

CHEMICAL INDUCTION OF MUTAGENESIS AND CARCINOGENESIS

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1. Introduction

It is taken for granted that man strives to control his environment by the extensive use of energy sources. Many of these energy sources are chemical and result in unstable intermediates in side products. Areas which require large energy outputs for industrial purposes, automotive uses and a variety of other needs may be expected to contain harmful concentrations of chemical side products.

Most industrialized cities and cities with extensive automotive traffic have large areas containing variable amounts of chemical impurities, at all times. More rural areas may have seasonal or transitory periods where field burning or some equivalent action results in high concentrations of air or water borne impurities. Local zones having very high concentrations of chemical impurities may also exist for variable periods. Notably, kitchens and bathrooms may be exposed to a variety of potentially hostile chemicals such as an almost infinite variety of aerosols, aromatics and "germ-killing" agents most of which have unknown long range effects on man and other organisms.

Fossil fuels are being used at a rapid rate such that we may expect a world-wide and ever increasing amount of atmospheric impurities over the next several years. The major sources of chemical impurities comes from hydrocarbon combustion, insecticides and herbicides, cosmetics and cleaning agents including dyes, food additives and perhaps, indirectly, the extensive use of inorganic fertilizers.

Before proceeding, I wish to state working definitions of the basic genetic conditions, mutagenesis and carcinogenesis. DNA is localized in the chromosomes of organisms and is composed of four small molecules arranged in triplet information bits. A set of triplets comprises a basic information unit called a gene or cistron. In cellular function, the information contained in a cistron is transcribed onto a complementary RNA segment which, in turn, is translated into one amino acid. The amino acids are assembled into the same sequence as was contained in the cistron. If the triplet code is intact, then the condition is referred to as *wild type*. On the other hand, if the triplet sequence, composition

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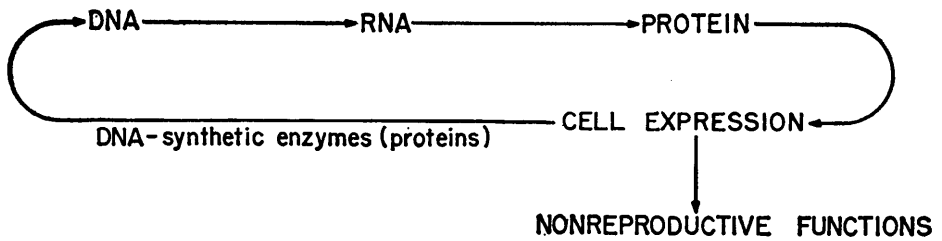


FIGURE 1

Cell cycle.

of each triplet, or number of triplets is altered, then the net information in a given cistron is altered and the condition is referred to as a mutation. There is usually a correspondence between a functional protein unit (enzyme) and a cistron; however, some proteins, such as hemoglobin, may be comprised of peptides emanating from more than one cistron. In such cases, a mutation in either peptide results in an altered protein and the relationship between protein and DNA is still functionally the same.

2. Mutagenesis

Mutagenesis results from the genetic transferable material in an individual undergoing a chemical alteration such that the genetic information in the altered cell contains some different information. In higher organisms we are only concerned with the reproductive cells; however, in lower forms such as one celled organisms generally all the cells are reproductive.

3. Carcinogenesis

Carcinogenesis results from an alteration in a cell or tissue such that the growth and functional properties of the cells are altered. An alteration of the genetic information in a cell is not a requirement. It is generally recognized by developmental biologists that every cell in higher organisms has the same genetic information in a given animal and that specific tissues have cells whose genetic expression is modified (reduced) such that a specific tissue performs limited functions. For example, brain cells do not normally divide in adult mammals; however, if some of the brain cells in an individual do start to divide (grow) a brain tumor is the result. It is generally believed that these cells have become de-repressed and in so doing have become more primitive such that they can now divide. Cell division is apparently inhibited by the repression of the appropriate genetic information in mature mammalian brain cells. Several mechanisms may account for the causes of cancer. I merely wish to establish that mutagenesis and carcinogenesis are not mechanistically the same, although

some carcinomas may be caused by mutagenic events. Certainly, many chemicals will cause either or both.

4. Chemicals which are reactive with nucleic acids

I define these as either causing changes in covalent bonding (Type I) or as having some noncovalent interaction (Type II). Type I would include base analogs, some chemicals which attack functional groups on nucleic acids and the chemical alkylating agents. Type II would be dyes and other chemicals which intercalate into DNA or in some way interfere with nucleic acid enzyme functions.

Some known mutagens have an incredible degree of specificity for a given cell type. A small chemical alkylating agent, ethylmethane sulfonate (EMS) has been used extensively in chemical and mutagenic studies and in the fruitfly, *Drosophila melanogaster*, it acts primarily on mature sperm [1]. EMS has very little effect on other stages of sperm development, somatic cells, or on female *Drosophila*, in general, at equivalent concentrations. Normally, *Drosophila* males are placed in vials containing a small piece of porous paper saturated with a solution of 0.003M EMS and exposure is for 24 hours. By contrast, haploid yeast is exposed to about 0.3M of EMS for an hour or less in solution and this concentration results in a relatively high mutation rate. The extent of contact is hard to compare but certainly much larger effective doses are used for yeast. Probably any cell is sensitive to EMS at some concentration. Therefore, the seemingly unique sensitivity of sperm (mammalian sperm, as well) may relate to permeability processes. The scheme shown in Figure 2 shows the

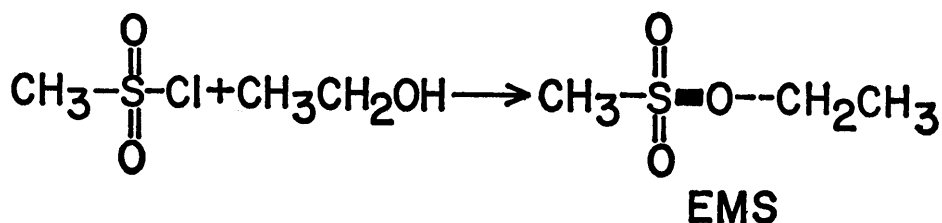


FIGURE 2

Synthesis of ethylmethane sulfonate (EMS).

synthesis of EMS and illustrates that the bond shown by the heavy line is formed during the synthesis. Conversely, the decomposition of EMS proceeds by breaking the bond shown by the dotted line (Figure 3). The alkyl group has a high affinity for nucleophilic sites such as those on the N₇ position of guanine or else is hydrolyzed by water to yield the original starting materials, methane sulfonic acid and ethanol (Figure 4). This reaction proceeds by the familiar S_N2 mechanism. Many known mutagenic agents are alkylating agents which alkylate by the S_N2 mechanism.

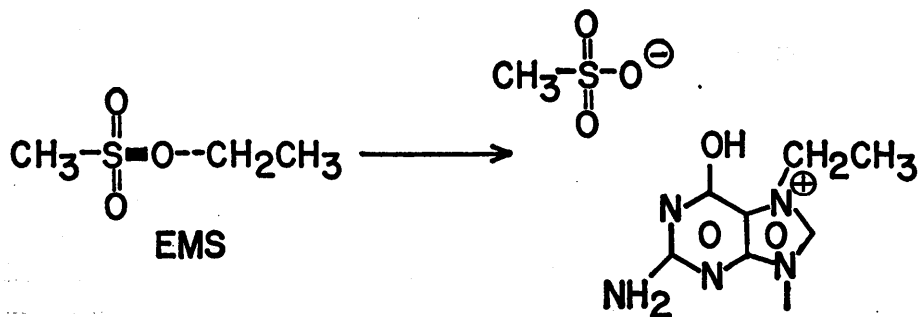


FIGURE 3

EMS alkylates guanine.

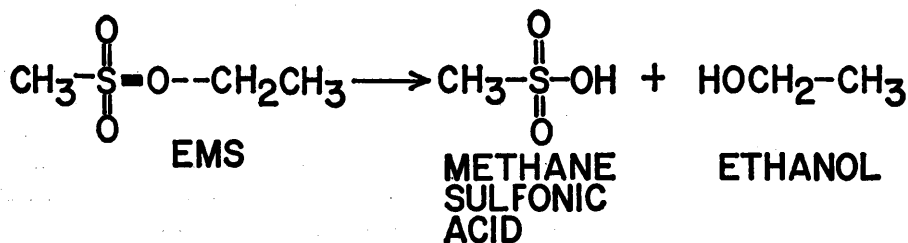


FIGURE 4

EMS decomposes in water.

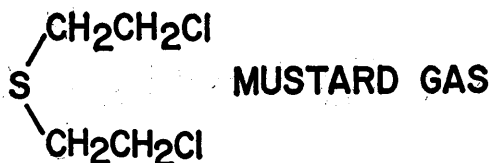


FIGURE 5

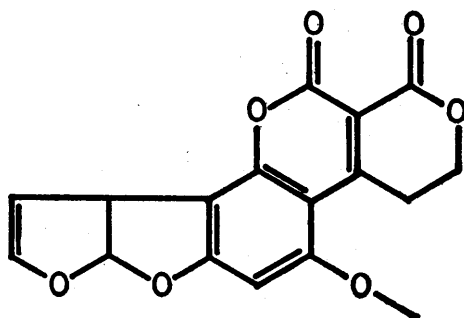
Another chemical mutagen.

Mustard gas is also a mutagenic and alkylating agent (see Figure 5). This compound has a different cellular specificity than EMS in that *Drosophila* late spermatogonia are sensitive to mutation while the mature sperm are resistant [2]. This is determined by observing that broods from virgin females mated on sequential days to the exposed male do not show appreciable mutations until about the fifth or sixth day, while treatment with EMS results in high mutation rates the first three days and then demonstrates a drastic reduction of mutation rate in subsequent days.

There is a large body of literature dealing with induced chemical mutagenesis. The two chemicals mentioned and a few other alkylating agents have had extensive research carried out on them and demonstrate that chemically unstable species are potentially extraordinarily dangerous. The myriad of chemicals human populations come in contact with, by and large, have not been characterized with respect to mutagenesis and carcinogenesis, especially on human subjects.

Many chemicals also cause carcinogenesis. Some are natural products and some are synthetic. For present purposes these fall into two groups. Those that are carcinogenic in their native state without requiring metabolism of the host organism. The other group must be acted upon by the host organism; therefore, the second group are detoxification products or products of enzyme-mediated reactions. In either case the carcinogen has some action on a given tissue such that the tissue becomes abnormal in its growth and/or function.

In the early 60's in England 100,000 turkeys died of cancer which had been caused by grain infected with the mold *Aspergillus flavus* which produce chemicals known as Aflatoxins. The Aflatoxins are among the most carcinogenic



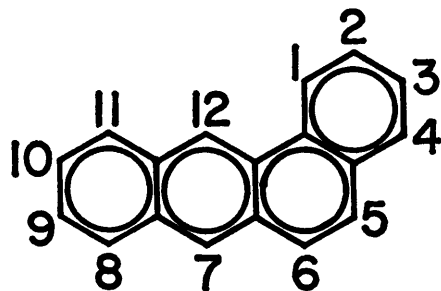
Aflatoxin G

FIGURE 6

A fungal metabolic product which is carcinogenic.

substances known. These chemicals in $\mu\text{g}/\text{kg}$ body weight quantities cause cancer with an extremely high rate. Whether a given chemical is carcinogenic is often not predictable and must be determined by experimental means on the organism in question. Therefore, while we know such chemicals as Aflatoxins cause cancer it was not possible to determine this by inspection of the chemical formula but only through experimental evidence.

The majority of research carried out on chemical carcinogenesis has been phenomenological; however, some work in recent years has been more analytical and allows some generalizations. Huggins and co-workers [3] showed how small chemical alterations on substituted benzantracene molecules drastically altered the frequency of induced carcinogenesis in rats. Methyl groups on carbons 7

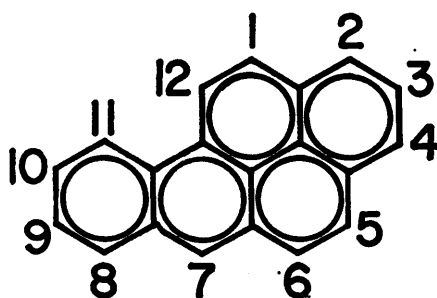


BENZANTHRACENE

FIGURE 7

A polycyclic aromatic hydrocarbon carcinogenic.

and 12 result in a compound which is among the most carcinogenic of all chemicals known. Ethyl groups on carbons 7 and 12 result in a compound which is comparatively harmless. These authors tested a variety of substituted benzanthracene compounds at different ring positions and concluded that the positions 6, 7, 8, and 12 were the most active sites. The exact molecular geometry is critical as to whether a given polycyclic aromatic hydrocarbon is carcinogenic. The consideration is also important as to whether the compound itself is carcinogenic or a metabolic product. If the latter is true, then we expect considerable variation based on small molecular geometry changes due to enzyme specificity as a function of molecular geometry. Cavalieri and Calvin [4], some years later, proposed that 7, 12-dimethyl-benzanthracene was a carcinogen due to an enzyme-mediated electrophilic attack on either carbons 6 or 8. They elaborated somewhat more on a related substance, benzapyrene. They proposed that benzapyrene is possibly mutagenic in rats due to enzyme-mediated electrophilic



BENZAPYRENE

FIGURE 8

A polycyclic aromatic hydrocarbon carcinogenic.

attack on carbon 6 by an oxygen atom causing positions 1 or 3 to become a reactive electrophilic center. The electrophilic zone would then be expected to react with a cellular site which would result in cellular transformation into a tumor type. The implications of these last two papers are that, an animal, in trying to protect itself from harmful chemicals may, in fact, produce new chemical species which are carcinogenic. Mammalian organs and tissues are capable of a great many enzyme-mediated oxidative reactions. The closest molecular analogs to the two polycyclicaromatic hydrocarbons just mentioned are probably sterol molecules of which cholesterol is an example. Mammals are



FIGURE 9

A natural product cyclic hydrocarbon in animals.

capable of both complete chemical synthesis and oxidative degradation of sterols. A hydrocarbon of similar structure could well serve as a pseudosubstrate. It is important to understand these metabolic relationships in consideration of the control of cancer.

Studies dealing with either mutagenesis or carcinogenesis are difficult to apply to humans or human populations. Some chemicals probably are mutagenic or carcinogenic to virtually all cells of all organisms. Among these would be the unstable chemical species such as alkylating agents and other reactive chemicals. A wide variety of other chemicals have wide variability dependent upon the species, age, or metabolic condition of a given organism. For example, compound A may be mutagenic to *E. coli*, bladder cancer inducing to man, cause liver cancer in the rat, and have no observable effect on the guinea pig. An ensemble of such compounds exists which have variable and species-dependent effects.

The statistician works with systems where partial ignorance exists. The amount of ignorance is great in consideration of present day cancer or mutation induction by chemical means; consequently, efforts must be made to reduce the number of variables on these systems. I also wish to emphasize that cancer frequency may be trivial to future generations, however frightening it appears to the current generation. Mutation frequency, on the other hand, may seem

(and truly be) unimportant to the present generation since germ cells are unimportant to the owner's personal physiology; however, the long range survival and quality of survival of man depends more on mutation frequencies.

Probably the only feasible way to monitor the possible effects of chemical impurities on human populations is to use microbes and higher organisms with shorter generation times than those of man. Appropriate sampling of impurities and controlled experimentation will allow high probability extrapolation to determine the effect on humans. Agencies having control over allowable limits of air and water impurities should realize that as the frequency of deleterious genes increases in human populations an increase in the frequency of monster births and defective offspring is not far behind. Some upper limit exists for the genetic load beyond which a species cannot survive.

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Discussion

Question: R. J. Hickey, Institute for Environmental Studies, University of Pennsylvania, Philadelphia

I believe you stated that there are chemicals which are carcinogenic but not mutagenic. Would you take the position that this will be a true statement for all time, and that it will never be demonstrated that such carcinogenic chemicals are not, in fact, also mutagenic?

Regarding the de-repressor concept, is it not possible that this phenotypic character of the cell could originate in part, and perhaps in large part, with the cell genotype? Could not modification of the cell genotype of some particular pattern effect repressor activity or function? I confess little knowledge in this area. I am merely curious.

Reply: A. Keith

It is simply a fact that some chemicals are carcinogenic in a given species and are not mutagenic in the same species. The way you have asked your question invites uncertainty. Of course, some chemicals which meet the criteria I have just mentioned may be mutagenic on some other species. The main point is that there is not a one to one correspondence between chemicals which are carcinogenic and ones which are mutagenic.

In answer to your second question, yes.

Question: Alexander Grendon, Donner Laboratory, University of California, Berkeley

Even though, as you pointed out, some chemical agents produce cancers and have not been shown to produce mutations and though your hypothesis as to their carcinogenic mechanism seems very plausible, have there, in fact, been any experiments that rule out the possibility that the process occurring during the formation of such cancers involves a change in the DNA of the affected cells?

Reply: A. Keith

I know of no such experiments.

Harold L. Rosenthal, School of Dentistry, Washington University

Your paper shows so beautifully what man can accomplish using his ingenuity and intelligence to develop that which is useful and to correct those things that may be harmful. At the same time, you make the statement that man cannot return to the land or give up the technology that may endanger our environment. I must take issue with the statement that we "can't" return. It may be necessary for man to return to the land if we don't use our intelligence and ingenuity to understand and combat the dangers of man's activities.