Hemoglobin: Molecular Structure and Function, Biosynthesis, Evolution and Genetics

The large and rapidly advancing field of hemoglobin research was discussed and reviewed at the 1962 Hemoglobin Workshop held 4–7 November at Arden House, Columbia University. Molecular structure and function, biosynthesis, evolution, and genetics were the four main topics of discussion.

That the newly honored Nobel laureates in chemistry, John C. Kendrew and Max F. Perutz had been scheduled as participants in the conference gave special significance to the meetings. Indeed, these two men led the way in the conference; their fundamental contributions initiated the discussion and provided a broad basis for the exchange of ideas in chemistry, physics, biology, and medicine.

Vernon M. Ingram (M.I.T.) was general chairman. One formal lecture, "Hemoglobin, Evolution, and Molecular Disease," was delivered by Linus Pauling (California Institute of Technology).

Of the many excellent papers and discussions, only a partial account can be given.

Reviewing the present state of his work on myoglobin, Kendrew indicated that of the 150-odd amino acids in the molecular chain, the positions of 120 are certain, of 20 probable, and of 8 to 10 uncertain. Myoglobin is a compact molecule held together by the nonpolar groups and the Van der Waals forces in its interior. His studies provide confirmation of the extraordinarily accurate predictions of Pauling and Corey on the coordinates of the alpha helix. They also demonstrate the great promise of the new techniques which permit resolution at 1.5 angstroms.

Showing newly constructed molecular models, Perutz presented data, still not completely worked out, on the structure of deoxygenated human hemoglobin. The evidence from amino acid sequence and from analysis of x-ray structure indicates close similarity between horse and human hemoglobin, so that the deoxygenated form of one can be compared with the oxygenated form of the other. The major change upon deoxygenation appears to be in two of the four polypeptide chains, the so-called beta chains. These two beta chains move out from the center of the molecule, and away from each other by a few angstrom units, thus increasing the distance separating the two heme groups embedded in the beta chains—the point of attachment of the oxygen molecules. There is also a slight change in the angle between two portions of the beta peptide chains. These findings represent a great step forward in our quest to understand the molecular basis of the most important property of hemoglobin, the ability to combine reversibly with oxygen.

However, Pauling pointed out that a great deal of work still remains to be done in this area of hemoglobin and myoglobin research, especially since we do not yet understand how the oxygen molecule is attached to the iron atom of the heme group.

The x-ray investigations and the determination of the primary chemical structure of hemoglobin (G. Braunitzer, Munich; R. J. Hill, Duke University; W. Koenigsberg, Rockefeller Institute; W. A. Schroeder, California Institute of Technology; and others) are resulting in a very detailed view of this protein. These findings, together with further reports by Braunitzer, Hill, and others, also support the view that the hemoglobin peptide chains have evolved from a common ancestral chain. Pauling, in his lecture, presented some frankly speculative calculations, deducing a possible amino acid sequence of the primitive peptide chain. On the physiochemical basis of oxygenation, there are still many unsolved problems; on this subject there was a vigorous debate between D. M. Gomez (Columbia) and F. J. W. Roughton (Cambridge). Gomez, in a mathematical analysis, suggested that in the closely packed intracellular arrangement of hemoglobin, the fourth constant of the Adair four-compound hypothesis might need to be modified because of some additional variable such as the behavior of carbon dioxide, while Roughton defended the original Adair hypothesis. He also presented some new data on the combination of carbon dioxide with hemoglobin.

Another controversy in the field of hemoglobin research, the "Bohr effect," has now been somewhat clarified. In a comprehensive survey, R. Benesch (Columbia) supported the view that the groups in the protein which cause the release of protons on oxygenation (Bohr effect), are imidazole groups of four histidine residues, one per peptide chain. The change in *p*K of these groups, the phenomenon of heme-heme interaction, and the effect of blocking the sulfhydryl groups on oxygenation should be interpreted in terms of subtle changes in the tertiary and quaternary structure of hemoglobin. E. Antonini (Rome) reviewed recent experiments with bromthymol blue which indicate that hemoglobin undergoes configurational changes during oxygenation. J. Wyman (Rome) discussed the oxygenation equilibrium of hemoglobin with respect to *p*H, ionic strength, and associated changes in molecular weight.

Leading the session on the evolution of hemoglobin and genetics, P. S. Gerald (Harvard) presented some of the theories for the pathogenesis of the thalassemias. The idea that thalassemia is a "hidden," or nonelectrophoretic, amino acid substitution of either the alpha or the beta chain has been tested by Guidotti and also by Stretton and Baglioni. Evidence was presented from both groups that careful amino acid analyses of "thalassemia" hemoglobin peptide chains failed to show anything but the expected amino acid composition, suggesting either that the substitutions are really inversions of sequence, or that perhaps thalassemia might be caused by a defect in the usual mechanism that activates the hemoglobin structural genes. In this connection, C. L. Conley (Johns Hopkins) described an unusual patient with persistent, fetal hemoglobin. In this child, homozygous for the defect, the production of both the beta and delta chains was blocked so that only fetal hemoglobin was produced. Conley explained this defect as an overlapping deletion that affected neighboring beta and delta genes. Others, including J. V. Neel (Michigan) and A. G. Motulsky (Seattle), preferred to view the defect as a mutation of an "operator" controlling both beta and delta chain genes.

An interesting type of human mutation was reported by C. Baglioni (Naples), as the result of work with hemoglobin Lepore. His evidence indicates that the abnormal peptide chain is the product of a gene which is part delta and part beta. The hybrid is thought to have arisen by nonhomologous crossing over.

In a review paper, H. Dintzis (Johns Hopkins) explained why protein biosynthesis can be so well studied in the reticulocyte. From his own work he described the assembly of the hemoglobin peptide chains as a sequential process, proceeding stepwise from the amino group at the end of the chain. In the reticulocyte only 1 to 2 minutes (at 37°C) are required for the synthesis of a whole chain. On the other hand, in cell-free systems prepared from reticulocytes, it appears that peptide chains are merely completed and that the synthesis of few if any new chains is begun. This point was disputed by R. S. Schweet (Kentucky).

A. Rich (M.I.T.) demonstrated that aggregates of reticulocyte ribosomes are needed for the active synthesis of hemoglobin. His electron micrographs do indeed show aggregates of approximately five ribosomes. Rich interprets these as being held together by a strand of messenger RNA. Each ribosome is synthesizing one or more peptide chains as it moves along the messenger RNA—a stimulating idea. The data of P. A. Marks (Columbia) also show that the aggregated ribosomes are those active in hemoglobin synthesis and that information for protein synthesis is contained in a relatively stable form in these particles. The role of soluble RNA as the "adaptor" in placing amino acids in sequence on the messenger RNA template was clearly demonstrated by G. von Ehrenstein (Johns Hopkins).

In another area of study, H. Borsook (California Institute of Technology) related hemoglobin production to the developing red cells (erythroblast series) in the bone marrow. Hemoglobin synthesis normally is completed at the orthochromatic stage. An interesting report was read by L. Bernini (M.I.T.) who showed that human bone marrow cells synthesize carbonic anhydrase in addition to hemoglobin A and A_2.

The three chairmen of the sessions, J. T. Edsall (Harvard), F. Lipmann (Rockefeller), and J. V. Neel (Michigan), were most effective, and much of the success of the workshop was

due to their guidance. The conference closed with a unique talk by H. Lehmann (London) who connected the ethnological distribution of the abnormal human hemoglobins throughout the world with certain unusual social customs.

The conference was sponsored by the Department of Medicine of Columbia University and generously supported by the National Heart Institute.

VERNON M. INGRAM
DICKINSON W. RICHARDS
ALFRED P. FISHMAN

Department of Medicine,
Columbia University, New York

Forthcoming Events

January

7–8. Ultra-High Energy **Nuclear Physics**, conf., Bristol, England. (Administrative Assistant, Inst. of Physics and the Physical Soc., 47 Belgrave Sq., London S.W.1, England)

14–16. **Radiation Research**, intern. conf., Natick, Mass. (Army Quartermaster Research and Engineering Center, Natick)

14–18. Association of **Surgeons** of West Africa, Ibadan, Nigeria. (V. A. Ngu, University College Hospital, Ibadan)

14–19. Atomic and Molecular **Quantum Theory**, symp., Sanibel Island, Fla. (D. W. Smith, Chemistry Dept., Univ. of Florida, Gainesville)

15–15 Feb. World Meteorological Organization, Working Group on **Meteorological Transmissions**, Paris, France. (WMO, 41 Avenue Giuseppe Motta, Geneva, Switzerland)

15–17. Association of **American Colleges**, annual, Atlantic City, N.J. (T. A. Distler, AAC, 1818 R St., NW, Washington 9)

15–17. **Sesame**, intern. conf., Maracay, Venezuela. (D. G. Langham, Sesamum Foundation, Milford, Conn.)

15–19. **Immunopathology**, intern. symp., La Jolla, Calif. (by invitation). (Science Information Div., National Foundation, 800 Second Ave., New York 17)

17–19. **Engineers' Training**, conf., Strasbourg, France. (Council of Europe, Avenue de l'Europe, Strasbourg)

17–19. Royal College of **Physicians and Surgeons** of Canada, annual, Edmonton, Alberta. (J. H. Graham, RCPSC, 74 Stanley Ave., Ottawa 2, Ont., Canada)

18–19. **Blood**, annual symp., Detroit, Mich. (G. F. Anderson, Dept. of Physiology and Pharmacology, Wayne State Univ., 1401 Rivard St., Detroit 7)

21–23. Chemistry and Biochemistry of **Seed Proteins**, intern. conf., New Orleans, La. (C. H. Fisher, Southern Utilization Research and Development Div., Agricultural Research Service, U.S. Dept. of Agriculture, P.O. Box 19687, New Orleans 19)

21–23. Institute of the **Aerospace Sciences**, annual, New York, N.Y. (IAS, 2 E. 64 St., New York 21)

21–24. American **Meteorological** Soc., annual, New York, N.Y. (R. L. Pfeffer, Lamont Geological Observatory, Columbia Univ., Palisades, N.Y.)

22. Infectious Diseases of the **Heart and Circulation**, conf., New York, N.Y. (C. A. R. Connor, New York Heart Assoc., 10 Columbus Circle, New York 19)

22–24. **Reliability and Quality Control**, natl. symp., San Francisco, Calif. (L. W. Ball, Boeing Co., P.O. Box 3707, Seattle 24, Wash.)

23–25. **Elevated Temperature Mechanics**, intern. conf., 3rd Navy Structural Mechanics Symp., New York, N.Y. (by invitation). (A. M. Freudenthal, 624 Mudd Bldg., Columbia Univ., New York 27)

23–26. American Assoc. of **Physics Teachers**, New York, N.Y. (R. P. Winch, Williams College, Williamstown, Mass.)

23–26. American Group **Psychotherapy** Assoc., annual, Washington, D.C. (AGPA, 1790 Broadway, New York 19)

24–27. American **Mathematical** Soc., annual, Berkeley, Calif. (AMS, 190 Hope St., Providence 6, R.I.)

26. Association for **Symbolic Logic**, Berkeley, Calif. (T. Hailperin, Dept. of Mathematics, Lehigh Univ., Bethlehem, Pa.)

26–28. **Mathematical** Assoc. of America, annual, Berkeley, Calif. (H. M. Gehman, Univ. of Buffalo, Buffalo 14, N.Y.)

27–1. American Inst. of **Electrical Engineers**, winter general meeting, New York, N.Y. (R. S. Gardner, AIEE, 33 W. 39 St., New York 18)

28–2. American **Library** Assoc., Chicago, Ill. (D. H. Clift, ALA, 50 E. Huron St., Chicago 11)

28–2. **Body Composition**, conf., New York, N.Y. (J. Brozek, Dept. of Psychology, Lehigh Univ., Bethlehem, Pa.)

30–1. Military **Electronics**, natl. winter convention, Los Angeles, Calif. (F. P. Adler, Space Systems Div., Hughes Aircraft Co., Culver City, Calif.)

31–1. American Soc. for **Engineering Education**, college-industry conf., Atlanta, Ga. (W. L. Collins, Univ. of Illinois, Urbana)

31–1. Society of **Rheology**, annual western regional meeting, Emeryville, Calif. (T. L. Smith, Stanford Research Inst., Menlo Park, Calif.)

31–2. Western Soc. for **Clinical Research**, annual, Carmel-by-the-Sea, Calif. (H. R. Warner, Latter-day Saints Hospital, Dept. of Physiology, Salt Lake City 3, Utah)

February

4–8. **Rice Genetics** and **Cytogenetics**, symp., Los Baños, Laguna, Philippines. (Inter. Rice Research Inst., Manila Hotel, Manila, Philippines)

4–9. Recent Trends in **Iron and Steel Technology**, symp., Jamshedpur, India. (Secretary, Indian Inst. of Metals, 31 Chowringhee Rd., Calcutta, India)

4–20. Application of **Science and Technology** for the Benefit of Less Developed Areas, U.N. conference, Geneva, Switzerland. (Science Conference Staff, Agency for International Development, 826 State Dept. Annex 1, Washington 25)

5–14. International **Radio** Consultative Committee, Plan Subcommittee for Asia, New Delhi, India. (V. Barthoni, 128 rue de Lausanne, Geneva, Switzerland)