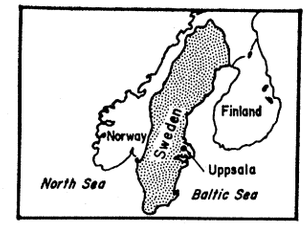
REPORT FROM EUROPE

Biochemical Laboratory at Uppsala



Uppsala, Sweden. The biochemical laboratory of Arne Tiselius at Uppsala is a leader in developing techniques for the chemical separation of proteins and other substances of biological interest. Tiselius himself won the Nobel prize in 1948 for his work on electrophoresis. More recently, his co-workers have developed another important fractionating technique, called gel filtration or exclusion chromatography, which has interested researchers in many fields [*Science* **141**, 13 (1963)].

Tiselius and his colleagues are quick to point out that they do much more than carry out research on techniques; they are users of their own separation methods. The techniques, which grew out of an interest in biochemical mechanisms, are being applied at the laboratory to studies of the structure and function of nucleic acids and proteins.

Early History

The department of biochemistry of Uppsala University had its beginnings in the late 1940's, about 10 years after Tiselius had become the first to hold a new professorship in biochemistry, for studying the physical and chemical basis of life. The professorship was the gift of the late Major Herbert Jacobsson and his wife Karin, of the Brostrom shipping family. Tiselius did much of his important early work in a few rooms in the department of physical chemistry, which was headed by The Svedberg, inventor of the ultracentrifuge and himself a Nobel prize winner. Money from the Rockefeller and Wallenberg foundations supported Tiselius's biochemical research for the first 5 or 6 years after he became professor. Had it not been for this support, he notes, the university would have hesitated to accept endowment for a chair in so new a field as biochemistry.

It was only after World War II that the Swedish parliament voted funds to run the department of biochemistry. The award of a Nobel prize to Tiselius accelerated government plans for constructing a building to house the department. In 1952 Tiselius and his collaborators moved into the new gray structure at the Kemikum, the complex of chemistry buildings west of the English Park.

Today, the department's laboratories, workshops, and offices, which occupy 2500 square meters on five floors of this building, have become crowded, not only because of expanding research activity but also because of expanding student enrollment in laboratory courses in biochemistry. The number of chemistry students choosing biochemistry has grown, and will grow very much faster now that changes in the curriculum of the Swedish gymnasium (equivalent to the last 2 years of high school and the first 2 years of college in the United States) have made biochemistry a required subject for all biology teachers. The crowding has caused the department to restrict the number of visiting foreign scientists, requiring it to choose those who can work independently and those who can come for short periods. Tiselius and his co-workers talk regretfully of this development, because there have always been many visitors and their work and later collaboration have been important to the laboratory. Construction of a new building for the biochemistry department is now planned as part of the center for basic medical research which will be built on the Artillery Field in Uppsala, but it probably will not be ready for 4 or 5 years.

Of the 62 people now working in the laboratory, 38 are researchers, ranging from graduate students up to senior scientists. The others are technicians. There are half a dozen visiting scientists, chiefly Americans. The total staff has been diminished recently because co-workers of Bo G. Malmström, until recently an enzyme chemist at Uppsala, are following him to the University of Göteborg, where he occupies a new chair of biochemistry.

The biochemistry department at Uppsala is now large enough to permit Tiselius to spend much of his time on problems of money-raising, money-handling, and staff changes. He is leaving much of the planning of the new building to Jerker Porath, a pioneer in the development of gel filtration, who will become an associate professor this summer. Every two weeks or so Tiselius calls a meeting of the senior researchers and of one or two of the younger scientists to discuss research leads, blind alleys, and other matters that govern the direction of the laboratory's studies.

Outside activities demand much of Tiselius's time. As early as 1946 he served as the first chairman of the Natural Sciences Research Council, one of several such councils created after completion of a World War II study of the best way to support scientific research in Sweden. He is now president of the Nobel foundation, which administers the funds yielding the prize money, and a member of Prime Minister Tage Erlander's scientific advisory council, established at the end of 1962. The advisory council has been studying ways to help establish priorities in public spending for science, pushing for better statistics on private and public support of science and on allocations of scientific manpower, setting up a new fund for technical development, and quashing plans for establishing a separate space-research council in Sweden.

Work on Gamma Globulins

Porath is still deeply engaged in research. A 43-year-old organic chemist and former pupil of Arne Fredga, he joined Tiselius's group in 1950 because, he says, he was "so bad at fractionating organic materials." Since 1951, when he spent a year in the California laboratory of C. H. Li, he has worked steadily in Tiselius's lab-

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The Kemikum. [American Swedish News Exchange]

oratory. Many of these years he spent in developing separation methods, gel filtration being the latest. Now, with a group of more than 15 people, he is working to purify both gamma globulins and specific blood group substances against which the antibodies act. He is particularly interested in the exact interaction between gamma globulins and the antigens of the blood group substances. His main collaborators are Hans Bennich of the biochemistry department and Johan Killander of the Academic Hospital in Uppsala.

Porath began his work on gamma globulins about a year and a half ago. His studies led to the separation, with visiting scientist Nobuo Ui, of subfractions of plasma having a sedimentation rate of 7*S*. With a series of glycine-rich solvents, he and his co-workers, by means of ultracentrifugation, zone electrophoresis, and gel filtration, separate fibrinogen, low-density lipoproteins, and fast-moving proteins from the plasma. The 7*S* gamma globulins, with a molecular weight around 150,000—probably an assembly of two pairs of sub-units—are the most numerous, and Porath's group is concentrating on them.

Now that a method of segregating gamma globulins has been developed, Porath and Bennich are investigating the structure of the rather small portions (peptide chains) of these serum proteins which take part in binding with the antigen. Porath also is about to collaborate with Bernard Witkop of the National Institute of Arthritis and Metabolic Diseases in Bethesda, Maryland, in studies on the use of Witkop's techniques of bond-breaking with such reagents as cyanogen bromide and *N*-bromo-succinamide. The cyanogen bromide is of interest because it attacks the bonds of the sulfur-containing amino acid methionine in polypeptide sequences.

Jore Kristianssen at the Uppsala biochemical laboratory has obtained fairly pure samples of blood group "A" substance from man and pig. "A" substance can be split into smaller subunits which are still active in inhibiting agglutination. These smaller units may be useful in studies of the model system Porath uses: "A" substance and the corresponding gamma globulin antibody.

Enzyme Structure and Function

Studies of enzyme structure and function engage the attention of present and former associates of the biochemistry department. Assistant professor Andreas Rosenberg, a native of Estonia, is using optical rotatory dispersion techniques to get a measure of the amount of alpha helix in the alcohol dehydrogenase of the liver. Despite difficulties with abnormal dispersion with absorption bands, Rosenberg and Takashi Yonetani of Hugo Theorell's enzyme study group at the Nobel Medical Institute in Stockholm feel that they have demonstrated a marked increase in the percentage of alpha helix when the enzyme is bound to coenzyme in a ternary complex. The number of turns of helix affects the dispersion of light directed at the enzyme.

Rosenberg, who recently worked for 2 years with Rufus Lumry at the University of Minnesota, is also using optical rotatory dispersion to study the alpha helices of carbonic anhydrase. This enzyme, with a molecular weight of about 30,000, is found in red blood cells. It speeds up a natural reversible reaction which either binds or releases carbon dioxide. Three forms are known —one bovine and three human. Workers at Uppsala have concentrated on form C of the human enzyme, and John T. Edsall's group at Harvard has studied the B form. The activity of form C is about three to five times that of form B, and the scientists are seeking a structural explanation of this difference. The work on alpha helix is related to many other studies of the active site and crystal structure of carbonic anhydrase. Malmström had a prominent part in these studies, and now that he has moved to Göteborg he will begin studies of the B form. Malmström, S. Lindskog, and P. O. Nyman studied the amino acid composition of the enzyme and showed the essential role of zinc in its activity. Substitution of other metals for zinc was found, in most cases, to reduce the enzyme's activity; cobalt is an exception.

Malmström, Nyman, Björn Tilander, and Bror Strandberg reported at the first meeting of the Federation of European Biochemical Societies, on 24 March, that the sulfhydryl (SH) group attached to the enzyme's single cysteine residue did not play a role in metal binding, and that the enzyme had an esterase activity. This reaction and the reaction with carbon dioxide are both inhibited by the mercury-containing sulfonamide groups used for x-ray diffraction studies.

Tilander, an organic chemist, and Strandberg, a crystallographer, began their collaboration 3 years ago when Strandberg returned from Cambridge,

Arne Tiselius

England, where he had worked in Kendrew's group. They succeeded in crystallizing the human C form of carbonic anhydrase; then Tilander prepared derivatives of the enzyme, and those, bound to three different sulfonamides, have been studied by x-ray diffraction, along with the native enzyme.

In the different derivatives the heavy mercury atom is in slightly different positions, and thus the derivatives give information about the phase of the x-rays, essential for interpreting the scattering patterns yielded by the bombarded crystals. Strandberg has just completed his work on two-dimensional pictures of the electron densities of the crystal, and he plans to construct a three-dimensional synthesis of the molecule to a resolution of 5.5 angstroms.

Partition Method of Studying DNA

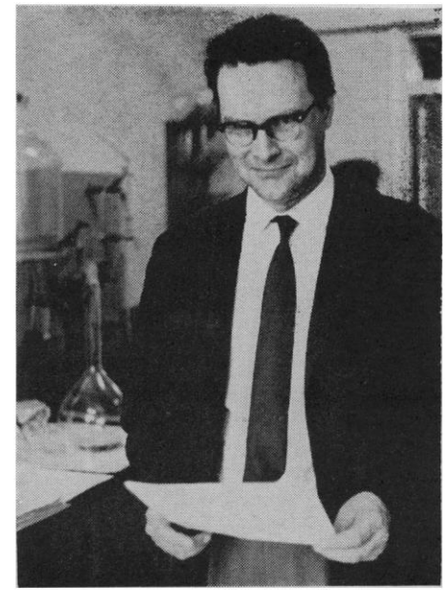
The youngest of the laboratory's assistant professors, 34-year-old Per-Ake Albertsson, is using a partition method he developed to study normal double-stranded DNA and heat-separated single-stranded DNA [*Science* **141**, 13 (1963); Albertsson, *Partition of Cell Particles and Macromolecules* (Wiley, New York, 1960)]. Research in 1961 with DNA extracted from calf thymus seemed to show that some DNA molecules are more sensitive to heat than others. When an ultraviolet light-density test indicated that the separation of strands was 50-percent complete, a partition test was run. The heat-denatured molecules migrated to one half of the partition system, the native DNA to the other. Instead of all the molecules' being half unwound, some were still complete, others entirely unwound. This phenomenon is now being studied in more detail by Albertsson and his collaborators. They are testing the simpler DNA of *Escherichia coli* and of bacteriophage T2. Albertsson has also experimented with artificial polyadenylic and polyuridylic acids.

Albertsson describes the discovery of the partition method, first announced in 1956, as the result of a combination of ignorance and accident. While seeking a surface-acting agent to free chloroplast-containing material tightly bound to calcium phosphate during a chromatographic process, he tried polyethylene glycol, more or less idly, not knowing that this compound is used as a precursor of detergents. Polyethylene glycol did cause the green matter to migrate to the top of a liquid layer above the calcium phosphate suspended in a phosphate buffer in a test tube. As a result, Albertsson conceived the idea of a liquid-liquid separation technique in which the often-used mixture of water with an organic solvent—a mixture frequently unsuitable for studies of biological products—would be avoided. One successful combination was polyethylene glycol, dextran, and water. In this mixture two distinct phases develop, each of them containing up to 90-percent water.

Recent Studies

The fractionation program of Tiselius's department has been of help to assistant professor Hans G. Boman's group in their recent studies of RNA. Boman heads an eight-person group in molecular biology. His work began under a National Institutes of Health grant in 1960 and continues with financing from the Swedish Natural Science Research Council. The group's research focuses on the methylation of RNA and on the resistance of *Escherichia coli* to penicillin.

Much of this work on the methylation of soluble RNA, the small RNA



Jerker Porath

fractions which bring amino acids to the point of assembly, was reported in 1963 [*J. Mol. Biol.* **7**, 254 (1963)]. Carried out in collaboration with Ingvar Svensson, these studies paralleled in part the work of the American researcher E. Borek. The methyl groups can convert the uracil of RNA to thymine and also bind to other sites in the molecule. They are donated by the amino acid methionine. The methylation of soluble RNA was first noticed during studies of the formation of methionyl RNA, an intermediate in protein synthesis.

More recently, Boman and his associates have collaborated with visiting researcher Julian Gordon on studies of the methylation of ribosomal RNA. They want to know if this methylation has a role in regulating the assembly of ribosomes. This work will be reported in New York in July at the 6th International Congress of Biochemistry.

Boman first became interested in RNA and protein synthesis while working in the laboratory of Fritz Lipmann at the Rockefeller Institute in 1958–60. Before this he had spent many years studying ion-exchange chromatography of enzymes. Like many other workers in Arne Tiselius's laboratory, he has moved from studies of chemical separation techniques to studies of biochemical processes in living things.

—VICTOR K. MCELHENY