonfils, 1964) for 1 h before halothane anaesthesia and blood sampling. Disodiumethylenediaminetetraacetate (EDTA) 1% w/v in physiological saline (10% of the blood volume) was used as anticoagulant.

Platelet-rich plasma (PRP) was obtained by low-speed centrifugation (120 g, 15 min) at room temperature. The platelets were separated from plasma by a higher-speed centrifugation (2700 g, 15 min) at 6°C and washed twice with modified ice-cold Tyrode buffer (Da Prada, Bartholini & Pletscher, 1965).

### *Measurement of catecholamines in platelets*

CA were extracted from washed platelets by lysis with  $\frac{1}{2}$  volume of 0.3 N perchloric acid. In most instances the platelet sediments derived from 2.5 ml PRP were therefore lysed in 0.5 ml of perchloric acid. After careful mixing, the denatured platelet proteins were separated by centrifugation from the perchloric acid extract, which contained more than 95% of the platelet CA. Ad, NA and dopamine were determined radioenzymatically in 100  $\mu$ l of the platelet extract (Da Prada & Zürcher, 1976).

### *Measurement of conjugated catecholamines in platelets*

Estimations of total CA were made on rat and rabbit platelet extracts submitted to acid hydrolysis (0.3 N perchloric acid at 100°C for 20 min) (Hoeldtke &

Sloan, 1970). The amount of the conjugated CA was calculated by subtraction of the pre-hydrolysis values from the total (post-hydrolysis) CA values.

### *Determination of 5-hydroxytryptamine in platelets*

Platelet 5-HT was determined fluorimetrically (Bogdanski, Pletscher, Brodie & Udenfriend, 1956) on separate aliquots of washed platelets.

### *Catecholamine assay in platelet-free plasma*

Blood was obtained in the absence of significant physical or psychic stress through an acutely inserted venous catheter in human volunteers and rabbits and through chronic indwelling jugular catheters in rats, as previously described (Bühler, Da Prada, Haefely & Picotti, 1978). Each blood sample was collected into a plastic syringe containing heparin (Liquemin, Roche, 10 NIH units/ml) and plasma-free platelets were obtained by sedimenting the blood cells using a Beckman Microfuge (Bühler *et al.*, 1978). Ad, NA and dopamine were determined radioenzymatically in 100  $\mu$ l of deproteinized plasma (Da Prada & Zürcher, 1976).

### *5-Hydroxytryptamine assay in platelet-free plasma*

Following centrifugation of PRP (2700 g, 15 min) 5-HT was measured by a highly sensitive radioenzymatic method (Saavedra, Brownstein & Axelrod, 1973) in aliquots of the supernatant plasma devoid of platelets.

### *Platelet counting*

Platelets were counted in diluted aliquots of PRP by phase contrast microscopy.

### *Measurement of catecholamines and 5-hydroxytryptamine in subcellular organelles of rabbit platelets*

The subcellular distribution of CA and 5-HT was studied in rabbit platelet homogenates subjected to density gradient centrifugation as previously described (Da Prada & Pletscher, 1968; Da Prada, von Berlepsch & Pletscher, 1972). Briefly, the platelets were sonicated, precentrifuged and then the platelet homogenate was ultracentrifuged in a continuous density gradient of Urografin (Schering). After ultracentrifugation, the density gradient above the sediment of pure 5-HT organelles was divided from the top to the bottom of the tube into two aliquots of 20 ml and five aliquots of 10 ml each.

The 7 fractions were then diluted with 0.9 w/v % NaCl solution (1:3 v/v) and subjected to an additional ultracentrifugation ( $1.31 \times 10^5$  g, 30 min).

The sedimented pellets, as well as the isolated 5-HT organelles (fraction 8) were re-suspended and assayed radioenzymatically for CA (Da Prada & Zürcher, 1976) and 5-HT (Saavedra *et al.*, 1973).

**Protein determination**

Proteins were measured colorimetrically (Lowry, Rosebrough, Farr & Randall, 1951) in platelets and in the particulate matter of the density gradient fractions.

**Statistical evaluation**

The data were analyzed statistically by the Kolmogorov-Smirnov test (two tailed) (Siegel, 1956).

**Drugs and animals**

Chemical used and their respective sources were: reserpine (Serpasil, Ciba-Geigy, Switzerland), Urografin (Schering, Germany). All other reagents used were Analytical Grade and obtained from commercial sources.

Inbred albino SPF rats of Wistar origin, Fawn-hooded rats (derived from a colony kept at the Roosevelt Hospital, New York), Swiss hare rabbits, cats and guinea-pigs of the Füllinsdorf strain were used.

**Results**

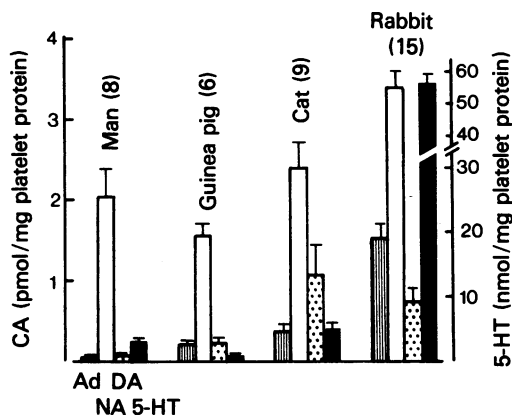
*Content of catecholamines and 5-hydroxytryptamine in human and animal platelets*

As shown in Figures 1 and 2, Ad, NA and dopamine were consistently detected in human and animal (cat, rabbit, guinea-pig and rat) blood platelets. The content of the three CA together was less than 0.1% of the 5-HT content, the highest absolute concentrations of both 5-HT and CA being found in the rabbit platelets.

In all platelet samples analyzed, including cattle and pig platelets (results not shown), the content of NA was higher than that of both Ad and dopamine. In human platelets, which had the lowest content of Ad and dopamine ( $0.05 \pm 0.01$  and  $0.08 \pm 0.01$  pmol/mg protein, respectively), the level of NA exceeded that of Ad and dopamine by a factor of about 20 fold and 30 fold, respectively.

*Content of catecholamines and 5-hydroxytryptamine in platelets of Wistar and Fawn-hooded rats under normal and stress conditions*

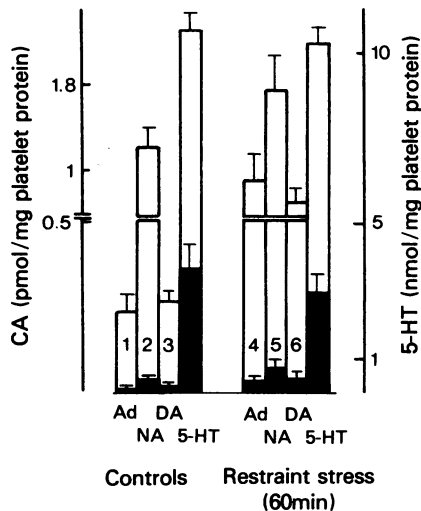
Figure 2 shows that platelets from Fawn-hooded rats contained extremely small amounts of CA and 5-HT



**Figure 1** Content of adrenaline (Ad), noradrenaline (NA), dopamine (DA) and 5-hydroxytryptamine (5-HT) in blood platelets of man and some animal species. The values are means; vertical lines show s.e. mean. The numbers of donors and animals used are indicated in parentheses.

as compared with platelets of Wistar SPF rats. Moreover, storage deficient platelets, in contrast to normal platelets, were unable to increase their abnormally low CA content even in conditions of high plasma CA concentrations (Table 1).

Wistar as well as Fawn-hooded rats exposed to stress showed a similar increase in the plasma CA



**Figure 2** Content of adrenaline (Ad), noradrenaline (NA), dopamine (DA) and 5-hydroxytryptamine (5-HT) in blood platelets of SPF (Wistar, open columns) and Fawn-hooded (solid columns) rats: control vs 1 h restraint stress values. Results are mean values for 6 animals; vertical lines show s.e. mean. Statistical significance: 1 vs 4; 2 vs 5:  $P < 0.01$ ; 3 vs 6:  $P > 0.05$ .

**Table 1** Effect of restraint stress (5 min) on the plasma adrenaline (Ad), noradrenaline (NA) and dopamine (DA) concentrations

Rat strain	Ad	NA (pg/ml plasma)	DA
SPF Wistar			
Freely moving	134 ± 86	535 ± 54	89 ± 24
Restrained	2968 ± 447*	3228 ± 290*	293 ± 33*
Fawn-hooded			
Freely moving	171 ± 5	485 ± 92	81 ± 38
Restrained	3708 ± 544*	2926 ± 218*	241 ± 67*

Blood was collected from chronic catheterized rats before and after stress. Results are mean values ± s.e. mean for 3 animals. Statistical significance: \* restrained different from respective freely moving,  $P < 0.01$

concentration (Table 1); however, only in Wistar rats was the elevation in plasma CA content accompanied by a significant rise in platelet Ad and NA concentration.

#### *Catecholamines and 5-hydroxytryptamine in the subcellular structures isolated from rabbit platelets*

The localization of Ad, NA and dopamine in rabbit platelet homogenates was assessed after isolation of the subcellular structures of the platelets by ultracentrifugation on Urografin density gradients. 5-HT, taken as a biochemical marker of the 5-HT organelles, as well as Ad, NA and dopamine were measured in the particulate matter sedimented from the 7 fractions of the density gradient and in the pellet consisting of pure 5-HT organelles (fraction 8).

Typical patterns of the subcellular distribution of these four monoamines are shown in Figure 3. The relative specific concentrations (% amine/% protein per fraction) (De Duve, Pressman, Gianetto, Wattiaux & Appelmans, 1955) for Ad, NA, dopamine and 5-HT were by far the highest in the 5-HT organelle fraction. The content of these monoamines was up to several hundred times higher in the 5-HT organelle fraction than in any other one.

#### *Content of catecholamine and 5-hydroxytryptamine in isolated 5-HT organelles versus whole platelets of rabbits*

The amounts of Ad, NA, dopamine and 5-HT found in the 5-HT organelles as well as in the intact rabbit platelets are illustrated in Table 2. In isolated 5-HT organelles and in platelets the absolute values of the three CA together were about 10,000 times

lower than that of 5-HT. However, the concentration ratio between 5-HT organelles and whole platelets did not markedly differ for CA and 5-HT, the isolated 5-HT organelles being about 300 fold more enriched in monoamines than the intact platelets (Table 2).

#### *Effect of reserpine on the content of 5-hydroxytryptamine and catecholamines of rabbit platelets and 5-HT organelles*

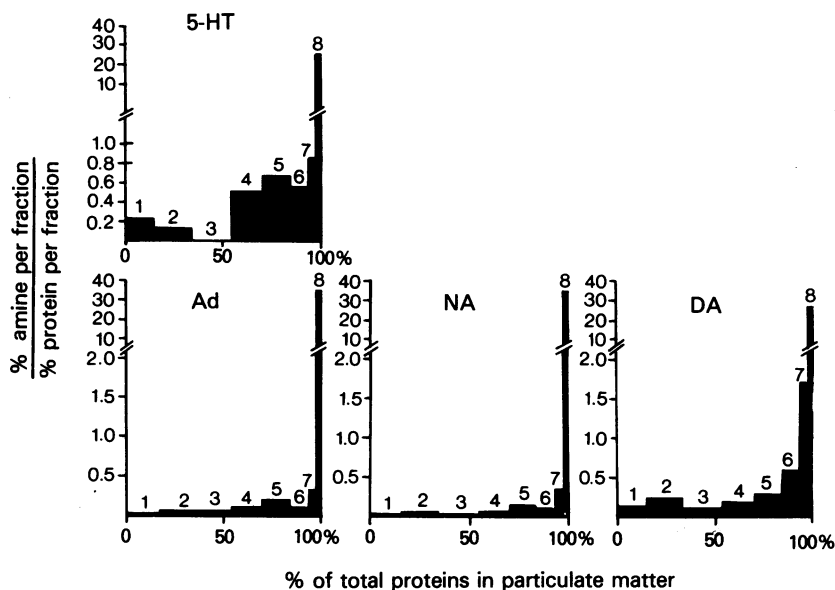
Reserpine pretreatment (5 mg/kg i.p., 16 h before exsanguination) decreased the level of CA and 5-HT in intact platelets as well as in isolated 5-HT organelles to between 1 and 15% of the pre-drug level (data not shown).

#### *Distribution of catecholamines and 5-hydroxytryptamine between platelets and plasma of humans and rabbits*

The concentration-ratio between platelets and platelet-free plasma was calculated in samples from humans and rabbits. In each sample CA as well as 5-HT were measured both in isolated platelets as well as in platelet-free plasma. The data presented in Tables 3 and 4 show the concentration-ratios platelet/plasma for 5-HT, Ad, NA and dopamine for man and rabbit, respectively.

In man, as well as in the rabbit, 5-HT was concentrated about 25,000 times more in platelets than in plasma. On the other hand, the CA were concentrated, at best, 800 times more in platelets than in plasma.

In man the concentration-ratio platelet/plasma was about 8 times higher for NA than for Ad and dopamine (Table 3) whereas in rabbit (Table 4) the Ad



**Figure 3** Subcellular distribution of adrenaline (Ad), noradrenaline (NA), dopamine (DA) and 5-hydroxytryptamine (5-HT) in the particulate matter of blood platelets of rabbits. Fraction number 8 consists of pure 5-HT organelles.

**Table 2** Content of monoamines in isolated 5-hydroxytryptamine (5-HT) organelles and intact blood platelets of rabbit

Constituent	5-HT organelles (nmol/mg protein)	Platelets (pmol/mg protein)	Ratio: organelles/ platelets
5-HT	21,000 ± 1,700	55,900 ± 3,200	376
Adrenaline	0.481 ± 0.021	1.540 ± 0.170	312
Noradrenaline	0.863 ± 0.060	3.400 ± 0.200	254
Dopamine	0.192 ± 0.010	0.740 ± 0.160	259

Results are mean values ± s.e. mean for at least 4 animals.

**Table 3** Distribution of 5-hydroxytryptamine (5-HT), adrenaline, noradrenaline and dopamine between blood platelets and plasma in man

Amine	Platelets <sup>1</sup> (pmol/ml)	Plasma (pmol/ml)	Ratio: platelets/ plasma
5-HT	972,000 ± 84,000	38 ± 9.7	25579
Adrenaline	22.8 ± 4.7	0.30 ± 0.05	74
Noradrenaline	740 ± 176	1.17 ± 0.11	631
Dopamine	34 ± 7.5	0.41 ± 0.07	83

Results are mean values ± s.e. mean for 5 volunteers.

<sup>1</sup>The volume (about 2 µl) of the platelets contained in 1 ml of human PRP was calculated assuming that a single platelet has a volume of 7 µm<sup>3</sup> (Paulus, 1975). One ml PRP contained 2.86 ± 0.16 × 10<sup>8</sup> platelets.

**Table 4** Distribution of 5-hydroxytryptamine (5-HT), adrenaline, noradrenaline and dopamine between blood platelets and plasma in rabbits

Amine	Platelets <sup>1</sup> (pmol/ml)	Plasma (pmol/ml)	Ratio: platelets/ plasma
5-HT	26,832,000 ± 1,540,000	971 ± 83	27,633
Adrenaline	739 ± 82	0.91 ± 0.21	815
Noradrenaline	1,632 ± 96	2.32 ± 0.20	703
Dopamine	355 ± 77	1.41 ± 0.15	251

Results are mean values ± s.e. mean for 6 animals.

<sup>1</sup>The volume (approx. 2.5 µl) of the platelets contained in 1 ml of rabbit PRP was calculated assuming that a single platelet has a volume of 5 µm<sup>3</sup> (Baumgartner, personal communication). One ml PRP contained 5 × 10<sup>8</sup> platelets.

and NA values were about 3 times more elevated than for dopamine.

## Discussion

In the present study the limits of sensitivity of the fluorimetric methods in measuring the low concentrations of plasma and platelet CA as well as of plasma 5-HT have been overcome by the use of highly sensitive and specific radioenzymatic assays. In particular, the use of a radiometric method which allows the simultaneous determination of fmol concentrations of CA has permitted, for the first time, a reliable measurement of the concentration of dopamine in platelets.

### Catecholamines in platelets

The platelet CA are likely to be of plasma origin since it is known that platelets lack CA synthesizing enzymes (Lahovaara, Paasonen & Airaksinen, 1968; Solomon, Spirt & Abrams, 1970; Boullin & Green, 1972).

The present results show that all three CA detectable in plasma are also present in platelets. Moreover, NA, which in normal conditions occurs in human and animal plasma in higher amounts than Ad and dopamine (Bühler *et al.*, 1978), is also the CA which is most concentrated in the platelets. In a few early studies, the assay of CA concentrations in blood platelets was hampered by the low sensitivity (detection limit about 5 ng) and poor specificity (insufficient discrimination between the fluorescence spectra of Ad and NA) of the fluorimetric methods (Häggendal, 1966). The platelet CA content has been reported for Ad (Born *et al.*, 1958), for Ad plus NA (Weil-Malherbe & Bone, 1958) and for total CA (Markwardt,

1967). A comparison of our data with those of the two pioneer studies dealing with CA content in platelets of man and cattle (Weil-Malherbe & Bone, 1957; 1958) as well as that reporting concentrations in pig platelets (Born & Hornykiewicz, 1957; Born *et al.* 1958), reveals that our CA values are much lower. For instance, if the Ad and NA concentration reported for man by Weil-Malherbe & Bone (1958) are converted to pmol/mg platelet protein (assuming that 10<sup>9</sup> platelets correspond to 3 mg protein) their data are about 50 and 4 times higher, respectively, than those found in the present study. For bovine platelets, too, the Ad and NA contents reported by the same authors are about 90 to 70 times higher respectively than our values (Da Prada, unpublished data). The inadequacy of the fluorimetric methods for measuring CA in blood is further supported by the fact that the same authors (Weil-Malherbe & Bone, 1957) reported values for Ad and NA in human and bovine plasma which are at least 20 times higher than those obtained by improved radioenzymatic assays (Callingham, 1975; Da Prada & Zürcher, 1976). More recent experiments with improved fluorimetric techniques (Markwardt, 1967) have given values for human and rabbit platelet CA (about 8 and 50 pmol/mg protein, respectively) which were only 5 to 7 times higher than those found in the present study (1.86 and 7 pmol/mg protein respectively).

### Catecholamines and 5-hydroxytryptamine in platelets of different animal species

The present data indicate that no correlation exists between CA and 5-HT levels in blood platelets of various species. While rabbit platelets exhibit the highest amounts of both 5-HT and CA, rat, cat, guinea-pig and human platelets, contain much less 5-HT but similar amounts of CA (see Figure 1).

*Catecholamines and 5-hydroxytryptamine in SPD rat platelets*

Results from different laboratories have shown that blood platelets of rats with SPD have a low content of 5-HT and adenosine 5'-triphosphate (ATP) (Holmsen & Weiss, 1970; Tschopp & Weiss, 1975) and that these abnormal platelets when seen with the electron microscope appear to contain only a few atypical 5-HT organelles (Da Prada, Pieri, Keller, Pieri & Bonetti, 1978; Da Prada, Richards & Lorez, 1978). In addition, previous data from our laboratory have shown that platelets from humans with SPD (Lorez, Richards, Da Prada, Picotti, Pareti, Capitanio & Mannucci, 1978) and from Fawn-hooded rats have a low storage capacity not only for 5-HT but also for mepacrine, a fluorescent probe which selectively accumulates in the 5-HT organelles (Lorez, Da Prada, Rendu & Pletscher, 1977). As previously reported for human SPD platelets (Lorez *et al.*, 1978) our findings indicate that Fawn-hooded platelets, also, store much less CA than normal rat platelets.

The fact that platelets of rats with SPD have an impaired storage mechanism for CA was further supported by exposing normal and Fawn-hooded rats to restraint stress, a procedure which dramatically increases the concentration of Ad and NA in plasma (Bühler *et al.*, 1978).

In contrast to 5-HT, the CA exhibit a relatively low affinity for the platelet 5-HT carrier mechanism; therefore, it is generally believed that *in vivo* they cross the platelet membrane mainly by passive, non-saturable transfer, even if at very high, unphysiological extracellular concentrations CA can be actively transported via the 5-HT carrier (Born & Smith, 1970; Gordon & Olverman, 1978; Pletscher, Laubscher, Graf & Saner, 1978). Thus it is likely that platelets accumulate substantial amounts of CA only in conditions of sustained and long-lasting high CA concentration in plasma. Our data show that prolonged restraint stress results in a large increment of the levels of CA in the platelets of normal rats, but not of Fawn-hooded animals. These findings indicate that, under *in vivo* conditions of long-lasting elevation of the CA concentration in plasma, there is a progressive increase in the CA level in the circulating platelets. Apparently, however, the process which allows uptake and accumulation of CA from the plasma operates only in normal platelets with unimpaired storage mechanisms. It was previously shown that plasma CA concentrations immediately reflect the state of sympathoadrenal activity (Bühler *et al.*, 1978). In contrast, the increase of the platelet CA level is delayed, obviously because of the low affinity of the CA for the platelet transport system.

Moreover, once accumulated and stored in the circulating platelets, the CA remain elevated even when

the plasma CA concentrations return to normal values.

Platelets from a patient with pheochromocytoma, a tumour secreting CA in paroxysmal bursts, contained high amounts of CA both in plasma and platelets. A few hours after surgical removal of the tumour (surrenalectomy) the high plasma CA concentrations became normal whereas the platelet CA levels attained normal values only after several days (Picotti, unpublished data). Thus it can be anticipated that the platelet CA concentration will provide a reliable and useful index for the assessment of previous paroxysmal and/or persistent CA discharges in the blood stream in pheochromocytoma and neuroblastoma tumours.

*Subcellular localization of catecholamines and 5-hydroxytryptamine in platelets*

Previous findings provided indirect evidence that CA might be accumulated together with 5-HT and ATP in the 5-HT organelles of the platelets. For instance, it was found that labelled CA injected into rabbits, were highly concentrated in a platelet fraction rich in 5-HT organelles (Da Prada & Pletscher, 1969b). In addition it has been shown that isolated platelets take up CA *in vitro* (Born *et al.*, 1958; Weissbach *et al.*, 1958; Weil-Malherbe & Bone, 1958; Born & Smith, 1970; Boullin & O'Brien, 1970) and that 5-HT organelles isolated from rabbit platelets are also able to concentrate labeled CA *in vitro* (Da Prada & Pletscher, 1969a). Born & Smith (1970) have demonstrated that reserpine, which reduces 5-HT uptake by acting at the level of the 5-HT granular membrane (Pletscher, Burkard & Tranzer, 1967; Da Prada & Pletscher, 1969b), also impairs Ad uptake in intact platelets.

Moreover, thrombin, which is known to release platelet 5-HT, ATP and calcium (typical constituents stored in the 5-HT organelles) was also able to release labelled Ad from platelets preloaded *in vitro* with this tritiated amine (Born & Smith, 1970). The present data obtained by measuring radioenzymatically the concentration of endogenous Ad, NA, dopamine and 5-HT in the subcellular structures isolated by subjecting rabbit platelet homogenates to density gradient centrifugation, directly demonstrate that 5-HT organelles are also the storage sites of the platelet CA. In fact 5-HT as well as Ad, NA and dopamine reached their higher concentrations in the fraction of the gradient which consisted of pure 5-HT organelles.

*Effect of reserpine in vivo*

The experiments with reserpine further support the notion that 5-HT, Ad, NA and dopamine are stored together in the 5-HT organelles. Reserpine which *in*

*vivo* releases 5-HT and CA from various tissues (Pletscher, Shore & Brodie, 1955; Carlsson, Rosengren, Bertler & Nilsson, 1957), when injected into rabbits, induced a pronounced depletion of platelet 5-HT and CA. Accordingly, 5-HT organelles isolated from reserpine-treated rabbit platelets contained only 5 to 10% of the 5-HT, Ad, NA and dopamine found in the 5-HT organelles isolated from normal rabbit platelets.

#### *Catecholamine conjugation*

Previous findings indicated that human platelets incubated with labelled Ad conjugate part of the Ad taken up (Born & Smith, 1970). However, in our experimental conditions both in rat and rabbit platelets, the CA concentration of platelet extracts did not further increase after acid hydrolysis. These findings, in contrast to those reported for human platelets (Born & Smith, 1970) indicate that, at least in rat and rabbit, platelet-bound CA are stored in the platelets essentially in an unconjugated form.

#### *Catecholamines and 5-hydroxytryptamine distribution between platelets and plasma*

At least four factors determine the distribution of Ad NA and dopamine between platelets and plasma, namely, the plasma CA concentrations, the transport process(es) at the cytoplasmic membrane, the storage mechanism(s) operating intracellularly at the 5-HT organelles and the metabolic CA degradation by monoamine oxidase and catechol-*O*-methyltransferase (Pletscher, 1968).

The circulating platelet is surrounded by a plasma environment which has been recently demonstrated to be richer in NA than in Ad and dopamine (Bühler *et al.*, 1978). This explains why the content of the three CA in the platelets *in vivo* reflect their relative concentration in the plasma, in spite of the higher affinity *in vitro* of dopamine than of NA for both platelets and isolated 5-HT organelles (Da Prada & Pletscher, 1969a).

A constant high level of plasma NA is available for diffusion into the platelets and for the subsequent storage in the 5-HT organelles. Recent *in vitro* findings with rat platelets show that 5-HT and dopamine compete for the same active transport mechanism. However, 5-HT has a much higher affinity than dopamine for this transport process (Gordon & Olverman, 1978). *In vivo* this competition is likely to occur since both in human and rabbit the concentrations measured in plasma were much higher for 5-HT than for dopamine (see Tables 3 and 4).

The relatively low concentration of Ad found in the platelets is likely to be accounted for by the fact that plasma Ad concentrations are elevated only occasionally and for short periods of time during stress.

According to our data, the concentration-ratio between platelet and plasma CA in man and rabbit attains, at best, a value of about 800 which is at least 30 times lower than that for 5-HT. These data clearly show that *in vivo* at physiological extracellular concentration the platelets have a much more efficient active transport mechanism for 5-HT than for CA.

Due to the unavoidable rupture of platelets occurring during the preparation of the plasma, it is possible that the real concentrations gradient for 5-HT between platelet and plasma is even more pronounced than actually determined.

With regard to the 5-HT concentration in human platelet-free plasma, the present value (38 pmol/ml plasma) is somewhat lower than that obtained by Crawford (1965) using fluorimetric methods (74 pmol/ml plasma).

#### *Multitransmitter storage mechanism*

The presence of an intact storage mechanism is an essential prerequisite for the formation and maintenance of a concentration gradient between platelets and plasma. Accordingly, an impaired storage process, as occurs in reserpine-treated and SPD platelets prevents monoamine accumulation.

The 5-HT organelles of the platelets which accumulate 5-HT, CA, histamine and octopamine (Da Prada, unpublished results) behave as a multitransmitter storage site resembling that operating in the sympathetic nerves of the pineal gland (Jaim-Etcheverry & Zieher, 1971; 1975). Previous equilibrium dialysis experiments provided evidence that 5-HT and histamine as well as CA interact with ATP (Berneis, Da Prada & Pletscher, 1974; Da Prada, Obrist & Pletscher, 1975).

The physico-chemical dynamic interaction between ATP, bivalent cations and monoamines in the interior of the 5-HT organelle is likely to represent the basic mechanism which permits the storage of various aromatic amines in the 5-HT organelles (Pletscher & Da Prada, 1975). It is likely that these storage sites create a concentration gradient between platelets and plasma even for CA, which are mainly transported into the platelets by diffusion.

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