Biochemical Pharmacology, 1960, Vol. 4, pp. 29–33. Pergamon Press Ltd., Printed in Great Britain.

THE INVOLVEMENT OF DEOXYRIBONUCLEIC ACIDS IN CHEMICAL MUTAGENESIS*

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Resumé—Des faits mettant en évidence la "nature génétique" de l'ADN chez les virus, les bactéries et les organismes supérieurs sont passés en revue et des informations obtenues par l'étude de la mutagenèse chimique sont présentées.

Bien que certaines substances soient capables de provoquer des mutations chez les micro-organismes mais non chez les organismes supérieurs et vice versa, l'universalité d'action de certains agents alkylants en tant que mutagènes, parait bien indiquer un mécanisme d'action commun. Les résultats biologiques et la chimie bien connue de tels agents alkylants rendent valable l'affirmation, qui doit être vérifiable sans trop de difficulté, que ce mécanisme est basé sur des réactions primaires avec l'ADN.

Tout essai d'explication de l'apparition de mutations comme conséquence d'une alkylation de l'ADN doit tenir compte de cinq questions posées ici.

THE GENETIC ROLE OF DEOXYRIBONUCLEIC ACIDS (DNA)

THE justification for considering deoxyribonucleic acids (DNA) as "genetic substances" derives from the evidence of bacterial transformation, the allied phenomenon of transduction, and the functional differentiation of the components of phage particles first established by HERSHEY and CHASE (1952) for T2. Supporting evidence comes from the association between genetic function and phosphorus-containing material revealed in various types of experiment involving the decay of incorporated ^{32}P; in particular, one may recall the relationship between order of failure of gene transfer during bacterial conjugation and extent of decay of incorporated phosphorus observed by JACOB and WOLLMAN (1958). Further evidence of the genetic prominence of DNA derives from experiments concerned with induced mutation which it will be the main purpose of this paper to consider. The *a priori* case for regarding the DNA as the carrier of genetic information in phage and in bacteria is thus a good one. The evidence, however, does not exclude the possibility of other substances playing a part and, *inter alia*, it must be remembered that the "germinal substance" (HERSHEY, 1955) of phage contains some protein. The case for considering DNA as the exclusive genetic determinant is such, nevertheless, that most workers in microbial genetics accept it, and interest is focused upon unravelling the coding systems which DNA may carry.

In "higher" organisms we know that the bearers of genetic information, the chromosomes, contain DNA, but certainly not exclusively, and it cannot presently

* This investigation has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

be assumed that this information is borne solely by the DNA moiety. WESTERGAARD (1959) recently uttered a plea for rejecting the assumption of DNA exclusiveness in chromosome-based organisms, arguing that to do otherwise is to go against some facts and to neglect evolution. Chromosome models of the type suggested by FREESE (1958) are, on the other hand, basically attempts to view the chromosomes of higher organisms as elaborations of the pattern visualized for viruses.

Our knowledge of the route of action of chemical mutagens is likewise incomplete, although the evidence of chemical studies favours the supposition that this is via DNA and, therefore, in turn, affords further support for the genetic importance of this material. It certainly seems much easier to interpret the chromosome changes produced by mutagenic substances on the basis of a "molecular" chromosome model of the Freese type.

MUTATION OF BACTERIA AND VIRUSES

For a long time little or no success was achieved in attempts to mutate extracellular viruses either chemically or by radiations. SILVESTRI (1949) claimed to observe an increase in host-range mutation of T2 following treatment with nitrogen mustard but failed to exclude the possibility of selection. We (LOVELESS and STOCK, unpublished) found that T2 and T2h *in vitro* showed identical patterns of inactivation by nitrogen mustard but obtained no significant mutation thereby. A number of apparent examples of ultra-violet induced mutation involved parallel or simultaneous irradiation of the host cells and it is probable that "mutation" was, in fact, the consequence of recombination between phage and bacterium (STENT, 1958). The first really convincing example of induced phage mutation was that afforded by LITMAN and PARDEE (1956) who employed bromouracil which was already known to be capable of assimilation into T2 DNA in place of thymine during phage synthesis (DUNN and SMITH, 1954). Unfortunately, it is not possible to assert that there is any relation between assimilation and mutation, and, since the bromouracil must be administered during the vegetative phase, one cannot entirely rule out the possibility of "indirect action". (KIHLMAN (private communication) concluded that the radiomimetic action of various purines could not be due to incorporation into DNA.) Unambiguous evidence of chemical reaction of phage *in vitro* leading to an enhanced mutation rate during subsequent multiplication was afforded by treatment of T2 with ethylating agents (LOVELESS, 1958, 1959) and with nitrous acid (VON VIELMETTER and WIEDER, 1959).

The results of the Tübingen group seem to establish that the deamination of nucleic acid bases is the essential chemical step determining increased mutability. Our own results with ethylating agents certainly implicated the germinal substance since the chemical reagents were not transferred to the host cells at a concentration showing measurable activity upon the complex. It cannot, formally, be excluded that reaction with the minority protein component of the germinal substance might have contributed to the mutational events. ROBERTS and WARWICK (1958 and private communication) have clearly established the extensive reaction of ethyl methane sulphonate with free or bound cysteine in the rat.

Many of the classical alkylating agents have been successfully used as mutagens in bacteria (DEMEREC, 1953, GLOVER, 1956) as also, recently, nitrous acid

(KAUDEWITZ, 1959). In addition other less readily classifiable agents, such as manganous chloride, have proved effective mutagens. We (LOVELESS and HOWARTH, 1959) have extended the study of the mutagenicity of ethyl methane sulphonate to *Salmonella typhimurium* and *Escherichia coli*; it proved very effective in the production of revertant and auxotrophic mutants in *S. typhimurium* and of phage-resistant mutants in *E. coli*. In none of these cases, however, are we entitled to assume that the mutagenic effect was due to an initial reaction between agent and DNA, whether of the gene(s) studied or not.

Whether this is or is not the case could conceivably be established by the use of transduction or recombination methods, but only in respect of a mutagen of very high activity such as ethyl methane sulphonate.

MUTATION OF HIGHER ORGANISMS

The whole study of chemical mutagenesis derives from the observation of induced mutation in a higher organism, to wit, *Drosophila*, by an alkylating agent, to wit, mustard gas (AUERBACH and ROBSON, 1947). Since that time this and many other alkylating agents have been shown to be mutagenic for *Drosophila*, moulds, flowering plants and other groups. A complete bibliography would be laboured and unnecessary but, RAPOPORT (see Bibliography in LOVELESS (1951)) and WESTERGAARD (1957) should not go unnoticed. In addition, many other chemical substances which cannot be considered as alkylating agents have been acclaimed as mutagenic; it is not my intention to itemize these. Again, a host of agents has been classified as "radiomimetic" on the basis of cytological studies, some unequivocally, others dubiously. Only a few of these can claim the description of "mutagenic" by reliable genetic tests. None, and this is the important issue, has shown a spectrum of mutagenic action comparable with that manifested by the alkylating agents. It must also be remembered that chemicals which are reactive for protein groups exclusively do not prove to be radiomimetic by cytological criteria, and have been found inactive or of restricted activity as mutagens. However, it could well be that a potential mutagenic action would not be observed owing to the absence of any basis for specificity in chromosomal protein.

DISCUSSION

Whilst retaining a moderate scepticism, the experimental evidence permits us to make the pragmatic assumption that the majority of chemically-induced mutations proceed from a direct association of the mutagen with genetically active DNA. This may be by direct reaction, e.g. alkylating agents and nitrous acid, transient incorporation, e.g. bromouracil and other base analogues, or some other type of association leading to atypical development, such as adsorption of the sort occurring with acridine derivatives. It is difficult to avoid the further assumption that the operative association is at a site identical with, or functionally related to, the actual locus studied. It should not be impossible, given the co-operation of geneticist and biochemist, to establish whether these assumptions are substantially correct, and, in the attempt, important information should be obtained concerning the *in vivo* configuration of DNA and the manner of its replication and function.

Any model purporting to describe the induction of mutations by DNA-reacting substances must provide answers to the following questions:

(1) Why do only a small minority of a treated population produce mutants though all must have undergone extensive chemical reaction? (This, for example, is the case with ethylated T2).

(2) How, on the one hand, can a treated cell give rise only to mutant progeny (DEMEREC's (1953, 1946) zero-point mutation)?

(3) How, on the other hand, can a mutation arise after a number of divisions of the treated cell (DEMEREC's end-point mutation)?

(4) What, in the latter case, is the nature of the pre-mutational state?

(5) How can a particular chemical agent bring about both forward and reverse mutation at a given locus?

SUMMARY

The evidence for regarding DNA as a "genetic substance" in viruses, bacteria and higher organisms is reviewed, and information obtained from studies in chemical mutagenesis presented. Although certain substances are capable of initiating mutation in micro-organisms which may not have this property for higher organisms, and vice versa, the universality of action of certain alkylating agents as mutagens is considered to indicate a common mechanism of action. The biological results and the known chemistry of such alkylating agents warrant the assumption, which should not be difficult of verification, that this mechanism is based upon primary reactions with DNA.

The proposition of any model purporting to explain the occurrence of mutation as a consequence of alkylation of DNA must take account of five questions posed.

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DISCUSSION

J. DANIELLI:

(1) You have pointed out that, in practically all cases, only a small minority of genes treated with a chemical mutagen (and known to have reacted) actually give rise to a mutation. May this not be due to the action of a mutagen being based upon a side-reaction, and not the characteristic reaction of the mutagen?

(2) May it not be that ethylated phage is not in fact itself "mutated", but merely acts as a convenient mechanism for carrying ethylated bases into cells, where, either as single bases or as abnormal polynucleotides the abnormal constituent produces mutation in the same way as any other chemical mutagen?

A. LOVELESS:

(1) I think this is true of the majority of the mutagenic alkylating agents which show a high toxicity and low mutagenic efficiency (based upon the population treated). In the case of ethylmethane sulphonate it may be that the 'typical" chemical reaction is equivalent to the "atypical" reaction of these in accord with its very low toxicity and high mutagenic efficiency.

(2) If one accepts DNA incorporation of unnatural bases (e.g. bromouracil) as underlying the mutational events, then the DNA constituted by the incorporation of an ethylated base or polynucleotide derived from the invading phage material would be likely to be indistinguishable from the latter and, therefore, still only in a pre-mutational state.

F. KASTEN: Do you consider that the mechanism of action of the potent mutagenic agents is the same as that which occurs when these agents are carcinogenic?

A. LOVELESS: I doubt it; I am not enamoured of the mutational "theory" of cancer.

W. PLAUT: To what extent could some of your questions be answered by assuming genetic multiplicity?

A. LOVELESS: The evidence adduced by DEMEREC in respect of "end-point" mutations, which, so far as I am aware, is still valid, rules out the possibility of "genetic multiplicity" playing this role in bacteria. In our own experiments with T2, single burst experiments revealed that mutants were always accompanied by wild-type phage in any one burst and the proportions of mutants varied greatly.

P. FREDERICQ: Some chemical mutagens are known to induce prophage development in lysogenic bacteria or colicin synthesis in colicinogenic bacteria. Do you know if it is by the same mechanism as the one by which they induce mutations?

A. LOVELESS: Our evidence is that it is not. We have tried by every obvious means to induce K12 (λ) by means of ethylmethane sulphonate, without success. Cells which fail to produce colonies consequent upon treatment neither increase in size nor lyse, thus excluding the possibility of the production of defective λ. Dr. OZEKI has likewise failed to induce the formation of colicin in *S. typhimurium.*