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How do in vivo mammalian assays compare to in vitro assays in their ability to detect mutagens?

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Mutations are detected in organisms by their phenotypic expression. Depending upon the complexity of the organization of the genetic material, the primary change leading to a mutation can vary. In prokaryotic systems, such as Salmonella and *Escherichia coli*, single base-pair changes or additions and deletions of single bases in the DNA can lead to mutations. However, large deletions and duplications cannot be tolerated by these organisms, as unrepaired or misrepaired breaks in DNA lead to lethality. In eukaryotic systems on the contrary, in which the genetic material is organized in the form of separate chromosomes which contain a complex of DNA with proteins, in addition to mutations resulting from single base-pair changes similar to bacteria, those due to small and large chromosomal rearrangements can be detected. This basic difference between prokaryotes and eukaryotes obviously makes a unified definition of mutations impossible and makes direct comparisons and extrapolations difficult.

While most of the directly acting mutagens can readily be detected in both in vitro and in vivo test systems, it is difficult to detect mutagens which require metabolic activation. This, for instance, can be achieved by employing a mammalian liver activation system in in vitro studies (Natarajan et al., 1976). This, however, cannot always fully replace an in vivo situation, and thus limits detection of certain classes of chemicals in vitro. In such cases in vivo tests would be appropriate. However, a successful mammalian in vivo test depends on the ability of the active metabolites of the mutagen to reach the target cells under study. Thus, mutagens with short-lived metabolites though very active, may not come out as positive in such in vivo tests.

Bacterial tests can be of predictive value for the ability of a compound to induce point mutations but a chemical which predominantly induces DNA strand breaks and thus chromosomal aberrations will not be detected in this test easily. Since chromosomal aberrations per se contribute to a significant proportion of detectable genetic damage in human it is important that the test systems (battery) employed should include the ability to detect chromosomal aberrations. Information is also needed with respect to tissue-specific responses of mutagens in germinal vs. somatic cells. In several instances a potent mutagen may turn out to be negative in a mammal in an assay measuring genetic damage in germ cells simply because the active metabolite does not reach the germ cells due to the existence of the blood–testis barrier. Though such a chemical may not pose a hazard for future generations it might induce changes in the somatic cells of the exposed individual, leading to somatic

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Dedicated to Dr. M.S. Swaminathan on the occasion of his 60th birthday.

mutations (cancer). In such cases the mouse spot test can be useful. Accumulating evidence points towards an involvement of specific chromosomal rearrangements in the etiology of different types of cancers in human, such as lymphomas, leukemias, Wilm's tumor etc. (Rawley, 1983).

In this review, we have compared the mammalian in vivo test systems against in vitro test systems utilizing pro- and eukaryotic cells. We have described briefly the test systems employed and the genetic end points they can detect. All the chemicals which have been tested in a mammalian in vivo germ cell test, namely, specific locus mutations, dominant lethals and heritable translocations, have been compared with regard to their ability to induce genetic changes in in vitro tests. Since the number of chemicals tested with germ cells in mammals is very limited, we have further compared the chemicals which have been studied in the mouse micronucleus test with their ability to induce mutations in bacterial assays. These limited data point towards some of the advantages and limitations of an in vivo mammalian mutagenicity test.

In the literature several attempts have been made to compare the results from bacterial tests with rodent tests as well as between rodent tests (Hollstein et al., 1979; de Serres and Ashby, 1981; Jenssen and Ramel, 1980; IARC, 1980; L.B. Russell and Matter, 1980). In another study the results of mammalian male germ cell assays were compared with those from different short term assays (ICPEMC Committee 1, 1983). We have used the information from these reports as well as the information generated by different Gene-Tox committees. However, for the comparative study reported here, it was difficult to use Gene-Tox reports as several of the committees involved have rejected a majority of published papers in this field as they did not fulfil the strict criteria used to evaluate the results. We have tried to group the tested agents into chemical classes in order to check whether we can detect any definite trends in a preferential ability of the mammalian in vivo test systems to detect (or miss) certain classes of chemicals in comparison to in vitro systems. For this purpose we have used the classification of chemicals adapted by Rinkus and Legator (1979) as well as Vogel et al. (1980).

Validated in vitro mutagenicity tests

(1) Bacteria

The standard bacterial assay to detect mutagens is the *Salmonella typhimurium* test using reverse mutations in the histidine locus developed by B.N. Ames and his colleagues (Levin et al., 1982). Though the Salmonella test is the most universally used, similar reversion tests using *Escherichia coli* have also been developed (Brusick et al., 1980; Mohn et al., 1984). The types of genetic damage detected by the different strains employed in these tests are point mutations such as transition and transversion of single DNA bases and frameshift mutations. In addition there are bacterial repair tests, which are based on the inability of special repair-defective tester strains in repairing damaged DNA, thus leading to killing or to reduced proliferation in comparison to wild-type strains. This test detects all types of DNA damage. Such sensitive DNA repair tests have been developed for *E. coli* and *Bacillus subtilis*. Most classes of mutagens tested with these strains (*E. coli, S. typhimurium*) come out positive in the repair test with few exceptions (De Flora et al., 1984). Aromatic amines and polycyclic aromatic hydrocarbons can be detected in the standard Salmonella assay with plasmid-containing strains (e.g., TA98) but are not efficiently detected in most of the other bacterial systems. Procarbazine and safrole are identified as mutagens in *E. coli* but not in Salmonella standard plate test.

(2) Yeast

Unlike prokaryotic cells such as bacteria, yeast represents an eukaryotic system. Eukaryotes have a nuclear membrane and chromosomes with the DNA associated in a typical nucleosome structure. Therefore, it is possible to detect chromosomal aberrations in addition to point mutations in this system. Other types of genetical alteration which can be detected with yeast are mitotic recombination and aneuploidy resulting from mitotic nondisjunction or chromosome breaks. In addition, growing yeast cells have some capacity to activate mutagens because they have the cytochrome P-450 system operating.

(3) In vitro cytogenetics

(3.1) Chromosomal aberrations

Since most mutagens are also known to induce chromosomal aberrations, the ability of a compound to induce chromosomal aberrations has been used as an index of mutagenic potential of an agent (Natarajan and Obe, 1982). Chromosomal aberrations can be detected in different types of proliferating cells of plant and animal origin. However, for routine screening mammalian cells are employed, such as established Chinese hamster cells or stimulated human peripheral lymphocytes. The types and the frequencies of aberrations induced depend on the cell cycle stage treated and the mutagen in question. Agents which directly induce DNA double-strand breaks, such as X-rays and bleomycin induce chromosome type of aberrations in G_1, chromatid type in G_2 and both types in the S phase of the cell cycle. These agents are considered as S-independent agents. On the other hand mutagens such as alkylating agents and UV light induce chromatid type of aberrations irrespective of the cell cycle stage treated and the cells have to go through an S phase before the aberrations can be visualized. These agents are classified as S-dependent agents.

(3.2) Sister-chromatid exchanges (SCEs)

SCEs are the outcome of exchanges of replicated DNA at homologous loci, a process involving DNA double-strand breaks and rejoining of such breaks between sister chromatids. Sister-chromatid differentiation to detect SCEs can be achieved by (a) labeling chromosomes with tritiated thymidine for one cycle followed by growing the cells for another cycle in cold thymidine, and (b) by growing cells for the first or the first and second cell cycles in a medium containing 5-bromodeoxyuridine (BrdUrd). The baseline frequency of SCEs using the BrdUrd method varies between 5 and 10 per cell, while the baseline frequency of chromosomal aberrations is between 0.0 and 2.0 per 100 cells (Vogel and Natarajan, 1982; Natarajan et al., 1984). The majority of the baseline frequencies of SCEs are most probably induced by incorporated BrdUrd. Incorporated BrdUrd itself is known to be mutagenic and therefore an evaluation of a chemical using the SCE test in reality reflects an additive or synergistic effect of two agents (Natarajan et al., 1984). The SCE formation is an S-dependent event and therefore all agents which require an S phase to induce chromosomal aberrations are efficient inducers of SCEs. More than 300 chemicals have been tested for their ability to induce SCEs (Abe and Sasaki, 1982). Chemicals which induce DNA strand breaks directly are not potent inducers of SCEs. On the other hand, with very few exceptions (3 aminobenzamide and other inhibitors of poly(ADP-ribose) synthetase) agents which induce SCEs do also induce chromosomal aberrations (Natarajan and Czukas, 1984; Natarajan et al., 1984).

(4) Point mutations in mammalian cells in culture

Several systems are available to detect point mutations in mammalian cells. These are usually characterized by resistance to selective agents such as 8-azaguanine, 6-thioguanine (hypoxanthine-guanine phosphoribosyl transferase), ouabain (Na^+–K^+-ATPase), bromodeoxyuridine and trifluorothymidine (thymidine kinase). Ouabain resistance is a dominant mutation and occurs spontaneously at very low frequency (10^{-8}). HGPRT mutations on the other hand occur at much higher frequencies (10^{-6}). Cytological analyses of HGPRT mutations induced by ionizing radiations, ethyl methanesulfonate as well as those occurring spontaneously indicate that part of the mutations are associated with chromosomal rearrangements involving the X-chromosome. This indicates that HGPRT mutations can arise as point mutations as well as chromosomal aberrations. A similar situation seems to be true for the thymidine kinase locus as well.

(5) DNA repair tests

The basic method is to detect incorporation of tritiated thymidine in the nucleus of the mutagen-treated cells which is not in the S phase of its cell cycle. Such an incorporation reflects the amount of repair of damaged DNA and this synthesis is called unscheduled DNA synthesis (UDS), as it occurs outside the main replicative DNA synthesis. UDS can be measured by microautoradiography or by scintillation counting. For this test it is

easier to use non-proliferating cells such as human lymphocytes and rodent hepatocytes. UDS does not reflect a mutagenic event per se, but rather a repairable damage to the DNA. There are several other in vitro tests which are being used in different laboratories, but the tests discussed above are the most frequently used and relatively well validated ones.

(6) Drosophila melanogaster: Sex-linked recessive lethal mutations

Next to the bacterial tests, only in the Drosophila sex-linked recessive lethal test (SLRL) a large number of chemicals have been tested for mutagenicity. The capacity of these flies to activate indirect mutagens like mammals and man makes this system very attractive. The SLRL test measures mutations in about 700 loci and these mutations represent a mixture of point mutations and deletions, the relative proportion of which depends on the mutagen under test.

A somatic mutation/mitotic recombination assay (SMART) has been developed recently, by which a variety of genetic events can be evaluated, by measuring the frequencies of single and twin spots in the eye (Vogel, 1985). This assay system appears to be very sensitive and less time consuming in comparison to the SLRL test.

(7) Plant systems

Several assay systems are available in plants. These include chromosomal aberrations and SCEs (*Vicia faba, Allium cepa*), point mutations (*Glycine mexicana, Zea mays*) as well as somatic recombination (*Glycine*). Though detection of chromosomal alterations is very easy and quick in plant systems, the detection of point mutations is more difficult and time consuming. For this reason this system has not been introduced in large-scale mutagenicity testing of chemicals. However, in cases where metabolic activation of chemicals mediated through plants is essential (atrazine, maleic hydrazide) plant systems become important. In view of the extensive cytological studies from the early 1930s, a large number of chemicals has been tested using chromosomal aberrations in plants. Already in a review from 1962, Rieger and Michaelis have reported 218 chemicals tested using root tip meristems of *Vicia faba*. A very sensitive test to detect low exposures to mutagens has been developed using Tradescantia staminal hairs, but large-scale testing data are lacking.

(8) Mammalian in vivo mutation-detecting assays

With the in vivo mutagenesis tests available in mammals, both point mutations (recessive and dominant) and chromosomal aberrations can be detected in somatic as well as germ cells.

(8.1) Mouse specific locus test

The test involves detection of mutations transmitted from treated germ cells and recovered in the offspring of the next generation. This test has been developed by W.L. Russell (1951) using coat color markers at 7 different loci, which can be screened simultaneously. Though these mutations are generally considered as point mutations, it is recognized that many of these mutations arise due to deletions of single or multilocus nature. About 30 chemicals have been evaluated with this test. All these tests have been made with male germ cells. Only 2 chemicals have been tested so far using oocytes (L.B. Russell et al., 1981). A selected list of 10 environmental chemicals tested failed to increase the frequencies of mutations in the male germ cells (W.L. Russell, 1984).

(8.2) Mouse spot test

This is an in vivo somatic mutation test which was originally developed by L.B. Russell and Major (1957) and later propagated by Fahrig (1978). The test detects the expression of recessive markers which are used as indicators in the specific locus test. In principle, heterozygotes are treated in utero and mutant spots on the skin of the newborn mice are scored. The spots can arise due to different types of genetic events, such as point mutations, duplications, deletions, non-disjunction or somatic recombination. In this test, in addition to mutations, embryonic and teratogenic effects of test chemicals can be evaluated. About 60 chemicals have been tested using this test so far.

(8.3) Dominant lethal test

The dominant lethal mutation test can be used as a quick in vivo test and represents a mutation in a gamete (egg or sperm) which is lethal to the zygote produced by these gametes. Dominant lethals reduce the litter size due to failure of the fertilized egg to implant or to develop after implantation. It is believed that most of the dominant lethals arise due to chromosomal breakage or loss. About 100 chemicals have been evaluated using this test (IARC, 1980). A compilation of 140 chemicals tested for induction of dominant lethals, has recently become available in the literature (Green et al., 1985) and these results are also included in our comparative tables.

(8.4) Heritable translocation test

The heritable translocation test is designed to detect partial or full sterility among first-generation male offspring of mutagen-treated mice. The partial sterility can be caused by the animal being heterozygous for a reciprocal translocation. This test also gives an estimate of transmissibility of induced chromosomal exchanges, if this test is done parallel to a study directed to estimate the induced translocations in male germ cells.

(8.5) In vivo chromosomal aberrations

Chromosomal aberrations induced in an animal can be evaluated in all proliferating cells. Bone marrow cells as well as spermatogonial cells are usually studied. When induced breaks are not included in the daughter nucleus the fragments usually form a small nucleus, termed as micronucleus appearing in the cytoplasm separated from the main nucleus. In the bone marrow, micronuclei formed in the erythroblasts are retained during maturation in contrast to the main nuclei which are extruded from the cells. Thus, in polychromatic erythrocytes, micronuclei can be recognized and scored. Because of the ease with which it can be performed, this test is widely used. All the cytogenetic tests involving bone marrow and germ cells can be done in one animal if an appropriate protocol is used (Tates and Natarajan, 1976). Because aberrations induced by chemicals are usually of chromatid type and the proportion of reciprocal translocations which are transmitted to further cell generations is rather low, it is difficult to get high frequencies of reciprocal translocations detectable at the diakinesis of metaphase I stage of the spermatocytes. In routine testing bone marrow chromosomal aberrations and micronuclei are usually employed.

A liver micronucleus assay has been developed by Tates et al. (1983), in which the treated animals are partially hepatectomized and the hepatocytes from the regenerating liver are analyzed for the presence of micronuclei: so far, 10 chemicals have been tested in this assay, using rats (Table 4).

(8.6) Sister-chromatid exchanges (SCEs)

SCEs can be detected in proliferative bone marrow and spermatogonial cells. In vivo SCEs are scored from cells which have gone through the first cycle in the presence of BrdUrd, due to the fast depletion of BrdUrd from the animal when BrdUrd tablets are implanted in the animal. SCEs can also be detected in lymphocytes from spleen or from peripheral blood which are stimulated in vitro in the presence of BrdUrd (Perry, 1981; Abe and Sasaki, 1982).

General discussion

(1) Factors limiting the sensitivity of mammalian germ cells to mutagens

In view of the large number of animals and high cost as well as time necessary to perform these tests only a limited number of chemicals has been screened using these tests. These chemicals are either established mutagens or suspected mutagens which have *direct human relevance*. ICPEMC Committee 1 (1983) considered the question as to how many of the in vitro short-term tests have predictive value for mammalian germ cell mutagenicity by using available data on 53 compounds with which one or more of the following tests have been conducted: (1) rodent dominant lethal test; (2) mouse specific locus test; and (3) mouse heritable translocation test. Though there were inconsistencies in the performance of certain compounds within these 3 tests (e.g. myleran, hycanthone, benzo[*a*]pyrene, which were positive in the dominant lethal test but negative in the specific locus test), most of the chemicals were positive in all tests in vitro. We have compared the perfor-

TABLE I

QUALITATIVE COMPARISON OF IN VIVO MAMMALIAN TESTS TO 4 SHORT-TERM TESTS

Chemicals	In vivo mammalian assays		Drosophila SLRL	Bacteria reverse mutations	Yeast mitotic recombination	In vitro cytogenetics (CA + SCE)
	Germ cells	Somatic cells				
Group I. aziridines						
Mitomycin C	+	+	+	+	+	+
Tepa	+	+	+	NT	NT	+
Thio-tepa	+	+	+	+	+	+
Trenimon	+	+	+	+	+	+
Triethylenemelanamine	+	+	+	+	+	+
Group II: epoxides						
Ethylene oxide	+	+	+	+	NT	+
Styrene oxide	−	?	+	+	−	+
Group III: haloalkanes						
Captan	?(+)	+	−	+	+	−
DDT	?	?	?	−	−	?
Dieldrin	?	?	NT	−	−	NT
Vinyl chloride	?	?	+	−	−	NT
Dichlorvos	?	?	−	+	+	−
Group IV: inorganics						
Sodium nitrite	−	−	+	+	+	+
Cadmium chloride	−	+	−	?	+	NT
Sodium bisulfite	−	−	−	−	−	−
Group V. N-nitroso compounds						
N-Nitroso-*N*-butyl urea	?	+	NT	NT	+	+
N-Nitroso-*N*-ethyl urea	+	+	+	+	+	+
N-Nitroso-*N*-methyl urea	+	+	+	+	+	+
N-Ethyl-nitroso-urethane	+	+	+	NT	NT	NT
N-Nitroso diethylamine	?	+	+	+	+	+
N-Nitroso dimethylamine	?	+	+	+	+	+
Group VI: N-, S,- O-mustards						
Cyclophosphamide	+	+	+	+	+	+
ICR 170	?	?	+	+	+	NT
Nitrogen mustard	?	+	+	+	+	+
Group VII: purine analogues						
Caffeine	−	?	+	−	NT	+
6-Mercaptopurine	+	+	NT	+	NT	+
Group VIII: polycyclic hydrocarbons						
Benzo[*a*]pyrene	+	+	+	+	+	+
7,12-Dimethylbenzanthracene	?	+	+	+	−	+
Group IX: sulfate, sulfonate						
Diethyl sulfate	?	+	+	+	+	+
Methyl methanesulfonate	+	+	+	+	+	+
Ethyl methanesulfonate	+	+	+	+	+	+
Iso-propyl methanesulfonate	+	+	+	+	+	+
Myleran	+	+	+	+	NT	+
Hycanthone methylsulfonate	?(+)	?	+	+	NT	+

TABLE 1 (continued)

Chemicals	In vivo mammalian assays		Drosophila SLRL	Bacteria reverse mutations	Yeast mitotic recombination	In vitro cytogenetics (CA + SCE)
	Germ cells	Somatic cells				
Group X miscellaneous						
Saccharine	?(+)	?	?	–	?	+
Chloramphenicol	?(+)	?	?	–	–	–
Dimethyl sulfoxide	?	?	–	–	+	–
Methotrexate	?	?	NT	–	+	+
Nitrofurantoin	?	?	NT	+	+	NT
Butylated hydroxytoluene	?	–	–	–	NT	NT
Isoniazid	?	?	NT	+	+	?
Trimethyl phosphate	+	+	+	+	NT	+
Ethylenethiourea	–(+)	–	+	+	NT	+
4-Nitro-o-phenylenediamine	–	–	+	+	+	NT
2-(2-Furanyl)-3-(5-nitro-2-furanyl)-2 propenamide	?	?	+	+	+	NT
Procarbazine	+	+	+	–	+	+
1,2-Dimethylhydrazine	?	?	+	–	NT	NT

Germ cell data include sperm abnormality.
(+), positive in dominant lethal assay.

mance of 4 short-term tests, namely, Drosophila SLRL, yeast mitotic recombination, reverse mutations in Salmonella and *E. coli*, and in vitro cytogenetics with the performance of in vivo mouse tests, including both somatic and germ cells. We tried to group the chemicals in different chemical classes in order to see if there is any relationship between the ability of any one system to detect any particular chemical class. With only a few exceptions all chemicals which are detected as positive in mammalian in vivo systems were also positive in the short-term tests under consideration (Table 1). There is a large number of chemicals in in vivo tests where the available results do not allow to draw firm conclusions about their mutagenicity (marked as ? in Table 1). This illustrates the inherent difficulty in using whole mammals in routine mutagenicity testing. If we confine ourselves to the data obtained in mammalian germ cells alone, the number of uncertainties is even higher (Table 1). Doing an additional sperm anomaly test does not seem to improve the picture. The inconclusive results obtained in the germ cells are most probably due to the inability of the mutagenically active metabolites to reach the testes in sufficient quantities to be effective. Inconclusive or negative results obtained following treatment with potent mutagens such as MNNG, DEN and DMN illustrate this point. However, it is possible that the germ cells are more resistant to mutagenic insult or are more efficient in repairing damaged DNA. Systematic molecular dosimetric measurements have to be conducted to solve this problem. An evaluation of induction of histocompatibility gene mutations may prove to be useful, but this system is not fully evaluated yet (Harnasch and Stumpf, 1984).

(2) Comparison of mouse in vivo somatic chromosomal aberrations (as revealed by micronuclei in the bone marrow) with the results obtained with the Salmonella typhimurium assay including those with liver microsome activation

Unlike the mouse germ cell assays with the micronucleus test many more chemicals have been screened in view of the ease with which this test can be performed. Jenssen and Ramel (1980) com-

TABLE 2

QUALITATIVE COMPARISON OF BACTERIAL TEST TO IN VIVO RODENT MICRONUCLEUS TEST

Chemical	Ames test	MN test
Group I aromatic amines		
(1) 2-Acetylaminofluorene	+	+
(2) 2-Aminofluorene	+	–
(3) Benzidine	+	+
(4) 2,5-Diaminotoluene	+	–
(5) 1-Naphthylamine	+	–
(6) 4-Nitro-*o*-phenylene diamine	+	–
(7) 2-Nitro-*o*-phenylene diamine	+	–
(8) *p*-Phenylene diamine	+	–
(9) 3,3'-5,-5'-Tetramethylbenzidine	+	?
(10) 4-Acetylaminofluorene	+	–
(11) Dinitrosopentamethylene tetramine	–	–
(12) 2-Naphthylamine	+	–
(13) 2-4-Diaminoanisole	+	–
(14) *O*-Toludine	?	–
(15) Acriflavine	+	+
Group II: aziridines		
(16) Ethyleneimine	+	+
(17) Metepa	+	+
(18) Mitomycin C	+	+
(19) Thio-tepa	+	+
(20) Trenimon	+	+
(21) Triethylenemelamine	+	+
Group III: biphenyls		
(22) 4-Chloromethylbiphenyl	+	–
(23) Benzylchloride	+	–
(24) 4-Hydroxymethylbiphenyl	+	–
Group IV. carbamyl-thiocarbamyls		
(25) Furyl furamide	+	+
(26) *N*-Hydroxyurethane	+	+
(27) *N*-4,5-Nitro-2-furyl-2-thiazolyle formamide	+	–
(28) Urethane	+	+
(29) Dimethyl carbamoyl chloride	+	+
(30) Dimethyl formamide	–	–
(31) Isopropyl-*N*-3-chlorophenyl carbamide	–	–
(32) Ethylenethiourea	+	–
Group V: DNA synthesis inhibitors		
(33) Aminopterin	–	+
(34) Cytosine arabinoside	–	+
(35) 5-Fluorouracil	+	+
(36) Hydroxyurea	–	–
(37) 5-Iodo-deoxyuridine	–	–
(38) 6-Mercaptopurine	+	+
(39) Methotrexate	–	+
Group VI. flavonoids		
(40) Quercetin	+	+
(41) Kaempferol	+	+
(42) Rutin	+	–
(43) Neohesperidin dihydrochalcone	?	+

TABLE 2 (continued)

Chemical	Ames test	MN test
Group VII haloalkanes and haloalkenes		
(44) Captan	+	–
(45) *p,p'*-DDT	–	–
(46) 1,2-Dichloroethane	+	–
(47) Ethylene dichloride tar	+	+
(48) Vinyl chloride	+	+
(49) Lindane	–	–
(50) Chloroethane	–	–
(51) Aroclor	–	–
(52) Chloroform	–	–
(53) 2,4-D	–	–
(54) 2,4,5-T	–	–
Group VIII: hydrazines		
(55) 1,1-Dimethyl hydrazine	+	–
(56) Formic acid hydrazide	+	–
(57) Maleic hydrazide	–	–
(58) Procarbazine (natulan)	–	+
(59) Hydrazine sulfate	+	–
Group IX. inorganic salts		
(60) Methyl mercury acetate	–	–
(61) Cadmium chloride	–	–
(62) Sodium nitrate	+	–
(63) Lead acetate	–	–
(64) Potassium dichromate	+	+
(65) Sodium chloride	–	–
Group X: lactones		
(66) β-Propiolactone	+	–
(67) β-Butyrolactone	–	–
Group XI: N-, S-, O-mustards		
(68) Cyclophosphamide	+	+
(69) Isophosphamide	+	+
(70) Nitrogen mustard	+	+
(71) Quinacrine mustard	+	+
Group XII: nitro-aromatic and nitro-imidazoles		
(72) 4-Nitroquinoline oxide (4NQO)	+	+
(73) 3-Methyl 4NQO	+	+
(74) Nitrofurantoin	+	–
(75) Nitrofurazone	+	–
(76) Metronimidazole	+	–
(77) Ronidazole	+	–
(78) Ornidazole	+	–
(79) Dimetridazole	+	–
(80) Azathioprine	+	+
(81) Niridazole	+	–
Group XIII: N-nitroso compounds		
(82) Dibutylnitrosamine	+	–
(83) *N*-Nitrosodiethylamine	+	–
(84) *N*-Nitrosodimethyl urea	+	+
(85) *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine	+	–
(86) *N*-Nitroso-carbaryl	+	–

TABLE 2 (continued)

Chemical	Ames test	MN test
(87) *N*-Nitrosoethylene thiourea	+	+
(88) *N*-Nitrosomethomyl phenol	+	–
(89) *N*-Nitrosomorpholine	+	+
(90) Diphenyl nitrosamine	?	–
Group XIV phenols		
(91) 2-Amino-4-nitrophenol	+	–
(92) *m*-Amino-phenol	–	–
(93) *p*-Amino-phenol	+	+
(94) Phenol	+	–
Group XV: polycyclic hydrocarbons		
(95) Aroclor 1254	–	–
(96) Benzo[*a*]pyrene	+	+
(97) 9,10-Dimethylanthracene	+	+
(98) 3-Methylcholanthrene	+	+
(99) Pyrene	+	–
(100) Anthracene	–	–
(101) Naphthalene	–	–
Group XVI. sulfates, sulfonates		
(102) Busulfan (myleran)	+	+
(103) Ethyl methanesulfonate	+	+
(104) Methyl methanesulfonate	+	+
(105) Isopropyl methanesulfonate	+	–
(106) Hycanthone methanesulfonate	+	+
(107) Propyl methanesulfonate	+	+
Group XVII: triazenes		
(108) 1-(4-Chlorophenyl)-3,3-dimethyltriazene	+	+
(109) 1-Phenyl-3,3-dimethyltriazene	+	+
Group XVIII: miscellaneous		
(110) Trimethylphosphate	+	+
(111) Ethylene oxide	+	+
(112) Epichlorohydrine	+	–
(113) Actinomycin D	–	–
(114) Adriamycin	+	+
(115) Bleomycin	–	–
(116) Chloromycetinsuccinate	–	–
(117) Cycloheximide	–	+
(118) Daunomycin	+	+
(119) Griseofulvin	–	–
(120) Azobenzene	+	+
(121) Acranil	+	+
(122) Ascorbic acid	–	–
(123) Azoxybenzene	+	?
(124) Auramine	+	–
(125) Colchicine	–	+
(126) Vincristine	–	+
(127) Vinblastine	–	+
(128) CA cyclamate	–	–
(129) Codeine phosphate	–	–
(130) BHT (butylated hydroxytoluene)	–	–
(131) Dimethyl sulfoxide	–	–
(132) Ethanol	–	+
(133) Epinephrine	–	–
(134) Hydrochinone	+	+
(135) D,L-Ethionine	–	+
(136) Methionine	–	–
(137) Diethyl stilbestrol	–	?
(138) Moca	+	?
(139) Sucrose	–	–
(140) DAB sulfonic acid	+	–
(141) Sodium phenobarbiturate	+	+
(142) Monosodium glutamate	–	–
(143) Nitrofurantoin	+	–
(144) Nitrofurazone	+	–
(145) Resorcinol	–	–
(146) Quinacrine dihydrochloride	+	+
(147) Aflatoxin B	+	+
(148) Benzimadazole	–	–
(149) Formaldehyde	+	–

pared micronucleus test and Ames test and found that they had about the same specificity and predictive value, while there was a significant difference in sensitivity in favor of Ames' test. In their review, no attempt was made to group the tested chemicals in specific categories. In Table 2 we have compared the results comprising 148 chemicals tested with both the micronucleus test and the Ames test. The chemicals have been grouped with regard to their nature and mode of action (Table 2). In Table 3 we have summarized the data in order to see if any distinct differences exist between the two tests with regard to the detection of any specific group of chemicals. Potent directly acting mutagens come out positive in both the tests (aziridines, triazenes, mustards, sulfonates). Inhibitors of DNA synthesis and DNA base analogues are picked up with the micronucleus test with greater efficiency. This is not unexpected as these chemicals are efficient chromosome breakers and do not induce point mutations effectively. Mutagens which require metabolic activation such as aromatic amines and some *N*-nitroso compounds are easily detected in the Salmonella test using S9 activation whereas most of them are not detected in the micronucleus test but can be detected in the liver micronucleus test.

TABLE 3

PERFORMANCE OF IN VIVO MICRONUCLEUS TEST AND BACTERIAL TESTS IN DETECTING DIFFERENT GROUPS OF CHEMICALS

Chemical classes	Number compared	Bacterial tests	Micronucleus test
(1) Aromatic amines	15	+(13)	+(3)
		−(1)	−(12)
(2) Aziridines	6	+(6)	+(5)
(3) Biphenyls	3	+(3)	−(3)
(4) Carbamyl, thiocarbamyls	8	+(6)	+(4)
		−(2)	−(4)
(5) DNA synthesis inhibitors	7	+(2)	+(5)
		−(5)	−(2)
(6) Flavonoids	4	+(3)	+(3)
		?(1)	−(1)
(11) Haloalkanes	11	+(4)	+(2)
		−(7)	−(9)
(8) Hydrazines	5	+(3)	+(1)
		−(2)	−(4)
(9) Inorganic salts	6	+(2)	+(1)
		−(4)	−(5)
(10) Lactones	2	+(1)	
		−(1)	−(2)
(11) Mustards	4	+(4)	+(4)
(12) Nitroaromatics	2	+(2)	+(2)
(13) *N*-Nitroso compounds	9	+(8)	+(3)
		?(1)	−(6)
(14) Nitroimidazoles	6	+(5)	+(1)
		−(1)	−(4)
(15) Phenols	4	+(3)	+(1)
		−(1)	−(3)
(16) Polycyclic hydrocarbons	7	+(4)	+(3)
		−(3)	−(4)
(17) Sulfates, sulfonates	6	+(6)	+(5)
			−(1)
(18) Triazenes	2	+(2)	+(2)
(19) Miscellaneous	50	+(22)	+(18)
		−(27)	−(29)
		?(1)	?(3)

This again should be due to the inability of the active metabolites to reach the target cells in the bone marrow, whereas hepatocytes are directly involved in the activation of these carcinogens. Nitroimidazoles seem to be ineffective in inducing micronuclei in bone marrow, but effective in inducing mutations in bacteria.

Chemicals which disturb the mitotic spindle apparatus such as vincristine and colchicine are positive in the micronucleus test and negative in Salmonella. Considering only the results which are either clearly positive or negative we have data for 148 chemicals tested in both these systems. Of these 50 were positive and 40 were negative in both the test systems. The two test systems responded differently to 58 chemicals. These discrepancies are not unexpected in view of the fact that we are measuring two different genetic end points, namely, point mutations and chromosomal aberrations and only one tissue in vivo, namely bone marrow cells. Chemicals which induce both these events will be picked up with both the systems. On the contrary chemicals which induce chromosomal aberrations preferentially as well as those inducing spindle defects will be picked up by the micronucleus assay and not by the bacterial assay. The reverse is true for chemicals which induce point mutations mainly.

In bacterial assays the test chemicals are in direct contact with the target cells at high concentrations whereas in mammalian in vivo assays the concentrations reaching the target cells are limited by various pharmacokinetic parameters. For this reason the sensitivity of this test system is

TABLE 4

PERFORMANCE OF LIVER MICRONUCLEUS TEST

(1) Dimethyl nitrosamine	+
(2) Diethyl nitrosamine	+
(3) Ethyl nitrosourea	+
(4) Ethyl methanesulfonate	+
(5) Methyl methanesulfonate	+
(6) Mitomycin C	+
(7) 2-Acetylaminofluorene	+
(8) 4-Acetylaminofluorene	−
(9) Benzo[*a*]pyrene	−
(10) Pyrene	−

Based on data by Tates (personal communication).

relatively low. However, employing an in vitro cytogenetic assay using a mammalian metabolic activation system one can detect most of the chemicals which are capable of producing point mutations as well as chromosomal aberrations, because cells can be treated with much higher concentrations of the test chemical than those employed for in vivo experiments.

(3) The relevance of chromosomal aberrations in mutagenicity testing

It is known that about 6 out of 1000 newborns have a chromosomal abnormality though there is no estimate of point mutations in newborn. Populations exposed to mutagens respond with increased frequencies of chromosomal aberrations and cancer rather than detectable point mutations. This demonstrates the importance of screening chemicals for their ability to induce chromosome aberrations in vitro and/or in vivo as an indicator of potentiality for mutagenicity and carcinogenicity in man. This brings us to the question of the choice of a test battery to detect mutagenicity of chemicals. While in general all potent mutagens induce chromosomal aberrations potent chromosome-breaking agents do not always induce point mutations efficiently e.g., X-rays, bleomycin. In a routine test for mutagenicity it is important to employ an in vitro cytogenetic assay as such an assay would detect chemicals which are not efficient inducers of point mutations but are of potential hazard to man by inducing chromosomal aberrations. Similar conclusions were arrived at by the analysis of the results of a study designed to compare short-term tests for mutagens and carcinogens (Ashby et al., 1985). The number of such chemical agents are increasing rapidly, e.g., benzene, resorcine and several flavoring agents (such as allylisothiocyanate, anisaldehyde, benzaldehyde, cinnamaldehyde, citronellal, heliotropin, vanilline etc.) (Kasamaki et al., 1982).

Concluding remarks

In a comparative survey of the ability of in vivo mammalian assays to in vitro assays to detect mutagens it is obvious that one is dealing with two related phenomena i.e., point mutations and chromosomal aberrations intermingled. The limited data available using in vivo mammalian assays (somatic as well as germ cells) indicate that the sensitivity of this system is not very high when compared to in vitro systems. This is in view of inherent characteristics of the in vivo assay, namely the complex activation and deactivation mechanisms that operate as well as the efficiency with which the active metabolite reaches the target cells under study. Nevertheless, with appropriate modifications of the protocol, for a micronucleus assay in bone marrow cells, as well as introduction of liver and germ cell micronucleus assay (Tates et al., 1983), the sensitivity of in vivo assays can be greatly improved.

In mutagenicity testing, in vitro and in vivo assays serve different purposes. For preliminary screening, in view of the increased sensitivity, the possibility to use higher concentrations of test chemical, and the short time involved, in vitro tests are ideal. These assays detect the genotoxic potential of the test chemical. However, to study the ability of a chemical to express the genotoxic potential in vivo, whole-animal test systems should be used (Ashby, 1983). A decision tree approach to detect possible human carcinogens has been proposed by Ashby (1983) in which it is clear that in vivo tests have a very important position, not at the detection stage, but at validation stage. It is clear that when a chemical is being considered to be released for large-scale public use, an in vivo mammalian assay should be mandatory.

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