PROTEIN BIOGENESIS

A COLLOQUIUM on "Specificity in Protein Biogenesis" was held in Louvain during June 8–9 by the Centre interuniversitaire de Recherches enzymologiques, a government-sponsored association grouping several Belgian biochemical laboratories. The aim of the organizers was to bring together scientists actively engaged in research on protein synthesis and to establish new contacts with the Belgian groups interested in this field. The meeting was arranged on an informal basis and the speakers were asked to discuss freely the current work of their laboratories ; the lectures will not be published.

Present ideas on the relationship between deoxyribonucleic acid and protein structures were lucidly summarized and discussed by C. Levinthal (Massachusetts Institute of Technology). Since deoxyribonucleic acid and protein are both linear polymers, it would seem that a simple relation should exist between the arrangement of the amino-acids in the protein and that of the nucleotides in deoxyribonucleic acid of the corresponding gene—probably a point-to-point correspondence between the two sequences. Some of the characteristics of the coding system serving to translate one sequence into the other can be deduced from what is known about deoxyribonucleic acid and protein structure. It seems feasible at present to check experimentally the idea of collinearity between the structure of the two types of polymers. Find an organism in which genetic maps can be established with great accuracy, choose an enzyme molecule of moderate size produced by this organism and devise a good selection principle for recovering the useful mutants. Isolate several mutants of the genetic locus of the enzyme, map the mutation sites, isolate the abnormal proteins, if any, which are produced in place of the normal enzyme by the mutants, and locate the differences within the protein molecules. This gives a test of the correspondence between deoxyribonucleic acid and protein fine structure.

C. Levinthal described the progress of his own work on the genetic control of the formation of a phosphatase in *E. coli*. Thirteen different mutants resulting from mutations within the locus of the phosphatase have been isolated so far ; the linear order of the mutation sites has been established by a very interesting use of the transfer of genetic material during bacterial conjugation. Unfortunately only one of the thirteen mutants produced a recognizable modified phosphatase protein. Both the normal and the abnormal enzymes have been isolated, and their structure is now being studied. Future developments along this line of research will be watched with great interest.

In bacterial systems, genetic analysis is ahead of chemical knowledge. In man, on the other hand, where genetic analysis is at a great disadvantage, brilliant success has been achieved on the chemical side. J. Hunt (Cambridge) reviewed the main results obtained by the Cavendish Laboratory group on the structure of abnormal hæmoglobins. This work will undoubtedly become a classic of genetics and biochemistry. Hæmoglobins *S* and *C* differ from the normal protein in only one detail : the replacement of one glutamic acid in the β chain by valine or lysine respectively. Hæmoglobin *E* differs from normal hæmoglobin by the replacement of another glutamic residue by lysine ; in hæmoglobin *G*, still another glutamic is replaced by glycine. Thus genetic differences presumably arising from mutations can result in the replacement of individual amino-acids at specified places in the polypeptides.

J. Hunt further described in detail his recent work on fœtal hæmoglobin. He has shown that one of the two polypeptide chains of fœtal hæmoglobin (chain α) is identical with that of normal adult hæmoglobin. This discovery opens new perspectives and will certainly give important information on the mechanism of the genetic control of protein structure, for here is a simple case of differentiation at the molecular level. The structure of fœtal hæmoglobin of infants carrying genes of abnormal adult hæmoglobins will be very informative. It might indicate whether the switch from fœtal to adult hæmoglobin during development results from the inhibition of the activity of a gene and the unveiling of another gene, or whether the change occurs somewhere between the gene and the protein-making system and consists in a change of expression of the same gene.

Other aspects of the control of the synthesis of specific proteins were discussed by B. Magasanik (Harvard University), who considered the phenomena of enzyme induction and repression and the function of ribonucleic acid in the synthesis of bacterial protein. B. Magasanik reported results obtained in his laboratory on various mutants which require amino-acids, nucleic acid precursors, or certain energy sources. By a very ingenious use of these mutants, several aspects of the correlation between ribonucleic acid content and level of protein synthesis were checked. Increased rate of protein synthesis goes together with an increased content of the bacteria in both soluble ribonucleic acid and ribosome ribonucleic acid. This is comparable to the well-known relation found in higher organisms. Studies on ribonucleic acid and protein synthesis during adaptation, especially in 'diauxie' experiments, indicate that the synthesis of new ribonucleic acid does not accompany enzyme adaptation. The results are compatible with a catalytic function of ribonucleic acid in protein synthesis, and with the view that induction and repression of enzyme synthesis rest upon the control of the activity of pre-existing protein-forming centres, rather than on the formation or destruction of such systems.

The existence of specific ribonucleic acid molecules capable of carrying some sort of genetic information is established by the discovery that pure virus ribonucleic acid is able to cause infection. Present knowledge on the structure of tobacco mosaic virus was summarized by H. Fraenkel-Conrat (University of California), who presented results of his current work on the molecular size of the virus ribonucleic acid. End-group determination in the virus acid by a combination of tracer methods with specific enzymic degradation and controlled chemical oxidation indicates that each virus particle might contain one single molecule of ribonucleic acid made up of some 6,000 nucleotides in one chain. The polypeptide chain of the virus protein contains only about 150 amino-acid residues. It would seem, therefore,

that the ribonucleic acid of the virus is large enough to carry much more information than that which is required for controlling the primary structure of the protein unit contained in the finished virus. This raises several problems for future research. H. Fraenkel-Conrat also reported very interesting results on a special state of the virus ribonucleic acid at the beginning of infection.

Another approach to the specific function of ribonucleic acid in protein synthesis is the artificial modification of the structure of the acid. This has been done by chemical means in the case of virus ribonucleic acid. In bacteria, composition of the acid can be changed by growing the organisms in the presence of analogues of the normal purines or pyrimidines. F. Gros (Institut Pasteur, Paris) gave a very clear account of research on the effects of fluorouracil on protein synthesis in *E. coli*. Incorporation of all the individual amino-acids does not respond in the same way to fluorouracil. For example, the incorporation of proline and tyrosine is depressed whereas that of arginine is stimulated. These changes appear to reflect qualitative as well as quantitative modifications in the protein equipment of the organism, indicating that the analogue may actually interfere with the agents which control protein structure. A phosphatase formed in the presence of the analogue has a normal enzymic activity although it contains less proline than the normal enzyme; thus it is probably slightly modified at a place which is not important for the catalytic properties of the protein. On the contrary, β-galactosidase synthesis is abolished and replaced by the formation of some related inactive protein. It is striking that fluorouracil specifically reduces the fixation of proline and of tyrosine on soluble ribonucleic acid at the same time as it reduces the incorporation of these same amino-acids into the proteins. This indicates that soluble ribonucleic acid plays an important part in the specificity of protein formation. These results also support current views according to which activated amino-acids are bound to soluble ribonucleic acid before condensing into polypeptides.

T. Hultin (Wenner Gren Institute, Stockholm) reported observations on animal tissues which indicate that another pathway of amino-acid incorporation might exist beside that passing through soluble ribonucleic acid. That the latter must also be operative was shown by several of his results which agree with the classical scheme. However, he obtained, by means of new techniques of isolation of ribosomes and by fractionation of supernatant preparations, a system in which amino-acids are incorporated into proteins in the particles in the absence of soluble ribonucleic acid. A protein of the supernatant is required for incorporation with this system. Protein synthesis which can occur in isolated mitochondria or nuclei appears to depend on the presence in these cell organelles of particles closely resembling the ribosomes of the cytoplasmic ground substance.

Of great benefit to the meeting was the presence in the audience of biochemists from different countries who had taken part a few days before in Brussels in the Solvay Conference on Nucleoproteins. All the lectures were followed by very good discussions. The meeting was closed by a general discussion which concerned the function of the various ribonucleic acid fractions, the transfer of information from gene to protein, and coding problems.

H. CHANTRENNE

PROGRESS IN GAS CHROMATOGRAPHY

AN informal symposium of the Gas Chromatography Discussion Group (associated with the Hydrocarbon Research Group of the Institute of Petroleum) was held at the University of Bristol on September 25 under the chairmanship of Mr. C. S. G. Phillips.

Dr. F. H. Pollard commented on the enthusiasm and free interchange of ideas among workers in this field which was undoubtedly responsible for the present advanced state of the art. Having regard to the success achieved by him in the field of inorganic separations by paper chromatography, it was not surprising that he should mention the possible separation of such materials by gas chromatography.

The outstanding feature of the meeting was the demonstration by Mr. R. P. W. Scott of the presentation of gas chromatographic data with a high-persistence cathode ray tube. With capillary columns it is possible to effect separations at speeds much greater than the response of conventional recorders, and in order to take full advantage of the technique in its application to kinetic and other studies a means of high-speed recording is essential. Mr. Scott, using a 70-ft. column, demonstrated separations of 100° C. boiling-range samples in less than 1 min. with his apparatus which, inclusive of automatic repetitive sample injection system, cost less than £80 for materials.

The discussion which followed a paper by Mr. C. L. A. Harbourn on quantitative determinations showed that this aspect of the subject is one that affects most users of the technique. As yet, however, if one uses the published literature as a guide, it would appear to have received very little attention. The well-prepared paper covered methods of peak measurement, sources of error and repeatability of calibration, internal standard, and normalization methods, and interpretation of unresolved peaks. Recent developments in integrators and the use of analogue computers and tape recorders were also discussed.

Some of the practical aspects of the measurement of retention volumes were dealt with by Dr. G. W. A. Rijnders, and Dr. C. R. Patrick mentioned some of the problems attached to 'scaling-up' analytical columns to sizes capable of handling up to 10-gm. samples. The values of height equivalent of a theoretical plate (H.E.T.P.) increase and the much higher volumetric flow-rates necessitate modifications to the design of hot-wire detectors. Mr. D. H. Desty read a paper by Dr. J. Janak, who unfortunately was unable to be present, describing the application of gas chromatography to the identification of structure of involatile substances by pyrolysis and subsequent analysis of the products.

Members attending, among whom were some from the United States and Europe, were able to inspect and see working a good selection of the commercial instruments now available for laboratory and process control work.

C. G. SCOTT