FUNCTIONAL AND NON-FUNCTIONAL MUTATION IN A BACTERIAL SPECIES

Thesis

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By

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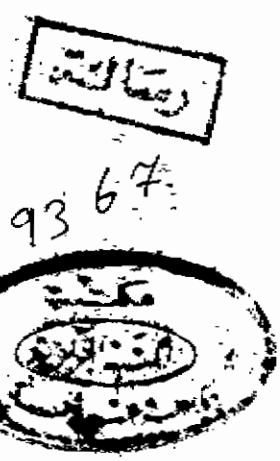

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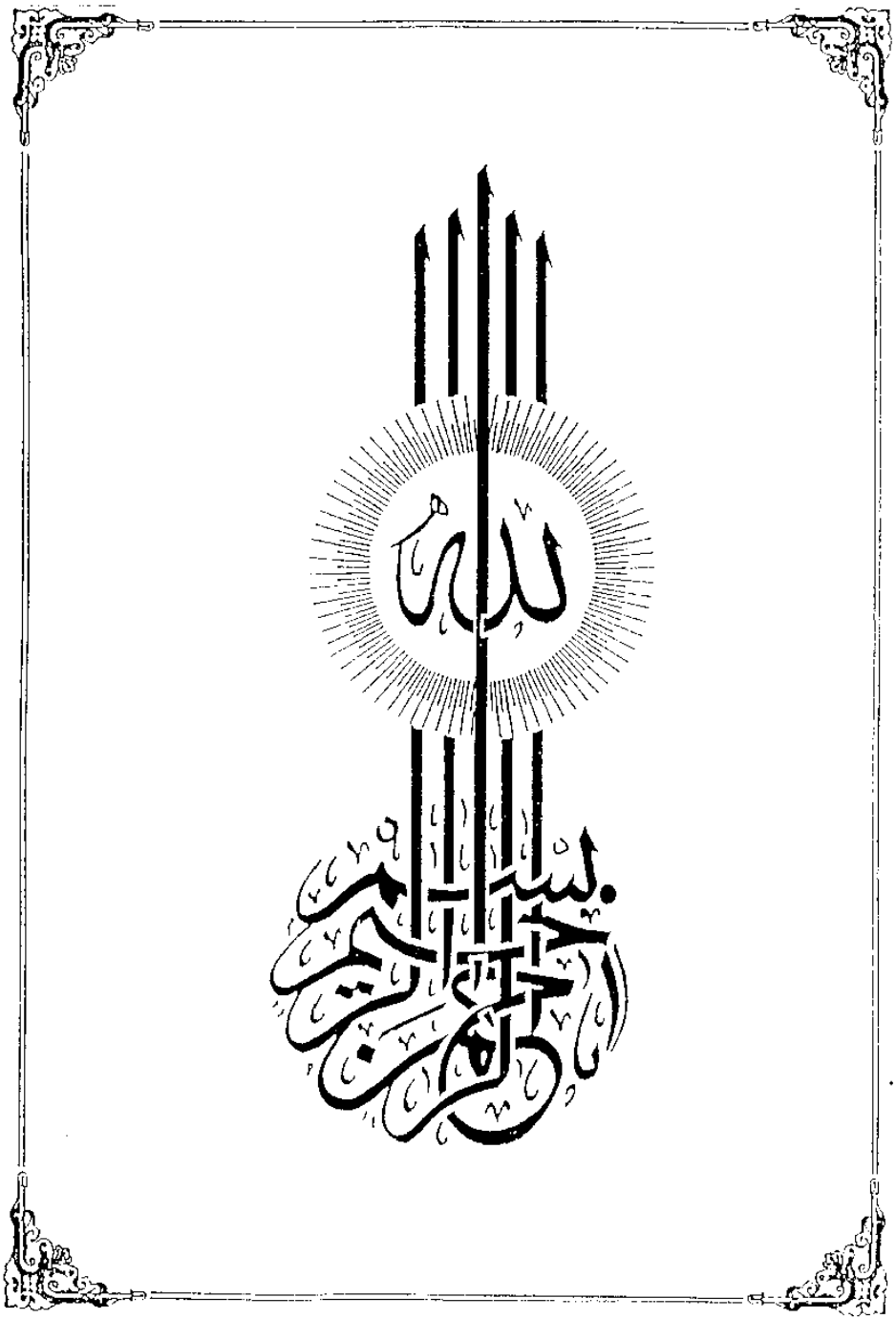

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INTRODUCTION

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Humans are being exposed to a wide variety of environmental chemicals that are mutagens or carcinogens (Hiatt et al., 1977).

Mutation is one of the most fundamental properties of the gene. According to Watsen-Grick model (1953) the DNA molecule consists of a complementary pair of poly nucleotide chains, held together by hydrogen bonds between adjacent purines and pyrimidines. The entire molecule can thus be pictured as a linear sequence of nucleotide pairs, the exact order of these pairs forms a code which contains the information necessary to govern the specific function of the cell. Any permanent alteration in the sequence of the nucleotide pairs would, according to this model, constitute a mutation. Therefore, studies of mutation are of great importance in the understanding of the nature of the genetic material and its function.

110.000 out of 550.000 deaths recorded in England and Wales are ascribed to the large of neoplastic diseases, characterized by the growth of new abnormal body

tissues knwon as cancer. Also, 210,000 deaths of the recorded aases due to the diseases of the circulatory system and only about 4.000 cases are due to the infective and parasitic diseases in the recent years.

The distribution of some forms of cancer is very far from uniform. For example, some counties in Wales show rates of stomach cancer about double those in counties of South-East England, while the reverse situation is found in the case of bladder cancer. Such differences are however, very small compared with those found on a world scale, where areas of high and low cancer incidence differ by a factor of 10 for a number of cancers and even 1000 for primary liver cancer.

Local differences can be very marked, particularly in relatively undeveloped areas with little movement of population and with largely local sources of food, but highly developed countries also differ greatly among themselves in their cancer patterns. Very high incidence of stomach cancer and the relatively low incidence of breast cancer in Japan, compared

with the United States and some countries of Western Europe. France showed a lower rate of stomach cancer and much higher rate of oesophageal cancer compared with West Germany, and the extremely high rate of lung cancer in Britain.

Cancer incidence can also change markedly with time. In Britain, as in various other industrialized coutries, the most spectacular change has been the long continued increase in cancer of lung and bronchi. Deaths in England and Wales from this cause have been increasing by about 900 annually and now account for nearly four times the number of deaths attributable to road accidents.

With such differences in cancer patterns it now seems inescapable that much cancer must have environmental causes which vary greatly from place to place and also with time, and the formerly widespread view of cancer as something inevitable has been giving way to a belief that 80% or more of human cancer is environmental and it should, at least theoretically, be possibile to prevent it.

Of the hundreds of known chemical carcinogens, a small number were originally identified through being causes of human cancer and the rest as a result of tests in experimental animals. To find out if a compound is carcinogenic, tests in animals are still essential, and are commonly made using rats and mice. Strong carcinogens are relatively easy to detect. Weak carcinogens, which may still, be important if present in the environment, hard to detect because of the small numbers of tumours incluced even by prolonged treatment and the need to distinguish these from spontanous tumours.

If appropiate tests show a compound to be carcinogenic in an experimental animal it is prudent to assume it will all be carcinogenic in man, but marked species differences have been found in the bioligical behaviour of foriegn compounds. The important carcinogen 2-naphthylamine, for example, induces bladder tumours in man and dogs but liver tumours in mice, breast tumours in one strain of rat and is inactive in rabbits.

Chemical carcinogenesis is a slow process related, at least roughly, to the life-span of the species concerned. The time between exposure to an industrial carcinogen and the appearance of cancer may be under 5 or even 40 years.

There is considerable evidence that a large proportion of human cancer may be caused by exposure to toxic chemicals in the environment, very few of which have been tested for carcinogenicity or mutagenicity (McCam *et al.*, 1975). A number of rapid *in vitro* systems for detecting these chemicals have been developed (Hiatt, *et al.*, 1977).

The purpose of the present investigation is an attempt to determine the mutagenicity and carcinogenicity of cyclohexanone which is commonly used as a solvent for many pesticides, in *Bacillus subtilis*.

REVIEW OF LITERATURE

An ideal test of the mutation hypothesis would to show by direct base sequence analysis that in the DNA from a gene which has suffered a single mutation, just one specific base-pair has been subatituted by another. Since this is technically impossible at present one must resort to indirect methods. Among the most promising of these is the use of certain groups of chemicals which are not only highly mutagenic but are also known to intereact with the purine and pyrimidine bases of DNA in very specific ways. These chemicals fall into three main categories. These are the base analogues which can replace the normal bases of DNA during replication, substances which chemically alter the bases of resting DNA, and those whose action is to remove DNA bases.

1) Base analogues:

These are close analogues, of normal nucleic acid bases which are, or can reasonably be expected to be, incorporated into DNA without destroying its capacity for replication. However, because, the

analogue differs from the normal base in the distribution or stability of its hydrogen atom, it has a greater tendency than normal to improper pairing. The most potent mutagens among the base analogues are 5-bromouracil (BU) and 5-bromodeoxyuridine which are analogues of thymine in which the methyl group at the 5 position is substituted by a bromine atom, and 2-aminopurine (AP) which is similar to adenine except that the amino group is attached to the 2, instead of the 6-position.

5 bromouracil is incoporated into the DNA of both bacteria and phage, replacing thymine quantitatively, so that a proportion of A-T base pair is replaced by A-BU pairs. Its mutagenic behaviour was thought to reflect its greater probalility than thymine of accidentally pairing with guanine, due to unstabilisation of its hydrogen atoms by the bromine atom which has a higher electronegativity than the substituted methyl group (Freese, 1959 a).

2) Substances whose chemical action alters the nucleic acid bases of resting DNA:

The most important of these substances is nitrous acid (HNO_2). Unlike base analogues whose mutagenic action depends in their incorporation during DNA replication, nitrous acid is highly mutagenic not only for free phage particles (Tessmens 1959 , Vielmetter and Schuster, 1960, Bautz-Freese and Freese, 1961) but also for isolated DNA in pneumococcal transformation (Litmann & Ephrussi-Taylor, 1952) and for purified RNA derived from tobacco mosaic virus (Mundry and Gierer, 1958). Nitous acid acts directly on nucleic acids by oxidatively deaminating their bases.

3) Substances or treatments which remove bases from D.N.A:

The most important of these are the alkylating agents which include the sulphur and nitrogen mustards and ethylene oxides, as well as much more and much less toxic mutagens as ethylethane sulphonate (E.E.S) and ethyl methane sulphonate (E.M.S) (Loveless, 1958,