Chemical Mutagenesis*

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Summary

Whereas the hazards of ionizing radiation for the human genetic material have been recognized early, the danger of chemical mutagenesis attracted attention only recently. The human environment is being contaminated with chemical compounds, whose influence on the genetic material is only insufficiently known. Research in chemical mutagenesis has been undertaken in view of eliminating such hazards. In this review a short account on molecular mechanisms of chemical mutagenesis is given as an introduction. Testing systems available to assess mutagenic activities in many different organisms from viruses to man are then described.

Many chemicals—in most cases drugs—have been tested in such systems. Some of the results are presented. Emphasis is then placed on chemicals of generalized use, like food contaminants, where whole populations are exposed to possibly noxious compounds. Whereas in the case of drugs with known strong mutagenic activity precautions are possible in order to diminish the genetic danger for possible offspring, this might be impossible for environmental pollutants. In many cases a connection between mutagenic and carcinogenic activity of a given compound exists. Mutagenic substances are therefore suspected of being also carcinogenic. At least the available experimental evidence points in this direction.

The main problem of mutagenicity testing lies in the extrapolation of the results from animals to man. In this context the need for more research into the fundamental biochemical processes of uptake, distribution, metabolism, excretion and molecular mechanisms of genetic activity is obvious. For only a thorough understanding of chemical mutagenesis will lead to the complete elimination of dangerous substances from our environment.

Introduction

Mutations are sudden heritable undirected changes in the genetic material. They can be of unknown origin (i.e. spontaneous mutations) or they can be artificially produced by a variety of agents. The influence of ionizing radiation on the genetic material has become known early[1], and the successful experiments of Auerbach and Robson[2] opened the way to the induction of mutations through chemical agents. After years of only academic interest in chemical mutagenesis the increasing concern over the environmental pollution focused attention also on possible genetic consequences of this situation. The magnitude of this problem can only be estimated by considering, that nearly 25% of the admissions to the Montreal Children's Hospital are either from genetic diseases or from congenital malformations[3]. Increasing the mutation frequency would add considerably to this load. It has also been shown, that more than 30% of the recognized spontaneous abortions are associated with chromosomal defects. In this case, too, the pollution of the environment with mutagenically active substances could increase this figure proportionately. Environmental pollutants may not be very active mutagenic substances, but one has to consider thereby, that the whole population is exposed to such agents, whereas in the case of drugs, which may be much more active agents, specific target populations, under medical supervision, are exposed for limited periods of time only. In this review we will describe evidence for the mutagenicity of drugs and environmental contaminants, and point out possible guidelines for the assessment of the danger to human health.

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Molecular mechanisms of chemical mutagenesis

Mutations in general can be divided into gene mutations (point mutations), where only submicroscopical changes take place, and into chromosome mutations, which are microscopically visible alterations of chromosome structure or number. Only for the former class of mutations molecular mechanisms can be described, whereas for the latter practically no experimental evidence exists at the moment on how they arise.

Desoxyribonucleic acid (DNA) is the carrier of inherited information. This information is stored as a sequence of only four different bases. After transcription into ribonucleic acid (RNA), translation of the information into the amino acid sequences of proteins is accomplished in the following way: The base sequence is read in groups of three bases (triplets), which specify the amino acid to be inserted. Each base triplet codes for only one amino acid, but as there are 64 possible triplets and only 20 amino acids, the code is "degenerate", i.e. each amino acid may be specified by more than one triplet. The high fidelity of information transmission, and thus the genetic stability, is due to the very stringent base pairing rules. Adenine pairs only with thymine (in DNA, and with uracil, in RNA, resp.), and guanine pairs only with cytosine (Fig. 1). This base pairing is necessary for the sequence recognition in transcription and translation. If a base pairs with a wrong partner, a point mutation may be the consequence of the altered base sequence.

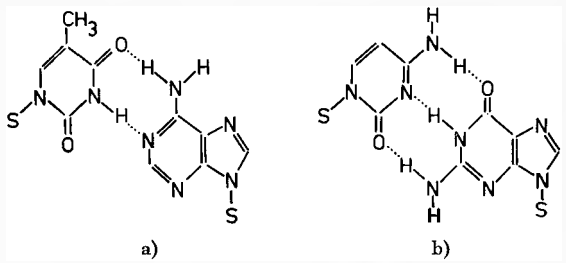

Fig. 1. Normal base pairing in DNA (S = desoxyribose). a) A-T pair, b) G-C pair

Depending on the type of chemical used two main categories of molecular mechanisms lead to point mutations: base substitution and intercalation. Base substitutions can arise from either a direct incorporation of a base analogue into the genetic material, or from a chemical reaction taking place at the DNA and altering one or more of the natural bases. The four bases, which constitute the genetic code, are to different degrees susceptible to such alterations. Alkylating agents, like diethylsulphate, methylmethane sulphonate etc., attack predominantly the N-7 position of guanine. This 7-alkylguanine has changed electronic properties and therefore a changed equilibrium between lactam and lactim form, which can lead to an erroneous pairing of this base (Fig. 2). Instead of pairing normally with cytosine the N-7-alkylguanine can form a pair with thymine, and will thus be replaced at the next replication by adenine.

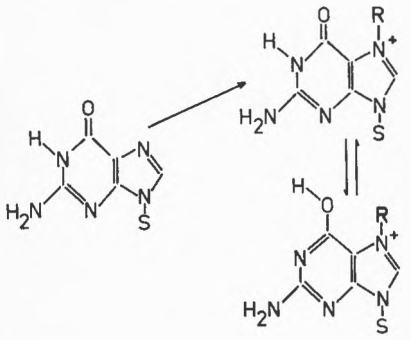

Fig. 2. N-alkylation of guanine and possible tautomeric shift

This in turn can lead to the insertion of a wrong amino acid into a polypeptide, whose function, e.g. as an enzyme, may be impaired by this exchange. A well known example for this kind of mutation are the several abnormal hemoglobins. Until recently this has been thought to be the main pathway for the mutagenicity of alkylating agents. Newer evidence[4] points to the O-6-alkylation, which occurs much less frequently than the N-7-alkylation, as the main mutagenic reaction, but the consequences—wrong base pairing—are the same (Fig. 3). Similarly base analogues, e.g. 5-bromouracil

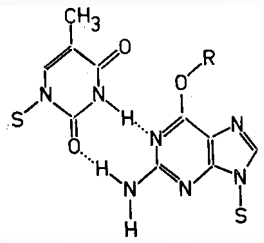

Fig. 3
O-Alkylation of guanine and wrong base pairing with thymine instead of cytosine

(BU), can be incorporated into DNA in the main tautomeric form, e.g. in the case of BU replacing thymine. Normally BU pairs with adenine, but occasionally it can also pair with guanine (Fig. 4). This again leads in the end to the introduction of a wrong amino acid into the protein coded for by the respective DNA chain.

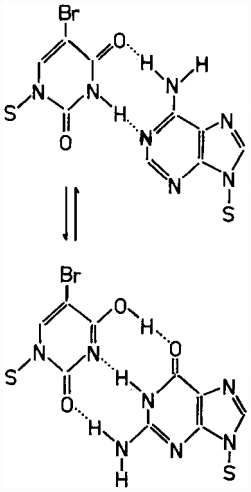

Fig. 4
Wrong base pairing induced by tautomeric shift in the base analogue 5-bromouracil

Another mechanism can lead to mutations, when chemical agents with special structural features bind physically to DNA. The basic structure of such an "intercalating" compound consists of a planar three membered heterocyclic ring system, as it is present in the acridines (Fig. 5). These agents have molecular dimensions enabling them to penetrate the DNA double helix between adjacent base pairs. The stacking of the base pairs remains undisturbed, but the helix is stretched to a small extent (Fig. 6), and this dilatation leads to mispairings during the next replication, the result of which are additions to or deletions from the DNA base sequence of one or more base pairs. Whereas the base substitution type of mutations leads to the insertion of only one

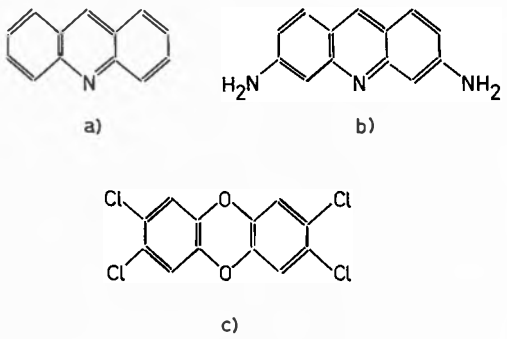

Fig. 5. Intercalating agents: a) Acridine. b) Proflavine. c) Tetrachlorodibenzo-*p*-dioxine

Fig. 6. Effect of intercalation on the DNA helix
- Sugar-phosphate backbone
- Base pairs
- Intercalated acridine

(After L. S. Lerman, *J. Cel. Comp. Physiol. 64* [1964] Suppl. 1, 1)

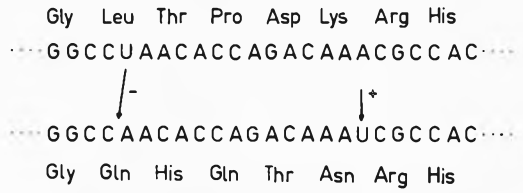

Fig. 7. Principle of reading frame shift mutations: deletion of one base in the sequence alters the corresponding amino acid sequence completely; addition of a base restores the original sequence

wrong amino acid into an otherwise unaltered polypeptide, additions or deletions of base pairs shift the "reading frame" in the translation process. The amino acid sequence of such a protein will—from the point of mutation on—be completely changed (Fig. 7), and such a protein will not only be impaired in its functions, but it will be completely non-functional. Such a mutation can even influence the next gene, in that the three codons used as punctuation ("stop reading" signals: UAG, UAA and UGA, resp.) will not be recognized, and the reading will be continued into the next gene.

These are in short the two main mechanisms responsible for the induction of point mutations by chemical agents. The existence of repair mechanisms completes the picture: damage within the DNA can be recognized and repaired by several mechanisms. These systems generally excise the damaged region of the DNA, synthesize this short DNA stretch according to the base sequence of the undamaged strand, and introduce it again into the whole DNA. Several enzymes are responsible for the exact execution of the diverse steps necessary for a successful repair. In bacteria two such repair systems are known, one of which repairs the damage with great fidelity, whereas the other system is much more "error-prone" and may introduce mutations by mis-repairing the damaged DNA. In eucaryotes the organization of DNA into chromosomes complicates the picture, but it has been possible to demonstrate the existence of repair mechanisms in mammalian cells, too[5].

For a more through treatise of these problems of molecular mechanisms the reader is referred to the excellent monograph of Drake[6].

Testing systems

The ability of a chemical compound to induce mutations can now be tested with a variety of methods. *In vitro* as well as *in vivo* test systems have been devised and used with organisms ranging from viruses to human cells. Inherent in all these systems is the incertainty of how to extrapolate the results to man. Although DNA is the same from bacterica to the elephant, this incertainty is in general the greater the lower the test organisms are. Higher organisms are considered to be more relevant to man, but even with cultures of human cells severe problems of metabolism and pharmacokinetics prohibit a clearcut interpretation of mutagenicity test results.

In addition all of the test systems currently used have their advantages as well as their disadvantages. A short description of a few generally used mutagenicity test systems will show their strong and weak points.

Submammalian systems are fast, sensitive and inexpensive. Bacteria, fungi, yeasts and insects have been used to determine the mutagenic activity of chemical compounds. The best known bacterial system are the many different histidine auxotrophic mutants of *Salmonella typhimurium*. The procedure of this bacterial plate assay may serve here as an illustrative example for the microbial test systems[7].

These bacteria, unable to carry out histidine biosynthesis, and thus dependent on an external source of this amino acid for growth, are grown in liquid culture to the desired cell density. They are then spread uniformly over an agar plate containing inorganic salts and glucose as the only carbon source. Histidine is added in very minute amounts, so as to allow the bacteria to grow for a few generations. This growth will allow the expression of mutagenic events evoked by the chemical agent to be tested, which is applied either as a solution or in solid form to the center of the petri dish. When the histidine is used up, only those bacteria can grow further and form colonies, which have mutated back to the wild type, able to synthesize histidine. Thus, after incubation, the number of colonies on the treated plates is compared with the number of spontaneously arising colonies on plates without added chemicals (Fig. 8). The particular set of bacterial mutants, which is used in this test, has another great advantage. The single strains have arisen from either a base substitution or an intercalation mutation; they can only be reverted by the same mechanism, i.e. a strain carrying a base substitution can only revert to the original state through another base substitution. Therefore testing a chemical with the whole set of tester strains not only indicates its mutagenic activity, but

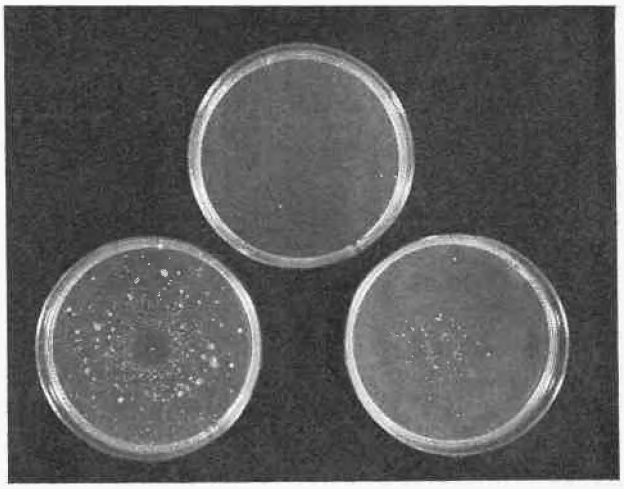

Fig. 8. Mutagenicity test with *Salmonella typhimurium:* Upper dish = spontaneous mutations, lower right dish = Mutations by 2-aminopurine, lower left dish = mutations by dimethylsulphate

also the type of mutation it induces. One great disadvantage of this simple assay is the missing of the mammalian metabolism. To avoid this shortcoming two different approaches have been tried. Either a liver homogenate is added to the agar plate in order to metabolize the chemical compounds added[8]. Another way of introducing mammalian metabolism into the simple microbial plate test is the so called "host mediated assay".[9] In this test microorganisms are injected into the peritoneal cavity of a laboratory mouse, whereas the chemical to be tested is introduced by another route into the animal. After some time the animal is sacrificed, the microorganisms are withdrawn, plated, incubated, and the colonies counted as usual. Besides of *Salmonella typhimurium* several strains of the genetically well known bacterium *Escherichia coli*[10,11] the fungus *Neurospora crassa*[12] and the yeast *Saccharomyces cerevisiae*[13] have been used in such test systems.

The most versatile submammalian system is the fruit fly *Drosophila melanogaster*, where not only single point mutations but also gross defects leading to dominant or recessive lethality can be detected. *Drosophila* has the further advantage that the fly's metabolism can perform most if not all conversions of chemicals which take place in the mammalian liver. As *Drosophila* is one of the genetically most studied organisms, many strains are in use, which are ideally suited for different purposes. A good review on the possibilities offered by *Drosophila* for mutagenicity testing has been given by Sobels[14].

Several mutagenicity tests are available which use mammals or mammalian cells; these include the dominant lethal test in mice, the specific locus test in mice, *in vivo* and *in vitro* cytogenetic tests and the above mentionned host mediated assay. The dominant lethal test[15] can be performed in males or in females. Ordinarily male mice are treated with single or repeated doses of the chemical and mated sequentially with several virgin females. The females are sacrificed on the 12th day of pregnancy and live and dead implants are counted. The ratio of these two figures indicates the mutagenicity of the compound in test, whereas the sequential mating demonstrates the sensitivity of the germ cells during different stages of spermatogenesis. This test is in widespread use in toxicological laboratories despite its disadvantages, among which are the relative insensitivity of the test and the—in many instances considerable—fluctuations in the control values. However, the use of this test diminishes the pharmacokinetic problems, as germ cells are involved, which allows the direct investigation of the mutagenic effects on the offspring.

Cytogenetic tests require generally highly trained specialists for evaluation; in this type of test, chromosomal aberrations are scored. The aberration can be structural, i.e. chromosomes can be broken, or pieces of one chromosome can be transferred to another chromosome (translocation), or it can be numerical, when one or several chromosomes, or the whole set of chromosomes is not present twice, as in normal cells, but only once (monosomy, monoploidy), or three or more times (polysomy, polyploidy). Several congenital diseases stem from such aberrations, among which Down's Syndrome (Mongoloidism), a trisomy of the chromosome number 21, is the best known. Cytogenetic tests can be performed *in vitro* or in *vivo*. After the application of the test compound cells are arrested during the cell division by the spindle poison colchicine (Fig. 9); the cells are then withdrawn, spread on microscopic slides, stained and examined for chromosomal aberrations in mitotic cells (Fig. 10).

A simpler and more sensitive version of the cytogenetic tests is the micronucleus test. During the mitoses of erythroblasts chromosome breaks lead to separate small nuclei[16]; these micronuclei are—for unknown reasons—remaining within the erythrocytes even after expulsion of the nucleus, and are thus easily scored (Fig. 11).

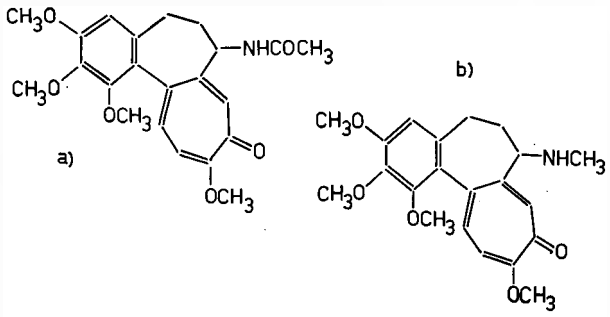

Fig. 9. Spindle poisons, a) colchicine, b) colcemid

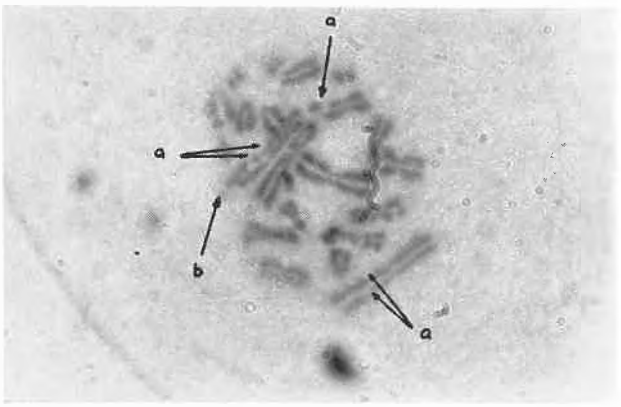

Fig. 10. Metaphase arrest of a bone marrow cell of the Chinese Hamster. The arrows point to structural aberrations, a) gaps, b) break with translocation

Further advantages are the short duration of the test, the small number of animals which has to be used and the fact, that the young erythrocytes (i.e. those less than 30 hours old) are staining blueish instead of red, thus allowing the scoring of mutations, which took place during this time[17].

The specific locus test allows the scoring of point mutations in mammals, in contrast to most of the mammalian test systems, which score chromosome mutations or

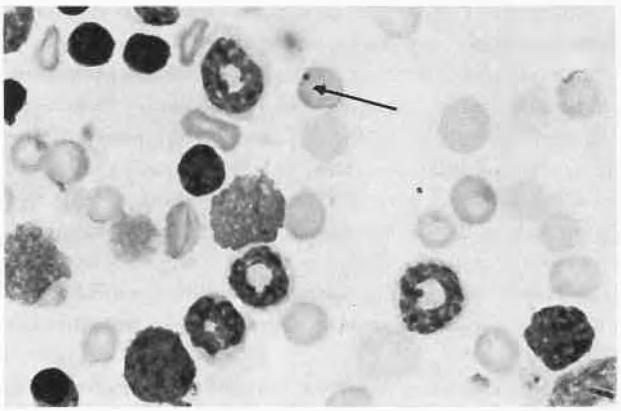

Fig. 11. Bone marrow smear of the laboratory mouse showing erythrocyte with micronucleus

other gross defects. Several gene loci specifying different coat colors are combined in a heterozygous tester strain. By mating these animals to treated mice of a normal strain mutations at any one of these genes is detected by color deviations in the offspring from "wild" to any one the specified types[18]. This test is time and space consuming, as several thousand animals have to be checked for such mutations in order to make the result statistically significant.

At the present "state of the art" it is impossible to recommend one test as the only suitable and relevant system, and this will certainly remain so. Therefore in its 1971 paper on the mutagenicity testing of drugs and other chemicals[19] a WHO Scientific Group stressed, that only a combination of different test systems is likely to give more or less definite answers on the mutagenic potential of chemical agents. This statement will be exemplified below in more detail.

Results and their Interpretation

A large number of drugs and other chemical substances have been tested in a variety of test systems[20, 21]. This body of evidence is not yet sufficient to draw definite conclusions as to the genetic hazard, but it can be used to show some general lines, and with a cautious interpretation it can lead to an estimate of the risks involved. To the most potent mutagenic agents belongs the class of cytostatic drugs. The drugs used in cancer chemotherapy are designed to act on reproductive mechanisms, for it is the unlimited growth of tumor cells, which is the major feature distinguishing them from normal tissues. They range from simple alkylating agents to chemicals acting on specific steps in the biosynthesis of DNA. Examples for the former are mustard gas, cyclophosphamide (Endoxan), alkyl sulfonates and ethyleneimines (Trenimon) (Fig. 12). In the latter series we find some of the newer anticancer drugs like mercaptopurines, cytosine arabinoside and folic acid antagonists (Methotrexat) (Fig. 13). For these substances the mutagenic activity in man can be directly investigated with cytogenetic methods *in vivo*, i.e. blood can be drawn from patients receiving such drugs and chromosome aberrations can subsequently be scored in lymphocyte cultures. All the above mentionned drugs have been tested and found to be mutagenic in man. They should therefore constitute a considerable genetic risk for the single exposed individual. It is clear on the other hand, that the benefit of treating and possibly curing cancer patients outweighs this risk. This is even more so because the risk for the population as a whole is neglibly small. As a point in question remains the genetic influence on possible offspring. Thus the need for genetic surveillance of children born from treated parents is obvious. On the other hand it has been demonstrated in the dominant lethal test in mice, that alkylating agents show a peak efficiency during post-meiotic stages of spermato- or oogenesis[22]. Either the cellular repair systems are able to correct these mutagenic alterations, or the defect cells are eliminated before they are used for reproduction. In this respect the exact knowledge of the genetic behaviour of the administered drug is vital; a prescribed time of reproduction-prohibition can alleviate, if not eliminate, the danger of mutagenic alterations in the offspring.

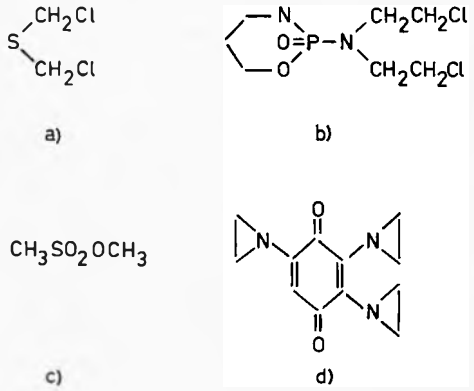

Fig. 12. Mutagenic alkylating agents: a) Sulfur mustard, mustard gas. b) Cyclophosphamid (Endoxan). c) Methylmethanesulfonate. d) Trenimon

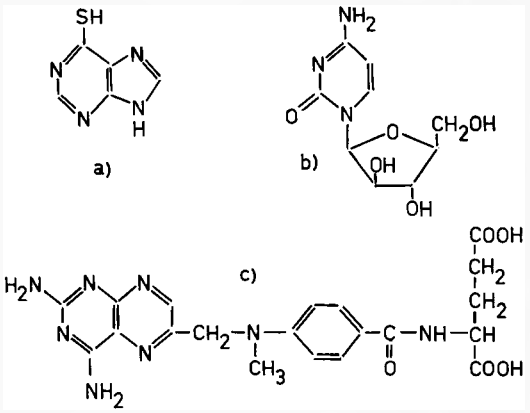

Fig. 13. Mutagenic antimetabolites: a) 6-Mercaptopurine, b) Cytosinearabinoside, c) Amethopterin (Methotrexat)

Other, and possibly more difficult problems are posed, when widely used drugs, food contaminants and pesticides are considered. Even when mutagenic, they may not be so active agents as the cytostatic drugs, but a weak mutagenic activity may produce considerable effects if the influenced population is very large. This danger is enlarged by the possibility of a weak mutagen going undetected through the test procedures. In this respect concern has been expressed over the heavy use of Isoniazid (INH), an antituberculosis drug, and of Hycanthone, an antischistosomiasis agent (Fig. 14). The principal metabolite of INH is hydrazine, whose carcinogenic and chromosome breaking activity is known:

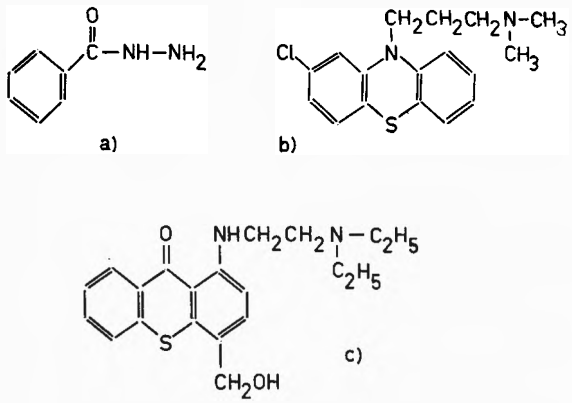

Fig. 14. Widely used drugs: a) Isoniazid. b) Chloropromazine. c) Hycanthone

furthermore INH has been shown to inactivate transforming DNA. In this case it can also be demonstrated that a significant fraction of the general population lacks certain enzyme mechanisms, which are used to detoxify hydrazines[23]; for such people a treatment with INH involves a clearly greater genetic risk than for the majority of the population. Hycanthone is used by the WHO in a large scale antischistosomiasis program. Schistosomiasis is a great problem in the tropics and the development of a drug with the efficacy, the ease of administration, and the low toxic side effects of hycanthone has generated widespread enthusiasm. Hycanthone and some other related substances have however been proved to be mutagenic in several test systems ranging from microorganisms to mammals[24]. Some negative results are available, too, e.g. in the specific locus test the chemical is non-mutagenic[25]. In a study with human cells *in vitro* chromosome aberrations were found only at concentrations of 5 mg/ml, which is far above the concentration reached in treatment[26]. Although this result seems to diminish the potential danger, it has to be taken into account, that large populations—literally millions of people—are treated with this substance. Most of these people are also undernourished and the influence of the general state of health and the dietary level on mutagenic reactions remains largely unknown. In this case, too, the benefit for the individual has been considered to outweigh the risk for the population as a whole. This need not be so in the case of chloropromazine, a tranquilizer, of the same chemical family. Fewer reports are available for this compound, but in the tranquilizer field several substitutes are available and the additional danger of drug abuse is present.

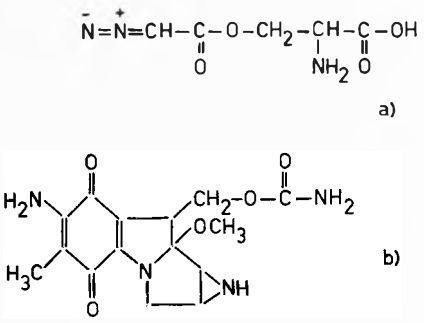

Fig. 15. Anticancer drugs: a) Azaserine, b) Mitomycin C

A considerable number of antibiotics with mutagenic properties has been detected. Most of them are used in cancer chemotherapy on account of their antimetabolite mode of action. Azaserine (Fig. 15) for instance, an antibiotic from a *Streptomyces* strain, is a glutamine antagonist which inhibits purine synthesis, and it is mutagenic in *Escherichia coli* and in plant cells. Also Mitomycin C, which has been used for the treatment of Hodgkin's disease, and Daunomycin, which is effective against acute leukemias, produce point mutations and chromosome aberrations in several test systems. For these antibiotics the same principles apply as outlined above for the alkylating cancer chemotherapeutics.

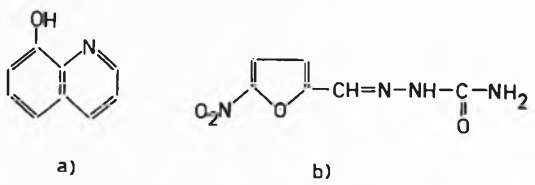

Fig. 16. Antibacterial agents: a) 8-Hydroxiqninoline, b) Nitrofurazone

Another class of drugs with mutagenic members are the antibacterials. Apart from the antibiotics several widely used compounds exist whose mutagenic properties have been assessed. 8-Hydroxyquinoline (Fig. 16) has been used for over sixty years as a bactericidal agent; nitrofurazone is used as a local disinfectant, but its use as feed additive in the United States and as a food preservative in Japan is much more important. Both compounds have mutagenic properties. The former is thought

to act through its chelating power, thus effecting a removal of essential metal ions; this has been given as an explanation for its bactericidal as well as of its chromosome breaking activity. Nitrofurazone is not only mutagenic, but also carcinogenic and its use or abuse as food preservative has aroused much concern about its effects on man.

With this example we are on the borderline between drugs and food additives; to the food additives we shall not only count those substances, which are deliberately added, but also such compounds which occur naturally in various foods, and chemical agents which have been used in the food production and whose residues remain in the product.

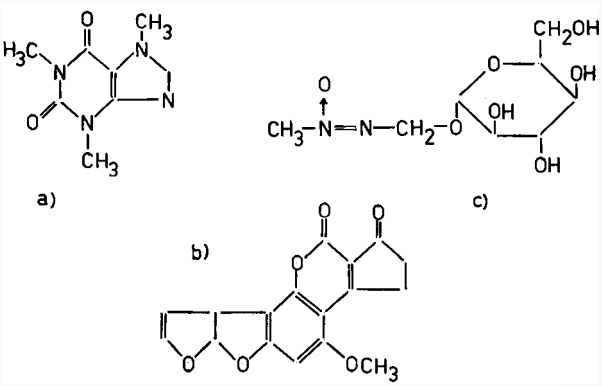

Fig. 17. Naturally occurring mutagens: a) Caffeine, b) Aflatoxin B, c) Cycasin (methylazoxymethane-β-D-glucoside)

Caffeine, aflatoxins and cycasin (Fig. 17) are the examples we will mention as naturally occurring mutagens; to the food additives *sensu stricto* belong the artifical sweeteners; pesticide residues of various kinds can be found in our food. The case of caffeine has evoked a great controversy[27]. Mutagenic as well as nonmutagenic effects have been found; *in vitro* caffeine produced chromosome breaks in cell cultures, but most extensive dominant lethal studies showed only negative results. Furthermore no synergistic effects between caffeine and known mutagens could be found. Despite the structural similarity between caffeine and the purine bases in nucleic acids, and despite the fact, that caffeine can penetrate blood-brain and blood-placenta barriers, we need not be anxious, at the present state of knowledge, about mutagenic effects of our cup of coffee.

Much darker is the picture with the two other compounds mentionned above. Aflatoxins have been shown to be active mutagens in a variety of test systems. The prime concern with this substance, however, stems from its carcinogenic potential, which has been connected with the increased occurrence of liver tumors in such parts of the world, where contamination of foodstuffs with molds is likely to occur. The high incidence of neurological diseases in areas, where cycad nuts are used as food or medicines, pointed to a constituent of this plant as the causative agent. It was found that the glycoside cycasin induces mutations in the host mediated assay with *Salmonella*. Its hepatoxic and carcinogenic activity in rats has also been reported; in this case cycasin is active only when given *per os*, and subsequently it has been demonstrated in *Drosophila* and *Salmonella* that the aglycon, methylazoxymethanol (MAM) is the active component. The toxicity of cycasin is therefore due to a β-glucosidase activity of the intestinal flora, which converts the inactive glycoside into its active form. MAM is then acting as a methylating agent, as increased amounts of 7-methylguanine were found in both hepatic RNA and DNA.

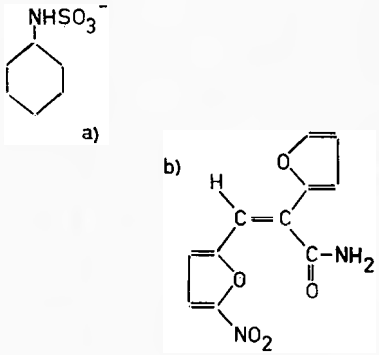

Fig. 18. Food additives: a) Cyclamate, b) AF-2

Everybody is still aware of the controversy over the addition of cyclamates (Fig. 18) as artificial sweeteners to soft drinks and other low-calorie-food. In large studies cyclamates had been found to induce an increase in the incidence of bladder tumors. It was demonstrated that cyclamate could be metabolically converted to cyclohexylamine in about 30% of the people consuming cyclamates. Cyclohexylamine is not only far more toxic than the parent cyclamate, but it has also been shown to induce chromosome breaks in germinal cells of rats. Prior to the banning of cyclamates, their consumption amounted to nearly 10000 tons with individual intakes of as much as 4 g per day.

Of more recent importance is the finding of the mutagenic activity of AF-2 [*trans*-2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide], a food preservative widely used in Japan. This situation is worsened by the fact that on one hand the concentration of AF-2 in food was high enough to cause very marked mutagenic responses in the tester organisms, and that on the other hand a strong correlation between mutagenicity and carcinogenicity in related nitrofurans leads to the expectation of AF-2 being also a strong carcinogen[28].

Today methods of food production involve the use of fertilizers and pesticides for higher yields. Such substances are in many cases present in the final product as residues. Other compounds are inadvertently introduced due to environmental contaminations. Such residues are generally small, but the ubiquitous presence of poten-

tially harmful compounds requires a careful examination of mutagenic, carcinogenic and teratogenic properties of those substances.

One of the major problems is the contamination of the environment with lead. Due to the addition of tetraethyllead to automobile fuel, large amounts of lead are deposited alongside the roads. This can either be taken up by plants and accumulated within, or it can remain deposited as dust on the leaves. In either case an increased lead uptake by people eating such food would be expected. Because of the mutagenic activity of several bivalent metal ions, lead has also been suspected of being mutagenic. This seems not to be the case, as a recent investigation failed to detect any increase of chromosome aberrations in the blood of severely exposed people compared with control blood of low lead exposure[29].

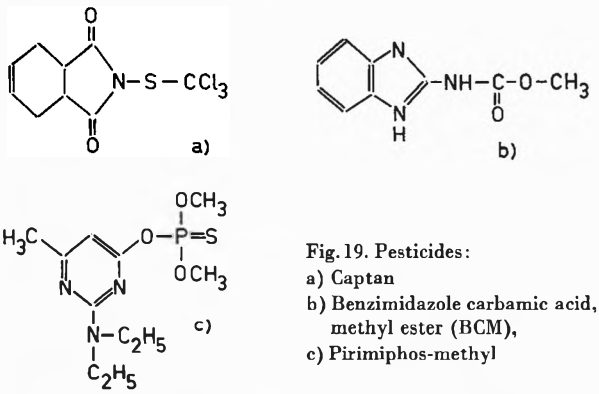

Fig. 19. Pesticides:
a) Captan
b) Benzimidazole carbamic acid, methyl ester (BCM),
c) Pirimiphos-methyl

In the pesticide sector reviews of mutagenicity have been published[30, 31, 32]. Many of the insecticidal organophosphates have been investigated for their mutagenic potential. They can act as alkylating agents, and it has been found that methyl esters are much stronger mutagenic agents than ethylesters, which is to be expected from the chemical reactivity of methyl- *vs.* ethylesters. As an example, Pirimiphos-methyl is shown in Fig. 19, an agent which is an extremely potent mutagen in *Salmonella typhimurium*. Equally important in agriculture as the insecticides are the fungicides. A member of this group is BCM (benzimidazole carbamic acid, methyl ester). The benzimidazole moiety has aroused the suspicion of being mutagenic, because of its structural resemblance to purine. It has been shown that benzimidazole and BCM, resp., are weak mutagens in *Salmonella typhimurium*[33]. Its fungitoxic action seems to be due to some interference with spindle formation[34], and indeed the incidence of micronucleated erythrocytes in bone marrow is enhanced by feeding the mice with BCM[35]. Other reports of its mutagenicity in fungi and mammals have been published[36, 37], and the rapid appearance of resistant fungi after the treatment of vineyards and orchards with BCM[38] may be regarded as another expression of its mutagenic potential. One other compound shall be described here in detail, because it shows especially well, how conflicting results can arise. The fungicide captan is widely used on several crops. Its structure has first been thought to resemble the teratogenic thalidomide, and indeed teratogenic effects could be found by injecting captan into egg yolk[39]. This test system however has several inherent weaknesses, especially the absence of the placenta barrier, and the results could not be reproduced in other more relevant systems. In bacterial tests, however, captan was found to be a very potent mutagen[31]. In cell cultures captan produced chromosome aberrations and mitotic arrest[40], whereas in *Drosophila* captan showed no mutagenic activity[41, 42]. Thorough investigations with several strains of *Escherichia coli* demonstrated then, that the mutagenicity of captan is ultimately due to a volatile hydrolysis product which reacts very quickly with different cell constituents[10]. It is therefore unlikely to reach the germ cells or their DNA, because its estimated half-life in serum is about ten seconds. This is an excellent case to demonstrate how different results can arise. Captan produces mutations in test systems from microorganisms to cell cultures; on the other hand it is completely inactive in *Drosophila* tests and in the mouse dominant lethal assay[43]. This difference can be explained by the extraordinary reactivity of this otherwise mutagenic compound towards sulfhydrylgroups. In general the pharmacologic and pharmacokinetic properties of a given substance can lead to very different answers in various test systems. It is obvious that one has to be very cautious in extrapolating test results from any given organism to man, when the differences in the biochemical handling of the compound are not known.

The Cancer Connection

One of the early theories of carcinogenesis was the "somatic mutation" theory[44]. Malignant growth was thought to be the result of an appropriate mutation in a somatic cell. The theory had to be abandoned, because a correlation between mutagenic and carcinogenic activities of chemical compounds could not be detected[45]. It is now known, however, that in many cases chemicals have to be transformed through metabolic activation to the ultimate or proximal carcinogens. With the increasing knowledge of such activation processes, more and more chemical carcinogens have been found to possess also mutagenic activity. The reader will have noticed the close association between carcinogenicity and mutagenicity in some examples of the previous chapter. The "somatic mutation" theory of carcinogenesis need not be literally true, but it is more or less accepted today, that chemical carcinogens act through a molecular form, which is also capable to induce mutations. Such a unifying theory has been proposed by Miller and Miller[46]. They interpreted the available data on mutagenic and carcinogenic acti-

vity of various compounds as to be dependent on the ability of the substance to be converted into an electrophilic compound. The reactivity of these electrophilic forms leads then to mutations, if DNA in germ cells is affected, or to cancer, if DNA or some other cell constituent in somatic cells is affected. They conclude that most, possibly all, chemical carcinogens are mutagens, and that most, but possibly not all, chemical mutagens are carcinogens.

Based on such hypotheses and on the available experimental evidence, it has been proposed to use microbial mutagenicity testing systems as a prescreening for carcinogenicity[47]. Such an approach might have considerable potential for the future, as it would cut the costs of carcinogenicity testing, if systems could be developed, where the probability of false negative results would be extremely low. Again the metabolic, pharmacologic and pharmacokinetic behaviour of the tested substances should be thoroughly investigated in order to be able to account for species differences. It can already be stated that chemical substances which are—at least after proper metabolic activation—mutagens, have to be suspected of being carcinogens, at least until the contrary has been doubtlessly proved.

Discussion and Conclusion

An array of more or less reliable, more or less theoretically understood, and more or less practicable test systems is now used to produce a wealth of data, whose consequences may be tremendous, if not interpreted in the right manner. We are faced with several questions, which are to be solved, before we can reliably compute the genetic risks for man caused by its chemical environment. Until then we are left with the task of estimating these risks and putting them in a relation to the possible benefits. Bridges[48] has advocated a three-tier system to evaluate chemicals for mutagenic activity. His third tier is reached by all those substances, whose potential genetic influence is proved, but who are of certain benefits. The authority has then to decide, whether the hazard of mutations may be regarded as small in relation to the benefit, which might be the case for drugs used only in special circumstances (e.g. anticancer drugs), whether the risk warrants special precautions and reservations (e.g. pesticides), or whether the risk *versus* benefit is too high to allow further use of this compound. Such decisions are to be taken on the latest available findings, and they could be overruled later, if newer evidence warrants it. To have this decision machinery more refined and to make the risk estimates more accurate, we need more research in chemical mutagenesis. We need therein the cooperation of molecular biologists, geneticists, pharmacologists and toxicologists. Three main routes will have to be pursued. In the first place we should gain more insight into the molecular events, which lead to mutations, and into the repair systems, which are able to cope with them. For this problem an excellent basis has been laid with microbial systems, but much research has to be performed in order to gain more insight into repair mechanisms in mammalian cells. This knowledge is essential for reliably extrapolating data from lower organisms to mammals. In the second place, also relevant to the problem of extrapolation, we have to investigate the biochemical, pharmacological and pharmacokinetic differences between various species. In toxicology the extrapolation from mouse to man is done with the aid of a "safety factor"; but can a single safety factor account for species differences or individual variations? This problem can best be illustrated by the comparison of micronucleus tests done on different animal species[49]; the incidence of micronuclei at the same dose level varied within nearly one order of magnitude. It is obvious that we need a better understanding of such basic things as blood transport, cell permeation, metabolic degradation of chemicals in relation to animal species. The third line of research will be the development of existing and new test systems to relevance.

We will probably never have a single test system affording us all pertinent information on every type of chemical mutagen, but we have to develop tests, which are able to answer specific questions, whose answers are reliable, and whose answers can also be understood theoretically. Mutagenicity testing has to lead to definite conclusions and recommendations. Between test results and legislative recommendations lies the broad field of interpretation. Sound and thorough basic research should narrow this field, but even now, with our limited knowledge we should try to eliminate hazardous substances from our environment, before irreparable damage has occurred.

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