Hemoglobin

Its Precursors and Metabolites

Edited by
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and
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Its Precursors and Metabolites

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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.
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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	GlN	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		5 THR
	15 GLY		6 TYR
	16 LYS		7 PHE
	17 VAL	CE—	1 PRO
	18 GLY		2 HIS
	19 ALA		3 PHE
AB—	1 ALA		4 ASP
	2 HIS		5 LEU
	3 ALA		6 SER
B—	1 GLY		7 HIS
	2 GLU		8 GLY
	3 TYR		9 SER
	4 GLY		10 ALA
	5 ALA	E—	1 GLU
	6 GLY		2 VAL

	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

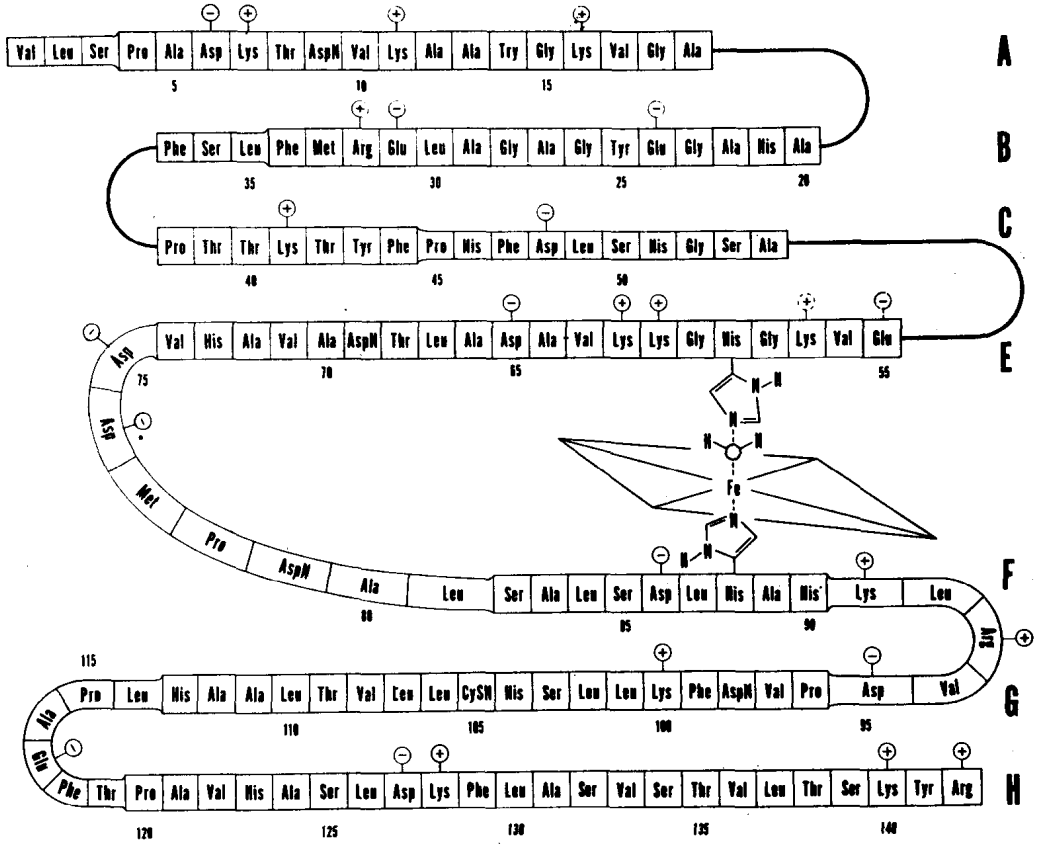

Fig. 1. The alpha chain of the normal human hemoglobin molecule.

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	GlN	Threonine	THR
Glycine	GLY	Tryptophan	TRY
Histidine	HIS	Tyrosine	TYR
Isoleucine	ILEU	Valine	VAL

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

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

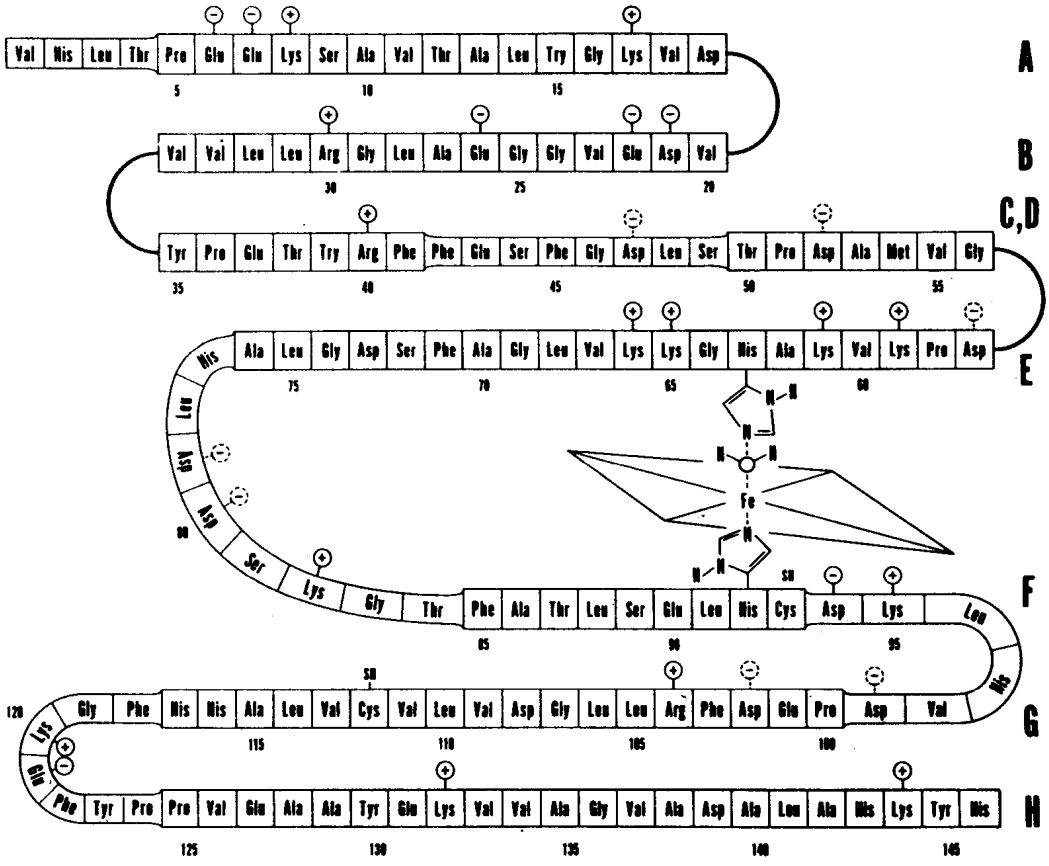

Fig. 2. The beta chain of the normal human hemoglobin molecule.

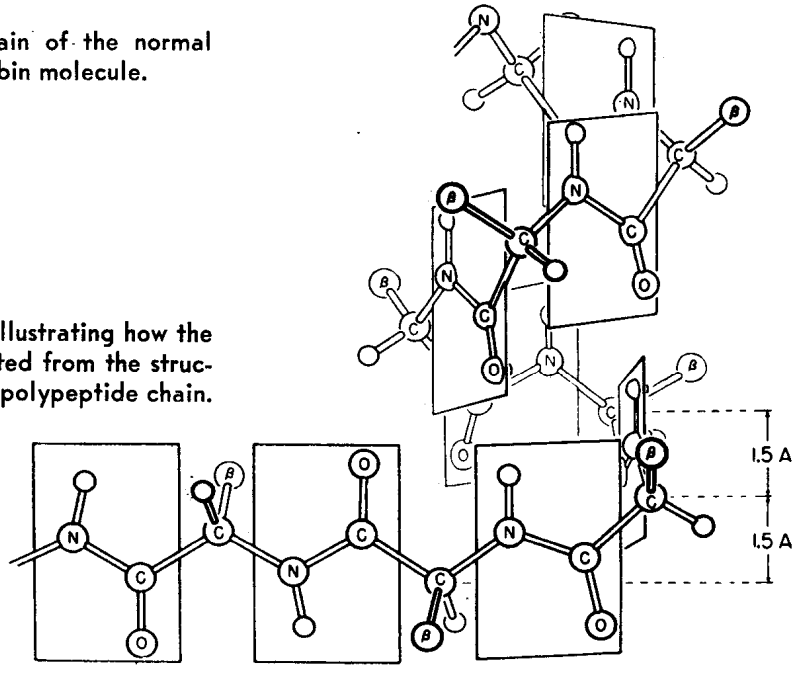

Fig. 3. A drawing illustrating how the alpha helix is generated from the structural elements of the polypeptide chain.

SECONDARY STRUCTURE—THE ALPHA HELIX

Linus Pauling in 1950[3] 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 ±0.01 Å and ±2°, 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 ± 0.12 Å. 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 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

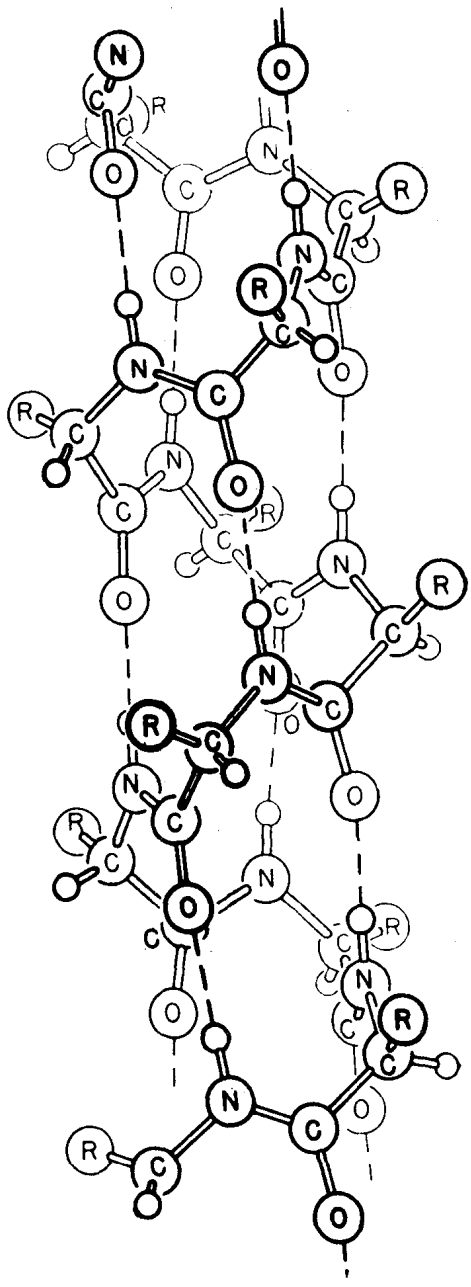

Fig. 4. A drawing of a portion of an alpha helix.

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 Å 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 Å 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 Å. 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 technic of x-ray crystallography, Kendrew

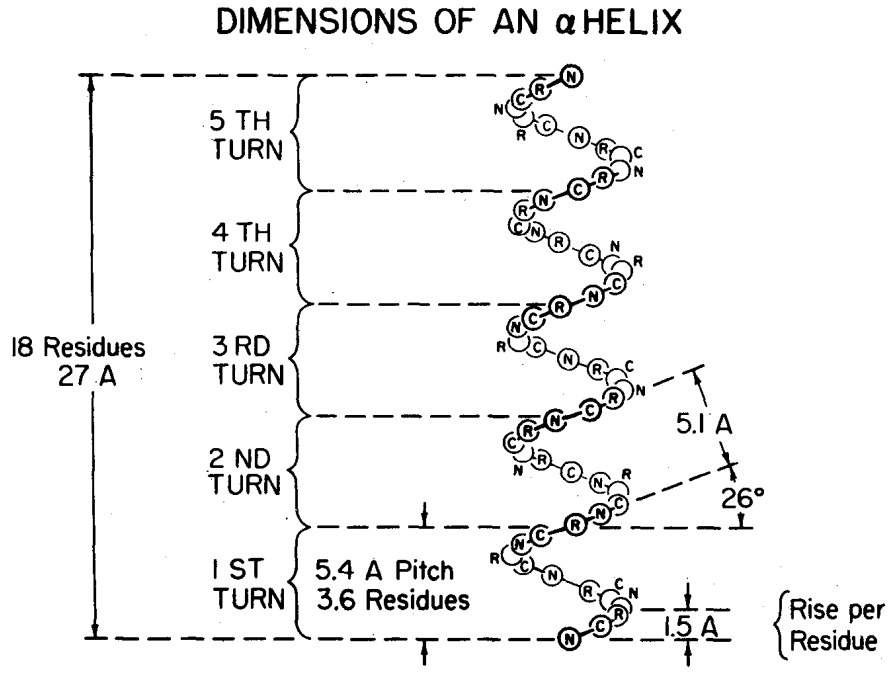

Fig. 5. A conventional representation of the alpha helix with 3.6 residues per turn.

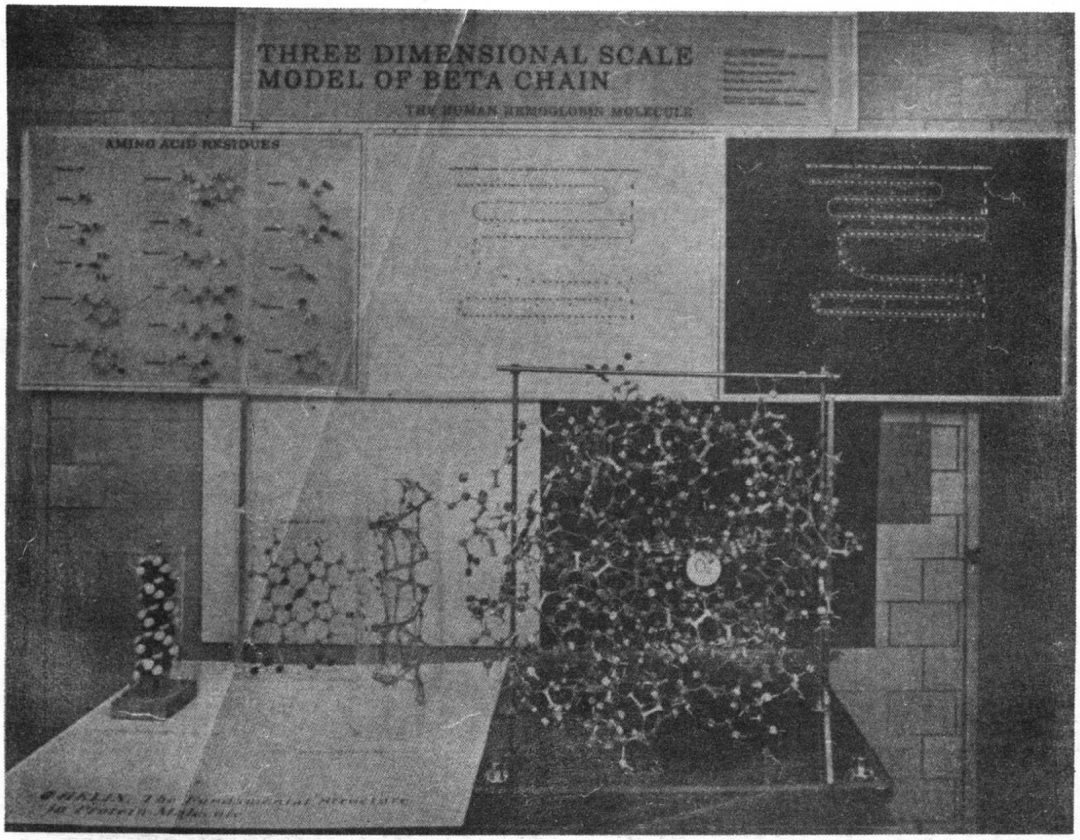

Fig. 6. Three dimensional scale model of the beta chain of hemoglobin.

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
Chain Segments	Black Chain	White Chain	
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 (O° to 27°) only about one half of the binding sites are available to mercuric ions. However, all of the binding sites (sulfhydryl groups)