Meetings

Mutagenic and Carcinogenic Agents

Molecular action of mutagenic and carcinogenic agents was the main theme of the annual symposium sponsored by the Biology Division of the Oak Ridge National Laboratory in Gatlinburg, Tennessee, 6–9 April 1964. The symposium provided an occasion for a thorough discussion of the status of our present understanding of these mechanisms. The results, as a whole, bore out our uncertainty about the nature of the mechanisms of these processes.

The action of acridine dyes and of alkylating agents in mutagenesis was examined. The role played in mutagenesis by the intercalation of dye molecules in between nucleotides of double-stranded DNA was discussed by L. S. Lerman (University of Colorado School of Medicine). While the evidence for intercalation appears convincing, its role in mutagenesis could not be demonstrated. The main difficulty lies in the existence of acridine derivatives which intercalate very effectively but are not mutagenic.

The most frequent alterations of DNA caused by alkylating agents are also well known. These alterations should lead either to CG → AT transitions or to the loss of a base, which then could be substituted randomly. However, the mutagenic role by the latter mechanism is dubious because there is little evidence that certain expected base substitutions, called transversions, actually occur. F. J. de Serres (Oak Ridge National Laboratory), testing a variety of mutagens on *Neurospora*, concludes that mutations in these organisms are produced predominantly by base pair substitutions.

The exciting recent discovery by R. B. Setlow (Oak Ridge National Laboratory) of an enzyme which excises thymine dimers from ultraviolet-irradiated DNA may have a profound impact on the study of mutagenesis and perhaps also of recombination. It reveals the existence of enzymatic mechanisms which remove altered nucleotide sequences and replace them with the correct ones. At the same time, however, this discovery pointed out the important difficulties in studying the molecular bases of mutagenesis. In fact, between the mutations which are formed at first in DNA and those finally detected, there exists a series of screens —error correction, degeneracy of the code, and variable influence of different amino acid substitutions on protein function. A case in point is again that of mutagenesis by acridines; it was reported by Setlow that these dyes interfere with the excision of thymine dimers. Thus they may cause mutagenesis by suppressing error correction.

An important question in mutagenesis is also its relation with recombination. Evidence presented by G. E. Magni (University of Parma, Italy) showed that in yeast mutation frequencies are increased in meiosis. The question whether recombination is involved in mutagenesis by acridines was also raised by J. W. Drake (University of Illinois) in his discussion of the *r*II cistrons of bacteriophage T4.

Sydney Brenner (Molecular Biology Laboratory, Cambridge, England) described a novel approach to the problem of polarity of fragments of the polypeptide chain produced in bacteriophage "amber" mutants.

Sensitization of bacteria to x-rays and ultraviolet rays was shown by H. S. Kaplan (Stanford University School of Medicine) to be greatly enhanced by incorporation into DNA of certain purine and pyrimidine analogues.

Great uncertainties exist about the molecular mechanisms of carcinogenesis. These mechanisms are most likely different from those of mutagenesis, because very few substances are able to cause both consequences. A really meaningful test of this point, however, must await a study of the two actions in the same cell type.

It was apparent from the discussion that it is not possible to conclude what the target of carcinogenic action is. On one hand, the study of the electronic configuration (Bernard Pullman, University of Paris, France) and of the reactivity (Charles Heidelberger, University of Wisconsin) of carcinogenic hydrocarbons suggests that they act by interacting with a protein. On the other hand, the study of carcinogenic alkylating agents tends to implicate an interaction with DNA (Peter Brookes and P. D. Lawley, Chester Beatty Research Institute, London, England). Unfortunately all carcinogens can interact with essentially all macromolecular components in the cells.

The striking relationship of carcinogenic hydrocarbons with a soluble protein of the skin was discussed by Heidelberger. This relationship has been known for some time; now its possible meaning for carcinogenesis has been reexamined. Models have been discussed which might explain carcinogenesis, at the level of regulation, as a stable shift in feedback systems. It has been suggested that the soluble protein may be a gene repressor which, upon interaction with the carcinogen, causes the discontinuation of its own production.

Hubert Chantrenne (University of Brussels, Belgium), in describing dramatic changes in RNA and protein synthesis of *Bacillus cereus* caused by incorporation of 8-azaguanine, discussed these effects with regard to regulatory mechanisms and the formation of abnormal enzymes.

The symposium ended with a brief discussion of viral carcinogenesis (Harry Rubin, University of California); this process can be considered as a genetic alteration of the cell caused by the persistence of viral genes.

This conference was sponsored by the Biology Division, Oak Ridge National Laboratory, and the U.S. Atomic Energy Commission. A publication of the symposium proceedings will appear as a supplement to the October 1964 issue of the *Journal of Cellular and Comparative Physiology*.

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Anaerobic Microorganisms in the Soil

Significant advances have been made in the study of anaerobic bacteria in rumen and sludge digestion microbiology, but the study of anaerobic and microaerophilic bacteria in soil micro-

biology has suffered from a lack of application of proper techniques and research approaches. In the hope of relating the techniques and known information of rumen and sludge digestion microbiology to soil microbiology, a round table discussion was held at the 64th annual meeting of the American Society for Microbiology in Washington, D.C., 3–7 May 1964.

L. E. Casida, Jr. (Pennsylvania State University) pointed out that only limited knowledge is available concerning anaerobic soil microorganisms and that it is not even known whether most soils maintain conditions anaerobic enough for the existence of anaerobic bacteria other than the spore-forming clostridia. Conventional techniques for the demonstration, enumeration, and isolation of anaerobic soil bacteria were discussed by Casida, and it was pointed out that these techniques do not show the existence of highly oxygen-sensitive anaerobes even if they are present in the soil.

An alternate approach was suggested to include these organisms. Dilutions of soil would be quantitatively applied to thin nutrient agar films of low O/R potential and then sealed between cover slips and slides. After incubation at suitable temperatures the dilutions would be observed microscopically for formation of microcolonies. Thus, anaerobes, which show no macroscopic evidence of growth in the laboratory when present techniques are used, might produce microscopically visible growth of a few generations in such a sealed chamber.

It was pointed out by D. Caldwell (U.S. Department of Agriculture, Beltsville) and P. H. Smith (University of Florida) that the anaerobic bacteria of the rumen and sludge digestion systems are extremely sensitive to oxygen. Therefore, their enumeration and isolation require complete exclusion of oxygen and maintenance of low O/R potential during all handling, including collection of sample and inoculum transfer. It was suggested that the same techniques be applied to soil anaerobes. In particular, it was suggested that soil in the field be impregnated with inert gas before it is tested, and that such soil then be transported to the laboratory and processed under a blanket of inert gas.

It is hoped that at least a few of the investigators who listened to or participated in this discussion will find their imagination and interest so sparked by the presentations that they will initiate investigations on the more oxygen-sensitive anaerobes of the soil.

This meeting was planned by the Soil Microbiology Section of the American Society for Microbiology.

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Neurospora

Researchers working with *Neurospora* in various disciplines and in various parts of the world gathered at the 2nd *Neurospora* Information Conference, Houston, Texas, 4–7 March 1964, for the informal exchange of ideas and information and for the comparison of methodological approaches to some of the more rapidly developing areas of research. No formal presentation of research papers was scheduled; there were, instead, five informal sessions, each of which was devoted to the discussion of one field of research.

Each session was opened by a chairman who presented a summary of the current status of knowledge in that field, an evaluation of the methods currently in use, and a formulation of the questions he felt could most profitably be explored through studies on *Neurospora*. An informal discussion of research programs and techniques followed each session. Relevant experimental data, when available, were presented.

The session on mutagenesis was chaired by Charlotte Auerbach (Edinburgh University, Scotland). She emphasized in her introductory remarks (i) the difficulties of quantitative evaluation of mutation data, particularly in regard to mutagen specificity, and (ii) the technical difficulties encountered in the study of mosaicism and delayed mutation. In the following discussion that was devoted largely to methodology, D. Stadler (University of Washington) described a new heterocaryon system for the detection and study of recessive lethal mutations. He reported some of the results obtained after using a heterocaryon containing the *cot*, igloo, and flat mutations.

Other discussions on mutations were presented. F. de Serres (Oak Ridge National Laboratory) reported results of an intensive study of mutations at the *ad-3* locus. Stadler described a method for the scoring of both forward and reverse mutations in the same genetic system with the use of 4-methyl tryptophan, and S. Gross (Duke University) described a system for scoring both forward and reverse mutations for sulfate requirement. J. Reissig (Universidad de Buenos Aires) discussed his method for detecting both forward and reverse mutations at the *pyr-3* locus. In the discussion on the measurement of reverse biochemical mutations, resistance mutations, and the problem of mutagen specificity, the technical difficulties and the need for new methods were again emphasized.

In the session on recombination (chairman, Stadler) it was noted how interallelic recombination in *Neurospora* and other ascomycetes yield characteristic patterns of results which could be explained if a chromosome were made up of a series of fixed recombination regions. It was suggested that when one of these regions is paired for recombination (as it is in perhaps 1 percent of the meiotic cells) reciprocal (2:2) segregation always occurs at the ends of the region. However, between the ends there may be multiple, nonreciprocal events. Recent hypotheses were discussed which attempt to describe recombination at the level of DNA molecules. Critical tests of these molecular models for recombination can be made with *Neurospora* and other organisms suited to tetrad analysis and the selection of rare recombinants.

The session on regulation and development (chairman, A. Sussman, University of Michigan) opened with reports on cytology and ultrastructure by R. J. Lowry and somatic mitosis in *Neurospora* by A. N. Namboodiri (both of University of Michigan). Then, the question of the spatial and temporal localization of enzymes during development was introduced; the localization of invertase (R. Metzenberg, University of Wisconsin) and the regulation of aryl β-glucosidase, cellulase, and cellobiase (B. Eberhart, University of North Carolina) were both noted. R. Wagner (University of Texas) described a class of isoleucine-valine mutants which may represent "organizational" mutants. Such mutants possess all of the enzymes necessary for the synthesis of these amino acids but are defective in the organization of these enzymes into a functional complex. A. Fox (University of Wisconsin) and N. Horowitz (California Institute of Technology) compared their disparate results on the tyrosinases of *Neurospora* and discussed possible reasons for the discrepancies. The remainder of this session evolved into a discussion of some of the hitherto neglected morpho-