ERYTHROPHAGOCYTOSIS

ITS RELATION TO AGGLUTINATION AND HAEMOLYSIS, PARTICULARLY IN ACQUIRED HAEMOLYTIC ANAEMIA

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CONTENTS.

The regulations of the University of Adelaide for the degree of Doctor of Medicine require:

(1) A declaration that the thesis is the writer's own composition. This declaration may be found on page 6.

(2) An indication of where the writer considers the thesis to advance medical knowledge or practice. This subject is contained in the Conclusion on page 102.

CONTENTS

CONTENTS

CONTENTS

PART I - INTRODUCTION.

An Outline of the Study Undertaken.

The phenomenon of erythrophagocytosis, as the name implies, refers to the ingestion of red blood corpuscles by living cells endowed with this ability. It is known that the spleen is abundantly supplied with phagocytic cells of the reticulo-endothelial system, but how great a part is played by erythrophagocytosis in the destruction of aged normal red cells is unknown. Exactly what changes occur in erythrocytes as they approach the end of their normal life span of 120 days, and what is the actual mode of their destruction, are problems which still remain unsolved.

In disease, however, certain observations have supplied evidence of the existence of agents in the circulating blood which directly or indirectly lead to red cell destruction. The agents or antibodies with which this thesis deals, exist in several forms which may be classified according to their effects on erythrocytes. Thus, there are agglutinins, haemolysins and incomplete or sensitising antibodies. The last group, although producing no directly detectable effects, sensitise erythrocytes to agglutination by antiglobulin sera.

In the past, evidence has been produced that an alteration of the surface of erythrocytes initiated by the action of antibodies is also one factor responsible for erythrophagocytosis, and the present thesis is concerned with this erythrocyte change, the types of antibodies which will produce it and the mechanism of the process of ingestion. It is particularly concerned with erythrophagocytosis in relation to the antibodies of acquired haemolytic anaemia. Because a comparison has been made between this and the two related phenomena of agglutination and haemolysis, a historical survey has been traced of all three in as far as they are

concerned in this disease. A summary is presented of those properties of the antibodies of acquired haemolytic anaemia which were known at the time when the experimental work embodied in the text was completed. Any account of the clinical aspects of the patients whose sera have been examined is beyond the scope of this work, but it might be mentioned here that they were classified by Dacie and de Gruchy (1951) into three main clinico-pathological syndromes: Group I, idiopathic acquired haemolytic anaemia; Group II, haemolytic anaemia following virus pneumonia; and Group III, chronic haemolytic anaemia with haemoglobinuria and Raynaud's phenomena. Serum from patients suffering from haemolytic anaemia of the Donath-Landsteiner type (paroxysmal cold haemoglobinuria) were also examined. The four clinico-pathological groups overlap the three main serological groups into which the antibodies that can be demonstrated in the sera of these patients, may be placed. An analogy can be drawn between these classifications and those of anaemia: (1) according to the more common clinical concept with regard to the aetiological factors and (2) according to the morphological and other characteristics of the erythrocytes that are involved.

Moreover, the work is confined to <u>in vitro</u> studies and is not greatly concerned with the numerous reports of erythrophagocytosis <u>in vivo</u>. It commences with the development of a technique whereby the agglutination, haemolysis and erythrophagocytosis which were to be studied, could be examined in material from the same tubes, and therefore under identical conditions. The results of titration experiments could then be strictly correlated. Supravital observations on erythrophagocytosis have been included and the mechanism of erythrophagocytosis discussed.

The method for the combined study of these three phenomena was

then applied to the investigation of normal sera and to the antibodies of acquired haemolytic anaemia. An unusual antibody combination has been described.

Similar investigations were extended to the following antibodies and antibody systems:

(1) The blood group specific antibody, anti-A. (2) The Rh iso-antibodies, anti-D and enti-e. (3) The naturally occurring and immune anti-human red cell antibodies of normal and immunised rabbits. (4) The reversible agglutinin of trypsinised erythrocytes.

Lastly, some other haemolytic systems were investigated in which haemolysis was not induced by antibodies. For these purposes it was produced by: (1) The tanning of erythrocytes, (2) The action of hypotonic saline solutions, (3) The action of normal serum, under the influence of a lowered pH, on the erythrocytes of a patient suffering from paroxysmal nocturnal haemoglobinuria.

In every instance it was determined whether erythrophagocytosis could be produced in vitro, what conditions of pH and which cell types were necessary for its demonstration, and what was the rôle that complement assumed in the reaction.

The Reasons for the Study.

During the early researches in immuno-haematology, there was a close correlation in the development and understanding of the basic mechanisms of agglutination, haemolysis and erythrophagocytosis. In recent years, however, a great deal of knowledge has accumulated on the antibodies of acquired haemolytic anaemia regarding their ability to produce agglutination, haemolysis and sensitisation to antiglobulin serum,

yet the subject of erythrophagocytosis had been relatively uninvestigated.

While on overseas study leave from the Institute of Medical and Veterinary Science, I spent some nine months at the Postgraduate Medical School of London working under Dr. J. V. Dacie in the Department of Haematology. It is largely due to the work of Dr. Dacie that the antibody patterns in acquired haemolytic anaemia have been so adequately described, and it was at his suggestion that Dr. Lawrence I. Schwartz, from Mt. Sinai Hospital, New York, and I commenced the study of erythrophagocytosis in respect of these antibodies.

When this work was almost completed and before it was accepted for publication, a somewhat similar paper appeared in the literature in which different methods were employed, but in which most of our work on the specific blood group systems was described. These authors (Jordan, Prouty, Heinle and Dingle; 1952) also touched on the antibodies of acquired haemolytic anaemia, but their work overlapped the present study only in respect of the antibody of paroxysmal cold haemoglobinuria (or the Donath-Landsteiner type of haemolysin).

At the time when the experiments embodied in this thesis were carried out, they represented an attempt to study a then partially uninvestigated aspect of the properties of many types of iso- and auto-antibodies, and a comparison was made between the related phenomena of agglutination, haemolysis and erythrophagocytosis.

Acknowledgements.

Over the last three years Dr. Dacie has collected a large number of pathological sera taken from patients suffering from acquired haemolytic anaemia, and I am deeply indebted to him for making this material available. Indeed, I believe that a similarly complete study

has not been carried out simply because other investigators have lacked access to such a full collection of sera and to so many appropriate patients. I am also grateful to him for allowing me to work in his department where Dr. Schwartz and I received his continual advice and encouragement, and where I had the opportunity to learn his methods and to become familiar with his work. He also encouraged me to publish this work in the form of a thesis.

I am grateful to Miss Marie Cutbush and Dr. P. L. Mollison, of the Blood Transfusion Research Unit of the Medical Research Council, who kindly supplied many of the anti-A and anti-Rh sera.

I would like to sincerely thank Dr. L. I. Schwartz, in intimate association with whom most of the work embodied in this thesis was carried out. Because of their knowledge of German, both he and Dr. S. Klaar, of the Institute of Medical and Veterinary Science, have been of great assistance in the translation of many papers written in that language to which reference has been made in the text. Appended at the beginning of this section is either the original or a copy of a letter from Dr. Schwartz in which he has given his written consent to the use of our joint investigations as a basis for this thesis.

Publications.

By far the great majority of the work was performed in London and an account of the combined studies of Dr. Schwartz and myself has recently been accepted for publication in 'Blood'. Still more recently, I have separately submitted another paper which has been accepted for publication in the same journal. It contains the report of a case of chronic acquired haemolytic anaemia associated with haemoglobinuria and Raynaud's phenomena. This patient's serum contains an auto-antibody

of unusual serological characteristics. Full reference to these findings is made in the text. Should reprints of these papers be available when the thesis is completed, they will be appended at the end.

The remainder of the experimental material has been undertaken in Adelaide since my return from Europe and America. Whether performed in London or Adelaide, I wish to state that the work is entirely original and that I personally conducted or assisted Dr. Schwartz in the performance of every experiment. The actual composition of the thesis is entirely my own. In conclusion, at the end of Part VI, it is stated where the work has been thought to advance medical knowledge.