MUTATIONS IN ESCHERICHIA COLI INDUCED BY CHEMICAL AGENTS[1]

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For over two decades geneticists have been interested in the possibility of inducing mutations with chemicals. Particularly, it has been hoped that mutagenic compounds might be discovered which, through their specificity of action, would lead to some understanding of the chemical basis of mutation, and ultimately of the structure and organization of the gene.

The first clearly successful attempt to induce genetic changes chemically was described by Auerbach and Robson (1944), who produced mutations and chromosomal abberrations in Drosophila by exposing the flies to mustard gas and related compounds. More recently, Demerec (1947 and unpub.) has shown that certain carcinogenic hydrocarbons (1, 2, 5, 6-dibenzanthracene, methylcholanthrene, beta naphthylamine, benzpyrene) are also effective in inducing mutations in Drosophila. These agents, like radiations, appear to be entirely nonspecific in the sense that the affected loci are distributed at random along the treated chromosomes. Nitrogen mustard has been shown to induce genetic alterations in Neurospora (Tatum, unpub.; Horowitz, Houlahan, Hungate and Wright, 1946), and in bacteria (Tatum, 1946; Bryson, unpub.), and at least one of the carcinogens, methylcholanthrene, is effective in Neurospora (Tatum, unpub.). These successful results seem to be the opening guns in what Muller called, a few years ago, "the coming *chemical* attack on the nature of the gene" (Muller, 1947). They suggest the need for a systematic survey to determine the distribution of mutagenic compounds among various chemical groups, and to lay the groundwork for subsequent analysis of their mode of action. This paper will deal with preliminary results obtained in tests of 4 substances, the first of a series to be investigated in an extensive survey.

Two methodological factors are of critical importance in an attempt to examine large numbers of compounds for mutagenic activity: the basis upon which the chemicals are selected for test, and the choice of biological material. Concerning the method of selecting chemicals, one sober if somewhat unimaginative approach is an indiscriminate raid on the nearest chemical shelf, which has the advantage of objectivity and avoidance of the hazards of premature preconceptions. On the other hand, it is far more tempting to extend oneself on the basis of present ideas concerning the possible organization of genic material, and to select chemicals which might reasonably be expected to affect, or fail to affect, the projected hereditary units. The approach used in these experiments has been to assume that nucleoproteins are somehow centrally involved in the genetic system, and, as a starting point, to investigate chemicals known to have some more or less well-defined chemical or physical effect on nucleoproteins or nucleic acids. It must be emphasized that this approach has no greater justification on *a priori* grounds than many others, and that the basis of selection may prove to be entirely spurious, since none of the chemicals tested thus far is specific in its action on nucleoproteins or nucleic acids.

The choice of biological material is obviously very important in this type of investigation. The primary requirements are 1) the availability of techniques for treating the organism with chemicals so as to be reasonably certain that they will reach and penetrate the critical sites, and 2) the availability of clear-cut genetic methods for detecting induced mutations. The penetration problem has been the most serious difficulty in the use of Drosophila for chemical induction, and although improved methods of treatment have been developed, the possibility remains that negative results may be due to the failure of some chemicals to penetrate the germ cells in sufficient concentration. The genetic techniques for detecting induced mutations in Drosophila are unparalleled in many respects, but for purposes of an extensive survey of the mutagenic action of chemicals, they are extremely laborious and slow. The problem of penetration is much less serious in microorganisms. Until recently, however, genetic methods analogous to the ClB and similar techniques in Drosophila have not been available for bacteria. At the present time, *Escherichia coli* provides promising material for a survey of the mutagenic activity of chemicals, and for detailed analysis of certain aspects of their mode of action.

Luria and Delbrück (1943) described mutants of strain B of *E. coli* which are resistant to one or more bacteriophages to which the parent strain is sensitive. These mutants arise spontaneously in cultures of the B strain at a rate of about 10^{-8} mutations per bacterium per generation, and can be detected easily by plating out samples of the culture in the presence of an excess of bacteriophage. The

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