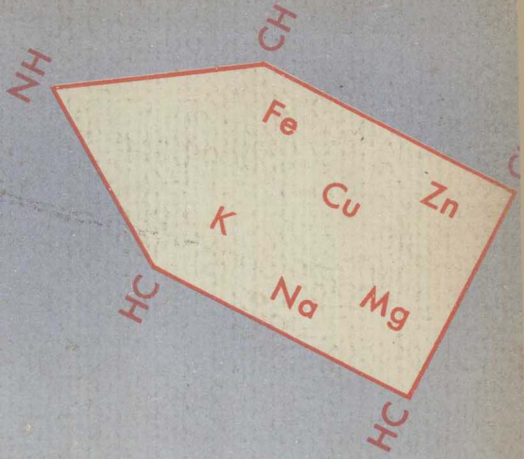
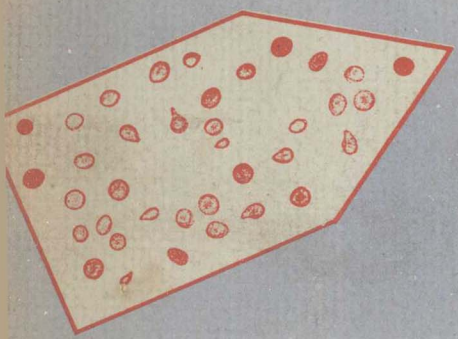
Chemistry of Erythrocytes

CLINICAL ASPECTS





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The PURPOSE of this book is ... to give clinically oriented guidance in the study of a subject which has long been a domain of physiologists but is now becoming of clinical importance.

This book might have been called THE CHEMISTRY OF RED BLOOD CELLS IN HEALTH AND DISEASE. It is written from the clinical point of view, and is intended primarily for medical students, physicians, clinical investigators and clinical pathologists.

Featuring the-
CLINICAL ASPECTS
ANEMIA • ALKALOSIS
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By

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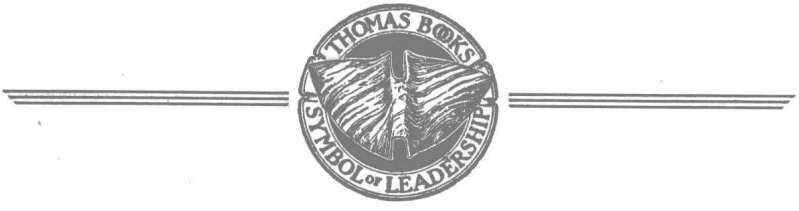

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Preface

The chemistry and functioning of the human erythrocyte, long the domain of physiologists, have recently become a matter of immediate concern to the clinician. The composition of the erythrocyte is assuming importance in the study of blood chemistry and there is now an extensive but widely scattered literature on the chemical constitution of erythrocytes and its relation to the composition of plasma in health and disease.

This volume represents an endeavor to give a clinically oriented account of the available knowledge in this rapidly developing field.

Neither in scope nor in depth does the discussion propose to compete with a biochemical treatise. The desirability of observing the boundaries commonly accepted for presentations of applied quantitative chemistry is recognized. I realize that the subject matter is not complete, but I have attempted to cover those more important facts and aspects which are likely to confront a steadily increasing number of physicians.

Vitamin and hormone substances have not been discussed in special sections, but were dealt with as the occasion arose. Methods of chemical analysis are not outlined in detail; their principles are described briefly, and appropriate references are given to the literature describing the technics.

The material constituting the substance of this book has been gathered through many years of active interest in the nature and pattern of intracellular blood constituents. The decision to gather my material into a monograph was spurred by the fact that so far no integrated description of the advances in erythrocyte chemistry has appeared.

The burden of my task was lessened by the ready encouragement and advice generously given by many colleagues and workers in the field. To all of them, I owe my sincere thanks. To Dr. S. Granick who read and criticized the entire manuscript, I am

particularly indebted and hereby acknowledge my debt with deep appreciation. Finally, my sincere thanks to Mrs. Natalie Friedheim for her editorial assistance.

New York, N. Y. H. BEHRENDT

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Structure, Chemistry, and Functional Organization

Architecture and Dimensions

Ever since Swammerdam, in 1658, and Leeuwenhoek, in 1673, first observed the existence of "orbicular particles" in the blood serum of frog and man, respectively, the shape of the human red blood cell has become known as globular in plane view and symmetrically biconcave in cross section. As Ponder (29a) sets forth so instructively, this form is maintained in the absence of any heterogeneous structure, i.e., of any material other than fluid. Physical forces of tension arising in the interior and on the surface of the erythrocyte were regarded for a long time as the cause of this peculiar configuration. During the last 25 years, however, a fundamental change has taken place in the approach to the problems of shape and structure. The essential factor in shaping cell structure in general, and the red cell's form in particular, has been recognized as a special arrangement of molecules. The architecture of the erythrocyte, therefore, is created not by a stroma of heterogeneous material but by an ultrastructure of molecules, and what distinguishes these molecular structures in physicochemical respect is the variability of their gelation.

Whether the specialized molecular arrangement exists only in the surface layers or also in the cell interior is so far an unsolved problem. Ponder suggests that "surface envelope and an internal structure are not mutually exclusive," and that the potential existence of both should be tentatively assumed. These ultrastructures, referred to as stroma, ghost, matrix material, and fixed framework, remain as residue when red cells are hemolyzed.

All the constituents of the erythrocyte are assembled within the

molecular frame of the surface structure, and possibly supported by a similar network in the interior. Water accounts for about 70 per cent of the erythrocytic volume, 25 per cent is occupied by hemoglobin, and only 5 per cent by all the remaining components (29b).

The average dimensions of a normal mature erythrocyte are indicated in Figure 1. The spatial relations between the hemoglobin molecules and one of the intracellular electrolytes are schematically demonstrated in a scale drawing by Ponder (Fig. 2). From these it may be seen that hemoglobin occupies by far the greatest portion of the interior, and that the small spaces left between and around the hemoglobin molecules contain all the other chemical constituents. Table 1 gives a number of figures to supplement the illustrations.

TABLE 1
MEASUREMENTS OF HUMAN ERYTHROCYTE (29b,e,f)

Surface ultrastructure, thickness	130.0 Å*
Hemoglobin molecule, radius	28.2 Å
Hydrated Na ion, radius	2.6 Å
Shell (atmosphere) surrounding hemoglobin molecule, thickness	10.0 Å
Cell, volume	87 μ^3
Cell, surface area	163 μ^2

* If hydration were allowed for, the value for thickness would be almost doubled.

Composition

Fixed Framework (Ghosts). The framework of the erythrocyte has its own characteristic chemical composition. First, there is a typical protein commonly referred to as stromatin, sometimes as fibrous or structural protein. It is very insoluble in water, forms gels easily, and is distinguished by the nature of its constituent amino acids. It differs from both keratins and collagens. Changes in the erythrocyte's shape, whether artefactual or spontaneous (spherocytes, sickle forms), are mediated by corresponding changes in the degree of stromatin gelation. Other protein substances identified in the framework include elinin (6), a-protein (33), and the "antisphering substance" (15)

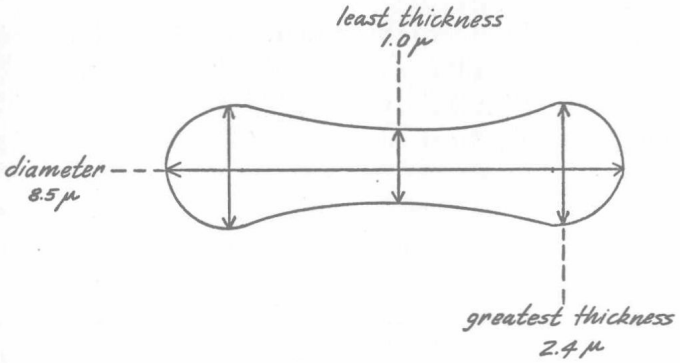

Fig. 1. Scale drawing of human erythrocyte in cross sectional view. From Ponder (29c).

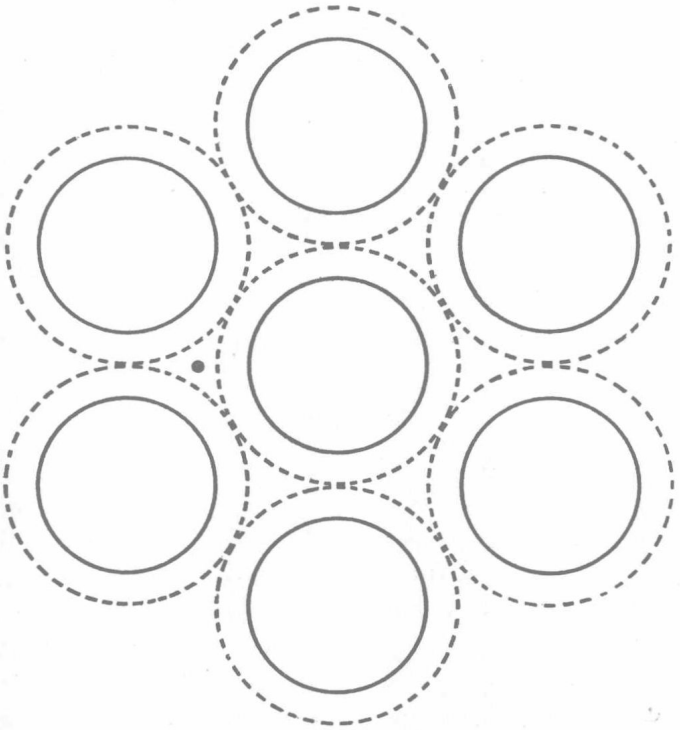

Fig. 2. Scale drawing showing proximity of hemoglobin molecules in concentrations in which they exist in erythrocyte's interior; small black object represents Na ion. From Ponder (29d).

A small amount of hemoglobin, about 2 per cent of the total cell content, is attached to the structural protein, held in chemical or physical combination (28,34). In this connection, two points are of practical importance: (1) the differentiation between hemoglobin protein and other proteins in the ghosts, and (2) the difficulty of detaching the hemoglobin from the ghosts.

TABLE 2

COMPOSITION OF RED CELL GHOST

Substance	*% of Total Residue*	*% of Total Lipid*	*% of Stromatin*	*% of Total N of Ghosts*
Hemoglobin	23			
Ash	5			
Protein	50			
Lipid (total)	11			
Phospholipid		65		
Free cholesterol		20		
Cholesterol ester		4		
Neutral fat		11		
Total nitrogen*			15.9	
Amino nitrogen*			11.6	
Histidin N				4.3
Arginin N				11.8
Lysin N				5.8
Tyrosin N				1.7
Cystin N				0.8
Methionin N				1.4
Tryptophan N				1.2
N not accounted for				27.0

According to Williams, Erickson, and Macy (35).
* From Ballantine (1).

Approximately 90 per cent of the total lipid content of the red cell must be assigned to the ghosts (29e); of the total dry residue of ghosts, lipids account for about 10 per cent (35).

As principle components of the surface structure the acidic lipids are held largely responsible for the well known fact that the isoelectric point of erythrocytes is shifted more or less to the acid side. According to recent measurements (12a) which may be considered as highly accurate with regard to technic and required corrections, suspended erythrocytes become isoelectric when the

pH of the suspending medium approaches 3.6. In contrast, hemoglobin is isoelectric at pH 6.9.

The figures on composition of ghosts vary considerably, depending on the analytic methods used. Furthermore, direct and indirect estimations often fail to agree. Normal figures, such as given in Table 2, are informative rather than standard values.

TABLE 3

COMPOSITION OF MATURE ERYTHROCYTES

A.

Constituents	*Gm./100 cc.**	10^{-12} *Gram/cell*	10^{-16} *Mole/cell*
Hemoglobin	34	29	5.7
Minerals	0.67	0.6	
Total lipids	0.48	0.43	
Non-Hb protein	0.87	0.78	0.05†
Glucose	0.83	0.075	3.7
Solids	35%		
Water	65%		

According to Ponder (29 g). * Added by the author. † Molecular weight taken as 200,000.

B.

Constituents	*mg./100 cc.*	*Source*
Urea	20	Houssay (22)
Creatine	2	Ponder (29g)
Creatinine	8	Ponder (29g)
Amino acids	30	Christensen (8)
Glutathione	70	Granick (19)
Adenine nucleotides	61	Granick (19)
Neutral fat	4	Erickson *et al.* (13)
Phospholipids	196	Erickson *et al.* (13)
Cholesterol	139	Brun (5)
Total reducing substances (as glucose)	114	Ponder (29g)
Glucose	74	Ponder (29g)
Phosphorus		
inorganic	2.5	Guest *et al.* (20)
organic acid-soluble	55	Guest *et al.* (20)
Chloride	186	Gram (18)
Bicarbonate	100	Ponder (29g)
Total nonprotein sulfur	2.9	Reed (31)
Sodium	37.5	Nichols (26)
Potassium	386	Lans (24)
Magnesium	4.8	Snyder *et al.* (32)