Molecular Biology: British Groups Push Enzyme-Structure Studies

Molecular biology in Britain has become diversified and decentralized since its pioneering days at postwar Cambridge. At a time when Britain's lead in the determination of the structures of large, biologically active molecules is being challenged from the United States, it is interesting to look at how work on the European side of the Atlantic is developing in one very important and exciting area, the structure of enzymes. Here the American challenge has been made direct and explicit by the publication in March, by David Harker and his colleagues at Buffalo, of a high-resolution version of the shape of the enzyme molecule ribonuclease. The Harker structure caps—and contradicts—a low-resolution model of the same molecule which was published only a month before by Harry Carlisle and his group at Birkbeck College, London. Most people may be inclined to find the Harker version more convincing. Carlisle remains confident, nevertheless, and thinks that 12 to 18 months must elapse before there is sufficient evidence available to settle the issue.

What follows is based on conversations with D. C. Phillips of Oxford and B. S. Hartley of Cambridge, and on a very brief chat with Pehr Edman in Prague. Phillips is a crystallographer concerned with elucidating the convolutions of protein molecules—"tertiary structures." Hartley is a chemist faced with the problem of determining the sequences of the 20 amino acids, as they form protein chains—now termed "primary structures."

International comparisons aside, the determination of ribonuclease is a matter for satisfaction, as we now have high-resolution pictures for two enzyme structures. It is not quite 2 years since Phillips reported to American colleagues on the first of these, lysozyme. For that major advance in molecular biology he had won a bottle of champagne from Sir Lawrence Bragg, "father" of x-ray crystallography and at that time director of the Royal Institution, London, where Phillips and his group were working. In 1960, when John Kendrew's team in Cambridge had determined the shape of the oxygen-storing protein, myoglobin, to an accuracy of 2 angstroms, Bragg had wagered that the achievement would not be matched before 1970. He himself had contributed to the research which cost him the champagne. Today, with several structural determinations being made in various countries, by the same powerful technique whereby heavy atoms, such as Hg, are introduced into the molecule, it can be seen to have been a rash wager, anyway.

Chemical "Teeth"

The most significant thing about the lysozyme work was the information it provides as to how the molecule operates as an enzyme. The striking feature of the molecule is its cleft that could plainly grip, jawlike, on the polysaccharide chains of a bacterium's wall, with chemical "teeth" suitably disposed to chew them up. Hence the antiseptic action of the enzyme ceased to be a mystery. There is great satisfaction in the discovery that ribonuclease, too, has a cleft. Similarities and differences between these molecules will be revealing, as will comparisons with the structures of other enzymes, including the now well-advanced work on the structures of carboxypeptidase (Harvard University), carbonic anhydrase (Uppsala), chymotrypsin (Cambridge), and papain (Groningen).

Phillips and some creative colleagues from the Royal Institution have recently moved to Oxford, forming a laboratory for molecular biophysics within the department of zoology. They have been joined by Dorothy Hodgkin, whose analysis of the shape of the hormone molecule insulin is now going well. Charles Bunn remains at the Royal Institution to work on rennin, while David Green has gone to Edinburgh to continue his investigation of beta galactoglobulin.

The study of lysozyme is continuing at Oxford. One of its aims is to ascertain atomic positions more precisely, but of equal or greater interest is the investigation of enzyme action. So far, the experimental binding of the substrates which the enzyme attacks has been confined to small saccharides; by model building it is possible to infer a more complete enzyme-substrate complex. Another trend, reflecting the molecular biologist's interest in species differences between key molecules, is comparison of the structural studies of hen-egg lysozyme with new studies on this enzyme obtained from goose eggs and from human tissues. Phillips is also interested in the very different lysozyme of bacteriophage and its involvement in the lytic process, and, on the other hand, in the functionally different alpha lactalbumin, a protein which shows interesting similarities to hen-egg lysozyme.

But Phillips' group does not want to be known only as "the lysozyme lab." Uppermost in Phillips' mind is a fundamental weakness of current molecular biology, that molecules are studied in isolation, giving, at least, a narrow and possibly a misleading impression of their functional roles in living systems. "We are still a long way from biology," he says. The artificiality may be less in the case of proteins like hemoglobin and lysozyme; the former binds oxygen and the latter attacks saccharides outside the cell. But many active proteins are enzymes working in the midst of complex sequences of reactions. As a start one might look for structural resemblances in the active sites of successive enzymes in a metabolic pathway. It is also possible that nature uses enzymes physically linked, in many cases, to facilitate transfer of substrate molecules along a conveyor belt. For example, two enzymes that take part in the utilization of glucose can be co-crystallized: glyceraldehyde-3-phosphate dehydrogenase (GPD) and aldolase. H. Watson at Cambridge is pushing crystallographic techniques to the limit in seeking the structure of GPD, which is four times as big as hemoglobin, the biggest molecule whose shape is known so far. If the structure of aldolase is also determined, the way will be open for the crystallographic study of the GPD-aldolase complex. Nevertheless, Phillips takes the view that molecular biologists will have to compromise increasingly with less simple biological systems and use techniques much less graphic than x-ray analysis, which do not depend on crystallization, techniques such as nuclear magnetic resonance. All in all, the laboratory for molecular biophysics is likely to be greatly influenced by its location in Pringle's department of zoology, in taking up studies of common interest, for

example, the flight muscle and cuticle of insects.

At the beginning of the decade there were hopes that the precision and power of x-ray techniques would become so great that, unaided, they could provide details of protein structure. It looked as though the sequence of amino acid residues comprising the primary chain of the protein might be "read off" from their individual shapes. That dream has not been fulfilled, but the improvement in chemical methods for the determination of amino acid sequences in complicated molecules has resulted in new triumphs reminiscent of the elucidation of the amino acid sequence in insulin by Frederick Sanger. Central to these developments of technique is the part played by B. Hartley, in Sanger's group at the Laboratory for Molecular Biology, Cambridge. "We are now doing real chemistry," Hartley observes, "compared with the hopeful pottering of 10 years ago." The new methods, which have been applied extensively to enzyme studies, depend on the shrewd use of simple reactions in selected parts of the complex molecules. Some information on Hartley's "diagonal electrophoresis" was presented in a report from Cambridge [*Science* **145**, 36 (1964)]. This method has now been taken a good deal further. It is based on the idea of identifying from the miscellaneous fragments of a digested protein those containing particular amino acids.

"Oblique thinking" is how Hartley categorizes the technique, and it has the simplicity that is the mark of true ingenuity. First, the protein is digested with enyzmes, and the peptides so obtained are spread in a line across a sheet of paper; an applied high voltage separates them into parallel bands by electrophoresis. A narrow strip that takes in all the bands is clipped from the paper, in the direction of the electric field. The strip is treated with an agent that alters only those peptides containing a particular amino acid. With a sewing machine, the strip is stitched to another sheet of paper. The electrophoresis is repeated, but this time with the field acting at right angles to the strip. The peptides move to positions along a straight line lying obliquely to the strip—that is, all except for the modified peptides.

Hartley first used this technique to identify fragments containing sulfur bridges formed between cystine residues brought close together by the convolutions of the chain. These bridges help to determine the shape of the molecule. When the protein is fragmented, these bridges persist in peptides consisting of two short lengths of chain, joined roughly in the middle. If, after the initial electrophoresis, the paper strip is exposed to performic acid vapor, that treatment breaks the bridges and introduces an extra negative charge in the place of cystine. The fragments therefore move away from the diagonal position when electrophoresis is repeated.

Peptide Identification

At the Cambridge laboratory, diagonal electrophoresis has proved convenient for identifying peptides containing the amino acids methionine or lysine. In these analyses also, specific chemical modifications cause changes in the electrophoretic charge on methionine or lysine and consequently on the electrophoretic mobilities of the modified fragments. Methionine is the rarest of the normal amino acids in protein. It alone reacts with iodoacetamide, in acid solutions, to form a positively charged side chain, so that peptides containing methionine become identifiable. Similarly, lysing-bearing peptides lose their positive charge when blocked by a trifluoroacetyl group and regain it when this group is removed by ammonia, so again the electrophoretic pattern is shifted. Better still, maleic anhydride reacts with lysine, converting a positively charged amino group into a negatively charged carbonyl group.

Of course, the amino acid sequences in the peptides have still to be analyzed, but that has been a less formidable task since the introduction of Hartley's highly sensitive fluorescent reagent "dansyl" (*l*-dimethylaminonaphthalene-5-sulfonyl chloride) for stepwise isolation and identification of the amino acids. Protein-sequence analyses are still not to be taken lightly, but they can certainly now be done more methodically, with smaller samples and on bigger molecules. The reaction with maleic anhydride promises to be important in another way: the alteration of the charges on the molecules helps to prevent denaturation during manipulation and also makes insoluble protein soluble. For example, the muscle protein myosin becomes soluble upon treatment with maleic anhydride. It seems reasonable, for the first time, to contemplate solving the amino acid sequence in myosin, a molecule almost ten times the size of chymotrypsinogen, which is the largest molecule fully analyzed so far (2000 amino acids compared with 245).

Meanwhile, Hartley and his colleagues have been using diagonal electrophoresis to supplement other "fingerprinting" techniques [notably the use of disopropyl fluorophosphate (DFP), radioactively labeled] for "quick looks" at parts of enzymes. The comparisons between different enzymes in the same species, or the same enzyme in different species, not only help to indicate how enzymes may work but also provide highly suggestive clues to the evolution of enzymes, and hence of genes. Hartley has already discussed the common evolution of the pancreatic enzymes trypsin, chymotrypsin, and elastase; and he has promoted the idea of spare genes created by accidental doubling, as providing the opportunity for nonlethal mutations in enzymes. Dennis Shaw has shown the recurrence of the same active sequences in the proteolytic enzymes of many kinds of animals, which can now be contrasted with the corresponding enzymes in molds and bacteria. Another colleague, Ieuan Harris, has been making comparisons of the glyceraldehyde phosphate dehydrogenases in species as different as rabbit, lobster, and yeast and finds similarities that cannot be coincidental.

Diagonal electrophoresis is proving particularly useful in current work of the kind just mentioned. The sulfur bridges between cystine residues, readily identified by diagonal electrophoresis, are probably of special functional and genetic significance because they help to fix the shape of the enzyme molecules. Deletion of a bridge could wreck an enzyme, and so it is likely to be conserved in evolution. Each of the pancreatic enzymes, trypsin, chymotrypsin, and elastase, have four bridges that secure three chemically significant regions of the molecules—the so-called "histidine loop," "methionine loop," and "serine knot." This observation is considered evidence of common ancestry. Similarly, studies of the cystine bridges, methionine, and lysine from the stomach enzymes pepsin and rennin has revealed a very high degree of homology. At present the Cambridge group is studying thrombin.

The dream of reading off the amino acid sequence from the shape of the protein molecule is being replaced by the hope of predicting shape from sequence. Here is common ground for the chemist and crystallographer. From thermodynamic calculations it may one day be possible to compute the lowest-energy configuration of the molecule—the one it can be expected to adopt.

Both Phillips and Hartley emphasize the current importance of empirical comparisons between known sequences and their observed foldings.

It now looks as if sequence analysis, even of the largest molecules, will outstrip the productivity of crystallography, which is still rate-limited in every case by the need to search for suitable means of introducing heavy-atom labels into the molecule. To refine "peptide surgery" the chemist can now add automatic sequence analysis. A most advanced machine for the latter purpose has just been installed by Pehr Edman of Melbourne in Borivoj Keil's laboratory at the Institute for Organic Chemistry and Biochemistry, Prague. On a recent visit to that laboratory, I observed that it was well instrumented, the American embargo on exports having encouraged the perfection (and marketing!) of homemade apparatus.

In Edman's apparatus, of which the prototype exists in Australia, a sample goes through a routine of about 30 operations to peel off each amino acid residue from a peptide; and the apparatus can go sequentially through 60 residues in one run, lasting about 4 days. That represents an impressive acceleration of such analytical procedures, and, hopefully, the instrument's powers will soon be demonstrated in new sequences announced from Czechoslovakia.—NIGEL CALDER

The author formerly was editor of the New Scientist. *In addition to the full-time correspondence of John Walsh, European correspondent for* Science, *Calder will provide occasional contributions as part of* Science's *expanded coverage of scientific affairs abroad.*

Appointments

Robben Fleming, chancellor of the University of Wisconsin, to president of the University of Michigan. . . . **Kermit Gordon**, vice president of the Brookings Institution and former director of the Bureau of the Budget, to president of Brookings. He succeeds **Robert D. Calkins** who has been appointed vice-chancellor for social sciences and professor of economics, University of California at Santa Cruz. . . . **Clark Kerr**, former president of the University of California, Berkeley, has accepted the position of chairman of the Carnegie Study of the Future of Higher Education, which is expected to take up approximately two thirds of his time. He has also accepted the part-time position of professor of industrial relations on the Berkeley campus. At the present time he is undertaking a number of lectureships, including Marshall Lecturer at Cambridge University, England, Pollack Lecturer at Harvard, and Davie Memorial Lecturer at the University of Capetown, South Africa. . . . **Robert Rathburn Wilson**, director of the Laboratory of Nuclear Studies, Cornell University, and recently appointed director of the proposed 200-Bev accelerator laboratory to be built at Weston, Illinois, to professor of physics at the University of Chicago. He will hold a joint appointment in the department of physics and the Enrico Fermi Institute for Nuclear Studies. . . . **Harry A. Towsley**, associate director of postgraduate medicine and professor of pediatrics, University of Michigan, to department chairman and professor of postgraduate medicine at the University. . . . **Peter E. Glaser**, senior scientist at Arthur D. Little, Inc., to president of the international Solar Energy Society. . . . **Ralph Hansen**, deputy director of the Agency for International Development's Regional Africa Office, Washington, D.C., to director of the U.S. Aid Program in Somalia. . . . **Albert J. Kelley**, deputy director of NASA's Electronics Research Center, Massachusetts, to dean of the College of Business Administration, Boston College. . . . **James T. Suter**, assistant chief of HEW's Division of Hospital and Medical Facilities' Research and Demonstration Grants Branch, to the Division's Health Facilities Services Branch. . . . **Ruth M. Davis**, staff assistant to the special assistant for National Intelligence, Department of Defense, to associate director for research and development, U.S. Public Health Service's National Library of Medicine. . . . **Richard R. Bond**, chief of party, Cornell University Project in Liberia, to vice president for academic affairs and professor of zoology, Illinois State University. . . . **William D. Lotspeich**, chairman of the department of physiology, University of Rochester Medical School, to executive secretary of the American Friends Service Committee. . . . **John E. Parker**, assistant professor and director of the department of medical art and photography, University of Minnesota Hospitals, to head of the department of medical illustration, Milton S. Hershey Medical Center of Pennsylvania State University. . . .

Recent Deaths

Charles F. Bond, 46; professor of zoology at the Universtiy of Vermont; 1 February.

James de Graaf-Hunter, 85; retired British geodesist; 3 February.

Harold A. Eggers, 52; head of the Navy's Torpedo Hydro-Propulsion Research Section; 27 February.

Paul F. Frank, 49; chief of the division of bacteriology, Naval Medical Research Unit No. 4, Great Lakes, Illinois; 3 March.

Hornell Hart, 78; professor emeritus of sociology at Duke University; 27 February.

Eight U.S. educators were killed in a plane crash in Vietnam on 23 March. They were on a tour for the U.S. Agency for International Development that was studying South Vietnam's high school and college problems. The educators were:

James H. Albertson, 41; president of Stevens Point State University, Wisconsin.

Harry F. Bangsberg, 38; president of Bemidji State College, Bemidji, Minnesota.

A. Donald Beattie, 45; dean of the School of Business and Economics, Whitewater State University, Wisconsin.

Vincent F. Conroy, 44; director of the center for field studies, Graduate School of Education, Harvard University.

Howard G. Johnsoy, 48; dean of academic affairs, Gustavus Adolphus College, Minnesota, and former assistant to the dean of International Programs, University of Minnesota.

Robert La Follette, 72; higher education adviser in the U.S. Operations Mission, Saigon, and former head of the department of social science at Ball State Teachers College, Indiana.

Arthur D. Pickett, 51; director of the honors program in the University of Illinois's Chicago division.

Melvin L. Wall, 54; head of the department of plant and earth sciences, River Falls State University, Wisconsin.

Erratum: In the report, "Thyroxine: effects of neonatal administration on maturation, development, and behavior," by S. Schapiro and R. J. Norman (10 Mar., p. 1279), the first line of Fig. 2 should read: "Fig. 2. Electroencephalograms from the cortex of thyroxine-treated and control infant. . . ."

Erratum: In the report "Mechanism of antibody synthesis: size differences between mouse kappa chains" by W. R. Gray *et al.* (27 Jan., p. 465), the headings of the final two columns of Table 1 should be reversed to read "Transversions" and "Transitions."