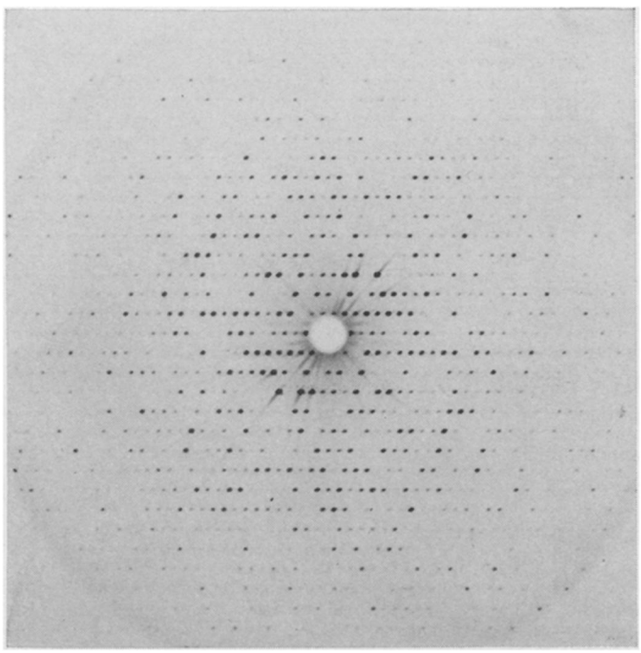
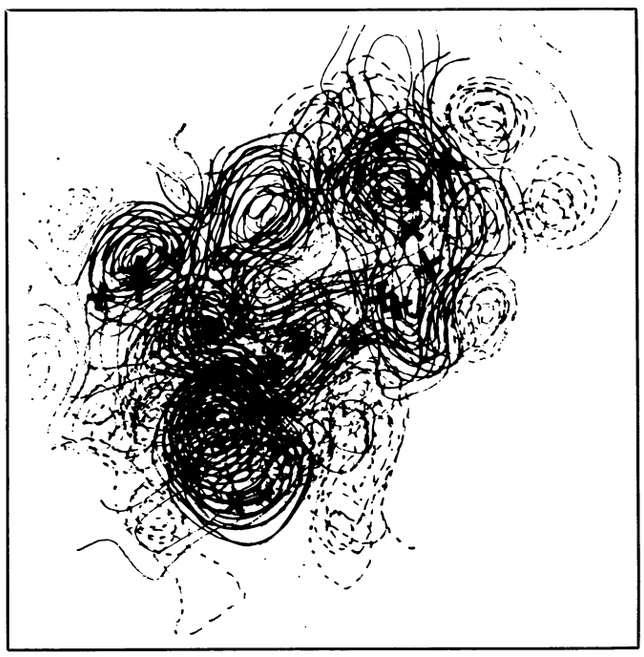
Neutron Analysis for Proteins



Dr. John Kendrew

Turning crystal and photo plate yields precession image.



Dr. Schoenborn

From this Fourier map, scientists locate amino acids.

by Barbara J. Culliton

Architecture, in the last decade, has become a subject of primary interest to molecular biologists intent on deciphering the chemical behavior of proteins, genes, hormones and other living substances. If they do not know how these materials are built, they cannot know how they work. Without knowledge of the atomic structure of a protein, without a precise model of its three-dimensional configuration, real understanding of the chemistry of life is impossible.

In biochemistry, form and function go hand in hand. Each protein's active sites are on the outer surface, where they are in a position to react with other proteins and body chemicals. The total configuration determines the location of that active site and controls the molecule's chemical behavior. Should the shape be altered, folding the active site into the interior, for example, the chemical behavior would be changed or even totally lost.

Until now, biologists in pursuit of atomic blueprints have had but a single tool with which to work: the technique of X-ray crystallography (SN: 9/21/68, p. 298). Protein crystals are bombarded by X-rays that strike the atoms in the giant molecule and are reflected and scatter in a characteristic pattern, identifying the position of these atoms within the protein. Eventually, after thousands of reflections have been made and computed, a map can be constructed showing the location of the atoms.

Valuable as X-ray crystallography is, the technique has limitations. For one, it picks up some atoms better than others; heavier atoms produce more diffraction than lighter ones. In the process of analyzing a molecule as large as a protein, that may have several thousand atoms, certain scatter patterns are inevitably lost. Hydrogen atoms are particularly susceptible to being lost because of their extremely low X-ray scattering. And if they are, elements essential to maintaining the three-dimensional shape of a protein or other molecule cannot be located.

The answer, says Dr. Benno P. Schoenborn of the Brookhaven National Laboratory in Upton, N.Y., is to supplement X-ray analysis with neutron diffraction analysis. He has tried it, and it appears to work.

Dr. Schoenborn reported, in the Oct. 11 NATURE, successful neutron analysis of myoglobin, the protein that carries oxygen in muscles. Previously, he comments, neutron analyses have been made of small molecules, perhaps of 50 or 60 atoms, but never of anything as large as myoglobin with its 2,400 atoms and 153 amino acid molecules.

"For some reason, many researchers thought neutron diffraction would be impossible," he observes. "Actually, it probably could have been done 10 years ago if anyone had tried."

In principle, neutron diffraction and X-ray diffraction are similar, but neutrons, instead of X-rays, are fired at the crystallized protein. The scatter patterns revealed by the two methods are quite different and easily distinguishable. The neutron procedure, however, offers an advantage in searching out hydrogen atoms; hydrogens have a strong scattering pattern after neutron bombardment.

For the diffraction experiment, large crystals of myoglobin from a sperm whale were mounted in quartz tubes, immobilized with quartz glass wool and subjected to neutrons from a germanium crystal. In all, 4,600 reflections were measured and a three-dimensional Fourier map was calculated, showing the position of the atomic nuclei.

A Fourier map looks like a skein of tangled yarn. It is really a stack of contour maps taken along a series of parallel sections of crystallized myoglobin. It shows the distribution of electron density within molecular subunits, such as the heme group, if the X-ray or neutron resolution is low, and of individual atoms if it is high. The hills and valleys of the contour maps signify regions of high and low density that can be correlated with the presence

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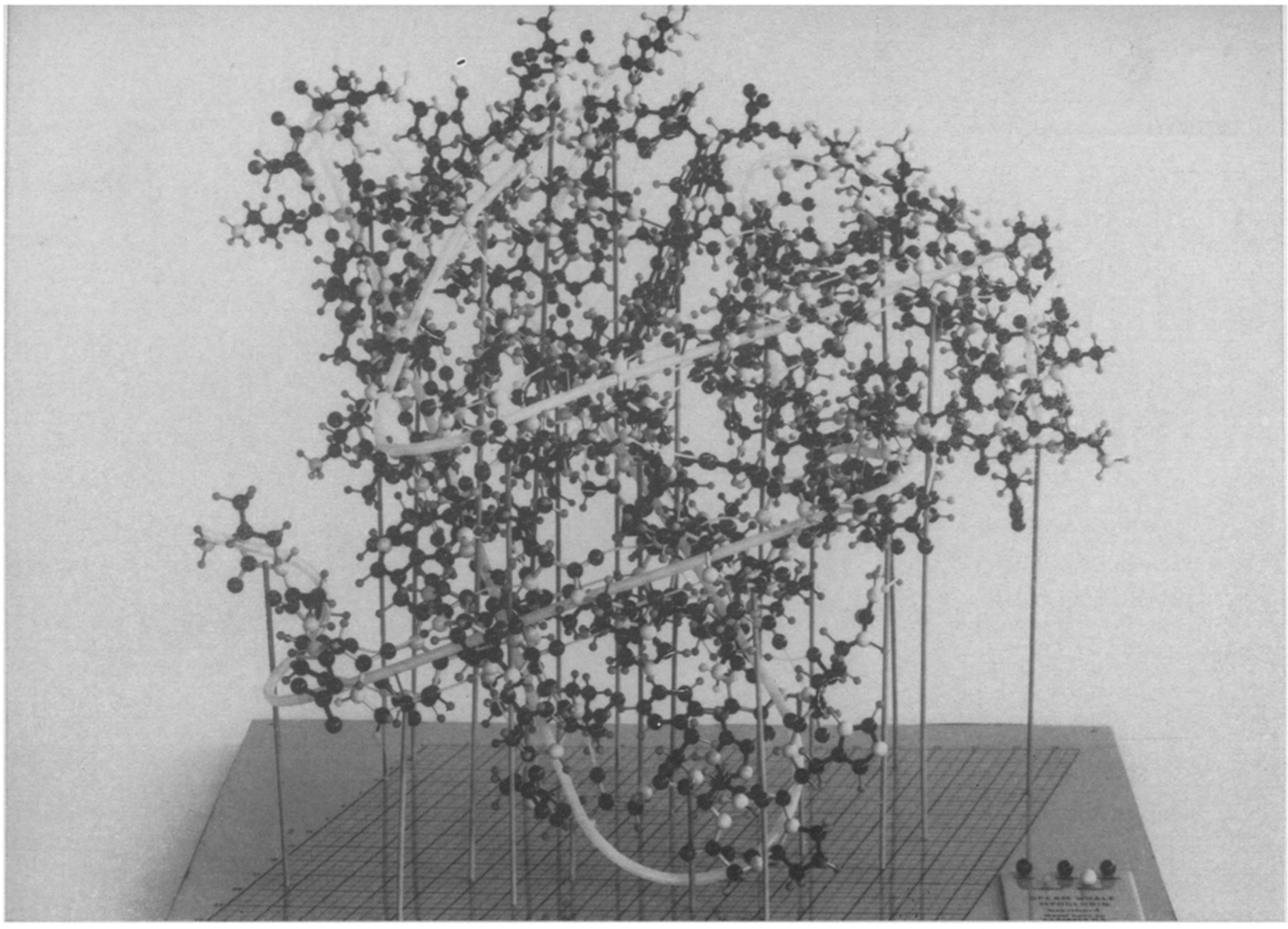


Dr. John Kendrew, Cambridge University, from R. Kretsinger

On the basis of X-ray analysis of crystals of sperm whale myoglobin, Dr. Kendrew constructed a Tinker Toy model.

of certain types of atoms. When the series of maps is compiled, it is possible to calculate, from the location of specific atoms, or groups of atoms, information about the three-dimensional configuration of the molecule.

"The myoglobin molecule," Dr. Schoenborn says, "is structurally well defined and, generally, atomic positions on the neutron Fourier map agree reasonably well with those determined by X-ray techniques."

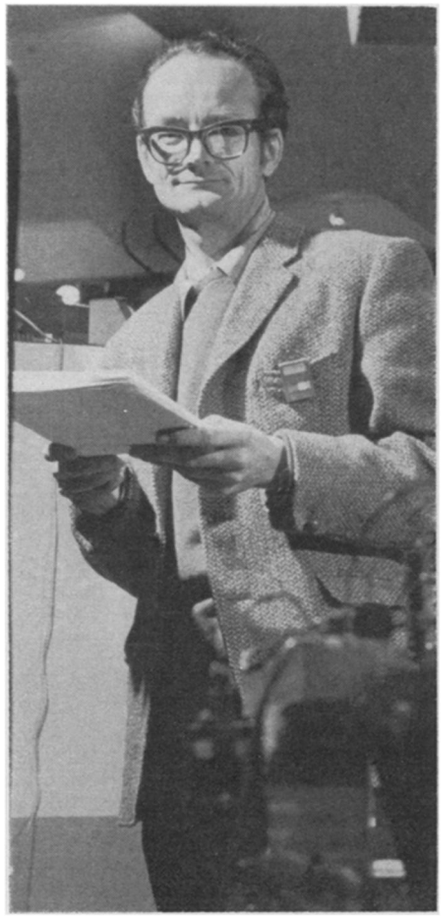
There were, however, some differences in addition to identification of the geography of the hydrogen atoms. Certain groups of amino acids, including phenylalanine, were more sharply outlined on the neutron Fourier than on previous X-ray Fourier maps, and the position of the heme group, thought to be the active site of action in the myoglobin molecule, was clear.

"We would like to know precisely how oxygen is stored and released in the muscle by myoglobin," Dr. Schoenborn says. "Further work will focus particularly on the hydrogen atoms in the heme group because a pattern of loss and gain of hydrogens underlies the chemical processes going on here."

Sperm whale myoglobin was selected for this initial neutron diffraction experiment because large crystals were available for analysis and because its three-dimensional structure had already been examined by X-ray crystallography. In fact, myoglobin was the first protein so studied, followed almost immediately by hemoglobin, the oxygen-carrying protein in blood.

This work, done in the late 1950's, won a Nobel Prize in 1962 for Drs. Max Perutz and John Kendrew of the Cavendish Laboratory at Cambridge University. Dr. Kendrew was able to assign a position in space to most of the heavy atoms in myoglobin and verified many major deductions previously made about its general configuration. Since then, X-ray analysis has revealed structures of lysozyme, an enzyme that destroys cell walls, ribonuclease, an enzyme that degrades ribonucleic acid (SN: 2/4/67, p. 119), and, most recently, of insulin (SN: 10/4, p. 307).

This year on the basis of information about structure developed by X-ray diffraction analysis, two teams of scientists successfully synthesized ribonuclease for the first time (SN: 2/1, p. 112), a goal applicable to these other architecturally known compounds as well. Also this year, several research teams almost simultaneously announced that they had grown crystals of transfer-RNA that were large enough for X-ray analysis (SN: 1/4, p. 9). Each of these substances could now be examined by neutron diffraction analysis in order to construct a blueprint complete in those details X-ray analysis is unable to resolve. □



Brookhaven National Laboratory

Dr. Schoenborn: No one tried before.