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IN VITRO METHODS OF TOXICITY EVALUATION[1]

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Toxicity testing has relied almost exclusively on the use of whole animal systems to describe pathologic consequences of chemical exposures, to elucidate mechanisms of action, and to ascertain hazards to human health. In the past decade, in vitro test systems have gained increasing popularity not only because of advantages in mechanistic investigations but also because of pragmatic considerations such as lower costs and shorter durations of experimentation. The wide variety of in vitro test systems falls into four categories: (*a*) microorganism systems, (*b*) mammalian cell culture systems, (*c*) tissue preparations, and (*d*) organ cultures. Because of their complexity and extensive literature bases, only the first two areas are reviewed in this chapter. The latter two categories will be treated in a future volume.

MICROORGANISM SYSTEMS

One of the more extensively investigated mechanisms for chronic irreversible toxicity is the interaction of the foreign compound with genetic material (DNA) to yield either somatic mutations that may be expressed as diseases such as cancer and atherosclerosis or germinal mutations that may be expressed as any number of physiologic and repair deficiencies and pathologic manifestations in the progeny of the exposed individuals. Although mutations may arise from differing mechanisms (e.g. either point mutations such as frameshifts and base-pair substitutions or chromosome aberrations such as breaks and sister chromatid exchanges), the commonality of a genetic event initiating the pathogenesis of some forms of chemical poisoning (1–3) focuses attention on the test systems able to select with sensitivity and reliability chemicals that produce gene mutations.

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Several test systems including various strains of bacteria and yeast have been developed to detect mutational events. A wide range of microorganisms have been used; however, relatively few have been evaluated for predictability and extrapolation to animals and man. The most commonly used test organisms are described, followed by analysis of data supporting their merit and deficiencies.

The measurement of point mutations has been achieved most effectively by the development (4–7) and application of unique mutant strains of *Salmonella typhimurium.* The genetic alterations of the tester strains have been characterized as base-pair substitutions and frameshift mutations, thus enabling the detection of reversions to prototrophy. Through selective deletion of one of the genes for the excision repair system, the likelihood of manifest genetic damage is magnified; and through the selective deficiency in the lipopolysaccharide which coats the surface of the bacteria, absorption of the test compounds into the organism is facilitated and the probability of interaction between xenobiotic and DNA is greatly enhanced. The tester systems are capable of recognizing not only the direct acting mutagens but also the indirect mutagens (i.e. promutagens) by introducing into the growth medium enzymes and cofactors from the postmitochrondrial fraction of mammalian liver that can generate electrophilic metabolites (5, 6). Generally the source of enzymes is the liver of animals treated with polychlorinated biphenyls (PCBs) which substantially increase the rate of most of the hepatic microsomal enzyme systems.

Strains of *S. typhimurium* have also been employed with whole animals in a modified host-mediated assay to detect chemical mutagens (8).

Several repair-deficient strains of *Escherichia coli* have been applied to measure in vitro mutagenic activity (9). For example, strains W3110 and p3498 measure a lesion at the locus for polymerase A_1 (*pol*A_1), whereas strains AB1157 and AB2436 measure repair deficiencies at the recombination locus A (*rec*A). Repair-deficient strains of WP2 are also employed as reversion assays for mutagenesis (10, 11).

As a result of intensive studies for many years, the genetics of the yeast *Saccharomyces cerevesiae* have been extensively characterized. Because yeasts are eucaryotic organisms containing a nucleus and cytoplasm and because the structural organization of the chromosomal material is comparable to that in mammalian cells, various strains of yeast have been extensively used to determine the deleterious genetic properties of chemicals (12, 13). Equally important is the well-characterized life cycle that provides mitotic and meiotic functions found in differentiated cells of multicellular organisms. In yeast, the study of chemically induced effects are facilitated by the ability to measure biochemical lesions, repair mechanisms, suppressions, and regulatory functions. By cultivating organisms selectively for the haploid, stable polyploid, and higher ploidy, yeasts are amenable to studies of forward and reverse mutations, of reciprocal and nonreciprocal mitotic recombination, and of mitotic crossing-over and gene conversion. In the evaluation of mutagen potential of chemicals in *S. cerevesiae,* strain D3 is used to measure mitotic recombination, and strains D4 and D7 are used for mitotic gene conversion (14–16). Consequently, a range of genetic phenomena can be investigated with the practical and versatile nature of the yeast. A drawback with this organism is the relatively thick cell wall which often impedes absorption and decreases access to the genetic material, thus

lowering sensitivity. As for the bacterial systems, a xenobiotic metabolizing system can be added to assay for mutagenic metabolites. An extensive discussion of the advantages and disadvantages of utilizing yeast for mutagenic screening is presented by Brusick & Mayer (17).

A number of repair-deficient strains of *Bacillus subtilis* have also been developed to measure mutagenic activity (9). Kada (18) found that strains having *rec*45 deficiency demonstrated sensitivity to the widest spectrum of chemicals. Thus this tester organism is often called the *rec-assay* and is frequently used as a prescreen to bacterial mutagenesis tests and carcinogenesis assays in intact animals. Since diverse chemical damage to DNA is repaired by cellular recombination functions in wild-type cells, but not in recombinant-deficient cells, most agents showing lethal activity on rec^- over rec^+ cells may have damaged cellular DNA and require investigations in more definitive test systems.

While heritable mutagenicity is a toxic endpoint of considerable import, evidence has been accumulating to indicate an association between mutagenesis and carcinogenesis. Consequently, the relationship does offer the possibility of screening for the latter with simpler and less costly test systems. Berenblum (1) and Farber (2) stated that somatic mutation is an essential event in the carcinogenic process. Mutations produced by chemical carcinogens include transitions (19), deletions (19–21), and frameshifts (19, 22, 23).

Several studies have been reported in which the in vitro systems have been evaluated for their differentiation between carcinogenic and noncarcinogenic chemicals. Poirer (24) described the results of an extensive correlation study with 66 carcinogens and 29 noncarcinogens. The compounds represented several chemical classes: aromatic hydrocarbons, aromatic amines, nitrosamines, alkylating agents, acylating agents, and heterocyclic and benzylic compounds. However, metals and promoters were excluded because they usually are not detected as mutagens in these systems. The in vitro microorganism systems included five strains of *S. typhimurium, pol*A$^-$ *E. coli,* and *S. cerevesiae* D3. Taken individually, yeast and *E. coli* gave positive responses with about 50% of the carcinogens and 25% of the noncarcinogens tested, whereas, with the *Salmonella* strains, 75% of the carcinogens and 11% of the noncarcinogens were mutagenic. By combining the results from *Salmonella* and from *E. coli pol*A$^-$, the correlation increased to 86% for carcinogens and to 28% for noncarcinogenic structural analogues. Seven carcinogens, six of which appear to require metabolic activation, could not be detected by either *Salmonella* or *E. coli.*

In a similar correlation study, Purchase et al (25) evaluated 120 compounds, 50% of which were carcinogens and the remainder noncarcinogens, in four in vitro (of which *S. typhimurium* was the only microorganism test) and two in vivo test systems. The *Salmonella* system accurately predicted 91% of the carcinogens and 93% of the noncarcinogens. The compounds tested were identified chemically as polycyclics, arylamines, and alkylating agents. The sensitivity of the *Salmonella* system was also supported by data from Herbold & Rohrborn (26). Of the nine mutagens studied, eight gave unequivocal positive results in the bacterial test with the metabolic system, results superior to one in vivo assay for mutagens.

Sugimura and co-workers (27) evaluated *S. typhimurium, E. coli,* and *S. cerevesiae* with several mutagens in the following classes: nitrofurans, hycanthone and its derivatives, nitrosamines, azobenzenes, and acenaphthenes. The authors concluded that most mutagens were also carcinogens (approximately 60) and that the number of nonmutagenic compounds that were carcinogenic was small (approximately 15). Very few compounds were noncarcinogenic mutagens. With the nitrofurans, *E. coli* was as sensitive at detecting mutagenic events as *S. typhimurium* TA100. Four strains of *S. typhimurium* were unable to detect the highly potent carcinogen AF-2. With dimethylaminoazobenzene derivatives, strains TA98 and TA100 of *S. typhimurium* predicted the carcinogenic compounds but also gave a high rate of false-positive responses.

In evaluating the utility of *B. subtilis* stains (*rec*-assay) in mutagenesis and carcinogenesis screening of chemicals, Kada (18) concludes that this organism has led to the detection of 20 previously unknown mutagens in the food additives and pesticides. The strain *rec*45 appears to be the most sensitive strain for detecting mutagens.

The major challenge currently is in the predictive value of the in vitro test methods in identifying the environmental mutagens and carcinogens. Recently the DHEW Working Group on Mutagenicity Testing (28) recommended an approach utilizing a battery of reliable in vitro systems to detect compounds with mutagenic properties. The subject of the predictability of mutagenicity tests for chemical carcinogens has been reviewed by several investigators (29–33). The microbial systems, particularly the strains of *S. typhimurium,* detect a relatively high percentage of carcinogenic substances that possess direct and indirect mutagenic activity. However, several problems still remain to be resolved in the application of these systems to the screening of compounds for carcinogenic activity. Point mutations do not appear to be sufficient to detect all mutagens, thus emphasizing the need to rely on a battery of tests rather than one specific species and/or strain. Clearly there are some mutagenic noncarcinogens that lead to a relatively low rate of false positives, whose long-term implications may be to unnecessarily restrict from the marketplace compounds of potential use to society. Purchase et al (25) attempted to address on a theoretical basis the issue of false-positives and concluded that a finite level of false-positives is inevitable and possibly very large in number. On the other hand, present data indicate a substantial number of false-negatives, which may lead to a failure to assess the potential for chronic irreversible toxicity in higher level test systems. While it is known that a number of carcinogens are mutagens from studies of known agents, it is still unclear what percentage of human or animal carcinogens are actually bacterial mutagens in the universe of compounds. Compounds such as metals, chrysene, urethan, and thioacetamide would not be detected by the most sensitive and accurate strains of *S. typhimurium.* Thus the nonmutagenic carcinogens pose the greatest challenge in the development of in vitro methods for detecting substances having potential carcinogenic activity in intact animals.

Of immediate concern is the interpretation of data generated from such systems. Extrapolation from in vitro systems to man appears at present to be untenable for several reasons. While DNA damage by a compound should signal concern that

diseases such as heritable mutations and cancer might be the outcome from exposure to these agents, the actual assessment of a hazard existing from the agent is a function of exposure of the organism and the target cells and molecules, the dosage (rate and concentration), metabolism within the organism, repair mechanisms and other factors, and innate sensitivity of the target species. Consequently, extrapolation of tolerable doses and of toxic manifestations such as chemical carcinogenesis can be done with higher probability when mammals are used to demonstrate mutagenic and carcinogenic effects than when indicator systems are used. The in vitro systems can be applied to make guarded judgments for possible risks and for the selection of compounds for more in-depth evaluations in intact animals. Coupled with the intact animal systems (34, 35), the in vitro systems can add a valuable dimension in the testing strategy of hazard evaluation in man.

CELL CULTURE

During the last quarter century, cultures of cells in vitro have become valuable adjuncts to whole animals in the investigation of toxic properties of chemical and physical agents. By contrast, toxicants have been used as tools to investigate basic biologic principles in in vitro systems because of the advantages afforded by simpler experimental models. Thus cell cultures offer the setting for observing the effects of compounds on cells in an artificial environment, without interference by nervous and humoral factors present in the intact organism. The nature of the culture systems enhances intracellular contact, with the greater likelihood of intracellular interaction with critical macromolecules and of response. Cell culture systems thus permit an analysis of the toxic responses of isolated cells to compounds.

Investigations of the toxic properties of agents are referable to (*a*) qualitative lesions (identification of target organs), (*b*) quantitative (determination of dose response and potency) toxicity, (*c*) mechanistic studies, and (*d*) a few specialized pathologic lesions such as carcinogenesis and mutagenesis.

Because of the restrictive source of the cells used in culture, such systems are not capable of predicting or identifying the more susceptible organs in the intact organism or determining how the intact organism, with protective mechanisms and repair processes, will respond to chemical insults to various organs. However, the measurement of toxic potency is quite feasible; and comparisons can be made to determine either differences in potency among compounds using the same cell system or various biological indices referable to the same agent among various systems (for example, a comparison of various cell lines with each other and with intact animal). In comparing cells with animals, one pitfall often exists: the method of measuring dose. For proper comparison, the dosage should be expressed as a standard unit such as "moles per cell." Estimates of cell populations in intact animals and various organs are available.

Proper utilization of tissue culture systems requires attention to a number of factors. Standardization of assay techniques including media components and serum enhances the possibility of reproducibility of results and is required to ensure valid comparability of data. Equally important is the chemical characterization of the test

substance, since impurities may be a substantial source of variability. Often overlooked is the influence of the vehicle or solvent system, which may have its own toxic properties in the bioassay system and which may structurally and physically alter the test agent. As a general rule, responses should be reported for cells in log-phase growth in contrast to lag or stationary phase cells. However, the selection of phases depends upon the parameters being measured: for example, cellular proliferation requires log-phase cells, whereas, biochemical modifications can be measured in either log or stationary phase cells.

For a number of years, a seemingly endless list of in vitro systems using isolated cells in culture has been developed for use in specialized applications for studying biological processes. However, if the criterion of predictability to animals and human toxicity is applied, the number of test systems is restricted substantially. Seeman (36) developed procedures to measure osmotic fragility of red blood cells to reveal the lytic effect of chemicals and to predict the related clinical responses in man and intact animals. Schindler (37) reviewed the use of cell culture in pharmacology with special emphasis on cytostatic agents and their mechanisms of action, antiviral agents, and carcinogenic chemicals.

Over the past 30 years, much effort has gone into the development and toxicity testing of biomaterials (for example, implants, supportive devices, and collection and administration and storage devices) for use in medicine, dentistry, and clinical pharmacology. Because these agents come directly in contact with tissue and with fluids that will eventually enter the body, the potential for releasing toxic components must be determined, preferably through the application of an appropriate battery of biological tests. For over two decades, *US Pharmacopeia* has described in vivo tests suitable for materials having specific medical application. More recently, in vitro tests were applied to the determination of toxicity of biomaterials. Guess et al (38) described a system employing a monolayer of mouse fibroblasts (L-cells) overlayed with agar and a vital stain for differentiation of lethality and viability upon insult with the test material. Rosenbluth et al (39), Autian (40), and Autian (41) describe similar systems. Comparisons of results (i.e. irritancy) from the in vitro versus the in vivo systems for a substantial number of biomaterials led to the conclusion that in vitro systems produce some false-positive responses but virtually no false-negatives. In this agar overlay method, not only can solids be studied conveniently and effectively but also liquids and extracts. Thus such cell growth inhibition tests have been found to be useful in the comparative toxicity testing of similar materials such as different polyurethanes for biomedical application. While the latter use of this test appears quite sensitive in determining leaching tendencies of biomaterials, the correlation with in vivo studies has not yet been established.

While the prediction of an endpoint such as irritancy appears well established with cell cultures, the prediction of systemic toxicity is far more complex and difficult. Early attempts to correlate acute systemic toxicity in mice with cell culture toxicity (both measured as lethality: LD_{50} in animals and ID_{50} in cells) (42, 43) led to equivocal results. Such an approach may have been oversimplified and emphasizes the need to obtain a comparable basis of dosage to take into account the

concentration of the test agent in the target cells as well as duration of cellular exposures in both systems. Equally pertinent is the characterization and standardization of the responses in the target cells in both in vivo and in vitro systems. If the expression of toxicity in intact animals is closely controlled by physiologic parameters such as transport, partitioning, selective distribution, and biotransformation rate and route, the probability of positive correlations of in vivo and in vitro toxicity will be most unlikely. However, if some of the intrinsic mechanisms of cellular toxicity are the same in culture and in whole animals, a positive correlation would be possible for some agents. One study (44) demonstrated that for two homologous series of compounds, acute toxicity (7 day LD_{50}) could be predicted from not only cellular toxicity (L-cells) data but also the lipophilicity (octanol: water partition coefficients) of the individual compounds. In addition to predicting acute lethal doses, the authors also generated data from the in vitro systems on the differing mechanisms of action and predicted changes in toxicity based on measured changes in rates of biotransformation. Whether the same conclusions will hold for other classes of chemical toxicants is unknown; however, these studies suggest the direction for reasonably predictive systems.

A similar, but less extensive, investigation was reported by Desi et al (45) who attempted to determine the differential sensitivity (i.e. acute toxicity) of in vivo (rat) and in vitro (primary monkey kidney cell line) test methods for a few chlorinated hydrocarbons and organophosphate pesticides. Gross comparison of the data leads to equivocal results, indicating a poor reliability that may be the result either of questionable comparison of dosages or of deficiencies in the assay system.

The extensive interest in cell culture toxicity work has led to a proliferation of human cell systems that are organ specific. Examples of these organ cell systems include the lung (46–49), the leukocyte (50–52), and the liver (53, 54). Organ-specific cell lines and cultures are available for most experimental species including hamster, mouse, rat, rabbit, guinea pig, pig, and monkey.

Mechanistic and cellular toxicity studies have seen the widest application of cells in culture, of which only a few examples will be noted. The toxicity of oxygen has been described on cultured human neonatal epithelium (55) in an effort to relate changes in ciliary function to squamous metaplasia and to degeneration of the surface epithelium. Cultures of HeLa cells and human embryonic fibroblasts have been employed to characterize the cytotoxicity of 14-membered heterocyclic compounds demonstrating a positive structure-activity correlation (56). Dietz & Flaxman (57) studied the toxicity of aromatic hydrocarbons on normal human epidermal cells in vitro and demonstrated morphologic and functional changes. Human lung fibroblasts (WI-38) were applied to the comparative investigation of platinum salts likely to be inhaled (58), while Ehrlich-Landschutz diploid ascites tumor cells were used to ascertain the comparative toxicity of organic solvents (59). The fate and mechanism of action in several classes of environmental chemicals have also been investigated in various cell culture systems. Because of the metabolic advantage of the liver, primary hepatocyte cultures have been generated that maintain their functional and biochemical integrity for sufficient time to perform cytotoxicity assays (60). Because of the ease of manipulation, cells in culture are amenable to

studies of interactive toxicity as demonstrated by Potter & Matrone (61) in which Chang's liver cells and mouse fibroblasts (3T3, clone 4A) were the experimental media in defining toxic interactions of mercury and selenium. Despite the relative ease and brevity in using cells in culture, some efforts have been directed to further simplify the assays and to increase their rapidity for screening of large numbers of compounds (62, 63). Perhaps one of the most ambitious applications of cell culture has been the attempt to develop procedures for the identification of teratogens including growing postimplantation mammalian embryos in culture and whole mouse embryos during several stages of organogenesis (64).

Cultured cells are particularly amenable to the study of metabolic processes. Control of lipid metabolism in WI-38 cells has been extensively analyzed by Bailey et al (65), whereas, phospholipid metabolism in HeLa and KB cells has been detailed by Rytter & Cornatzer (66). Changes in cyclic nucleotides in cultures of fetal rat liver have been delineated (67). In contrast to studies with natural substrates, reports are extensive on xenobiotic metabolism in cell culture, with special emphasis on polycyclic hydrocarbons (68-72) in attempts to explain various chemical-biological interactions related to chemical carcinogenesis. Phillips (73) described a two-compartment system of measuring in vitro drug activation and cytotoxicity as a means of screening for the presence of toxic metabolites.

The substantial resources and commitment of time required to determine the induction of cancer by compounds in experimental animals led to the quest to find more rapid and simplified and at least as reliable short-term test for predicting those agents that will produce malignancies in intact animals. Many of these tests apply an in vitro approach. Among the various test methods, the following deserve attention for assessment of their merits and possible application: (*a*) the acridine test, (*b*) the photosensitization action of hydrocarbon carcinogens on *Paramecium caudatum,* (*c*) bacteriostatic effects of carcinogens, and (*d*) malignant transformation of mammalian cells.

Szent-Gyorgyi & McLaughlin (74) noted the existence of a correlation between potent carcinogenicity and the reaction with acridine. When a test compound is reacted in equimolor concentration with acridine and a strong yellow color appears, the compound is called a *carcinogen.* The relationship is questionable since urethan, a potent carcinogen, gives a negative reaction and since 1,2-dimethylbenzanthracene yields only a weakly positive response.

Epstein et al (75) proposed an in vitro method for demonstrating carcinogenic properties using *Paramecium caudatum* based on the observations of Mottram (76) that 3,4-benzpyrene altered the growth rate of the organism. The basis of the technique is the observation of immobilization and death of the organism with treatment of the test agent followed by nontoxic, long-wave ultraviolet radiation. In general, the phenomenon of photodynamic toxicity represents a biologic reaction which is relatively common for a variety of organic compounds with and without carcinogenic properties. Consequently, it appears unlikely that such a nonspecific reaction will prove of predictive utility as a short-term test for potential polycyclic carcinogens (77, 78).

As demonstrated by Buu-Hoi (79) with a large number and variety of chemical carcinogens, a correlation exists between carcinogenic properties and bacteriostatic qualities. However, since many noncarcinogenic compounds also possess bacteriostatic effects these observations are not a sound basis for an effective screening method for chemical carcinogens.

The data of Mecke & Schmahl (80) suggested an in vitro colorimetric assay based on the cleavage of the azo-bridge with resulting decoloration of the azo dyes by yeast. The effectiveness of the reaction was inconsistent. While amino- and hydroxy-azobenzenes were, in general, completely decomposed by yeast, the phenomenon did not apply to azo-benzenes, methylated azo-benzenes, and sulphonated azo-benzenes. Thus no definitive correlation could be established.

A number of promising cell systems have been developed as in vitro models for the study of chemical carcinogenesis. These systems can be classified for discussion into (*a*) murine cell lines, (*b*) viral cell lines, (*c*) Syrian hamster cells, and (*d*) human cells. Only those systems having established value as a tool in carcinogenesis investigations are discussed. The basic principles underlying neoplastic transformation are that normally growing cells may be converted to cells that grow in a disorganized and proliferative pattern as do malignancies in situ and that the clones of transformed cells produce cancers when transplanted in syngeneic hosts. In addition, a cell culture assay measuring sister chromatid exchange in Chinese hamster ovary cells has been suggested for detecting chemical mutagens (83).

Murine cell lines include the cloned line of C_3H mouse embryo cells designated as 10T1/2 (81, 82) and the BALB 3T3 cloned cell line (82). Both cell lines are subject to postconfluence inhibition, and their growth characteristics are well defined. Under controlled conditions, the incidence of spontaneous transformation is exceedingly low, facilitating the quantitation of malignant transformation. Accurate and predictable dose-response relationships are produced with each line, since they follow one-hit kinetics for transformation. Because both lines are derived from well-characterized inbred strains of mice, correlations of in vitro response can be made with in vivo carcinogenesis with pathologic confirmation. Since 3T3 cells are readily amenable to transformation (40–50%) when infected with SV4O and murine sarcoma virus (MSV) (84) but not the feline sarcoma virus (FSV), the cell line may prove effective in elucidating interactions between chemical and viral oncogens. In addition to measurements of transformation rates in the cell lines, biochemical parameters such as DNA damage, fixation, and repair may be readily assessed. Some of the drawbacks of these lines include the relatively long periods of time (approximately 4 weeks) for analysis, the aneuploid state of the 3T3 (85) versus the hypertetraploid state of 10T1/2 (86), and the inhibitory influence of amphotericin B on 10T1/2 with simultaneous exposure to polycyclic hydrocarbons (87).

For those carcinogens and cocarcinogens that activate integrated viral genes to produce in vitro transformations, the sarcoma positive-leukemia negative (S+L–) cells derived by infection with sarcoma virus yield ideal model systems. These cells possess genetically stable markers such as viral core proteins of murine leukemia virus (MuLV) and interspecies gs-3 antigents (88), and they release C-type viral

particles and yield reverse transcriptase (an RNA polymerase). Generally, these cells are pleomorphic and refractile, with loss of contact inhibition and growth to high densities (89). Moloney murine sarcoma virus (MMSV) can infect 3T3 cells, whereas, murine sarcoma virus (MSV) requires concomitant infection with its replicating "helper" leukemia virus (89, 90). S+L– cell lines from human amnion and lung have also been developed (91) and can be used to study leukemogenic properties. The induction of tumor viruses by ionizing radiation and chemical carcinogens and mutagens has been reported for the AKR mouse embryo cell line (92); chicken cell (93); clonal lines of mouse (94, 95), rat (96, 97), and cat (98, 99); and human tumor cells (100, 101).

Fetal Syrian hamster cells are also amenable to studies of susceptibility to oncogenic transformation by chemical carcinogens. Since cell growth can be synchronized, this model can be used to determine differences in susceptibility of the various phases of cell cycle (102). This system has successfully produced transformations by chemical (103, 104), physical (105), and viral oncogenic agents (106). As with other transformation systems, those using Syrian hamster cells have demonstrated a low rate of spontaneous transformation either in the presence or absence of rat embryo feederlayers and can be administered to isogeneic hosts to establish the oncogenic potential of the transformed cells. The excision repair capacity for the primary cell cultures is believed by Hart & Setlow (107) to be intermediate between mouse and man. Of substantial significance is the ability of this system to respond to chemical promoters (e.g. phorbol myristate acetate) of carcinogenesis and mutagenesis (108) thus offering the opportunity to investigate cocarcinogenesis in vitro.

Human cells offer a unique advantage since they originate from the host of primary interest. In addition to the obvious benefit of evaluating compounds with the ability to transform human cells, these cell cultures afford an opportunity to investigate metabolic pathways and thus supply insight into species differences in carcinogenic susceptibilities. Human foreskin (e.g. strain called Detroit 550) has been used to determine transformation by chemicals and viruses (109, 110). Although epidemiologic studies have identified many agents as probable or potential human carcinogens, direct experimental demonstration of carcinogenic activity in human cells has not yet been accomplished. One avenue for the establishment of a toxicologic screening system for potential human carcinogens should be further explored: the development of the human liver cell culture that is deficient in the repair of DNA damage (e.g. from *Xeroderma pigmentosum* patients).

With the confirmation in a syngeneic host that transformed cells of some lines produce malignancies, there appear to be strong prospects for systems that will predict carcinogenic responses directly in whole animals. Purchase et al (25) evaluated 120 compounds in six short-term tests, of which cellular transformation of Syrian hamster kidney fibroblasts and human diploid fibroblasts or human liver cells were a part. Their results indicated that the cell transformation assays were highly predictive of known carcinogens (91%) and known noncarcinogens (97%). Cell transformation in culture was judged as predictable overall as the bacterial mutagenesis assay using *Salmonella typhimurium.* More limited studies by Marquardt et al (111) indicated a favorable correlation between in vivo and in vitro tests.

Similarly, a recent review (112), comparing the effects of chemotherapeutic agents on in vivo and in vitro systems concluded that a strong correlation exists for predicting carcinogenic activity using in vitro systems of transformation. From these studies, the actual rate of false-negatives is not precisely defined. Additional studies are required to substantiate and validate such model in vitro systems for their application as substitutes for testing in intact animals.

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