

PLATELETS

AND

ASCORBIC ACID

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INTRODUCTION

The importance of the role played by platelets in haemostasis and thrombosis has been well established. Intensive investigations are now being carried out by many workers to elucidate further the mechanisms by which platelets take part in these processes. Since it is likely that a complete understanding of the role of platelets will be at the molecular level many of these studies have been devoted to the investigation of the biochemistry of these elements. Since the early 1950's the development of methods for the separation of platelets from whole blood coupled with the development of micro-chemical techniques suitable for application to small amounts of biological sample has allowed the estimation of a large number of platelet constituents.

One substance which exists in a high concentration in platelets is ascorbic acid. Until recently the only report of the ascorbic acid content of platelets was by Barkhan and Howard (1958) who estimated that the ascorbic acid concentration in platelets was some twenty times as high as that in the plasma. Wilson et alii (1967) have now reported values which are some three hundred times those stated by Barkhan and Howard.

Taken with the finding of a high concentration of ascorbic acid in platelets the severe purpura which occurs in scurvy

suggests that ascorbic acid may be important in platelet metabolism. That platelet function is in fact abnormal in scurvy has been shown recently by studies demonstrating an impairment in the ability of platelets from humans and guinea pigs suffering from scurvy to stick to a glass surface (Born and Wright, 1967; Wilson et alii, 1967).

The essential role which ascorbic acid plays in tissue metabolism is emphasised by the fact that animals which cannot synthesise their own ascorbic acid will die of scurvy if deprived of vitamin C in the diet. Yet, despite an enormous amount of work devoted to this subject, the nature of this role remains unknown. Ascorbic acid does not appear to act as a specific cofactor in enzyme reactions as do the other vitamins, and though its function may be related to its action as a redox potential buffer, this remains to be proven. Since it is likely that the function of ascorbic acid in platelets is either related to or the same as its function in other cells, studies of its function in platelets could eventually lead to a better understanding of its function in other tissues. Because platelets are readily isolated from whole blood and are easily handled in vitro they provide a convenient model for the study of ascorbic acid metabolism.

An essential tool in the study of ascorbic acid is a method for its estimation in biological samples. A review of the

literature revealed that methods commonly used have the disadvantage that they are not specific for ascorbic acid. Those methods which depend on a measurement of the reducing power of the sample also measure other reducing substances present; methods which depend on the formation of the 2,4-dinitrophenylhydrazone derivative of dehydroascorbic acid are subject to interference from other substances which form hydrazone derivatives. It is surprising that, in view of the obvious importance of ascorbic acid in metabolic processes, better methods have not been devised.

A property of ascorbic acid which lends itself to further investigation is its ability to enhance the absorption of iron from the intestine (Moore et alii, 1939). It is usually claimed that this is due to the reduction of ferric ions to ferrous ions (Hahn et alii, 1945); ferric ions, unlike ferrous ions, are insoluble at the alkaline pH in the duodenal lumen from which site iron is absorbed. This does not explain, however, the fact that ascorbic acid also enhances the absorption of ferrous ions (Greenberg et alii, 1957). It has been shown that iron can also be maintained in solution in the duodenum by the formation of metal-chelate complexes between iron and ascorbic acid (Charley et alii, 1963; Davis and Deller, 1967), and that substances which can complex iron in this way can alter the rate of iron absorption (Stitt et alii, 1962; Davis and Deller, 1967). If complex

formation occurs when ascorbic acid is added to iron, this could explain its effect on iron absorption.

Because of the importance of platelets in haemostasis and thrombosis and the importance of ascorbic acid in metabolic processes, the investigations described in this thesis were devoted to the study of some aspects of the ascorbic acid content of platelets. The main objects of this work were:

1. To develop a more specific method for the estimation of ascorbic acid.
2. To determine the ascorbic acid content of normal platelets and to compare these results with the values previously reported.
3. To study the effect of variation in the dietary intake of ascorbic acid on the platelet ascorbic acid content.
4. To determine whether the ascorbic acid content of platelets differs from normal in diseases in which the platelets are thought to be affected.
5. To study the mechanism by which platelets maintain a higher concentration of ascorbic acid than in the surrounding plasma.

6. To explore the possibility of a relationship between ascorbic acid and adenosine diphosphate induced platelet aggregation.
7. To decide whether ascorbic acid or dehydroascorbic acid will form a stable complex with iron.