# PROFILLING OF PLATELET POLYAMINES BY A NOVEL HPLC XTRACTION TECHNIQUE

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## ABSTRACT

*Purified Human Blood Platelets were incubated in the presence of Tris-buffer, 6-diamino-hexane at 37$^0$C for 5minutes. Polyamines were subsequently extracted by sonification and pH adjusted to 4 with 1M KOH. Extracted polyamines were derivatised with dansylchloride and separated by high performance liquid chromatography (Hplc) at a flow rate of 1ml min using 70% phosphate buffer and 30% acetonitrile in methanol. The most abundant polyamines extracted were Spermidine with a mean value of 0.190 ± 0.03nmol mg protein, followed by Putrescine (0.153 ± 0.02nmol mg protein), Spermine (0.12 ± 0.02nmol mg protein) and finally Cadeverine (0.100 ± 0.03 nmol mg protein). For the first time we have been able to establish a novel approach to the extraction and separation of important biogenic polyamines in human blood platelets using high performance liquid chromatography without subjecting them to hydrolysis or any rigorous chemical modification prior to their analysis. We suggest that the presence and extent of these polyamines in purified resting platelets may be important in further understanding of platelet function and thus further help us to understand disease processes in which platelets has long been implicated.*

**Keywords:** *Polyamine, Spermidine, Putrescine, Spermine, Cadaverine*

## INTRODUCTION

The polyamines, putrescine, spermidine, spermine and cadaverine represent a group of naturally occurring compounds exerting a bewildering number of biological effects[1-3], yet despite several decades of intensive research work, their exact physiological function(s) still remain obscure. Early studies employing crude biological preparations have implicated their connection with cell growth[2] Other studies have also shown that, the levels of polyamines increase in blood and urine of cancer patients[4]. Polyamines have also been shown to act as glucocorticoid mediators of the synthesis of the postulated vascular permeability inhibitory protein that does not inhibit phospholipase $A_2$ activities[5]. However, no direct studies have been made on purified healthy human blood platelet relating to their ployamine content and function. Prompt by these observations and knowing the imminent role of platelets in the aetiology of diseases such as atherosclerosis and thrombosis, we investigated the cellular components and contents of purified human blood platelets polyamines and attempted to postulate their probable physiological properties and function in platelets. We report here the Hplc profilling of purified human blood platelets and suggest their possible role.

## MATERIALS AND METHODS

Whole screened pack ACD (acid citrate dextrose) anticoagulated blood was obtained from the North London Blood Transfusion Centre, UK. This was aliquoted into 50ml polystyrene tubes and centrifuged at 1000 rpm for 15 minutes to obtain platelet-rich plasma (PRP). Platelets were sedimented by centrifuging at 20,000

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rpm at 4°C for half an hour and washed twice with Tris-buffered saline pH 7.4. To 5 ml portions of washed platelets was added 100 ul of 0.1 mg/ml, 6-diamino-hexane (polyamine internal standard) and incubated at 37°C for 5 minutes. The tubes were sonicated for 2 minutes and centrifuged for 10 minutes at 13,000 rpm. Supernatants from each vial were collected individually and freeze-dried overnight. The resulting powdered samples were redissolved in up to 200ul of 0.1M HCL and subsequently derivatized (dansyl derivatization provided sufficient fluorescent detection of the individual polyamines). This was accomplished by mixing 100 ul of extracted sample, 200 ul of saturated $Na_2CO_3$ and 200 ul of dansyl chloride reagent (1 mg/ml in acetone). The mixtures were vortexed, sealed and incubated at 65°C in a preheated oven for 10 minutes. The derivatized samples were kept in an aluminium foil ready for Hplc analysis.

The Hplc analysis was carried out by injecting 20 ul (3x) of sample into a Spherisorb Silicon SW10 (4 x 250 mm) column (from Phase Sep, Deeside, UK) maintained at a flow rate of 1 ml/min. with a gradient of 70% mobile phase A (10 mm phosphate buffer) and 30% mobile phase B (185 ml acetonitrile and 15 ml methanol) gradually increasing the mobile phase A to 90% over a total run time of 50 minutes. The sensitivity was set at 0.02 scale using a fluorescence detector.
All other reagents including polyamine standards were obtained from Sigma (UK) and were of high analytical grade.

## RESULTS

Figures 1a and 1b depict the Hplc profiles of standard and platelet polyamines respectively. The dansyl derivatization provided sufficient fluorescent detection of the individual polyamines as shown in the chromatograms. The recovery of the polyamines was estimated from the recovery values of the known amount of internal standard added. The mean concentrations of ten such analysis expressed in nmol/mg protein are represented in Table 1. It could be seen from the data presented that the most abundant polyamine extracted from the purified resting platelets was Spermidine accounting for approximately 33.74 % of the total polyamines extracted. This was followed by Putrecine which accounted for 27.10% of the total extracted polyamine content in the platelets. Spermine and Cadaverine the other two occurring ployamines accounted for 21.30 % and 17.76 % respectively. A similar trend has been demonstrated in erythrocytes from crude biological preparations[1].

## DISCUSSION

Apart from the presence of large amount of other biogenic amines such as serotonin (5-hydroxy tryptamine), purified human blood platelets have also been shown in this study to contain polyamines namely, Spermidine, Putrescine, Spermine and Cadaverine[3]. It is not clear at this stage to ascertain whether the trend of polyamine distribution in the purified resting human platelets was a reflection of platelets age. Changes in polyamine content due to age of erythrocytes has been reported[6] Thus, the polyamine content as observed in this study may be important in understanding platelet cell ageing and therefore help in the characterisation of polyamine function in human platelets. In the light of the evidence that the preponderance of red cell constituents (including polyamines) lost during ageing are membrane associated[6], the results from the platelets in this study may reflect membrane-associated polyamines lost during fragmentation of platelets from the megakaryocytes. A further study in this area is recommended. Even though the exact mechanism which might be responsible for such process was not addressed in this study, it is quite likely that such changes in the megakaryocytes maturation could influence the polyamine inter-conversion system possibly via conjugation, degradation or cellular compartmentation and as a consequence result in an altered platelet survival[7]. This will have an important clinical significance since it has been reported that platelets from patients with hypercholesterolemia have shortened survival rates[8]. Polyamines have also been shown to be highly associated with phospholipid and fatty acid content, and membrane stabilisation of rat erythrocytes[9-10] It is interesting in the light of this to note

that increased plasma cholesterol levels have been associated with an increased sympathetic nervous system reactivity, as indicated by an elevated norepinephrine levels in plasma and in platelets of patients with hypercholesterolemia[11] This increase may in turn contribute to the platelet hyperaggregabvility observed in this metabolic disorder

## CONCLUSION

The work has thus proved that by combining fluorescence dansylation and Hplc tecniques, biogenic amine could successfully be extracted from intact body tissues/cells without subjecting them to hydrolysis or any vigorous chemical modification prior to their analysis. From this work also, we have been able to show the trend of content of the individual polyamines that occur in intact human blood platelets. Although the exact mechanism of synthesis and release of these biogenic amines was not the subject of this study, we suggest that the content of these polyamines in platelets may play a very important role probably as an indicator of the presence of a young and defective platelet population in the work-up of undiagnosed abnormality in the blood. The actual mechanism underlying their release and cellular compartmentation in disease processes in which platelets have been implicated need to be further elucidated

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Table 1: Polyamine content of intact human blood platelets
Concentrations are expressed in nml/mg protein

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| **Putrescine** | **0.153 ± 0.02** |
| **Cadeverine** | **0.100 ± 0.03** |
| **Spermidine** | **0.190 ± 0.03** |
| **Spermine** | **0.120 ± 0.02** |

* Values represent the mean ± SD of three Hplc analyasis