
Hemoglobin
●
Its Precursors
and
Metabolites

Edited by
F. W. Sunderman
and
F. W. Sunderman, Jr.

Hemoglobin

Its Precursors and Metabolites

Edited by

F. WILLIAM SUNDERMAN, M.D., Ph.D., Sc.D.

*Director, Division of Metabolic Research
and Clinical Professor of Medicine
Jefferson Medical College, Philadelphia, Pa.*

F. WILLIAM SUNDERMAN, JR., M.D.

*Associate in Medicine, Jefferson Medical College, Philadelphia;
Consultant in Clinical Pathology, Harrisburg Hospital, Harrisburg, Pa.,
City of Kingston Laboratory, Kingston, N Y., and
U.S. Naval Hospital, St. Albans, N. Y.*

Applied Seminar of the Association of Clinical Scientists



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F. WILLIAM SUNDERMAN, M.D., Ph.D.,
Sc.D.
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Pa.



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Jefferson Medical College, Philadelphia, Pa.
- FRANK TIETZE, Ph.D.
National Institute of Arthritis and Metabolic Diseases, NIH, Bethesda, Md.
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Jefferson Medical College, Philadelphia, Pa.
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Preface

This book contains the edited proceedings of an Applied Seminar on the Clinical Pathology of Hemoglobin, Its Precursors and Metabolites, held in Washington, D.C. under the auspices of the Association of Clinical Scientists. In organization and format this volume is similar to the published proceedings of three previous seminars—*Lipids and the Steroid Hormones in Clinical Medicine*; *Measurements of Exocrine and Endocrine Functions of the Pancreas*; and *Evaluation of Thyroid and Parathyroid Functions*.

The topic of the present seminar was selected to acquaint clinical scientists with the rapid advances in knowledge of hemoglobin metabolism within the past five years. In editing the proceedings for this seminar a judicious selection of material has been attempted. Although a number of the analytical procedures contained in this book are not currently being undertaken in most clinical laboratories, it is our opinion that many of them will assume an important role in future years for the diagnosis of disturbances of hemoglobin metabolism. For example, clinical recognition of the numerous hemoglobinopathies will depend in large measure upon the availability of specific laboratory procedures.

Our appreciation is expressed to the lecturers and assisting physicians who have generously contributed their time and energies to the success of the Applied Seminar and to the preparation of these proceedings. Our thanks are given to our publishers, and particularly to Mr. J. Brooks Stewart and Mr. Stanley A. Gillet for their gracious cooperation.

F. WILLIAM SUNDERMAN, M.D.

F. WILLIAM SUNDERMAN JR., M.D.

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The Chemical Structure of Hemoglobin

MAKIO MURAYAMA, Ph.D.

“Amino acids in chains
Are the cause, so the x-ray explains,
Of the stretching of the wool
And its strength when you pull,
And show why it shrinks when it rains.”

So wrote A. L. Patterson nearly 30 years ago. His name is intimately associated with a method of x-ray analysis of protein structure. Today the primary structure of a few proteins is known, including that of human hemoglobin. The primary structure refers to the amino acid sequence of the protein. The secondary structure refers to the alpha-helix, which was described by Linus Pauling in 1950. The tertiary structure is synonymous with the 3-dimensional structure; it is now being vigorously investigated by Perutz of Cambridge University, using the x-ray diffraction technic.

PRIMARY STRUCTURE

The amino acid sequence study of the normal adult hemoglobin was elaborated by the group of workers in Munich under the leadership of Braunitzer at the Max Plank Institute for Biochemistry.¹ The alpha chain was found to contain 142 amino acid residues; the amino terminal residues are Val. Leu . . . and the carboxyl terminal residues are Tyr. Arg. (Table 1 and Fig. 1). The beta chain contains 146 amino acid residues; the amino terminal

TABLE 1. AMINO ACID SEQUENCE OF
α CHAIN

Alanine	ALA	Leucine	LEU
Arginine	ARG	Lysine	LYS
Aspartic Acid	ASP	Methionine	MET
Asparagine	AspN	Phenylalanine	PHE
Cystein	CySH	Proline	PRO
Glutamic Acid	GLU	Serine	SER
Glutamine	GIN	Threonine	THR
Glycine	GLY	Tryptophan	TRY
Histidine	HIS	Tyrosine	TYR
Isoleucine	ILEU	Valine	VAL
A—			
1	VAL	7	ALA
2	LEU	8	LEU
3	SER	9	GLU
4	PRO	10	ARG
5	ALA	11	MET
6	ASP	12	PHE
7	LYS	13	LEU
8	THR	14	SER
9	AspN	15	PHE
10	VAL	C—	1 PRO
11	LYS		2 THR
12	ALA		3 THR
13	ALA		4 LYS
14	TRY	CE—	5 THR
15	GLY		6 TYR
16	LYS		7 PHE
17	VAL		1 PRO
18	GLY	AB—	2 HIS
19	ALA		3 PHE
AB—	1 ALA		4 ASP
	2 HIS		5 LEU
	3 ALA		6 SER
	1 GLY	B—	7 HIS
2	GLU		8 GLY
3	TYR		9 SER
4	GLY		10 ALA
5	ALA	E—	1 GLU
6	GLY		2 VAL

2 The Chemical Structure of Hemoglobin

3 LYS	5 AspN	6 LEU	3 VAL
4 GLY	6 ALA	7 LEU	4 HIS
5 HIS	7 LEU	8 SER	5 ALA
6 GLY	F— 1 SER	9 HIS	6 SER
7 LYS	2 ALA	10 CySH	7 LEU
8 LYS	3 LEU	11 LEU	8 ASP
9 VAL	4 SER	12 LEU	9 LYS
10 ALA	5 ASP	13 VAL	10 PHE
11 ASP	6 LEU	14 THR	11 LEU
12 ALA	F— 7 HIS-Fe	15 LEU	12 ALA
13 LEU	8 ALA	16 ALA	13 SER
14 THR	9 HIS	17 ALA	14 VAL
15 AspN	FG— 1 LYS	18 HIS	15 SER
16 ALA	2 LEU	GH— 1 LEU	16 THR
17 VAL	3 ARG	2 PRO	17 VAL
18 ALA	4 VAL	3 ALA	18 LEU
19 HIS	5 ASP	4 GLU	19 THR
20 VAL	G— 1 PRO	5 PHE	20 SER
EF— 1 ASP	2 VAL	6 THR	21 LYS
2 ASP	3 AspN	H— 1 PRO	22 TYR
3 MET	4 PHE	2 ALA	23 ARG
4 PRO	5 LYS		

ALPHA CHAIN contains 142 of the amino acid links in the massive hemoglobin molecule

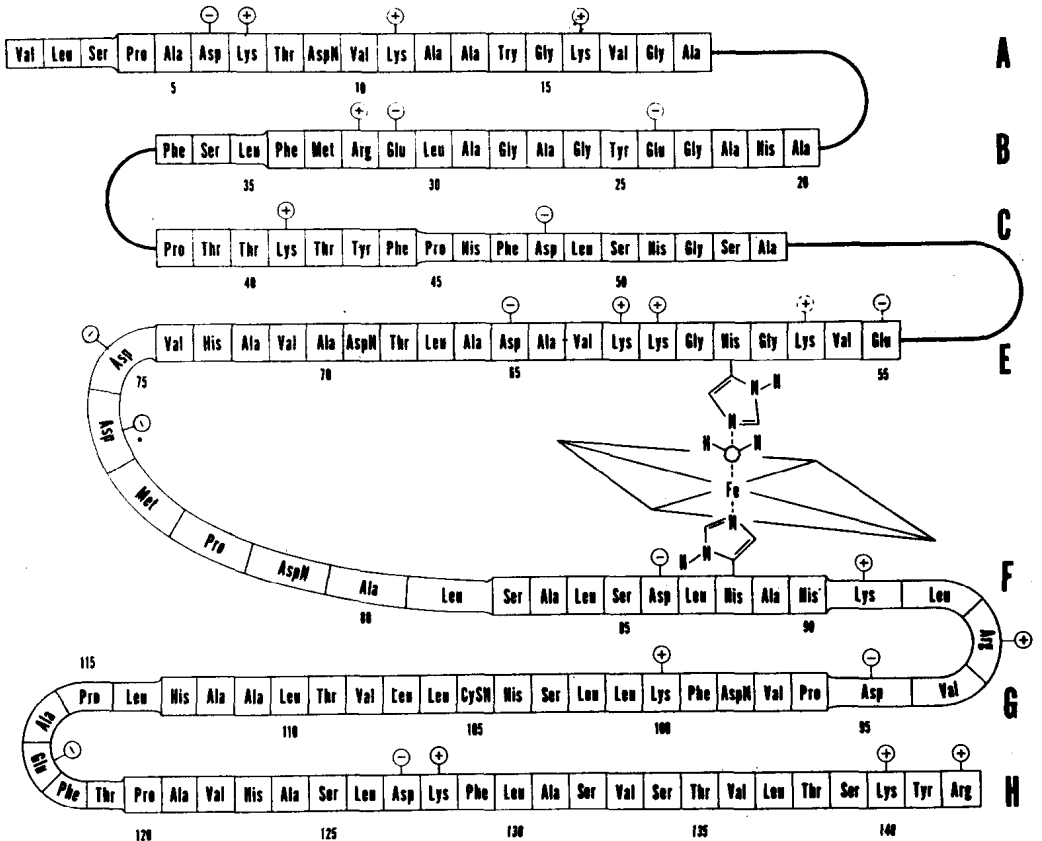


Fig. 1. The alpha chain of the normal human hemoglobin molecule.

residues are Val. His. Leu . . . and the carboxyl terminal residues are Tyr. His (Table 2 and Figs. 2 and 3). It is now possible to calculate the molecular weights of the alpha and the beta chains as well as the total molecular weight as follows:

MOLECULAR WEIGHT OF HUMAN HEMOGLOBIN AND ITS COMPONENTS
(Calculated from the chemical data)

	α Chain	β Chain	$\alpha_2\beta_2$	Hb-A
No Amino Acids	142	146	576	576
Mol. Wt.	15,228	15,870	62,196	64,660
			66-68,000*	

* From Ultracentrifuge Data

TABLE 2. AMINO ACID SEQUENCE OF
 β CHAIN

Alanine	ALA	Leucine	LEU
Arginine	ARG	Lysine	LYS
Aspartic Acid	ASP	Methionine	MET
Asparagine	AspN	Phenylalanine	PHE
Cystein	CySH	Proline	PRO
Glutamic Acid	GLU	Serine	SER
Glutamine	GIN	Threonine	THR
Glycine	GLY	Tryptophan	TRY
Histidine	HIS	Tyrosine	TYR
Isoleucine	ILEU	Valine	VAL

A— 1 VAL	17 LYS
2 HIS	18 VAL
3 LEU	19 AspN
4 THR	AB— 1 VAL
5 PRO	B— 1 ASP
6 GLU	2 GLU
7 GLU	3 VAL
8 LYS	4 GLY
9 SER	5 GLY
10 ALA	6 GLU
11 VAL	7 ALA
12 THR	8 LEU
13 ALA	9 GLY
14 LEU	10 ARG
15 TRY	11 LEU
16 GLY	12 LEU

13 VAL	5 GIN
14 VAL	6 LEU
15 TYR	F— 7 HIS-Fe
C— 1 PRO	8 CySH
2 TRY	9 ASP
3 THR	FG— 1 LYS
4 GIN	2 LEU
5 ARG	3 HIS
6 PHE	4 VAL
7 PHE	5 ASP
CD— 1 GLU	G— 1 PRO
2 SER	2 GIN
3 PHE	3 ASP
4 GLY	4 PHE
5 ASP	5 ARG
6 LEU	6 LEU
7 SER	7 LEU
8 THR	8 GLY
D— 1 PRO	9 AspN
2 ASP	10 VAL
3 ALA	11 LEU
4 VAL	12 VAL
5 MET	13 CySH
6 GLY	14 VAL
7 ASP	15 LEU
DE— 1 PRO	16 ALA
E— 1 LYS	17 HIS
2 VAL	18 HIS
3 LYS	GH— 1 PHE
4 ALA	2 GLY
5 HIS	3 LYS
6 GLY	4 GLU
7 LYS	5 PHE
8 LYS	6 THR
9 VAL	H— 1 PRO
10 LEU	2 PRO
11 GLY	3 VAL
12 ALA	4 GIN
13 PHE	5 ALA
14 SER	6 ALA
15 AspN	7 TYR
16 GLY	8 GIN
17 LEU	9 LYS
18 ALA	10 VAL
19 HIS	11 VAL
20 LEU	12 ALA
EF— 1 ASP	13 GLY
2 ASP	14 VAL
3 LEU	15 ALA
4 LYS	16 ASP
5 GLY	17 ALA
6 THR	18 LEU
7 PHE	19 ALA
F— 1 ALA	20 HIS
2 THR	21 LYS
3 LEU	22 TYR
4 SER	23 HIS

BETA CHAIN contains 146 of the amino acid links in the massive hemoglobin molecule

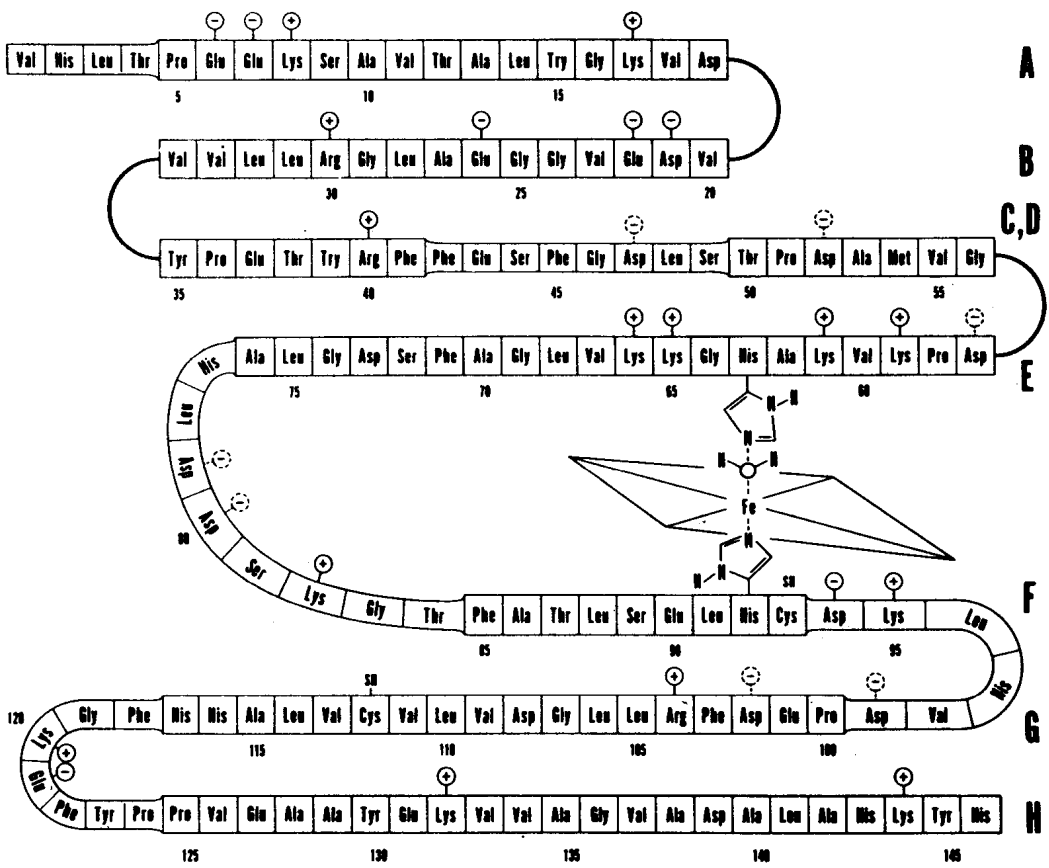
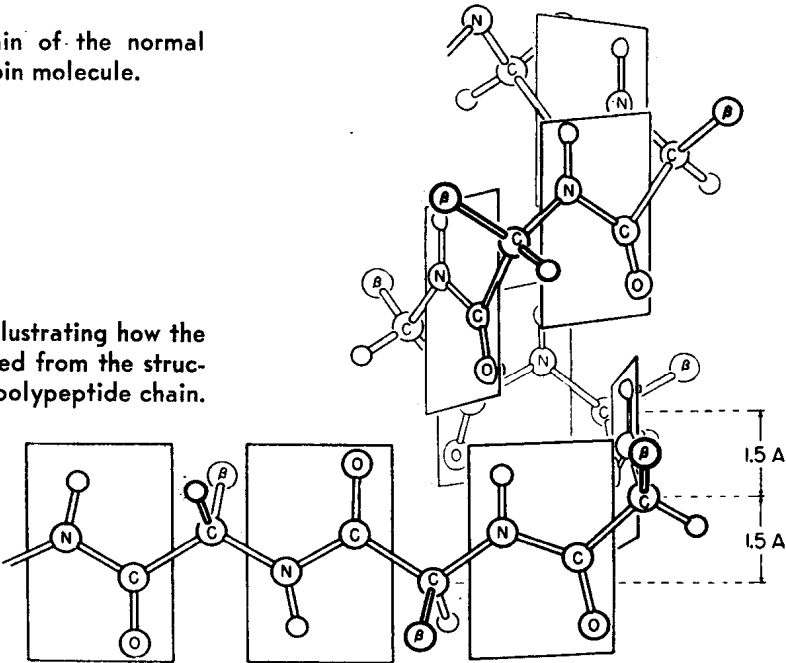


Fig. 2. The beta chain of the normal human hemoglobin molecule.

Fig. 3. A drawing illustrating how the alpha helix is generated from the structural elements of the polypeptide chain.



SECONDARY STRUCTURE—THE ALPHA HELIX

Linus Pauling in 1950³ formulated the fundamental dimensions and the structural principles of polypeptide chains. Pauling and his group solved the problem in an indirect way, namely, through the determination of the dimensions of amino acids and simple peptides and the formulation of structural principles which, together with the experimentally determined dimension, could be used with confidence in the derivation of acceptable configurations (conformations) of polypeptide chains.

From a series of determinations of crystal structure many data pertinent to polypeptide chains were derived. First, the dimensions, the bond lengths and the bond angles associated with the amide group were obtained and are now known within a probable error of $\pm 0.01 \text{ \AA}$ and $\pm 2^\circ$, respectively. Second, the planarity of the amide group, predicted by Pauling, was confirmed experimentally. The lengths of the C—N and the C—O bonds correspond to about 40 and 60 per cent double-bond character in these bonds, respectively. Therefore, theoretical considerations would ascribe the planar configuration to the amide group. This prediction was amply confirmed by the crystal analyses. All of the crystals of amino acids and peptides analyzed were characterized by the formation of the maximum possible number of N—H . . . O hydrogen bonds. Most of these bonds have the length $2.70 \pm 0.12 \text{ \AA}$. There appears to be a strong tendency of N—H . . . O hydrogen bonds to be essentially linear.

In 1950 all of this structural information was used to derive the alpha helical structure for polypeptide chains. In the derivation of these configurations the following basic assumptions were made by Pauling *et al.*:³ (1) the dimensions of the polypeptide chain are those derived from

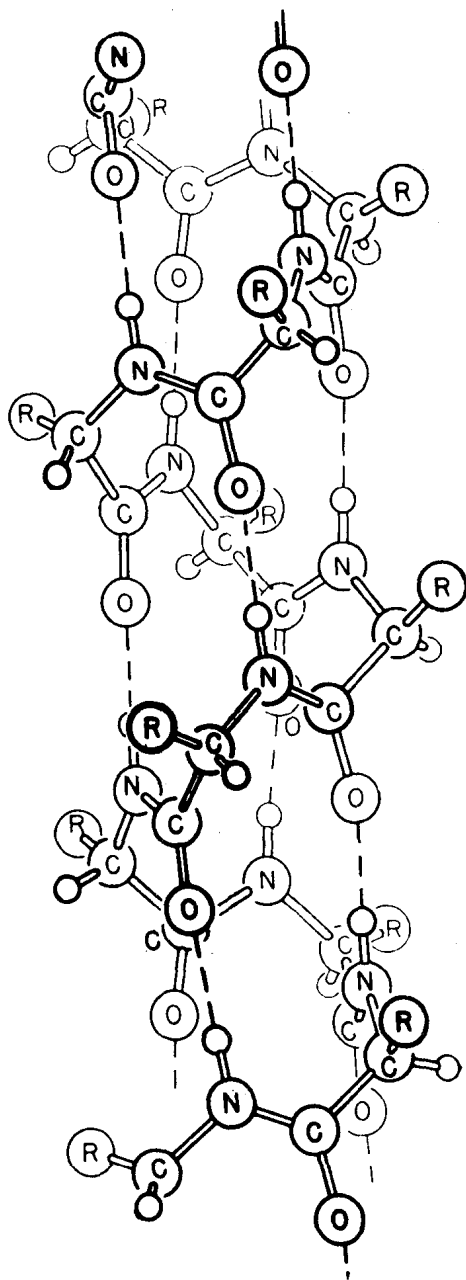


Fig. 4. A drawing of a portion of an alpha helix.

crystals of amino acids and simple peptides, (2) the amide group is planar and is in the *trans* configuration, (3) the maximum number of N—H . . . O hydrogen

bonds are formed. The additional condition was imposed that, except for the nature of the side chains, all acid residues were to be structurally equivalent. The most general set of operations that will convert an asymmetric element into its geometric equivalent is a rotation around an axis and a translation parallel with that axis. Repetition of this operation generates a helix, so that structures developed in this way would be helical. When this investigation was carried out only two configurations were found which were compatible with the assumptions.

One of these configurations was designated the alpha helix. The way in which the alpha helix is generated from the polypeptide chain is illustrated in Figure 3. The elements of the chains (the planar amide group and the tetrahedral alpha-carbon atom) are successively rotated 100° around a vertical axis and translated 1.5 \AA parallel with the axis. A drawing of

a portion of an alpha helix is shown in Figure 4. In this helix each amide group is connected by hydrogen bonds to the 3rd amide group from it along the polypeptide chain. There are about 3.6 residues per turn of the helix, and each residue is spaced about 1.5 \AA above or below its neighbor in the direction along the axis of the helix. The total rise of the helix per turn—the pitch of the helix—is 5.4 \AA . A model of the helix is shown in Figure 5. In this model the polypeptide chain represents polyglycine, since there are no side chains attached to the alpha carbon atoms. The helix is packed very firmly, and there is no space at its center. Nearly 70 per cent of the hemoglobin molecule is now known to be in alpha helix conformation.

THE TERTIARY STRUCTURE

By the isomorphous replacement technique of x-ray crystallography, Kendrew

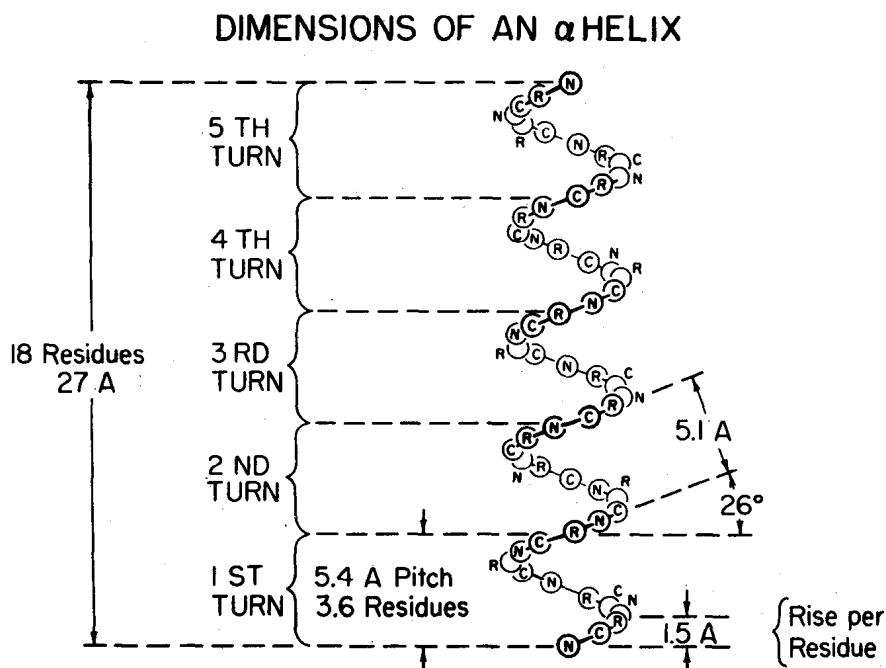


Fig. 5. A conventional representation of the alpha helix with 3.6 residues per turn.

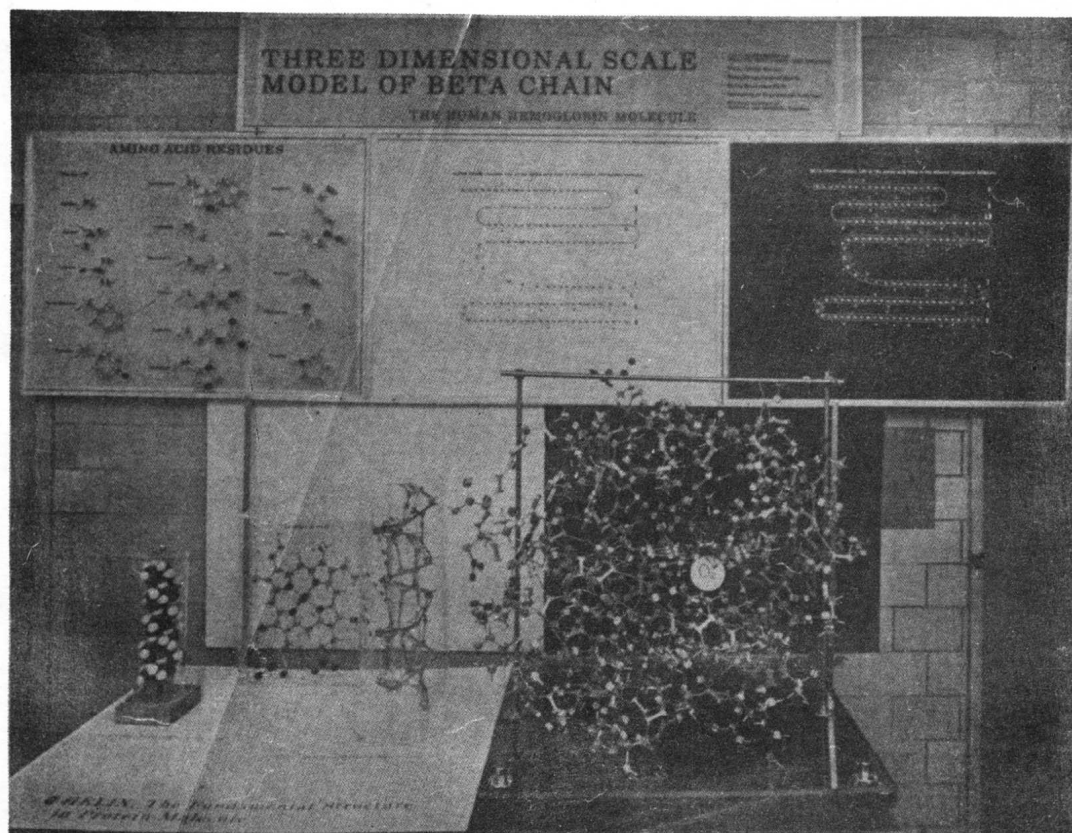


Fig. 6. Three dimensional scale model of the beta chain of hemoglobin.

succeeded in elucidating the tertiary structure of sperm whale myoglobin.² By this technic the 3-dimensional molecular structure of sperm whale myoglobin is brought into sharp focus. The alpha helix is now known to have the right-hand configuration (i.e., its screw sense is right-handed). Some of the larger amino acid residues like tryptophan, lysine, phenylalanine, etc., are clearly visible. The position of the heme is well delineated. The 3-dimensional picture of horse hemoglobin is still blurred; it will take some time before Perutz can obtain a sharply focused picture. Nevertheless, it is useful enough to obtain much of its detail by homology; the position of the heme appears to be identical with that in myoglobin. Angles between chain segments have been calculated by Perutz,⁴ as follows:

ANGLES BETWEEN CHAIN SEGMENTS

	HEMOGLOBIN		MYOGLOBIN
	Black Chain	White Chain	
Chain Segments			
GB	125°	127°	127°
GH	158°	156°	162°
HB	68°	67°	67°
AB	90°	94°	102°
BE	117°	117°	103°
GE	89°	97°	92°

The isomorphous replacement technic of x-ray crystallography requires at least 2 heavy metal atoms per molecule of protein. It was found in our laboratory that at low temperatures (0° to 27°) only about one half of the binding sites are available to mercuric ions. However, all of the binding sites (sulfhydryl groups)