PHYTOCHROME

New Ideas in Eretria

from a Correspondent

IT is now widely accepted that progress towards understanding the regulation of morphogenesis depends increasingly on cooperation between specialists in many different fields. Such cross-fertilization was the keynote of the NATO Advanced Study Institute on phytochrome held in Eretria, Euboea, Greece, between August 29 and September . 17 and organized and directed by Professor K. Mitrakos of the University of Athens.

The problems of the photoregulation of plant metabolism and morphogenesis were intensively discussed by photochemists, plant physiologists and biochemists and the general view was that significant progress can be expected in the very near future. The Advanced Study Institute was chiefly an educational exercise, although many speakers took the opportunity to present new information; further, associated with the institute was a two-day symposium for the presentation of original work.

Opening the symposium, Dr H. A. Borthwick (US Department of Agriculture, Crops Research Division, Beltsville) reminded the audience that the existence of phytochrome as a red/far red photoreversible pigment was postulated about twenty-one years ago to account for a vast mass of physiological data. Great progress has been made since then, particularly in understanding the molecular nature of phytochrome. The latest advances in this area were presented by Professor W. R. Briggs (Harvard University), who has shown that undegraded phytochrome from oats and rye has a molecular weight of 120,000. The previously reported values of between 15,000 and 60,000 were due to degradation of the native molecule by a protease which seems to co-purify with the phytochrome. The 120,000 dalton molecule seems to exist as a dumbbell-shaped structure as deduced by electron microscopy and negative staining; furthermore, there is evidence for an association of two molecules to give a unit with a molecular weight of 240,000 which might exist in significant proportions *in vivo*.

The chemical nature of the photoconversions was discussed in detail by Professor W. Rüdiger (University of Munich), who presented evidence that during the conversion of P_R to P_{FR} a proton is effectively removed from one of the terminal pyrole rings of the bilitriene chromophore. This would probably result in a movement of the protein in the environment of the chromophore so as to neutralize the resultant charge. Evidence for a *cis-trans* isomerization based on circular dichroism studies of purified phytochrome was reported by Dr M. Burke (University of Minnesota) who suggested that, at least at low temperatures, the photoconversions proceed through a pseudoporphyrin conformation. Flash spectroscopy studies on the purified phytochrome of 120,000 daltons by Professor H. Linschitz (Brandeis University) showed that there are separate and parallel pathways between P_R and P_{FR}. The simplest interpretation is that each molecule has two slightly different chromophores which photoconvert through different intermediates.

Consideration of the time courses of the *in vivo* light and dark transformations by *in vivo* spectrophotometry led to considerable discussion—it is now clear that no single model can account for all the available observations. Dr R. Marmé and colleagues (University of Freiburg) presented evidence for two populations of phytochrome with different kinetics of photoconversion in mustard seedlings. Furthermore, the proportions of phytochrome in the two populations, and the rate constants of their phototransformations, varied with the age of the mustard seedlings. The localization of phytochrome within the cell was also studied by this group using polaroid light beams. When the light was polarized parallel to the long axis of *Avena* coleoptiles, 25 per cent more photoreversibility was recorded by spectrophotometry than when the light was polarized normal to the long axis. Marmé and his colleagues have taken this to indicate that the pigment is located in the plasmalemma, and indeed differential centrifugation techniques showed a concentration of phytochrome in the plasmalemma fraction as identified by ability to bind naphthylphthalamic acid. Professor L. Pratt (Vanderbilt University) described an immunochemical technique for the cytochemical localization of phytochrome. Initial results indicated that phytochrome is associated with membrane, as was shown decisively for the alga *Mougeotia* by Professor W. Haupt (University of Erlangen-Nurnberg) by the use of polarized microbeams.

In the final sessions, considerable interest was generated regarding the manner in which phytochrome regulates enzyme activity in plants. The general view was that it is no longer possible to hold to a single model based on the regulation of gene transcription by phytochrome. Professor H. Smith (University of Nottingham) showed that the effects of light on phenylalanine

Nomenclature for Human Chromosome Banding

from a Correspondent

THE rapid developments in using quinacrine fluorescence and various modifications of Giemsa staining to produce specific banding patterns on human chromosomes led to the holding of the IVth Standardization Conference on Human Cytogenetics at Rungis, Paris, from September 2–4. In the tradition of the first three conferences at Denver, London, and Chicago, the aim of the meeting was to try to agree on a standard system for chromosome identification and nomenclature in relation to banding patterns.

The patterns produced by quinacrine fluorescence are to be used as the standard for reference on the basis of the numbering system proposed by Caspersson and his colleagues (*Hereditas*, **67**, 89 ; 1971). This involves a departure from the Denver system; it was agreed that the chromosome trisomic in Down's syndrome as defined by its fluorescent pattern should retain the number 21, although measurement of quinacrine-identified chromosomes shows it to be smaller than 22. The various laboratories represented were able to agree on the major quinacrine bands for each chromosome pair and to relate the Giemsa and reverse-Giemsa bands to the quinacrine bands. Each human chromosome pair can now be identified by its specific banding pattern, an advance which must open the way to a great increase in the precision of future human cytogenetic and linkage studies.

The conference was unable to reach a decision on the optimal method for numbering the bands. Two systems were considered. The first is to number from the centromere outwards for each chromosome arm; the second is a consecutive numbering system for the whole chromosome set starting at the end of the short arm of chromosome number 1. The conference appointed a standing committee (Dr B. Dutrillaux, Dr H. J. Evans, Dr C. E. Ford, Dr F. Ruddle, Dr J. Lindsten, with Dr J. L. Hamerton acting as coordinator) charged with the immediate task of making recommendations on this issue. It is intended that the report of this conference should be published by the National Foundation early in 1972 and it is hoped that it will include the standing committee's recommendations on an internationally acceptable numbering system. Any suggestions or comments should be sent to Dr J. L. Hamerton, Department of Genetics, Children's Hospital of Winnipeg.

ammonia-lyase levels in several plants include multiple effects on enzyme activation, enzyme inactivation and enzyme synthesis probably regulated at the translation level. Dr P. Schopfer (University of Freiburg), on the other hand, presented evidence that the regulation of gene transcription by phytochrome is a likely mechanism for the control of several enzymes in *Sinapis alba*. Perhaps the most striking contribution to the conference was read in the absence of its author (Professor Y. Yamamoto, Nagoya University) which reported evidence for the photoregulation *in vitro* of NAD kinase activity in cell-free extracts. This is the first report of an enzyme activity being associated with, or regulated by, phytochrome outside the plant cell and will undoubtedly stimulate many workers to attempt confirmation of the results.

DIFFERENTIATION

Models or Molecules?

from a Correspondent

THE most impressive feature of the first international conference on cell differentiation, held in Nice from September 13 to 16, was the paucity of novelty; there were hardly any new concepts and few new results. In part this was due to the hysterical pace. Speakers were allowed, on average, fifteen minutes for their presentations. Most responded to this challenge by describing their techniques and results in great detail. Of course, there were some outstanding exceptions, but the general impression was of the failure of indifferent molecular biology to provide any insight into the fundamental processes of differentiation, let alone to describe it adequately.

Dr F. H. C. Crick (Medical Research Council Laboratory of Molecular Biology, Cambridge) presented his new theory of eukaryotic chromosome structure, one of the most welcome aspects of which, as Dr S. Brenner (MRC Laboratory of Molecular Biology) pointed out, is that it will stop people talking about reverse transcriptase. He suggested that the chromosome contained "globular" (looped) DNA and "fibrous" DNA. The globular portions correspond to the bands of polythene chromosome, the fibrous portions to the interband regions. The interband regions contain genetic information for messenger RNA synthesis, the bands are control elements. On being questioned after his presentation, Dr Crick successfully destroyed all attempts to introduce facts as counter evidence by demonstrating, or by just stating, that the facts were predicted, indeed demanded, by his model.

Dr M. H. Cohen and Mr A. D. J. Robertson (University of Chicago) presented a mixture of theory and experiment, supported by two fascinating films, to explain some features of the aggregation of cellular slime moulds. Their most important result is the demonstration that aggregation can be artificially controlled by pulses of cyclic AMP released periodically from a microelectrode. As Dr Brenner put it, the work seemed interesting enough to become controversial.

Dr S. A. Newman (University of Chicago), in a theoretical paper, showed that in a multi-enzyme system stability could be achieved if each enzyme had a monotonically increasing activity with increasing substrate concentration; non-monotonicity, such as in the characteristic bell-shaped activity function found *in vitro* leads to instabilities. This correlates well with known activities of multi-enzyme systems *in vivo*.

Dr N. K. Jerne (Basle Institute for Immunology) introduced the section on immunology with a review of some recent research work on antigen receptor sites in lymphocytes. There are about 10^4 per cell. Although they are usually randomly distributed, appearing to "float" in the membrane, on binding with receptor antibody they aggregate into islands containing about 10^3 molecules. On incubation at 37° C the islands merge to form a cap which is then ingested; this might act as a trigger for lymphocyte multiplication. Other work Jerne described emphasized the enormous number, at least a thousand, of idiotypes (molecules with identical binding properties but different electrophoretic mobilities) that can be produced by cells of an inbred mouse strain to the same antigen. This is a further indication of the incredible flexibility of protein tertiary structure.

POLYPEPTIDE HORMONES

Membrane Interactions

from a Correspondent

THE relation between the structure of polypeptide hormones and their function was the subject of a colloquium held on September 2 at the University of Sussex and attended by members of the Biochemical and Chemical Societies. In the morning session which was devoted to insulin, Dr T. Blundell and other members of Professor Hodgkin's group at the University of Oxford described how the earlier work on the three-dimensional structure of insulin is now being followed up. The species variations in the primary structure of at least twenty insulins are now known, so that it is possible to speculate on which groups of residues are significant in biological and immunological reactions, and it is becoming feasible to identify those residues which when modified give rise to conformational changes. It now seems likely that some non-polar residues towards the terminus of the β chain may be responsible for interaction with membrane receptors.

In the same session, Dr P. T. Grant (University of Aberdeen) suggested that zinc may stabilize the storage form of the hormone. Dr H. Zahn and his colleagues (Aachen Technical University) discussed how they synthesize analogous insulin. Several modified insulins have now been made and their biological and immunological activities have been tested. In the last contribution of the morning session, Dr E. R. Arquilla (University of California) described the work of his group on reactivity of modified insulins with antisera. Again the emphasis was on how alterations in the C terminal groups of either chain might alter conformation and hence immunological activity.

One important conclusion which emerged from the morning session was that in spite of exciting progress, knowledge of the state of aggregation of insulin in biological fluids will be essential for defining just how insulin acts at membranes.

The afternoon session was devoted to papers on the structure-activity relationships of several other polypeptide hormones. In an excellent summary of the hormonal polypeptides of the upper intestine, Professors Mutt and Jorpes (Karolinska Institutet, Stockholm) compared the amino-acid sequences of gastrins, secretins and pancreozymin, and pointed out some interesting similarities between the glucagon and calcitonin sequences. Dr W. Rittel (Ciba-Geigy Ltd, Basle) discussed the effects of chemically modified corticotropins on adrenal stimulation and on lipolysis in adipose tissue. Structural modifications cause parallel alterations in activity in these two differing tissues. Dr M. Wallis (University of Sussex) gave an interesting contribution on growth hormones, prolactins and placental lactogens. He speculated on how rates of evolution might be determined by comparison of differences in the primary structure of hormones from related species.

Finally, Dr M. Rodbell and his colleagues (National Institutes of Health, Bethesda) suggested a possible model for the interaction of small polypeptide hormones such as glucagon with cell membranes. Rodbell postulated that the binding of glucagon takes place by non-covalent bonds to the membrane, perhaps through the C terminal 6 residues, whereas the N terminal histidine of glucagon is essential for biological activity. The membrane-bound glucagon then undergoes a conformational change, such that it activates adenylate cyclase.