EUROPEAN MOLECULAR BIOLOGY LABORATORY

ANNUAL REPORT

1975

Note: This report also includes an account of research carried out prior to 1975.

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1. Introduction

1.1 This is the first of a series of annual reports on the work of the European Molecular Biology Laboratory. It is, however, anomalous in that it describes the research activities not only during the year 1975 but also prior to that year from the beginning of EMBL. One such activity - the research using synchrotron radiation at DESY in Hamburg - in fact started, under other auspices, as long ago as 1970 and was later taken over by EMBL. Apart from this, the scientific beginnings were much more recent.

Although the idea of a European laboratory of molecular biology had been discussed in scientific circles since the end of 1962, it was not until 10 May 1973 that nine Governments, and a few days later a tenth (Austria, Denmark, France, Federal Republic of Germany, Israel, Italy, the Netherlands, Sweden, Switzerland, United Kingdom) signed the Agreement establishing it, and on 4 July 1974 that this Agreement was ratified by a sufficient number of Member States and the Laboratory took on a legal existence and was in a position to recruit scientific staff.

The first scientist joined the Heidelberg staff on 1 September 1974 and therefore, with the exception of the above-mentioned group at DESY for which special staffing arrangements had been made, the research activity described in this Report covers the period September 1974 until December 1975. Many of the research groups whose work is summarized have in fact been working for a much shorter time (two groups joined in 1974, three in the first half of 1975 and four in the second half of 1975).

1.2 In advance of the signature of the Agreement a site for the Laboratory in Heidelberg had been generously offered by the German Government, active planning had begun, and an architect had been selected and, in collaboration with the committees of EMBO, had drawn up a plan for the Laboratory building. Thus it was possible to begin actual building operations as early as October 1975, nevertheless the Laboratory will not be able to occupy its permanent home until late in 1977 or early in 1978.

It was thought important to begin scientific work before this, so steps were taken to borrow existing laboratory accommodation in Heidelberg in order that a start could be made. As everywhere else, such accommodation is hard to find; nevertheless through the courtesy of three local institutions - the Max-Planck-Institutes für Kernphysik und für medizinische Forschung the Deutsche Krebsforschungszentrum and the University of Heidelberg - a total of some 1,800 m² of laboratory and office space was secured.

It had originally been hoped that enough temporary accommodation could be found to enable the Laboratory to build up steadily towards its planned full size early in 1978 when the permanent building was due to be completed. Unfortunately these hopes were disappointed; the temporary accommodation amounts to only about one quarter of the space that will be available in the permanent building. After a rapid build-up beginning late in 1974 and completed in mid-1976, the staff complement must perforce remain virtually constant until the building is ready. Fortunately the completion date is likely to be some months earlier than originally forecast.

2 The research policy of the Laboratory during the initial phases of build-up

2.1 The philosophy underlying the proposal made by the Council of EMBO early in 1970* for the foundation of a European Laboratory was later accepted by the ten Member States in all essential details, with the exception that, for financial reasons, the size of the plan was cut by some 25%. To summarize this philosophy very briefly, the purposes of the Laboratory are:

a to carry out scientific research of the highest quality in molecular biology and related fields

b to engage particularly in activities that are difficult or impossible to pursue in the national laboratories

c to provide various kinds of services which will benefit the development of molecular biology in Europe, and which will complement, rather than rival, the activities of national laboratories.

2.2 The plan put forward by EMBO, and designed to fulfil these objectives, included the following elements:

a A very strong Instrumentation Division with the functions of developing the increasingly sophisticated kinds of instrument increasingly needed in biology, and which could not easily be developed in national biological institutes with their generally very small resources of workshops and design staff.

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* CEBM/70/12 "Revised proposal for a European Laboratory of Molecular Biology", February 1970.

b a Division of Cell Biology, which should concentrate on newly emerging fields (for example, animal cell genetics) rather than well-trodden areas (bacteria, bacteriophage), and which should where possible choose projects requiring the development of new instrumentation.

c a Division of Biological Structures, concentrating on more complex structures (for example, cell organelles, membranes and viruses) rather than on the study of relatively less complex structures (for example, proteins) in which many European centres were already very active; and, as in the case of cell biology, providing challenges for the development of new instrumentation.

d special laboratories designed to make available to biologists throughout Europe certain very important facilities that were either unique, or were otherwise available in few other places. These included the two outstations at DESY in Hamburg (which provides very high-intensity x-ray beams) and at ILL in Grenoble (providing facilities for neutron diffraction).

e facilities for advanced training whether by providing temporary staff positions or space for postdoctoral fellows, or mounting special training courses.

f in a more general way, the carrying out of activities that would encourage the European biological community to participate either for short or long periods, thus providing a focal centre of European biology in the interests of better communication and cooperation across national boundaries.

2.3 Given the very limited temporary accommodation available to the Laboratory there have been many difficulties in the way of implementing this plan during the build-up phase. The effects of shortage of space have been accentuated by the fact that the staff have had to work in three different laboratories in different parts of the city. In this situation, it was difficult to achieve the necessary "critical mass" to enable particular activities to "take off". In particular, work in instrumentation, intended to be the largest single element in the Laboratory, had to be begun at a very low level since it was impossible to provide all the necessary special workshops and technical facilities. No laboratory space has been available for teaching purposes. Activity at the Hamburg outstation has been delayed by the need to construct special laboratories and by the inadequacy of workshop capacity to construct the instrumentation needed. Arrangements for inaugurating the work of the Grenoble outstation were only completed late in the period under review.

2.4 The activity which has actually taken place, as described in this Report, is the result of policies that have been frequently and intensively discussed between the Director-General and the

members of the Scientific Advisory Committee, whose help and advice have been of crucial importance throughout. In these discussions it became clear that it was not possible to implement the original policies in a thorough-going way during the period of build-up. Rather, it seemed better to establish on a small scale a number of activities within the chosen areas; these would hopefully point the way towards the development of the final pattern that could only clearly emerge when the Laboratory attained its full size in its permanent quarters.

3 The scientific research of the Laboratory

3.1 Research activities in the outstation at DESY, Hamburg

(K C Holmes see also pp 26 and 50)

3.1.1 X-ray diffraction is one of the most important techniques for the study of biological systems, not only of molecules such as proteins and nucleic acids, but also of cell organelles and tissues - muscle fibres, connective tissue, membranes and many others. A major limitation in the past has been that most biological structures consist mainly of water, and therefore diffract x-rays very weakly. Even using the most powerful commercial x-ray tubes exposures of many hours or even days are required under some circumstances. An essential characteristic of life is motion; living organisms are dynamic and one would wish to study biological structures as a function of time - ideally to make successive x-ray diffraction photographs of a muscle fibre, for example, in milliseconds during the process of contraction. Even if, in other systems, static studies are adequate, the very long exposure times demanded in conventional techniques represent a very serious limitation on what can be achieved.

3.1.2 Electron synchrotrons were developed by high-energy physicists for their own purposes. It is an inescapable consequence of the fundamental laws of physics that a stream of electrons, constrained to move in a curved path as in a synchrotron, must emit powerful beams of radiation including x-radiation. From the physicists' point of view the radiation represents an inevitable loss of energy; for the biologist, as it turns out, the x-radiation emitted by an electron synchrotron represents an immense new potentiality of the x-ray diffraction technique - because its intensity is many times greater than that obtainable from the most powerful conventional x-ray tube. It makes possible the performance of experiments that are difficult or impossible to carry out by conventional methods.

3.1.3 These potentialities were first appreciated by Professor K C Holmes and Mr G Rosenbaum of the Max-Planck-Institut für Medizinische Forschung in Heidelberg as long ago as 1969, and

since that time they and their colleagues have carried out experiments using the DESY synchrotron, with financial assistance from the Deutsche Forschungsgemeinschaft. At a later stage Professor Holmes most generously decided to hand over this project to EMBL, and it was already incorporated into the 1970 EMBO plan for the Laboratory. The idea was to build up in Hamburg a facility that could be used by molecular biologists coming from all over Europe, a facility more powerful than any other available elsewhere; and to create an in-house research group which, carrying out its own program, would at the same time provide the services needed by visiting workers. With generous special help from the German authorities the necessary laboratories have now been built and the required special instrumentation is being constructed in the EMBL workshops in Heidelberg. This development is precisely in the spirit of the basic philosophy enunciated in paragraph 2.1 above ("to engage particularly in activities that are difficult or impossible to pursue in the national laboratories"), and in its present phase represents the most active line of research being pursued by the EMBL, and the one to which the highest priority has been given.

3.1.4 A more detailed account of the research at DESY is to be found on p. 26 . In addition, and in view of the long and interesting history of this development before the official foundation of EMBL, Professor Holmes has contributed a historical account which is annexed to this report (p. 50). The Laboratory is much in the debt of Professor Holmes not only for his early enthusiasm for the project, and for handing it over to EMBL, but also because in more recent times he agreed to act part-time as Head of the Outstation until a permanent Head could be found.

3.2 Research activities in the Central Laboratory

3.2.1 Biological membranes

3.2.1.1 At an early stage the (Provisional) Scientific Advisory Committee expressed the view that one or two research groups should be established in the Laboratory in the field of membrane studies. This field is of the greatest importance and interest at the present time. On the one hand the functions of biological membranes are vital to every living cell - in separating the cell from the world outside, in providing subdivisions between the organelles within the cell, in regulating the passage of materials across themselves - actively and selectively - in providing sites for the synthesis of proteins, for energy conversion, for hormone receptors, and in many other ways. On the other hand the structures of membranes, though known in general outline, are not understood in detail; and therefore their mode of functioning cannot yet be fully understood in molecular terms.

In the present context it was thought that membrane studies would provide a useful bridge between the two main research divisions of the Laboratory. In terms of function, they are important in understanding fundamental problems of cell biology; as subjects for studies of structure, they fit precisely into the proposed specifications for the Division of Biological Structures.

In order to select appropriate topics for EMBL work in this field, a Workshop on Biological Membranes was organized at Schönau near Heidelberg in October 1973. This was attended by several members of the (Provisional) Scientific Advisory Committee. As a consequence the two research groups whose work is described below were established in the Laboratory. The first of these groups perhaps fits most appropriately within the Division of Cell Biology, the second in the Division of Biological Structures; but because such classifications are in any case arbitrary, the two are here considered together.

3.2.1.2 Research on virus membranes

(K. Simons; see also p. 29)

In many important animal viruses (including those responsible for influenza, smallpox, yellow fever and measles) the genetic nucleoprotein component is assembled in the cytoplasm of the host cell and then acquires an outer membrane by budding out through the host cell plasma membrane. Thus the eventual virus membrane is of great interest not only in its own right but because of its evident close relationship to the plasma membrane of the host cell. In the present research Semliki Forest Virus has been chosen as the experimental subject.

By the action of various detergents the principal membrane proteins can be separated and their interactions studied by physico-chemical, immunological and electron-microscopical methods. Studies of the reassembly of the virus components into infectious virus particles are in progress. This research should throw light on the structural make-up of the membrane and on the mechanism of virus assembly *in vivo*; there may also be the possibility of developing vaccines since the membrane proteins carry the antigenic determinants responsible for immunity to viral infection.

3.2.1.3 Research on cytochrome b from the mitochondria of Neurospora crassa

(H. Weiss; see also p. 30)

The mitochondria of cells, complex organelles made up of highly convoluted membranes, are the normal sites of oxidative phosphorylation, the complex chain of chemical reactions

responsible for supplying the cell's requirements for energy. These reactions are mediated by a large number of molecules, especially enzymes and cytochromes, located in the mitochondria. A very important example of such a molecule is cytochrome b, a protein forming part of the mitochondrial membrane.

The work of this group is directed towards improving methods of extracting cytochrome b from the mitochondrial membrane in pure form, studying its structure and amino-acid sequence, and drawing conclusions about its biosynthesis. This research has involved the development of new techniques for the separation of the water-insoluble proteins which are found as components of membranes, and which cannot be handled by the normal techniques of protein chemistry. Thus, besides throwing light on the energy-producing systems common to most types of cell, this research is likely to result in the development of new technologies of importance in handling water-insoluble proteins in general.

3.2.2 Division of Cell Biology

3.2.2.1 Four research groups have been established, in three important areas of cell biology.

The first of these areas, and the work of two of these groups, concerns the structure of chromosomes and the regulation of gene expression. The characteristics of living organisms are determined by the nature of their genes - lengths of nucleic acid in which the sequence of four different bases carries a genetic message encoded in the genetic code. Successive genes form parts of very long nucleic acid molecules which are condensed with many different types of protein molecule to form the microscopically-visible structures known as chromosomes. The way in which the nucleic acid is organized through the mediation of structural proteins (histones) is the subject of very active research in many other laboratories. The work at EMBL has concentrated on another area of research, namely the role of the very many other proteins forming part of the chromosomes (non-histone proteins) in the superstructure of chromosomes, in enzymatic processes and in processes of regulation which determine whether particular genes are "switched on" or "switched off" in different cells at different stages of development.

The second area is morphogenesis. All the cells of a multi-cellular organism are derived by successive divisions from a single fertilized egg-cell, and all contain the same set of genes. Yet in the adult organism there are many different types of cell forming parts of many different structures, performing different functions and making different products. How does this process of diversification, or differentiation, come about? What are the mechanisms of control? This is the central problem of morphogenesis.

The third area is the structure of the nervous system. As is well known, the nervous system of a higher animal is the most complex of all forms of living organization; and as such its structure is least of all understood at the molecular level. The study of the structure of the nervous system might therefore seem premature in a laboratory of molecular biology, which is the branch of biology particularly devoted to the study of living systems at the level of the molecules making them up. Nevertheless, because of the intrinsic importance and fascination of getting insights into the mechanism of these extremely complicated structures, because the central nervous system of man represents the crowning achievement of life - but also because some recent developments elsewhere have shown that indeed some aspects of the functioning of nervous systems can be interpreted in molecular terms - it seemed appropriate to establish some research activity in this field.

3.2.2.2 Research on chromosome structure and gene regulation

(U. Plagens; see also p. 32)

This work uses as experimental material the chromosomes of the salivary glands of insect larvae. These chromosomes have many advantages; for example, they can be separated by micro-techniques, they are metabolically active and exhibit "puffing", they consist of thousands of parallel nucleic acid strands so they are very much larger in size than those of normal cells. Non-histone proteins have been extracted from these chromosomes in quantities sufficient for separation and chemical examination, and for studies of the differences between proteins from different parts of the same chromosome, or from the same part in different states of activity.

3.2.2.3 Research on non-histone chromosomal proteins

(E. Jost; see also p. 34)

The development of methods for the separation and identification of non-histone proteins makes it possible to examine the role of these proteins in various transformations and changes undergone by the nuclear material in different processes; for example, studies of their evolutionary conservation, correlations between the induction of specific messenger RNAs with the appearance and disappearance of chromosomal proteins, the role of these proteins in determining the preferential loss of chromosomes from interspecific hybrids, their relationship to the action of intercalating agents, their connexion with the inertness and reactivation of the erythrocyte nucleus, and the study of potential differences between the non-histone proteins of normal and malignant cells.

3.2.2.4 Research on the control of morphogenesis in hydra

(H. C. Schaller; see also p. 37)

Hydra is a very simple multicellular organism that can be used for studies of morphogenesis and of the way in which pattern formation and differentiation may be controlled by the presence of gradients of specific chemical substances. It appears that in hydra the number of such substances is small, and at least one of them is chemically quite simple, being a peptide containing around ten amino acids; furthermore, the same (or a very similar) peptide is present in the hypothalamus of mammals. In hydra it stimulates the formation of the head and of new buds, and has other activities as well. The peptide is available in extremely small amounts, and the determination of its amino-acid sequence, now being attempted, is a challenging problem at the limit of the available techniques.

3.2.2.5 Research on the neuronal arrangements which mediate vision in insects

(N. Strausfeld; see also p. 37)

The neuronal arrangements in the visual region of insects have been chosen as a technically convenient system. Their study calls for extensive light-microscopical and electron-microscopical surveys of the insect brain, and special staining techniques have been developed for marking individual neurons. The object of these studies is to reconstruct, from an extremely large number of serial sections, the very complex three-dimensional structure of the relevant region of the insect brain; both the sheer quantity of data, and the problem of conceptualizing the three-dimensional structure, require the use of a computer equipped with graphics facilities, and the development of this facility will be carried out in collaboration with the computer group. A basic knowledge of the neuroanatomy will make it possible to undertake studies of normal development and the establishment of connectivities between neurons, and also of abnormal development by investigating the neuronal errors found in behaviourally mutant insects.

3.2.3 Division of Biological Structures

3.2.3.1 Since there is already substantial activity in the field of biological structures at the two outstations, it was decided that, in view of the very limited temporary accommodation available in Heidelberg, the development of research in this area at the central Laboratory should initially be accorded a lower priority than that given to cell biology.

Research in biological structures can most conveniently be classified in terms of the techniques used. These include (a) diffraction (x-ray and neutron), (b) electron microscopy, (c) nuclear magnetic resonance, (d) other spectroscopic and physico-chemical techniques, (e) biochemical techniques (for example, determination of amino-acid and nucleotide sequences). Given the limited space available, and the fact that the diffraction techniques were being actively pursued at the outstations, the obvious choice for an initial project in the Division of Biological Structures at the central Laboratory was electron microscopy, and this for two principal reasons - first, that other research groups working in the Laboratory required electron microscope facilities to be available (for example, virus membranes and neurobiology) and second, that the development of Scanning Transmission Electron Microscopy (STEM) had been selected as an initial project for the Division of Instrumentation, and close collaboration between the two projects could be foreseen.

Further activity in this Division, in x-ray diffraction, protein chemistry and sequencing, has been planned for 1976 and on a larger scale after the completion of the permanent building.

3.2.3.2 Research on High Resolution Electron Microscopy

(K. Leonard; see also p. 42)

This group was installed only just before the end of the period under review. It intends to establish a fully-equipped electron microscopy laboratory using as principal instruments a Phillips 401 conventional transmission electron microscope and the STEM under development in the Division of Instrumentation. An optical diffractometer and a computer image processing system will be available.

Besides collaboration with other groups in the Laboratory, the group plans to concentrate on developing better specimen preparation techniques, on low dose imaging techniques, and on assessing the applicability of different detector systems in the STEM to biological problems.

3.2.3.3 Plans were already made in 1975 to instal a second electron microscope group, under H. Delius, early in 1976. This group will concentrate on the use of electron microscopy as a tool in nuclein acid research. This, together with the above-mentioned group of K. Leonard, the STEM development in the Division of Instrumentation, and the electron microscopical work of the neurobiology group of N. Strausfeld, will put the Laboratory in a position to mount a very strong activity in the biological applications of electron microscopy.

3.2.4 Division of Instrumentation

3.2.4.1 As has already been pointed out, space limitations have so far made it impossible to initiate more than a very restricted activity of what will eventually become the largest Division of the Laboratory. It was decided to concentrate in the first instance on providing the basic workshop and computing facilities needed by other groups, and in addition to embark on one single instrumentation project - the development of the scanning transmission electron microscope (STEM) - which could be initiated without the presence of other extensive facilities.

3.2.4.2 Workshops

A small mechanical workshop (under H. Flösser) was established at the beginning of the year, and an electronic workshop in August. The main activity of these workshops was the construction of a new optical bench for the outstation at DESY, together with the associated electronics; and in addition to provide assistance to the other groups in the Laboratory.

3.2.4.3 Position-sensitive detectors

(A. Gabriel)

These detectors were originally developed for applications in nuclear physics, but it has emerged that they can be highly efficient as detectors of neutrons and x-rays in some types of diffraction experiment. A. Gabriel who has great experience in the design of these detectors, and who is at present located with the outstation at Grenoble, has embarked on a program of developing detectors of this type in a two-dimensional form both for neutrons and for x-rays. These detectors will be of great importance for the biological work at Grenoble and Hamburg, and in addition Gabriel has been providing assistance and equipment to other laboratories in Europe wishing to use them.

3.2.4.4 Computer group

Plans were already made in 1975 for the establishment, early in 1976, of a Computer Group under R. F. Herzog. This group would, in the first instance, equip the Laboratory with mini-computer systems for data acquisition and processing of STEM images, for the collection and analysis of x-ray images, and for the manipulation of graphical information in the neurobiology project. It would also be responsible for the general supervision of computer activities at the two outstations, and for the provision of their computer facilities at Heidelberg. At a later stage it plans to become involved with other projects for the application of computers to biological research in general.

3.2.4.5 Development of Scanning Transmission Electron Microscopy (STEM)

(A. V. Jones; see also p. 43)

The STEM is a new type of electron microscope containing high resolution with the potential to reduce the susceptibility of specimens to electron-beam induced degradation, to decrease specimen contamination, and to provide images of high contrast - all these being limiting factors in the biological applications of conventional transmission electron microscopy. Besides this, a number of alternative modes of operation and detector systems are possible, and the value of these in biological research is yet to be explored. The resources of the Laboratory are still inadequate to make practicable the design of a completely new instrument, and it is therefore planned to purchase a commercial instrument (Vacuum Generators HB 5) with which a major program of instrumentation and computer development will be undertaken, and the very promising potentialities of this type of electron microscope in biological research can be explored.

3.3 Research activities in the outstation at ILL, Grenoble

3.3.1 It was proposed in a document submitted to the European Molecular Biology Conference in 1971* that the Laboratory should establish an outstation at the Institut Laue-Langevin in Grenoble. This Institute possesses a high-flux neutron reactor which is unique in Europe. It has already been known for some years that high-intensity neutron beams have very important applications in biological research. As an alternative to x-ray diffraction, neutron diffraction can reveal details of biological structures that are impossible to establish by x-ray methods, especially the positions of hydrogen atoms; neutron scattering can give important information about the structures of non-crystalline organelles such as ribosomes; and by varying the heavy water/water ratio in the circumambient solution the contrast between a biological structure and its surroundings can be changed.

3.3.2 Very many European biologists are already carrying out programs of research at the ILL. The purpose of EMBL in establishing an outstation there was to provide on the spot the facilities needed for biological research in an institute otherwise devoted to work in physics and chemistry. These facilities would include biochemical laboratory accommodation needed for the preparation of labile biological specimens that cannot be transported over long distances, and for the biochemical

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* CEBM/Lab/71/32 "Preliminary proposal for an EMBO neutron diffraction facility at Grenoble" October 1971.

manipulation of specimens during a research project; special instrumentation; x-ray diffraction equipment for checking specimens before exposure to the neutron beam; electron microscopy; computer facilities; and expert advice on neutron methods for biologists without previous experience of the technique. As in Hamburg, such services cannot be provided unless a strong scientific group with its own program of research, and requiring the same services, is working on the spot.

3.3.3 A number of problems had to be solved before the outstation could become a reality, especially that of laboratory accommodation which could not be provided within the ILL. Through the courtesy of an adjoining institution, the Centre d'Etudes Nucléaires de Grenoble, excellent laboratories were eventually secured on loan and, with the enthusiastic cooperation of the Institut Laue-Langevin the outstation was eventually established in September 1975 under Dr. A. Miller, who had been seconded by the University of Oxford. Up to the end of the period under review, although some research was begun the main activity of the outstation was to settle into its laboratories and to begin establishing the services and facilities needed for its own use and for the use of visiting groups of biologists.

3.4 Other activities of the Laboratory

3.4.1 Postdoctoral fellows and students

The Laboratory was already able in 1975 to receive a few postdoctoral fellows for research in various groups. These are listed on p. 16, and it is hoped that many more will be received in future years. It should be pointed out in this connexion, that the Laboratory has extremely limited funds of its own for paying the stipends of postdoctoral fellows, and it will be necessary for the great majority of these to come to the Laboratory with fellowships awarded by outside bodies.

3.4.2 Visiting workers

It was an essential part of the original plans for EMBL that the Laboratory should provide hospitality for substantial numbers of visiting workers (whose personal expenses and salaries could, however, not be met by the Laboratory). A number of such visitors have already been received both by the Heidelberg Laboratory and by the Outstation at Hamburg in spite of the limited accommodation at present available, and the names of these are listed on p. 17. As in the case of postdoctoral fellows, it will be the aim of the Laboratory to increase the number of visiting workers very greatly in the future, as accommodation and facilities permit.

3.4.3 Seminars

In addition to internal seminars at which members of the Laboratory discuss their work, a series of public seminars was inaugurated in May 1975, the first most appropriately being delivered by Dr. Max Perutz, Chairman of the M.R.C. Laboratory of Molecular Biology in Cambridge and the first President of EMBO. The public seminars given in 1975 are listed on p. 49.

3.4.4 Library

The nucleus of the Laboratory's library was established towards the end of the year. An early start with assembling the library was thought advisable, both in the interest of the staff already appointed, and also because the rapidly increasing cost of books and journals made it financially beneficial to acquire standard works and back numbers of journals as rapidly as possible.

3.4.5 Relations with EMBO and EMBC

The original idea of founding a European Laboratory of Molecular Biology came from EMBO, a private organization of biologists with which the Laboratory has no legal connexion, and which depends for financial support on the European Molecular Biology Conference, an intergovernmental body within which the plans for founding the Laboratory were first discussed by governments, but which is also legally quite separate from the Council of the Laboratory.

In spite of these legal separations between EMBL on the one hand, and EMBO and EMBC on the other, the purposes of the organizations in fostering the development of molecular biology in Europe, are just the same though the means they adopt are different, the activities of EMBO (with financial support from EMBC) consisting of schemes for fellowships, travel grants and lectureships, and of workshops and training courses. It therefore seemed scientifically important to maintain as close connexions as possible between the three organizations.

In this spirit, the Laboratory has been extremely glad to provide accommodation in Heidelberg for the Executive Secretary of EMBO, Dr. J. Tooze. Secondly, the Laboratory has welcomed the inauguration of an annual series of EMBO symposia held in the neighbourhood of Heidelberg; the first of these took place in April/May 1975 at Hirschhorn on the subject of "Developmental Genetics", and it is hoped that later symposia will provide opportunities for EMBO members and others to get to know the staff of the Laboratory and the activities going on there. Third, arrangements had

already been made in 1975 for the holder of an EMBO fellowship to work at the Laboratory and it is hoped that others will follow. Finally, the regular meetings of the EMBC and of the Laboratory Council now take place in Heidelberg on successive days, most of the Government delegates to the two bodies being the same individuals.

4 The future

4.1 As has already been indicated, several new research groups have been established in 1976. These include those of H. Delius (electron microscopy of DNA), R. Herzog (computer group), D. Marvin (structure of filamentous viruses) and R. Leberman (structures of protein factors important in the biosynthesis of proteins and their complexes).

4.2 In addition, late in 1975 Council authorized the construction of a containment facility for work on recombinant DNA. This facility will be available for the use of visiting groups that do not have similar facilities in their national institutions, and there will also be in-house research groups working on recombinant DNA. These and other developments will be described in the Annual Report for 1976.

DIRECTOR-GENERAL

John Kendrew

(Secretary) Anne Saunders

STAFF AND VISITORS

The following lists include all those who have worked for some period at the Laboratory and its Outstations up to the end of 1975

CENTRAL LABORATORY, HEIDELBERG

DIVISION OF CELL BIOLOGY

Scientific Staff

Henrik Garoff
Ari Helenius
Erich Jost
Ulrich Plagens
Chica Schaller
Kai Simons
Nick Strausfeld

Technicians

Louise Edlund
Elizabeth Ericsson
Kristine Flick
Cornelia Francke
Bodil Holle
Malu Obermayer
Hilkka Viirta

Postdoctoral Fellows

Erik Fries (Uppsala)
Ronald Lennox (Oxford)
Andrew Ziemiecki (Birmingham & Wageningen)

Visiting Workers

Oreste Acuto (Zürich)
Stefan Berking (Tübingen)
José Campos-Ortega (Freiburg)
Charles David (Albert Einstein, U.S.A.)
Klaus Hausen (Tübingen)
Sirkka Keränen (Helsinki)
Henry MacWilliams (Albert Einstein, U.S.A.)
Yochi Toprover (Tel Aviv)
Leslie Williams (Seewiesen)

Student

Tobias Schmidt (Heidelberg)

DIVISION OF BIOLOGICAL STRUCTURES

Scientific Staff

Kevin Leonard
James Torbet
Hanns Weiss

Technicians

Ingeborg Gillmeier
Brigitte Juchs

Visiting Worker

Cees Oosthuizen (Amsterdam)

Student

Barbara Ziganke (Munich)

DIVISION OF INSTRUMENTATION

Scientific Staff

Arthur Jones

Mechanical Workshop

Hans Flösser (Head)
Walter Schmidt
Otto Wernz

Electronic Workshop

Wilfried Muck
Alfons Riedinger
Siegfried Winkler

LIBRARY

Mary Holmes (part-time)

ADMINISTRATION

Director of Administration

Marc Delauche (to 30 September 1973)
Daniel Guggenbühl (to 31 August 1974)
Jack Embling (to 31 May 1975) (part-time)
Bernard Bach (from 1 June 1975)

(Secretary) Waltraud Ackermann

Finance Section

Eckart Weis
Dieter Ebser
Albert Stegmüller

Personnel Section

Gianni Giorgi-Alberti
René Guy
Konrad Müller

Purchasing Section

Torben Poulsen
Friedrich Wagenblass

Building project manager

Ottokar Beer

Meetings Secretary

Frieda Leenart

Secretaries

Anke von Böhl
Catherine Heath
Nelly van der Jagt
Christina Kjär
Monique Mary
Pamela Needham

MISCELLANEOUS SERVICES

Laboratory assistants

Inge Hauck
Annette Krebs
Waltraud Kühnle
Angelika Wegmann

Driver

Erich Honig

OUTSTATION AT DESY, HAMBURG

Head of Outstation

John Barrington Leigh (until February 1975)
Ken Holmes (part-time)

(Secretary) Jeanne Graf

Scientific Staff

Arnold Harmsen
Zofia Rek
Gerd Rosenbaum

Technicians and Engineers

Peter Bendall
Rolf Chors
Viktor Renkwitz
Bernd Robrahn

Visiting Workers

Robin Goody (Heidelberg
Jack Lowy (Aarhus)
Hans-Georg Mannherz (Heidelberg)
J. Rosenkranz (Bochum)
Richard Tregear (Oxford)
Jean Witz (Strasburg)
John Wray (Aarhus)

OUTSTATION AT ILL, GRENOBLE

Head of Outstation

Andrew Miller

Scientific and Engineering Staff

Andrê Gabriel
Defendente Tocchetti

Technical Staff

Carmen Berthet
Joseph Sedita

CONSULTANTS

The following have acted as consultants to the Laboratory

Ekke Bautz (Heidelberg)

Giorgio Bernardi (Paris)

Sydney Brenner (Cambridge)

Hermann Bujard (Heidelberg)

Mark Darlow (Porton)

Henry Harris (Oxford)

Klaus Heckl (Heidelberg)

Reuben Leberman (Heidelberg)

Hans Noll (Evanston)

Jeffries Wyman (Rome)

SCIENTIFIC ADVISORY COMMITTEE

The following served as members of the Scientific Advisory Committee* up to the end of 1975:

Max Birnstiel (Zürich)

Sydney Brenner (Cambridge)

Manfred Eigen (Göttingen)

Paolo Fasella (Rome)

Marianne Grunberg-Manago (Paris)

Henry Harris (Oxford)

Niels Jerne (Basel) (Chairman, 1971-June 1974)

Aharon Katchalsky-Katzir (Rehovot) (deceased)

Aaron Klug (Cambridge)

Vittorio Luzzati (Gif-sur-Yvette)

Ole Maaløe (Copenhagen) (Chairman, December 1974-)

Peter Reichard (Stockholm)

Werner Reichardt (Tübingen)

Arthur Rörsch (Leiden)

René Thomas (Brussels)

Hans Tuppy (Vienna)

Victor Weisskopf (Cambridge, Mass.)

Charles Weissmann (Zürich)

* known as the Provisional Scientific Advisory Committee until June 1974

WORKING GROUPS

The following Working Groups have met on various occasions:

Computer Policy Working Group:
to discuss the Laboratory's policy in computer applications

S. Brenner (Cambridge) (Chairman)
A. Borsellino (Camogli)
K. C. Holmes (Heidelberg)
L. de Maeyer (Göttingen)
A. C. T. North (Leeds)
J. G. White (Cambridge)

Nuclear Magnetic Resonance Working Group:
to discuss the possibility of an NMR project for the Instrumentation Division

R. Richards (Oxford) (Chairman)
I. D. Campbell (Oxford)
R. Freeman (Oxford)
M. Guêron (Paris)
U. Haeberlen (Heidelberg)
A. Klug (Cambridge)
P. Servoz-Gavin (Grenoble)
D. Stehlik (Heidelberg)

Recombinant DNA Working Group:
to discuss the construction of a containment facility and the program of a recombinant DNA research program

G. Bernardi (Paris)
S. Brenner (Cambridge)
H. Bujard (Heidelberg)
H. M. Darlow (Porton)
R. Leberman (Heidelberg)
V. Pirrotta (Basel)
H. Schaller (Heidelberg)
J. Tooze (EMBO)
H. Zachau (München)

THE LABORATORY COUNCIL

Chairman:	Professor A. Engström	(Sweden)	
Vice-chairmen:	Mr. E. Andres	(Switzerland)	(until December 1974)
	Professor P. Fasella	(Italy)	
	Mr. Y. Saphir	(Israel)	(from July 1975)

Chairman of the Finance Committee: Dr. C. Zelle (Federal Republic of Germany)

Vice-chairman of the Finance Committee: Mr. J. G. Duncan (United Kingdom)

The following lists all delegates and advisers who have taken part in meetings of the Council and Finance Committee during the period under review

Denmark

N. O. Kjeldgaard
N. O. Gram
N. Groth

Germany (Federal republic of)

C. Zelle
H. Zachau
W. Sandtner
E. Weis
E. Stertz
J. Dörr

France

A. Alline
J. P. Ebel
R. Monier
L. Amigues
F. Normand
F. Gros
M. Marchand

Israel

Y. Saphir
M. Sela
U. Littauer
T. Grizim

Italy

P. Fasella
B. Purificato
G. Cortellessa
A. Borsellino
L. Battaglini
C. M. Moschetti
E. di Mattei
A. Martinazzoli

Netherlands

W. Hutter
A. Rörsch
E. C. Slater
J. A. M. Goemans
F. Heyn
V. Ch. Ravensloot

Austria

W. Grimburg
A. Schacher
H. Tuppy
G. Zamostny

Sweden

P. Richard
A. Engström
M. O. Ottosson
L. Philipson
I. Agrell

Switzerland

E. Andres
N. Roulet
J. O. Quinche
U. Hofer
H. Lauri

United Kingdom

S. G. Owen
J. G. Duncan
K. Levy
M. Wood
D. C. Phillips
A. E. Turner

OBSERVERS

EMBO

N. K. Jerne
M. Sela
J. Tooze

EMBC

A. Rörsch

Spain

A. Duran-Miranda

Greece

P. Papadimitropoulos
D. Stathakos

Ireland

F. Winder
P. Freeney

Norway

A. Andersen
S. Laland

Belgium

J. Traest
L. Vandendriessche

RESEARCH REPORTS

Research using synchrotron x-radiation: the work of the outstation at DESY, Hamburg

Head: J. Barrington Leigh (until February 1975), K. C. Holmes

Scientific Staff: A. Harmsen, Z. Rek, G. Rosenbaum

Visiting Workers: R. Goody, J. Lowy, H. G. Mannherz, J. Rosenkranz, R. Tregear, J. Witz, J. Wray

Technical and Engineering Staff: P. Bendall, R. Chors, V. Renkwitz, B. Robrahn

Electron accelerators used in high energy physics are often ringformed since this allows the particle energy to be increased by passing repeatedly through the same set of accelerators. However, charged particles moving on a curved trajectory emit electromagnetic radiation and if the energy of the particle is of the order of 10^9 electron volts and the radius of curvature is of the order of 10 metres then the maximum of the emitted radiation lies in the x-ray region. In fact, electron synchrotrons emit copious amounts of spectrally continuous soft x-ray radiation. The radiation is thrown off tangentially at each bending magnet and is confined to the plane of the synchrotron. It was shown by Rosenbaum, Holmes and Witz that by the use of x-ray focussing elements it was possible to monochromatize and focus some of the radiation so as to use it for x-ray diffraction. In subsequent work, a beam with excellent optical properties has been produced. Since then it has been shown that the resulting beam is very well suited to the problem of recording the fibre diffraction pattern from muscle fibres giving at the same time very high intensity and very good angular resolution. Furthermore, recent experiments with protein crystals have demonstrated that existing beams were useful for protein crystal diffraction from crystals where radiation damage or large unit cells make a very well collimated and strictly monoenergetic beam desirable, although the advantages over conventional sources are not so great. Developments presently in progress (on DORIS) will improve this situation dramatically in the next few months and will in addition make many new types of scattering experiments possible. For example, low angle x-ray scattering will receive a new impetus when beams more than a 1000 times stronger than currently available from conventional sources make possible the combination of stop-flow techniques with low angle scattering. The very high beam intensities will certainly make it possible to extend the methods of structural molecular biology into the realms of kinetics and will, one hopes, help to lay the basis for a dynamical description of some mechanisms. Furthermore, synchrotron radiation opens up completely new ways of gathering structural data. For example, a new spectroscopic technique EXAFS (extended x-ray absorption fine structure analysis) may

be carried out using the intense continuous spectrum. The method is based upon registering the small changes in the x-ray absorption as the wavelength is varied in the neighbourhood of an absorption edge. The backscattering of the photoelectron from the neighbouring atoms has a readily measurable effect on the absorption observed. The nearest-neighbour distances may be accurately determined and the method should be of general applicability in enzymology since the "reporter" atom may be freely chosen by selecting the correct wavelength.

In an electron synchrotron the electrons are injected into the ring at relatively low energy and are accelerated in repeating sequences, the cycle time of each being normally 20 milliseconds. At the end of each sequence the electrons are ejected from the ring into the appropriate experiment. Within the 20 millisecond repeating period the energy of the electron beam is high enough to emit synchrotron radiation for about three or four milliseconds, therefore the source is pulsed. The intensity of the emitted radiation depends critically on the electron energy and is also sensitive to the radius of curvature. Typical values are 4-6 GeV energy and 20-30 metres radius of curvature. A second kind of device employed by high energy physicists, namely the "storage ring", offers even more interesting possibilities. In storage rings the electrons are not accelerated but are held at a constant high energy. The aim is normally to get the circulating electron beam current as high as possible. Thus, while an average beam currents of 10-20 mȧ are normal for a synchrotron, beam currents of between 300 mȧ and 1 ȧ are available from storage rings. The intensity of x-ray radiation emitted by storage rings is prodigious.

Initially the experiments to determine the feasibility of using synchrotron radiation as a source for biological x-ray diffraction were carried out on DESY. The beam coming from a tangent point of the synchrotron is conducted down a vacuum pipe into the experimental area which may be some distance away along a tangent from the synchrotron (ca. 30 metres). The beam is first reflected in the vertical plane at grazing incidence by a long fused-quartz mirror. This removes the hard radiation. The mirror is slightly bent to focus the reflected beam. The synchrotron radiation contains a very wide spread of wavelengths extending from hard x-ray to infra-red. For most purposes it must be monochromatized. Therefore, the beam is next reflected in the horizontal plane by a quartz crystal monochromator. This is also bent so that the beam is focused to a point (diameter ca. 200 μ) in the plane of the detector (film or counter). Together with slits, vacuum pipes, and specimen holder, this comprises the x-ray camera. All movements must be controlled remotely since the radiation levels within the experimental area are dangerously high when the beam shutter is open. The distance between the monochromator and the detector is typically 2-3 metres making it possible to work with up to 1 metre specimen film distance, thereby allowing very high angular resolution in recording the diffraction pattern. Using this arrangement photon fluxes at 1.5 Å of 10^8 photons/sec have been routinely recorded. Under the best machine conditions (7.2 GeV 11 mA) 5 x 10^8 photons/sec are available in the focus spot (the angular aperture of the beam in ca. 3 mrad in the horizontal plane

and 1 mrad in the vertical plane). Quartz is not the ideal monochromator since the band-pass from quartz (ca. .004%) is very narrow; Ge (111) is now used since it has a greater band pass (greater than .01%).

The major use of the apparatus to date has been on the structure and mechanism of insect flight muscle. Bundles of fibres from the flight muscles of the giant water bug Lethocerus maximus after treatment with glycerol solutions (to render the membranes permeable) provide a very convenient system for examining the mechanico-chemical conversion of the energy of hydrolysis of ATP. This system has been developed and characterised by Pringle and his group. In the absence of nucleotide ("rigor" state) the cross bridge projections from the myosin (thick) filaments bind tightly to the actin (thin) filaments to produce a highly crystalline hexagonal array. The unit cell of the semi-crystalline muscle fibre measures 520 x 520 x 1160 Å, which present serious technical problems for a conventional x-ray camera. Optimised conventional equipment giving adequate angular resolution is a factor of 50 weaker than the apparatus presently in use at DESY. To obtain pictures of comparable quality would take over 800 hours.

The addition of some non-hydrolysable ATP analogues, particularly β,γ imido ATP (hereafter referred to as AMPPNP), brings about large changes in the diffraction pattern of the fibres without bringing about any alteration of the fibre stiffness. The ratio of the two strongest equatorial reflexions alters on binding AMPPNP. These reflexions may be recorded in a few seconds using a position sensitive proportional counter and the DESY synchrotron so that it is relatively easy to produce a titration curve based upon the ratio of two x-ray reflexions. The binding constant is approximately the same as is found by biochemical methods. This example illustrates one area where synchrotron radiation diffraction is a useful adjunct to studies of enzyme mechanism. X-ray diffraction is an ideal way of reporting conformational changes.

One of the motivating aims of the work on synchrotron radiation was to try and record the structural changes taking place when a resting muscle is activated. Glycerinated insect flight muscle fibres when bathed in ATP, Ca^{++}, and Mg^{++} and when mounted in a machine which periodically stretches them, do work. This periodically oscillating system is well suited to stroboscopic examination by x-ray diffraction since the cyclic operation of the myosin cross bridges is largely synchronised. Experiments made some years ago using conventional rotating anode x-ray tubes by Miller and Tregear showed that the intensity of the two strong equatorial reflexions alters as the phase of the observation is varied with respect to the stretch. These experiments extended conventional technology to its limits. The more recent work with AMPPNP has demonstrated that regions of the diffraction pattern other than the equator are sensitive indicators of cross bridge orientation. However, the intensity of such reflexions is 50 times weaker than the equatorial reflexions so that the synchrotron x-ray source becomes a necessity for such experiments. At present experiments of this kind are being conducted by Rosenbaum and Tregear on DESY. Such experiments, in fact, demand the even more powerful x-ray source DORIS.

The storage ring DORIS is expected to outstrip DESY by a factor of 20. A new camera designed by Rosenbaum and coworkers at EMBL is at present being commissioned for DORIS. This camera is built in the form of a spectrometer allowing considerable freedom in the choice of wavelength. It is also built so that any or all of the 60 motorized movements may be computer controlled leading to (we hope) trouble-free alignment procedures. The first results from this new x-ray source are now eagerly awaited.

In parallel to these developments it is clearly necessary to have good detectors. An active program of development of area detectors with high counting rates is at present underway as a collaborative enterprise between the two EMBL outstations at DESY (Hamburg) and the high flux reactor at ILL (Grenoble).

Other uses to which the DESY source has been put include diffraction from invertebrate muscle, collagen, rhodopsin, chromatin, and protein crystal diffraction. We expect this list to get much longer as the new DORIS facilities come into operation.

Research on virus membranes

Members: K. Simons, H. Garoff, A. Helenius

Postdoctoral fellows: E. Fries, A. Ziemiecki

Visiting Workers: O. Acuto, S. Keränen, Y. Toprover

Technical assistants: B. Holle, H. Viirta

One central area of molecular biology today is the study of the structure and function of cellular membranes. Membrane research is, however, greatly impeded by the complexity of most cellular membranes. Experimental membrane models are therefore needed which are more amenable to detailed study. We have chosen to study a very simple biological membrane: the envelope of Semliki Forest virus. Our aim is to unravel the structure of this membrane and how it is assembled in the host cell. Semliki Forest virus is composed of a spherical nucleoprotein which is surrounded by a membrane. The nucleoprotein is assembled in in the cytoplasm and acquires its envelope by budding out through the host cell plasma membrane. Many other animal viruses are assembled by similar mechanisms. Some of these viruses produce widespread disease in humans (e.g. small pox, yellow fever, influenza and measles). However, Semliki Forest virus is apathogenic to man. This virus has one additional advantage as an experimental model system in that it is one of the simplest in structure among the membrane viruses.

We have studied in detail how three different detergents (SDS, Triton X-100 and deoxycholate) solubilize the virus membrane. All the detergents solubilized the virus membrane in a similar sequence of events (prelytic binding, lysis, solubilization and delipidation of the membrane proteins) which seems to be the same also for other biological membranes (see review article: Helenius and Simons, Biochim. Biophys. Acta 415, 29-79, 1975).

The major dissimilarity in the action of the detergents appears to lie in their effects on the proteins. SDS denatured and dissociated the virus membrane proteins E_1, E_2 and E_3 into monomeric form. Deoxycholate also dissociated the polypeptides from each other, E_1 and E_3 being solubilized as monomers and E_2 as octamers. The haemagglutinating activity of the virus was found to be associated with E_1. Triton X-100 solubilized E_1 and E_2 as separate dimers with E_3 apparently bound to E_1. These easily aggregate to form a homogeneous complex containing 8 copies of E_1, E_2 and E_3 each. The subunit structure of proteins in the virus membrane is still open, the most likely arrangements being $(E_1\ E_2\ E_3)_2$ as one unit or $(E_1\ E_3)_2$ and $(E_2)_2$ as separate units. These studies are based on the combined use of protein crosslinking reagents, antibodies against E_1 and E_2 and detergent solubilization.

The Semliki Forest virus proteins can be isolated in a monodisperse and water-soluble form virtually free from lipid and detergent. This was done by adding enough Triton X-100 to the virus to dissociate the lipids from the membrane protein. The mixture is sedimented through a zone of Triton X-100 into a detergent-free sucrose gradient. The lipids stay in the Triton zone, the nucleocapsids sediment to the bottom of the tube and the membrane proteins are recovered as a homogeneous complex in the middle of the gradient. The membrane protein complexes obtained have a sedimentation coefficient of 29.1S and a molecular weight of 9×10^5 and they contain 8 copies of E_1, E_2 and E_3 each. This water-soluble preparation is virtually free from lipid and detergent and is stable for weeks at 4°C. As the membrane proteins of the membrane viruses carry the antigenic determinants responsible for host immunity to viral infection this type of a protein preparation appears a promising candidate for a protein subunit vaccine. Such tests are in progress. In electron micrographs the 29.1S complexes can sometimes be seen to form paracrystalline arrays which appear to be suitable for more detailed structural studies.

Other studies under way include a systematic investigation on the optimal conditions for reassembly of the solubilized viral components into infectious virus particles.

Research on cytochrome b from the mitochondria of Neurospora crassa

Member: H. Weiss

Visiting Worker: C. Oosthuizen

Student: B. Ziganke

Technical assistants: I. Gillmeier, B. Juchs

Objectives

Investigation of cytochrome b from *Neurospora crassa* mitochondria with respect to biogenesis, protein structure, arrangement in the membrane and interaction with other membrane proteins.

Development of new techniques for purification of membrane proteins.

Results

To purify cytochrome b the mitochondrial membranes are separated using an amphipathic chromatographic system. The stationary phase of this system is polymethacrylic acid resin, some of the carboxylic groups of which are linked to oleylamine by means of an amide bond. A solution of the detergents cholate and deoxycholate is used as mobile phase. Thus, the mobile phase and the resin phase both contain weakly acidic carboxylate groups and hydrophobic aliphatic chains. Mitochondrial membrane proteins solubilized with deoxycholate are resin-bound, the stationary carboxylate and oleyl groups replacing the detergents. On elution with gradients of increasing detergent concentration and increasing salt concentration in the presence of detergent, cytochrome b appears well separated from cytochrome aa_3, cytochrome c_1 and from a number of proteins which do not contain haem.

Purified cytochrome b is a dimeric haem protein (M_r 50-55 000) consisting of two haem-binding subunits (M_r 25-28 000). It is soluble only in the presence of deoxycholate and salt, binding 0.3 g detergent per g protein. Its high content of nonpolar amino acids and very low content of basic amino acids suggest that the bulk of the protein is integrated into the membrane.

The two cytochrome b subunits can be separated by hydroxyapatite chromatography in the presence of dodecylsulfate. They show differences in their isolectric pH value (6.8 and 7.2), and probably in their lysine contents (5-6 lysines and 7-8 lysines), as well. The pattern of fragments obtained by cyanogen bromide cleavage after gel filtration in 80% formic acid seem to be indistinguishable, however.

Both cytochrome subunits are translated on mitochondrial ribosomes and most probably coded by mitochondrial DNA. The similarity of their polypeptide fragment pattern suggests that they are derived from the same gene but modified after translation.

This assumption is supported by preliminary observations that ^{3}H-leucine, added as a pulse to exponentially growing cells, appears delayed in cytochrome b compared to the average cell protein, following different time curves for each subunit.

Work in progress and future plans

The two cytochrome b subunits are compared in greater detail by means of two-dimensional "finger prints".

Sequencing of cytochrome b protein has begun in cooperation with E. Wachter, W. Machleidt and W. Sebald of the Institut für Physiologische Chemie der Universität München.

The precursor polypeptide, assumed to be common to the two subunits, will be isolated by immunoprecipitation using an antibody against cytochrome b.

The arrangement of the cytochrome b subunits in the mitochondrial membrane will be studied by labelling cytochrome b _in situ_ with surface probes.

It is planned to try to construct an ordered sheet from cytochrome b and lipids, suitable for high resolution electron microscopy studies.

New amphipathic systems are being tested for the chromatographic separation of membrane proteins: micro-beads of glass or silicate or controlled porous glass are coated with a layer of covalently linked ionic groups and long aliphatic chains. Such beads are brought together with a suspension of mitochondrial membranes and phospolipids or artificial lipids. Experimental conditions are being sought which will enable the different protein components of the membranes to distribute themselves independently between the stationary and the mobile lipid layers.

Research on chromosome structure and gene regulation

Member: U. Plagens

Technicians: E. Ericsson, C. Francke

The genetic information of eukaryotic cells is contained in DNA molecules which are complexed with proteins and some RNA to form "chromatin". During the cell cycle chromatin undergoes considerable changes. In mitosis chromatin forms highly condensed ordered structures, the chromosomes, which are metabolically inactive, in interphase, on the other hand, the visible order of the chromosomal structures is lost and the chromatin is decondensed, but becomes very active in transcription.

For the purpose of studying gene organisation and regulation these cyclical changes present considerable obstacles, which can be overcome in certain systems in which the chromatin is highly ordered and at the same time metabolically active. Polytene chromosomes of insects combine both qualities and have therefore been chosen for the study of chromosome structure and gene regulation. The special features of this system can be summarized as follows:

1 The salivary glands of insect larvae can easily be explanted and kept _in vitro_ for several hours without deterioration.

2 The mass of DNA per salivary gland cell is equal to that of up to ten thousand diploid cells, with a consequential enormous increase in the size of their so-called polytene chromosomes. The specific arrangement of the genetic material allows the resolution of up to five thousand bands per chromosome set (four chromosomes in the case of _Chironomus_); these are most probably identical with complementation groups (gene loci).

3 In contrast to metaphase chromosomes the polytene chromosomes exhibit metabolic activity, mostly accompanied by extensive decondensation (puffing) of the corresponding bands. Furthermore the activity level of the bands can be manipulated by external stimuli such as moulting

hormones, temperature shocks, or inhibitors.

4 Optically controlled microtechniques make it possible to isolate specifically gene products, groups of genes, single chromosomes etc. These can then be subjected to biochemical analysis on a macroscale because, for example, some hundred isolated chromosome sets are equivalent to the genomes of one million diploid cells.

5 The existence of established diploid cell lines in Drosophila makes possible the combination of biochemical and cytochemical data, to an extend which has not been possible before.

Description of the project

The work is concentrated on the analysis of the structural and functional aspects of the proteins of polytene chromosomes, including specific modifications. The species Chironomus tentans was chosen, because the four chromosomes show characteristic features: while chromosomes II and III possess a nucleolus and chromosome IV is characterized by three large puffs, chromosome I shows neither nucleoli nor large puffs. High resolution analysis of separated chromosomes should therefore make it possible to identify the proteins of nucleoli, puffs (i.e. active genes), and non-puffed regions, and furthermore to determine which proteins are modified during activation or repression of genes. Another purpose of the work is to determine which of the proteins are present in polytene chromosomes, and absent in normal metaphase chromosomes or in repressed interphase chromatin (in collaboration with E. Jost).

Work accomplished

Isolation methods have been developed for the fast separation of chromosomes on a small scale, and it has been established that the procedure does not significantly alter the RNA content either qualitatively nor quantitatively, in comparison to sister glands which have been fixed and treated by micromanipulation in the conventional way. An iodination procedure has also been developed that permits the analysis of the proteins of some fifteen to twenty five chromosomes of each type on one-dimensional gels. As a result characteristic quantitative differences between the protein patterns of different chromosomes have been found, but the resolution must be improved by employing two-dimensional techniques in order to establish qualitative differences. The same resolution problem occurred when phosphorylated and ^{14}C-labelled proteins were compared. In collaboration with A. Greenleaf from the Laboratory for Molecular Genetics in Heidelberg (Director: Professor E. Bautz), a method has been developed for squashing Drosophila salivary glands after fixation with formaldehyde in order to preserve the location of proteins. By employing indirect immunofluorescent techniques with antisera against Drosophila RNA polymerase B (enzyme and sera prepared by Greenleaf) it was shown that, as anticipated, regions of high RNA synthetic activity (puffs) fluoresce quite brightly, but unexpectedly most of the other bands (not in proportion to their mass) do so likewise. The interpretation can only

be that RNA polymerases are located nearly everywhere in the bands and have only to be activated to start RNA synthesis. This might mean that the initiation of puffing need not include a first step in which the DNA has to be made available for external RNA polymerase molecules. This hypothesis is now being tested.

Research on non-histone chromosomal proteins

Member: E.Jost

Visiting worker: R. W. Lennox

Technician: L. Edlund

This group only began work in September 1975, and the present report covers projects in progress (A, B, D) and in the planning stage (C, E); so far only project A has been partially completed.

A cell from a higher organism contains a number of more or less well defined subcellular organelles, the most important of which are the chromosomes. Chromosomes contain the genetic information of the cell and are mainly composed of DNA and proteins. It is apparent that progress in understanding cellular growth, differentiation or malignancy can be made more easily if our knowledge about the structure and function of chromosomes increases. Research in this field concentrates (1) on the superstructure of DNA in chromosomes (2) on the number and function of proteins in chromosomes (3) on the interaction between DNA and proteins. In chromosomes DNA is associated with a large number of protein molecules, the histones and the so-called non-histone proteins. The latter fraction contains molecules which are necessary for the structural organization of the DNA in the cell, enzymes, and regulatory proteins such as repressor molecules. We are studying certain aspects of the protein composition and arrangement in chromosomes by using affinity chromatography of proteins on various preparations of DNA as a model system. A more detailed description of our various research projects is given in the following sections.

A. Characterization of nuclear structures containing superhelical DNA

(This work was done in collaboration with P. R. Cook, University of Oxford)

Structures resembling nuclei but depleted of protein may be released by gently lysing cells in high salt and detergents. These nucleoids sediment through gradients containing intercalating agents in a manner characteristic of DNA that is intact, supercoiled and circular. It was concluded that nucleoid DNA was subject to the same kind of topological constraint restricting rotation of one strand of the duplex about the other as that found in circular DNA molecules. The concentration of salt present during isolation of the nucleoids affects their protein content. When made in 1.95 M NaCl they lack histones and most of the proteins characteristic of chromatin; in 1.0 M NaCl they contain variable amounts of histones. Some of the proteins which are still present in

nucleoids show the same electrophoretic mobilities as proteins from isolated nuclear envelopes. The possible involvement of these proteins in maintaining the superstructure of the DNA is further investigated.

B Protein-fingerprints of non-histone chromosomal proteins and purification of major components

In recent years we have used electrophoretic techniques developed for ribosomal proteins in order to characterize chromosomal proteins with affinity for DNA. We were able to increase the resolution for non-histone DNA-binding proteins about two to three fold as compared to conventional SDS-PAGE techniques. About a hundred polypeptides could be separated. This allowed us to compare chromosomal proteins from various sources and enabled us to conclude that non-histone DNA binding proteins are evolutionarily conserved. This may be the molecular explanation for the compatibility of interspecific somatic cell hybrids. It is argued that in man-mouse hybrids with only one or a few human chromosomes human DNA has to be complexed with murine proteins in order to be replicated and transcribed. Recently, further improvements have been made by other groups in separating chromosomal proteins in two-dimensional systems. We are currently combining the advantages of the various two-dimensional separation systems. By using this technique we try to answer the following questions.

1 Is the preferential loss of chromosomes of one parent in interspecific hybrids caused by the evolutionary heterogeneity of the chromosomal proteins?

2 Is it possible to correlate the induction of a specific messenger RNA with the appearance or disappearance of chromosomal proteins? We try to answer this question by comparing the chromosomal proteins from *Chironomus* chromosomes which were isolated before and after puff induction. (This work is being done is cooperation with U. Plagens.)

C Reassociation of chromosomal proteins with DNA in the presence of "reporter"-molecule

Non-histone chromosomal proteins and histones were reassociated with DNA which had been complexed with intercalating agents. Those used were ethidium bromide, actinomycin D, acridine orange and proflavine. Ethidium bromide and actinomycin D when intercalated into DNA prevented non-histone proteins with high affinity for DNA from binding to the drug-DNA complex. Proflavine and acridine orange, which do not contain bulky side groups, affect the binding of histones, but to a lesser extent that of non-histones with high binding affinity for DNA. Proteins can also be released from protein-DNA complexes by intercalating agents. The results can be interpreted in terms of the binding and arrangement of non-histone proteins along the histone-DNA structure. One class of non-histone protein seems to bind to DNA by recognizing the DNA structure in the groove believed to enclose the phenyl- and ethyl groups of bound ethidium bromide or the peptidic component of

bound actinomycin D. The presence of histones in a protein-DNA complex reduces the binding sites available to intercalating agents and thus, indirectly, the amount of non-histone proteins liberated from protein-DNA complexes by such agents. Binding sites for non-histone proteins along the histone-DNA fibre may therefore be on DNA associated with the histone subunit. At present we are studying the liberation of non-histone proteins by intercalating agents from protein-DNA complexes with different histone contents. These experiments should show whether non-histone proteins are exclusively associated with DNA which is stabilized by histones. The effect of primary and secondary binding of intercalating agents on the binding of non-histones to DNA is also investigated in more detail.

D Characterization of nuclear proteins in the erythropoietic cells of the chick embryo and in chick embryo erythrocyte nuclei undergoing reactivation in heterokaryons

The erythrocyte of the chick embryo is essentially biochemically inert; no DNA and almost no RNA synthesis take place within the erythrocyte nucleus. The erythrocyte nucleus resumes both of these activities when it is introduced into the cytoplasm of a normal tissue culture cell by Sendai virus-induced cell fusion. During this process of reactivation the erythrocyte nucleus increases in size, and this increase in size is due partly to the specific accumulation of proteins by the erythrocyte nucleus.

Methods have now been developed for the isolation and purification of erythrocyte nuclei from heterokaryons. The proteins which accumulate in the erythrocyte nucleus are being isolated, fractionated by affinity chromatography on columns containing DNA and analysed by one and two dimensional polyacrylamide gel electrophoresis. We hope to identify those proteins which bring about the resumption of nucleic acid synthesis in the erythrocyte nucleus.

The process of reactivation reverses the process of differentiation which led to the formation of the inert erythrocyte. Precursors of the chick erythrocyte which are synthesizing DNA and RNA are being examined in order to determine if the disappearance of particular proteins during the cessation of nucleic acid synthesis in erythropoiesis can be correlated with the appearance of the same proteins during the resumption of nucleic acid synthesis in reactivation.

E Chromosomal proteins from normal and malignant mouse cell lines

We have compared DNA-binding proteins from normal and malignant mouse cell lines in the hope of finding a biochemical marker which can be followed through the segregation analysis using cell fusion. No qualitative alterations were found among the major 80 DNA-binding proteins. These comparisons will be continued using electrophoretic techniques which allow a higher resolution of complex protein mixtures.

Research on the control of morphogenesis in hydra

Member: H. C. Schaller

Student: T. Schmidt

Visiting workers: S. Berking, C. David, H. MacWilliams

Technician: K. Flick

Hydra is used as a model system to understand how in embryology pattern formation and differentiation is controlled at the molecular level. So far only very few systems exist in which it could be shown that biologically defined gradients of properties such as induction or inhibition are due to a graded distribution of substances. We have presented evidence that in hydra these gradients of activation and inhibition are controlled by only a few (probably 4-5) substances. The general aim of our present research is to isolate and characterise these substances, to study their action at the cellular level and to see how they interact.

One of these morphogenetically active substances, the head activator, is a peptide with a molecular weight of approximately 1000 daltons. It is produced by nerve cells and is stored there in neurosecretory granules. A similar or identical peptide is present in the hypothalamus of mammals (rat and cow). This peptide has the following properties: (1) it is active as a morphogen by stimulating head and bud formation in hydra. (2) at the cellular level it is active a) as a mitogen or growth hormone by stimulating cells to divide, and b) as a determining factor by stimulating the differentiation of uncommitted stem cells to nerves and inhibiting that to nematocytes.

Present research program:

1 The head activator is available only in nanomole quantities. We are trying to improve or apply existing micromethods to analyse its primary structure.

2 One of the other morphogens, the foot inhibitor, is under investigation.

Research on the neuronal arrangements which mediate vision in insects

Member: N. Strausfeld

Visiting workers: J. A. Campos-Ortega, K. Hausen, L. Williams

Technician: M. Obermayer

The basic neuronal arrangements of the visual regions (which are reminiscent of mammalian and avian brain) are quite well documented[1,2]. It suffices to say here that their principal structural features comprise columnar relays of nerve cells that map the retinotopic mosaic onto precise configurations of large-field motion or form

sensitive neurons[3]. These are arranged in planar strata across the mosaic. At one particular level of the system we already know that defined sets of neurons mediate the three main components for reflex navigational behaviour: upward and downward motion detection, detection of motion from back to front (and vice versa) and positional recognition[4,5]. Our aim is to understand the structure and development of such a well-defined system, which also has the added advantage of showing plastic changes[6]. Possibly these may be rather similar to the kinds of phenomena involved in learning; and having once traced out the functional connectivities of this motion-sensitive system we may possibly be in a position to investigate membrane configurations in those nerve assemblies which mediate the plasticity.

Taking advantage of the extraordinarily precise geometries of insect neuropils we are already well under way towards elucidating some of the main channels onto motion-sensitive neurons and some of the main output pathways to body musculature. In addition, it is also possible to exploit the very stereotyped behaviours of these animals in order to screen for mutants; and one of our first technical developments is to devise ways by which behavioural mutants can later be screened anatomically for gross neural errors. Initial success in developing such a technique is reported under section I. Methods for behavioural screening will be largely the domain of G. Geiger who is joining the group in August 1976.

Our research entails the processing and data storage of large amounts of visual (graphics) information. For this and for structural reconstitutions we shall be working in close cooperation with the EMBL Computer Group, to be led by R. Herzog. Drs. J. White and S. Brenner at the MRC Laboratory of Molecular Biology, Cambridge, have also offered their good advice for this part of the project.

Report

The construction and equipment of the light-microscopical and electron-microscopical laboratory and photographic rooms were initiated in October 1975 and completed in January to March 1976. Actual research began in December 1975.

I Neural identification and preparation for electron-microscopy

Up to the present time a neuron could be identified both for the light and electron microscope by intracellular staining with silver. This is the classic Golgi method which completely, and at random, fills a small fraction of the total nerve cell population. This is its main advantage; and the surface structures of a stained neuron may be investigated by high voltage electron microscopy[7] and possibly by freeze fraction. This latter procedure will, it is hoped, be attempted after we move into the new buildings. The main disadvantage of the silver method is that in the electron microscope the interior of a marked cell is completely obscured by the silver chromate-proteinate deposit. Thus, only its postsynaptic relationships can be visualised.

Neurons can, though, be completely filled by organic procion[8] dyes. Usually these are injected into the cell by iontophoresis; and after appropriate fixation and section cutting the cell can be visualised by fluorescent microscopy. The method's main advantage is that it can be used in conjunction with electrode recordings; and for the present project K. Hausen, at the Max-Planck-Institut für biologische Kybernetik, Tübingen, is cooperating by providing single filled cells. The drawback of this method is that marked cells cannot be visualised by conventional transmission electron microscopy (TEM). However, electrons will elicit fluorescence from procion dyes and this should, in principle, be detectable by appropriate probes in a scanning transmission electron microscope (STEM). At present we are investigating reasonable combinations of fluorochromes and fixation for future use in the EMBL STEM, in conjunction with A. Jones. If successful, this may be a powerful technique because procion dyes leave sub-membrane structures intact, and with appropriate image storage, superimposition of STEM and TEM images may identify particular profiles as belonging to a particular species of nerve cell.

A third marking procedure is that of filling neurons with cobalt chloride and subsequently converting the translucent chloride to the opaque sulphide[9]. We are at present investigating the applications of this procedure for electron microscopy. At present our initial (light microscopy) findings can be summarised as follows:

1 Both cobalt and nickel chloride will diffuse into very small nerves. However, both salts tend to clump and do not completely fill all the ramifications of a cell.

2 Recently, it was reported that addition of bovine albumin to macerated vertebrate nervous tissue resulted in agglutination of neurofilaments[10]. We have introduced albumin with cobalt chloride into nerves and find that the active diffusion rate is accelerated ten-fold: the cobalt chloride seems to be bonded to internal structures throughout the cell's ramifications. We are now developing suitable preparations of this material for electron microscopy so as to determine whether formation of neurofilaments with bound cobalt is induced throughout the neuron. If this were to be the case then, in principle, introduction of albumin alone, bonded to fluorescent marker, would suffice to identify a neuron, both in the light microscope and TEM.

3 When $CoCl_2$ diffuses slowly at low concentrations and at low temperatures then the salt will migrate across membranes into other neurons.

These findings have given us the most excitement because the cobalt will jump only between contiguous neurons. That is to say, if neuron A is primarily filled then under the above conditions neurons A1, A2 which impinge on it will also take up the salt. If B is primarily filled then neurons B1 etc will be secondarily filled! This observation is consistently observed and our first electron microscopy observations of A and A1 show the latter to be presynaptic to the former.

4 Enhancement of low concentrations of cobalt sulphide can be made by flooding the brain with silver nitrate simultaneously with a reducing agent. The sulphide acts as a catalyst for the deposition of silver and the amount deposited is proportional to the initial sulphide concentration. Thus, primary filled neurons will afterwards appear darker than secondary filled elements. With prolonged diffusion, at low concentrations and at low temperatures, $CoCl_2$ and nickel chloride will migrate retrograde across three contiguous neurons (third order diffusion). From electrophysiological studies, combined with procion dye identification of neurons[5], the successive arrangements of filled neurons are those expected to be in synaptic (functional) contiguity.

Since low concentrations of $CoCl_2$ appear to migrate transneuronally our plan for the immediate future is to determine whether cobalt will jump enterograde by introducing it into elements, whose subsequent synaptic connexions are already known from conventional electron microscopy.

II <u>Developmental studies</u>

Taking advantage of the reiterative patterns of neurons in the retinopic mosaics, and the constancy of neuron positions between individuals of the same species, we shall undertake studies of the development of neural connectivities, in particular those leading to the formation of the "lobula plate". This part of the brain contains giant cells that provide one link in the motion-sensitive relays. As its name suggests, the neuropil is a plate-like structure that consists of functionally and structurally well-defined strata[1,3,5]. We shall screen for behavioural mutants and then examine these for gross mutations of neural patterns. Subsequently we shall use this as a morphogenetic marker for investigating the sequence of events which lay down the retinotopic map and, most significant for behaviour, which lay down unique giant neurons in the map. Our transneuronal cobalt diffusions already indicate that in the chalky mutant of <u>Calliphora</u> there is an abnormal map of vertical motion-sensitive neurons.

III <u>Graphics</u>

In electron-microscopical reconstructions only a fraction of a nerve cell can be sampled by serial sections. And this fraction has to be reconstituted into a three-dimensional structure which can be recognised as part of a neuron that was previously known from light microscopy; it must also be best fitted, with its synaptic loci, into a three-dimensional jig-saw which consists of other reconstituted neurons. This procedure involves quite complex reconstructions including averaging of three-dimensional images and the design of simple branching schemata which incorporate synaptic loci. On the one hand we shall be providing material for computer graphics and developing simple preprocessing devices, and on the other Geiger will be involved in devising methods for three-dimensional holographic reconstructions of whole cells, derived from light optical sections.

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Research on High Resolution Electron Microscopy

Member: K. R. Leonard

The work of this group began only in November 1975, and the present Report summarizes plans for the future.

Equipment

It is planned to instal a high-resolution conventional transmission electron microscope (Phillips EM400), and the group will also use the scanning transmission electron microscope (Vacuum Generators HB5) under development in the Division of Instrumentation. (In addition an Phillips EM 301 will be installed for the use of the neurobiology group and of the nucleic acid electron microscope group which is due to become active in 1976).

Supporting facilities will include normal E/M preparation facilities with a general purpose vacuum evaporator (Edwards 306) and subsequently a freeze-etch evaporator; an optical diffractometer for image analysis of electron micrographs; darkrooms; and at a later stage computer facilities for image processing.

Research projects

1 Collaboration with A. Helenius of the Division of Cell Biology to obtain electron micrographs of negatively-stained SFV proteins.

2 Conventional imaging methods

Efforts will be made to refine the present methods of image contrast enhancement (heavy metal staining, metal shadowing, etc.) to obtain better specimen preparation and higher resolution.

3 Low Dose imaging methods

Emphasis will be given to introducing the low-dose imaging techniques of Unwin and Henderson. This will place quite strong demands on the computer facilities required and on the need to prepare large well-ordered thin crystalline specimens.

4 STEM

As there is, as yet, no wide ranging experience with the use of scanning transmission electron microscopy in biological research, it is not known which area of operation will yield the best results. In the early stages, work will be concentrated, in collaboration with A. V. Jones of the Division of Instrumentation, on assessing the applicability of different detector systems in imaging (dark field, bright field, secondary X-ray etc.) and on the feasibility of using the STEM as an on-line data collecting system for direct image processing.

5 Computer Analysis

It is intended to build up a full system for computer image processing of electron micrographs. In the case of TEM this will be carried out off-line using images digitised by a computer linked high speed densitometer. In the case of STEM it will be possible to carry out some on-line procedures using a direct computer link. Both types of processing will be carried out using expanded minicomputer systems with hardware courier processors.

6 Specimen Preparation Techniques

New techniques will be required to assist in the preparation of specimens for low dose work (see above) and also ultra clean specimens for STEM, where contamination presents a problem. Work may also be carried out on specimens at low temperatures or in the hydrated state, to preserve structure.

Development of Scanning Transmission Electron Microscopy (STEM)

Member: A. V. Jones

The basic factors limiting the resolution attainable in biological electron microscopy are

a the susceptibility of biological material to electron-beam induced degradation

b specimen contamination, both from instrumental sources and resulting from the preparative procedures

c the inherently low contrast of biological specimens.

Instrumental development on the STEM is aimed at minimizing all three limitations.

The basic microscope, a Vacuum Generators HB5, will be delivered at the end of April 1976. It will be operable in both transmission and reflexion modes with a minimum probe diameter of $\lesssim$ 5A° (transmission) and 15A° (reflexion).

The microscope has already been modified to EMBL specifications to facilitate further instrumental development. It incorporates double differential pumping to permit low contamination conditions in the specimen region ($< 10^{-8}$ Torr) or the use of environmental cells. A newly-developed airlock system permits automatic insertion of transmission or reflexion specimens without degrading the vacuum condition.

A modified pole-piece and specimen cartridge permits simultaneous imaging in bright-field, dark-field and x-ray modes, while a modified deflection system makes possible selected angle dark-field operation.

The control electronics have already been modified to provide preselection of the magnification and the scanned area and image storage facilities will be added to reduce specimen irradiation and consequent damage to a minimum.

To improve specimen contrast, emphasis will be placed on the development of new detector systems to take advantage of imagine modes not available in conventional electron microscopy and on computer processing of the image data. The serial nature of the data permits on-line computer operation and software compatibility will enable all programs developed for the conventional TEM to be used on-line by the STEM.

Research on neutron diffraction and scattering: the work of the Outstation at ILL, Grenoble

Head: A. Miller

Scientific and engineering staff: A. Gabriel, D. Tocchetti

Technical Staff: C. Berthet, J. Sedita

The EBML outstation at Grenoble was started in September 1975 with the aim of making it easier for biologists to use the neutron facilities at the High Flux Beam Reactor at the Institut Laue-Langevin. In the last two or three years neutron diffraction studies have been shown to have special value when applied to biological materials. Basically neutron diffraction is a supplement to x-ray diffraction and is employed when the x-ray method is impractical or of little value. The location of hydrogen atom positions in crystalline materials has been a special use of neutron diffraction. Now it has proved applicable to the determination of the molecular arrangement in cell organelles such as ribosomes even when the ribosomes cannot be crystallized. This is because the large difference in neutron scattering between the hydrogen and deuterium isotopes makes it possible to label molecules differently. A second application of neutrons to biological materials involves the use of different D_2O/H_2O mixtures to vary the contrast between a structure and its surroundings. This has been applied to connective tissue, muscle, ribosomes, viruses, chromatin and complex membrane structures.

The outstation is being equipped with a range of biochemical and biological equipment to permit biologists from other countries to carry out preparative and assay procedures close to the neutron source at the ILL. These facilities should come into operation during 1976. Biologist users of the neutron source come from a range of European countries and they have been consulted about the kind of equipment which will be of maximal use in the outstation.

The staff of the outstation will, in addition to assisting the neutron users, carry out research. Initially this will be mainly on connective tissue and muscle and appointments are being made with a view to

bringing together scientists with a width of experience in neutron and x-ray diffraction, electron microscopy and image analysis, computing and biochemical methods. Research is also being carried out on the development of new applications of neutrons to biological systems. An example of this is the collaborative work between members of the outstation and ILL on inelastic neutron scattering.

A series of EMBL/ILL Biology Seminars has been started.

Professor E. Kellenberger (Basel) and Dr. K. A. Piez (N.I.H., Bethesda) were the first two speakers.

PUBLICATIONS BY MEMBERS OF THE LABORATORY

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WORKSHOPS

The following workshops were arranged to discuss the future program of the Laboratory

4-7 November 1972	Workshop at the Hotel Haarlass, Heidelberg, to discuss the future program of the Laboratory, and attended by some 60 scientists including potential staff members.
17 October 1973	Workshop at Heidelberg, to discuss the development of position-sensitive detectors.
18-22 October 1973	Workshop at Schönau, near Heidelberg, to discuss research projects for EMBL in the field of membranes.

SEMINARS

The following public seminars were given in Heidelberg during the year:

M. F. Perutz (Cambridge)	Allosterischer Mechanismus des Hämoglobins
	Effekt des Quartärstruktur des Globins auf das Hämen
K. Timmis (Stanford)	DNA replication studies with hybrid plasmids constructed *in vitro*
H. MacWilliams (Tübingen)	Pattern formation in hydra regeneration
C. Cohen (Brandeis)	Protein assemblies in the cell
S. Berking (Tübingen)	Kontrolle der Nervenzelldifferenzierung und Knospung bei Hydra

ANNEX

Notes on the Hamburg EMBL Outstation

for the exploitation of synchrotron radiation

The project grew out of a discussion between G. Rosenbaum and K. C. Holmes (Abteilung Biophysik, Max-Planck-Institut für Medizinische Forschung, Heidelberg) in the autumn of 1969. Preliminary experiments were carried out by Rosenbaum and J. Witz (University of Strasbourg) in 1970-71 in the radiation bunker of the DESY Group 41 (Rosenbaum, Holmes and Witz, 1971). The results proved that a considerable gain in intensity for biological x-ray diffraction would be obtained by using synchrotron radiation from the DESY synchrotron and that dramatic gains could be expected from the storage ring DORIS. On the basis of these experiments, planning of a synchrotron radiation facility for the exclusive use for x-ray diffraction from biological samples was started. The plans for an EMBL radiation bunker (Lab 2) came just in time to be included into the final building plans of the storage ring DORIS. The construction of an additional radiation bunker on the synchrotron (Lab 1) was also possible, because it could be erected at the same time as the connecting tunnel between synchrotron and storage ring without causing an interruption of the high energy physics program. This first building phase took place in 1972.

A small group consisting of J. Barrington Leigh and Rosenbaum, financed by the Deutsche Forschungsgemeinschaft and operating out of the Abteilung Biophysik, started work in the radiation bunker of DESY group F 41 in the middle of 1972. By early 1973, the first bunker (EMBL Lab 1) had been built onto the DESY synchrotron. At the same time, two walls of the projected EMBL Laboratory on the storage ring (DORIS) were built. In this period the group received invaluable guidance and assistance from Prof. Teucher, Dr. Degêle and many other members of the DESY staff. The group operated officially as a guest group within F 41 (synchrotron radiation group). From the beginning the group received much encouragement and help from Prof. Haensel, Dr. Kunz and many other members of F 41.

The first remotely controlled optical bench was built by Rosenbaum in collaboration with W. Gebhardt (electronics) and H. Wagner (mechanical design) from the Abteilung Biophysik. This was first installed in the radiation bunker of the DESY group F 41 in 1972. The bench was much improved (Barrington Leigh and Rosenbaum, 1974) and was moved to Lab 1 in late 1973. During 1974 serious work was often interrupted by building activities, since at this time the upper story of Lab 1 was being built. The building was undertaken by the Baubehörde Stadt Hamburg (Herr Seeland) and was supervised by Barrington Leigh who was also responsible for much of the scientific planning. At one stage, the whole of the optical bench had to be encapsulated to protect it from running water! By autumn 1974, serious work with the optical bench could start. Initially the group had trouble with the alignment of the beam pipe. This and other teething problems were out of the way by the autumn of 1974. At this time, Barrington Leigh and Rosenbaum became the first scientific staff members of EMBL. They were joined

by R. Chors as resident engineer . H.-G. Mannherz and R. Goody (Abteilung Biophysik) started working in Hamburg on the changes in the cross bridge orientation of insect flight muscle induced by ATP analogues (Goody et al., 1975). R. Tregear (Department of Zoology, University of Oxford) and Rosenbaum started collecting equatorial data from vibrating insect flight muscle stroboscopically using a position-sensitive detector and electronics designed by A. Gabriel who was the first member of the Grenoble outstation of EMBL. The experiment was arranged to take 6 to 12 time slices in synchrony with the oscillating muscle (frequency of oscillation about 5 Hz). These results were technically good, but did not extend the earlier published work of Miller and Tregear.

In November 1974, the group was joined by B. Robrahn as technical assistant in charge of the optical bench. The reliability of the optical bench and experience in adjusting it were rising steadily, so that some sort of service could be offered to visiting groups. The first two scientific groups active on site were those previously cited. The Heidelberg group were able to produce quite dramatic results in June 1975 by taking rather long exposures (by synchrotron radiation standards), namely 24 hours, of insect flight muscle in rigor and various other intermediate states produced by adding ATP analogues. The pictures produced were of magnificent quality and when shown at the Gordon Conference in the same year evoked great interest. Initially the real strength of the synchrotron radiation source was the ability to take very high definition x-ray diffraction fibre diagrams, but with 20 times shorter exposure times than with the most intense fine focus tube (Elliott GX 13 x-ray tube). All experiments were done at a wavelength of 1.5Å, this being a design limitation in the first optical bench. A. Miller (Grenoble outstation) and Rosenbaum (and later A. Harmsen) also collaborated to take some rather fine collagen pictures. The angular resolution was good enough to enable Miller to verify the non-meridional nature of certain important but weak reflexions which had previously been in doubt. Later in the year, J. Lowy's group from Aarhus joined in and carried out experiments on catch muscle. J. Wray started work on lobster muscle, which is rather similar to insect flight muscle. Some preliminary experiments on the changes in the mitotic spindles during mitosis in sea urchin eggs were carried out by J. Rosenkranz from Bochum. Later Rosenkranz started some investigations on retinal rods. Goody and Tregear (with the assistance of Rosenbaum) were able to determine the binding constant of an ATP analogue to insect flight muscle fibres from alterations in the intensities of low-angle x-ray reflexions. This is the first example of an x-ray titration. Experiments of this kind were impossible with x-ray tubes because of the long exposure times. It also was the first experiment where the use of a position-sensitive photon detector was imperative.

In the spring of 1975, Barrington Leigh resigned from EMBL and his place as acting group leader was taken by Holmes. At the beginning of 1975, the group was joined by P. Bendall as computer engineer.

Most of 1974 and beginning of 1975 were a time of great building activity. Barrington Leigh and Chors were mainly responsible for the building work. During 1974, the design of the main bunker (EMBL Lab 2) on the storage

rings, the so-called bunker 4, was finalized and the top storey of bunker 2 (EMBL Lab 1) was also planned. The building started in 1974 and went through until the summer of 1975. The building was finished somewhat earlier than planned, namely early summer 1975, and the summer was spent in buying furniture and getting equipment into the new laboratories.

The computer system, a PDP 11/45, was delivered in June 1975, and installed in its final resting place, the builders having managed to get out one week before. The purposes of the computer system are three-fold: first, it will act as the main data depository for the two-dimensional detectors, which are presently being developed by Gabriel and Rosenbaum. Secondly, it will allow the automatic control of the optical benches. Experience has shown that the alignment of the optical benches is not an operation which can be sensibly left to untrained or semi-experienced scientists. It is hoped by computerizing the main part of the alignment that the training time for the operation of these benches and for their subsequent adjustment may be drastically reduced. This problem will become much more pertinent when the new bench, with its variable wavelength facility, which at present is being commissioned in Lab 2 (Storage ring), comes into operation. The third purpose of the computer is the general computation of problems associated with the processing and evaluation of diffraction data.

A large new technological program was initiated in 1975, namely the design and building of the new optical bench for the storage ring laboratory. The design was undertaken by Rosenbaum in collaboration with Herrn Flösser (EMBL) for mechanical design and with members of the EMBL electronic workshop for the control. This impressive and elegant optical bench is now in Hamburg, although its installation and the build-up of the control functions will take until late 1976. It will be possible to control any one of its 60 motorised drives by computer or by hand according to the level of human interaction required.

Harmsen joined us in the summer of 1975. His first task was to develop reliable beam sensing and measuring devices and for this purpose he has built a number of 1 cm think transmission ionisation chambers. These have already proved very useful. The number of photons used in a particular experiment may now be recorded (this is a real need since the synchrotron radiation intensity from DESY fluctuates. On DORIS we do not expect this to be a problem). Zofia Rek in collaboration with Harmsen has started a through-going program for the evaluation of monochromator crystals. They are also investigating the effect of surface doping on the band widths of crystals. Harmsen in collaboration with R. Leberman and G. Schulz undertook a preliminary evaluation of synchrotron radiation as a source for protein crystal diffraction (Harmsen, Leberman and Schulz, 1976).

The beam intensity measured by Harmsen, Leberman and Schulz (6×10^7 photons/sec at 1.5 Å and 6.5 GeV and 10 ma current in the synchrotron) is no higher than can be obtained from an Elliott rotating anode tube used with a collimator as already calculated in the very first report (Rosenbaum, Holmes and Witz, 1971). However, more recently we have recorded up to 5×10^8 photons/sec. The optical properties

of the synchrotron radiation beam are naturally much better than those of a collimated nickel-filtered beam from a rotating anode tube. However, the optical properties of the beam are not important unless one has problems involving the resolution of spots or of high background. In such situations the synchrotron beam offers real advantages. This is mainly the case for low angle diffraction, fibre diffraction or diffraction from crystals of protein complexes. In this case we have to compare the synchrotron with focused radiation from a tube with mirror-monochromator or mirror-mirror optics. Then the fluxes from even the GX 13 rotating anode are more than one order of magnitude lower than the synchrotron radiation collected by the optical bench in its present state (see Barrington Leigh and Rosenbaum, 1976, p. 252).

The measured flux is far from the limit we can get from the synchrotron. Measurement of the reflectivity of germanium as a monochromator demonstrate a gain of 2-3 over quartz. (Replacement has recently taken place.) Another avoidable limitation is the short length of the mirror. A factor of at least 3 would be possible if one employed the better design used in the new optical bench for DORIS.

A further factor of 30 is to be expected from the storage ring DORIS because of its high current. We know from theory and observation that the intensity at shorter wavelengths (.7 Å) on the synchrotron DESY is an order of magnitude greater than 1.5 Å. This strongly suggests we should start a new program - short wavelength x-ray diffraction from biological samples. Accurate data collection with hard radiation from protein crystals could be a way of improving atomic coordinates considerably.

The main emphasis at present is low angle diffraction from fibres. Recently it has been demonstrated that the DESY optical bench can be applied to solution low-angle scattering. The intensity gain over a point collimation system using a mirror-quartz monochromator is between 20 and 100 fold. The extra factor of 30 available from DORIS makes it possible to envisage collecting data in a matter of seconds using existing designs of camera. New developments, utilizing a much wider band pass, could easily push the time resolution down to milliseconds.

At the beginning of 1976 J. Hendrix is due to join the group and to take over the design and setting up of a low angle scattering experiment suitable for stop-flow.

The other main technological development is concerned with two-dimensional detectors. The design chosen is a multi-wire proportional counter. The anode consists of an array of 10-25μm wires spaced at present 2 mm (later hopefully down to 0.5 mm, using wires as thin as 5μm). There are two cathode planes, each consisting of closely spaced wires on each side of the anode plane which sense an event on the anode wires by induction. The direction in which the two sets of cathode wires run are mutually perpendicular. The cathode wires are connected respectively to two delay lines at right angles, so that by time measurement of the arrival of pulses at both ends of the two delay

lines the two-dimensional position of the event may be calculated and stored in a computer. This development is being made by Gabriel and Rosenbaum in collaboration with Hendrix, Gebhardt and Bendall.

Since the summer of 1975 the Hamburg outstation has been housed in the two new laboratories. This has made possible the build-up of on site service groups such as the computer group already discussed. Visitors may now be offered beam time. There are also now quite respectable biochemical laboratories, so that specimen preparation and manipulation are no longer the makeshift affairs of previous years. A good mechanical workshop, (V. Renkwitz and W. Behrens) plays an increasingly important role in the massive technological build-up which still has to be made in Hamburg. Much remains to be done (e.g. the EXAFS project) but the outstation has gone through the pioneering period and looks forward to a period of consolidation and exploitation.

K. C. Holmes

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