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THE CHO/HGPRT MUTATION ASSAY. IV. PROGRESS WITH QUANTITATIVE MUTAGENESIS AND MUTAGEN SCREENING[a]

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Running head: Progress with CHO/HGPRT mutagenesis

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Introduction

In the previous presentations about the CHO/HGPRT system, we have presented the development and definition of the experimental protocol (O'Neill and Hsie, 1979a), the genetic basis of 6-thioguanine resistance (TG^r) (O'Neill and Hsie, 1979b), and the adaptation of the system for mutagen screening (O'Neill and Hsie, 1979c). In this article, we summarize our work on the development and application of this mutation system to study mutagenicity of various physical and chemical agents, some of which bear profound environmental concerns.

Experimental Procedures

All studies to be described employed a subclone of CHO K_1 cells designated as CHO-K_1-BH_4 (Hsie et al, 1975a) unless otherwise specified. Conditions for culturing the cells, treatment with chemicals, quantitation for the effects of mutagen on cellular cloning efficiency and mutation induction to 6-thioguanine (TG), have been described earlier in this conference (O'Neill and Hsie, 1979a, b, c).

Results and Discussion

Development of a protocol for quantifying specific gene mutagenesis

Many investigators have reported the induction and isolation of various mutants different from the phenotype of the parental mammalian cells (Reviews by Chu and Powell, 1975; Puck,1972; Thompson and Baker, 1973; Siminovitch, 1976).

Generally their procedure for mutation induction does not take into consideration the quantitative aspects of the mutagenesis since the purpose of these studies was aimed solely at obtaining a particular type of phenotypic variant for either genetic or biochemical or molecular analysis. Furthermore,

in many instances, only a small fraction of the presumptive variants was the bona fide mutants with the desired stable phenotype. Due to the intrinsic characteristics of each gene mutation assay, factors required to quantify mutagenesis need to be established individually. As presented earlier (O'Neill and Hsie, 1979a), in the case for the CHO/HGPRT assay, many factors which affect quantitative mutagenesis to TG^r must, first, be defined. These include:

(1) Mutagen treatment and the physiological state of cell growth. Except those experiments designed for cell cycle study, we treat cells during the exponential growth state because some mutagens may act preferentially on the proliferating cells.

(2) Medium for cell growth and mutant selection. The growth medium used should not allow preferential growth of wild type over mutants, and vice versa. This is especially crucial for such mutants as TG^r which required long phenotypic expression time in which a small fraction of mutants need to be co-cultured with a highly excessive proportion of the wild-type cells in a continued proliferative state. Thus, a slight advantage or disadvantage for mutant growth will grossly distort the observed mutation frequency. During mutant selection with TG, the selective medium is devoid of hypoxanthine because TG competitively inhibits hypoxanthine transport across cell membrane (O'Neill et al, 1977), and the reverse is likely true.

(3) TG concentration. Optimum TG concentration should be used to select for phenotypic variants of mutational origin without being either leaky or epigenetic in nature.

(4) Cell density for selection. To fully recover a small fraction of TG^r mutants from an excess of wild-type cells, consideration should be directed towards the ratio of mutants/wild-type cells in such as that the mutants will

escape the cytotoxic effects of the purine analogue metabolites converted from TG through the HGPRT activity in the wild-type cells.

(5) Phenotypic expression time. Since the selection of the mutants is based on the loss of the HGPRT activity, a period of delay for expressing the TG^r phenotype is expected to allow mutation fixation to complete and to permit diluting out the preexisting enzyme and mRNA coded for HGPRT. We have found that maximum stable expression of the TG^r phenotype is reached 7-9 days after mutagenesis and remains constant thereafter irrespective of the nature and dose of the mutagen (O'Neill et al, 1977a; O'Neill et al, 1977b; O'Neill et al, 1978a).

Characteristics of the CHO/HGPRT system: Evidence of the genetic basis of mutation at a specific locus

Conclusive, direct proof of the genetic origin of mutations in somatic cells should theoretically rely on demonstration that the affected hereditary alteration has resulted in a modified nucleotide sequence of the specific gene, causing modified coding properties which result in the production of altered protein with changes in the amino acid sequence. In the absence of such proof, one must rely on indirect criteria which are consistent with the concept that the observed phenotypic variations are genetic in nature. Such criteria include stability of altered phenotype, mutagen-induced increase in occurrence of stable variants, biochemical and physiological identification of the variant phenotype, chromosomal localization of the affected gene, etc. (Reviews by Chu and Powell, 1975; Baker and Thompson, 1973; Hsie et al., 1978c).

Over the past 5 years, we have used this assay protocol and have found in approximately 600 experiments that the spontaneous mutation frequency lies in the range of $0\text{-}10 \times 10^{-6}$ mutant/cell. A detailed fluctuation analysis of spontaneous

mutation was carried out by taking consideration of the problem of phenotypic delay and found that the spontaneous mutant phenotype arises in a random manner, as would be expected for a mutational event (Luria and Delbruck, 1943). The mean values for the mutation rate is 7.0×10^{-8} (F_o method) and 32.6×10^{-8} (mean method) mutations/cell/generation (O'Neill et al, 1979d). Various physical and chemical agents are capable of inducing TG resistance. Among all chemical mutagens examined, mutation induction occurs as a linear function of the concentration (Couch and Hsie, 1976; Couch and Hsie, 1978a, 1978b; Couch et al, 1978; Hsie et al, 1975a, 1975b; Hsie et al, 1977a, 1977b; Hsie et al, 1978a, 1978b, 1978c; O'Neill and Hsie, 1977; O'Neill et al, 1977a, 1977b; O'Neill et al, 1978a, 1978b). For example, mutation frequency increases approximately linearly with EMS concentration in the near-diploid CHO cell line, conforming to the expectation that mutation induction occurs in the gene localized at the functionally monosomic X chromosome. However, in the tetraploid CHO cells, EMS does not induce an appreciable number of mutations, even at very high concentrations, as predicted theoretically (Hsie et al, 1977b).

We have been unable to detect any spontaneous reversion with 13 TG-resistant mutants, all of which contain low, yet detectable, HGPRT activity. More than 98% of the presumptive mutants isolated either from spontaneous mutation or as a result of mutation induction are sensitive to aminopterin, incorporate hypoxanthine at reduced rates, and have less than 5% HGPRT activity (O'Neill et al, 1977a). Studies in progress have also shown that mutants containing temperature-sensitive HGPRT activity can be selected, suggesting that mutation resides in the HGPRT structural gene (O'Neill and Hsie, unpublished observations).

The CHO/HGPRT system appears to fulfill the criteria for a specific-gene locus mutational assay and should be valuable in studying mechanisms of

mammalian cell mutagenesis and determining the mutagenicity of various physical and chemical agents.

The interrelationships of mutagen-mediated cellular lethality and mutation induction.

When EMS was employed as a mutagen, mutation induction occurred over the entire survival curve, which includes both the shoulder region, where there is no appreciable loss of cell survival, and the exponential portion, where cell killing increases exponentially with increasing mutagen concentrations (Hsie et al, 1975a; Hsie et al, 1977b; O'Neill and Hsie, 1977). Apparently, there is no threshold effect of mutation induction with EMS. It appears that mutation induction is a more sensitive parameter for genetic toxicity for agents such as EMS.

Later experiments show that many physical and chemical agents such as X-ray (O'Neill et al, 1977a), UV light (Hsie et al, 1975b), ICR-191 (O'Neill et al, 1978a) and isopropyl methanesulfonate (iPMS) (Couch et al, 1978), diethylsulfate (DES) (Couch et al, 1978), exhibit "EMS type" curves of interrelationships of cell survival and mutagenesis. However, there are agents, typified by MNNG (Couch and Hsie, 1978b; O'Neill et al, 1977a) and N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) (Couch and Hsie, 1978b) which do not exhibit appreciable shoulder regions in the survival curve and for which mutation induction always occurs concomitantly with the loss of cell survival ("MNNG type").

As our studies on chemical mutagenesis progress, we have found other types of interrelationships of mutagenicity and cytotoxicity. Most pro-mutagenic agents are neither toxic nor mutagenic to the cells in the absence of S_9-mediated metabolic activation. With S_9, benzo(a)pyrene [B(a)P] is mutagenic and cytotoxic with the "EMS type" cell survival curve (Brimer, O'Neill, San Sebastian and Hsie, unpublished). A direct-acting mutagen, 4-nitroquinoline-1-oxide is highly

cytotoxic and mutagenic to the CHO cells, and its cytotoxicity and mutagenicity decrease when treated with S_9 (San Sebastian and Hsie, unpublished). S_9 also greatly decreases the mutagenicity and cytotoxicity of ICR-191 (Fuscoe, O'Neill and Hsie, unpublished). As will be seen later a slight modification of the structure of the chemical may effect either the cytotoxicity and/or mutagenicity further demonstrates that the cytotoxicity and mutagenicity of chemical mutagens are separable.

Quantitative analysis of chemical induced cellular lethality and mutagenesis.

(1) A study of EMS exposure dose.

Earlier, we found that EMS-induced mutation frequency to TG resistance in cells treated for a fixed period of 16 h is a linear function over a large range of mutagen concentrations, including both the shoulder region and the exponentially killing portion. Further studies using varying concentrations (0.05-3.2 mg/ml) of EMS for 2-12 h showed that the manifestation of cellular lethality and mutagenesis occurs as a function of EMS exposure dose in that the biological effect is the same for different combinations of concentration multiplied by duration of treatment which yield the same product. From these studies the mutagenic potential of EMS can be described as 310×10^{-6} mutants $(\text{cell mg ml}^{-1} \text{ h})^{-1}$ (O'Neill and Hsie, 1977). Thus, the CHO/HGPRT system appears to be suitable for dosimetry studies which are essential for our understanding of the molecular mechanisms involved in mammalian mutagenesis.

(2) Relationship between DNA alkylation and mutation induction to TG^r by N-methyl- and N-ethyl-N-nitrosourea.

The direct-acting alkylating chemicals transfer electrophilic alkylating residues to nucleophilic centers of the DNA double helix. Taking advantage of

the quantitative nature of the alkylating mutagenesis in the CHO/HGPRT system (Hsie et al,1977a; O'Neill and Hsie, 1977; Couch and Hsie, 1978b; Couch et al, 1978), we have investigated the relationship of DNA alkylation and mutation induction in cells treated in an identical manner by a range of concentrations of N-methyl-N-nitrosourea (MNU) or N-ethyl-N-nitrosourea (ENU). We found that alkylation by both MNU and ENU increases linearly with increasing nitrosamide concentrations over the ranges tested. Similar linearity is found for mutation induction by MNU and ENU. The data show that, on an equimolar comparison, MNU has 15 times the DNA alkylating activity of ENU, but only 3.3 times the mutagenic activity. Thus, in terms of mutation induction per unit alkylation, ethylation of DNA by ENU appears to result in a 4.5-fold greater fraction of mutagenic lesions than does methylation by MNU. From this comparison of MNU and ENU effects, it appears that ethylation results in a higher proportion of mutagenic lesions than does methylation. This may reflect either a higher frequency of miscoding events such as O-6-guanine alkylation or an effect of ethylation per se (Thielmann et al, 1979).

(3) Mutagenicity, Cytotoxicity and DNA-binding of Pt(II)chloroammines.

cis-$Pt(NH_3)_2Cl_2$ is a widely used inorganic antitumor agent. This compound also exhibits such diverse biological activities as enhancement of prophage induction, inactivation of transforming DNA, selective inhibition of DNA synthesis and induction of chromosomal abnormalities. In our previous study, we have found that cis-$Pt(NH_3)_2Cl_2$ is cytotoxic and mutagenic in the CHO/HGPRT assay (O'Neill et al, 1977a). We have extended our work to include other Pt(II)chloroammines in the same system. Based on the slope of the linear dose-response, the mutagenicities expressed as mutants/10^6 cells per μM of Pt(II)chloroammine are: cis-$Pt(NH_3)_2Cl_2$, 47; trans-$Pt(NH_3)_2Cl_2$, 0.35; K_2PtCl_4, 0.69; $[Pt(NH_3)_4]Cl_2$, 0.01. The relative cytotoxicity follows the same order

and similar magnitude. The mutagenicity and cytotoxicity of these compounds in CHO cells correlate well with other reported biological activities. Using radioactive ^{195m}Pt, the amount of Pt bound to cellular DNA was measured under identical conditions for mutagenesis studies. We observed that, after incubation for 16 h, cis-$Pt(NH_3)_2Cl_2$ binds linearly over a concentration range of 0.4 - 1.7 μM with a slope of 7.9×10^{-11} μmol Pt bound per nucleotide per μM of cis-$Pt(NH_3)_2Cl_2$ added. Preliminary results indicate that trans-$Pt(NH_3)_2Cl_2$ binds to DNA to a similar extent. It appears that the chemical nature of the lesions produced in the DNA rather than the numbers of molecules bound to DNA is responsible for the differential biological potency of these Pt compounds (Johnson et al, 1979).

Structure-activity relationship of direct-acting chemical mutagens.

(1) Alkylating chemicals (total of 11)

Since EMS mutagenesis is quantifiable, it appears that the CHO/HGPRT system should be useful for studying the relationship between structure characteristics of the chemical mutagens and their mutagenicity.

The dose-response relationships of cell killing and mutation induction of two alkylsulfates [dimethylsulfate (DMS) and DES] and three alkyl alkanesulfonates [methyl methanesulfonate (MMS), EMS, and iPMS] have been compared under identical experimental conditions. We observed that cytotoxicity decreased with the size of the alkyl group: DMS > DES; MMS > EMS > iPMS. All agents produced linear dose response of mutation induction with the following order when comparison was made based on mutants induced per unit mutagen concentration, DMS > DES; MMS > EMS > iPMS. However, when comparisons were made at 10% survival, the relative mutagenic potency was: DES > DMS; EMS > iPMS > MMS (Couch et al, 1978).

Similar comparative studies were extended to two nitrosamidines (MNNG and ENNG) and three nitrosamides [N-methyl-N-nitrosourea (MNU), N-ethyl-N-nitrosourea (ENU), and N-butyl-N-nitrosourea (BNU)] differing in the nature of their alkylating group. Based on mutants induced per unit mutagen, the following order of relative mutagenic potency was observed: MNNG > ENNG > MNU > ENU > BNU. This is the same order when comparisons were made at 10% cell survival (Couch and Hsie, 1978b). All of these 10 alkylating agents are known to be carcinogenic, and are mutagenic in our assay. Nitrosation appears to be essential for exerting mutagenicity of nitrosoamidines since N-methyl-N'-nitroguanidine (MNG), a noncarcinogenic analogue of MNNG, is not mutagenic even at concentrations 50,000 higher than its nitroso analogue, MNNG (San Sebastian and Hsie, unpublished).

(2) Heterocyclic nitrogen mustards (ICR compounds) (total of 19).

Structure-activity relationship of antitumor agents would be of interest to screen for desirable chemotherapeutic drugs. We have studied the cytotoxicity and mutagenicity of 19 ICR-compounds (ICR-449, 217, 220, 191, 191-OH, 170, 170-OH, 283, 171, 372, 372-OH, 340, 340-OH, 342, 371, 355, 292, 292-OH, 368). They contain structural similarities and differences which allow a study of the role of heterocyclic nucleus and the alkylating side chain in their biological acitivity. Thirteen of these compounds are mutagenic. At equimolar concentrations, the compounds with the 3^{o}-amine-type side chain (ICR 217, 340, 355, 368, 170, and 292) are more mutagenic than the compounds with the 2^{o}-amine-type side chain (ICR 449, 371, 191, and 372). All 2^{o}-amine types show a "plateau" in their concentration-dependent mutagenesis curves at 3 to 4 μM. Shortening of the side chain by one carbon (ICR 171) results in a reduced mutagenicity. Substitution of a sulfur atom for a nitrogen in the side chain (ICR 342) increases both mutagenicity and cytotoxicity. The

presence of two 2-chloroethyl groups on the side chain (ICR 220) also results in greatly increased cytotoxicity and mutagenicity. When the 2-chloroethyl group of ICR 340, 372, 292, 191, or 170 is replaced by a 2-hydroxyethyl group (ICR 340-OH, 372-OH, 292-OH, 191-OH, 170-OH), a mutagenically inactive compound results which remains toxic indicating that the 2-chloroethyl group is required for mutation induction (O'Neill et al, 1978a, 1978b; Fuscoe et al, 1979). In addition, it also suggests that cytotoxicity is dissociable from mutagenicity. Replacement of the amine linkage with an ether linkage (ICR 283) also yields a mutagenically inactive compound.

Tumorigenicity data are available for 6 of these compounds. Four, found to be highly mutagenic in our studies (ICR 170, 340, 292, and 342), are carcinogenic, and the other 2 (ICR 191 and 191-OH) are reported to be non-carcinogenic in a mouse lung tumor system (Peck et al, 1976a, 1976b). ICR 191-OH is nonmutagenic in the CHO/HGPRT system, but ICR 191 is a potent mutagen not only in our system but also in several others (Deluca et al, 1977; McCann et al, 1975). Apparently, ICR 191 is a mutagenic noncarcinogen. Perhaps the lack of carcinogenicity of ICR 191 in the mouse lung tumor system can be explained by its being inactivated in the mouse at the target site (lung) or before it reaches the lung, since the addition of S_9 metabolic activation system greatly reduced the mutagenicity and cytotoxicity of this compound (Fuscoe and Hsie, unpublished observations).

(3) Metallic compounds (total of 19).

$MnCl_2$, $FeSO_4$, $CoCl_2$ and $K_2Cr_2O_7$ are mutagenic, while $NiCl_2$, $BeSO_4$ are weakly mutagenic. Determination of metal mutagenicity is apparently complicated by the ionic composition of the medium, e.g. the mutagenicity of $MnCl_2$ was abolished by the excess of $MgCl_2$. The unusual environment required for

demonstration of mutagenicity of $MnCl_2$ may account in part for varying results obtained in studying the mutagenicity of $AgNO_3$, $CaCl_2$, $Pb(CH_3COO)_2$, RbCl, H_2SeO_3, $TiCl_4$, and $ZnSO_4$ (Couch and Hsie, 1978a; Couch, Tan, Forbes, Tindall and Hsie, unpublished). As mentioned earlier, cis-$Pt(NH_3)_2Cl_2$ is mutagenic while trans-$Pt(NH_3)_2Cl_2$, K_2PtCl_4 and $[Pt(NH_3)_4]Cl_2$ are not (Johnson et al, 1979).

(4) Miscellaneous compounds (total of 19).

Found non-mutagenic are three commonly used solvents (acetone, dimethylsulfoxide, and ethyl alcohol), four metabolic inhibitors (cytosine arabinoside, hydroxyurea, caffeine, and cycloheximide) and N^6, $O^{2'}$-dibutyryl adenosine 3':5'-phosphate. The following 11 compounds are mutagenic, i.e. hydrazine, hycanthone, ethylene oxide, ethylene dibromide, ethylene dichloride, ethylene chlorobromide, mitomycin C, myleran, captan, and folpet (Tang, Brimer and Hsie, unpublished observations). Some experiments are preliminary in nature and remained to be confirmed.

Structure-mutagenicity studies of promutagenic chemicals.

CHO cells, like many other organisms used in the mutagenicity testing, are unable to transform promutagenic compounds to their active metabolites. The CHO/HGPRT assay can be coupled to the host (mouse)-mediated system (Hsie et al, 1978a) or the S_9 microsomal activation system (O'Neill et al, 1977a). Because of the relatively high cost and low sensitivity of the host-mediated CHO/HGPRT assay, only limited studies have been conducted using this technique. Although a time- and concentration-dependency of mutation induced by DMN and EMS could be demonstrated (Hsie et al, 1978a), the mutagenicity of benzo(a)pyrene [B(a)P] is detectable only at its near saturation concentration in pure corn oil (Hsie, Machanoff and Holland, unpublished data).

Thus, most of our studies on the mutagenicity of promutagens were conducted through S_9-coupled CHO/HGPRT assay. The optimal conditions for the use of an S_9 microsomal preparation to mediate activation of promutagens has been investigated. The S_9 fraction was prepared from Aroclor 1254-induced male Sprague-Dawley rat livers, and two model procarcinogens B(a)P and DMN utilized for the initial studies. With these two compounds, several differences were found in the conditions which yielded maximal mutation induction. Over a S_9 protein concentration range of 0.1-1.5 mg/ml, the mutagenicity of a constant amount of DMN increases with increasing protein, while that of B(a)P increases at low protein concentrations, followed by a decrease. This may reflect a change in the balance between activation and inactivation with different compounds, and limits the range of protein concentrations at which comparisons of mutagenic potency are valid at least with these two compounds. In addition, DMN requires high concentrations of $CaCl_2$ for maximal mutagenic activity, while B(a)P does not. These results complicate the development of a single S_9 protein mix which would be useful in routine mutagenesis screening and in the quantification of the mutagenic potential of compounds for comparative studies.

With this limitation in mind, we have determined the mutagenicity of various classes of promutagens under conditions which are near-optimal for quantifying the mutagenicity of both DMN and B(a)P in which the S_9-mix contains $MgCl_2$ and $CaCl_2$ at 10 mM each (O'Neill, Machanoff and Hsie, 1979).

(1) Polycyclic hydrocarbons (total of 27).

We have studied the mutagenicity of benzo(a)pyrene [B(a)P] and its 19 metabolites, benzo(e)pyrene [B(e)P], pyrene, benz(a)anthracene (BA), and 4 related compounds. The carcinogenic polycyclic hydrocarbons B(a)P, BA, and 7,12-dimethyl-BA require metabolic activation to be mutagenic. The weak

carcinogen B(e)P is less mutagenic than B(a)P. The non-carcinogenic polycyclic hydrocarbons pyrene and anthracene are non-mutagenic even with metabolic activation. B(a)P-4,5-epoxide and B(a)P-7,8-diol, 9-10-epoxide are mutagenic. Since CHO cells cannot activate procarcinogens such as B(a)P, these cells appear to be most useful in screening for the mutagenicity of metabolites such as those of B(a)P (Hsie and Brimer, unpublished).

(2) Nitrosamines and related compounds (total of 16).

Nitrosamines generally require metabolic activation to be cytotoxic and/or mutagenic. All 9 carcinogenic nitrosamines (dimethylnitrosamine, diethylnitrosamine, 2-methyl-1-nitrosopiperidine; 3,4-dichloro-1-nitroso-piperidine; nitroso-pyrrolidine; 3,4-dichloronitrosopyrrolidine; 1,4-dinitro-sopiperazine; 1,5-dinitrosohomopiperazine; nitrosomorpholine) are mutagenic and all 4 non-carcinogenic nitrosamines (2,5-dimethylnitrosopiperidine; 2,5-dimethylnitrosopyrrolidine; 1-nitrosopiperazine; nitrosophenmetrazine) are non-mutagenic. Formaldehyde and sodium nitrite are non-mutagenic, and dimethyl-amine is mutagenic at high concentrations (San Sebastian and Hsie, unpublished). Variable carcinogenicity data on the latter 3 chemicals existed in the literature.

(3) Quinoline compounds (total of 5).

Quinoline, a known carcinogen, is mutagenic with metabolic activation. Another carcinogen, 4-nitroquinoline-1-oxide, is highly mutagenic; its mutagenicity decreases when assayed in the presence of the activation system. The carcino-genicity of 8-dydroxy-, 8-amino-, and 8-nitroquinoline is not known, but these compounds exhibit variably weak mutagenicity in preliminary experiments (San Sebastian and Hsie, unpublished).

(4) Aromatic amines (total of 5).

The carcinogens 2-acetylaminofluorene and its N-hydroxy and N-acetoxyl derivatives are mutagenic, while fluorene, a non-carcinogenic analogue, is

non-mutagenic. 1-hydroxy-2-acetylaminofluorene appears to be mutagenic at a very high concentration in preliminary experiments (Hsie, Sun, and Brimer, unpublished).

(5) Miscellaneous promutagens (total of 4).

Three chemicals which are mutagenic to mice, i.e. natulan, cyclophosphamide, triethylenemelamine are mutagenic in our system (Tan and Hsie, unpublished data). The mutagenicity of the artificial sweetener saccharin appears to be variable; its determination is complicated by the requirement of high concentrations to yield any biological effect (O'Neill, San Sebastian and Hsie, unpublished).

Quantitative analyses of radiation-induced lethality and mutagenesis (total of 7 agents).

The cellular lethality and mutagenicity of ionizing and nonionizing agents has also been studied with this assay. Ultraviolet irradiation does not appear to be significantly detrimental for clonal growth up to $\sim$ 8 J/m^2; it induces mutation linearly from $\sim$2 up to $\sim$26 J/m^2, including the shoulder region of the survival curve (Hsie et al, 1975b; Riddle and Hsie, 1978). Fluorescent white, black, and blue lights are weakly lethal and mutagenic. Sunlamp light is highly lethal and mutagenic, exhibiting these biological effects within 15 sec of exposure under conditions recommended by the manufacturer for human use. Lethal and mutagenic effects were observed after 4 min of sunlight exposure; responses varied with hourly and daily variations in solar radiation. The cell-survival and mutation-induction curves generated by exposure of cells to both sunlamp and sunlight were similar to those obtained by the use of a standard far-uv lamp (Hsie et al, 1977a). The mutagenicity of high-dose X-ray irradiation is clearly demonstrable; the precise dose response remains to be established after factors affecting ionizing mutagenesis are clarified (O'Neill et al, 1977b).

Correlation of mutagenicity in the CHO/HGPRT assay with reported carcinogenicity in animal tests.

Among a total of 132 chemical and physical agents studied at different stages of completion 84 have been reported to be either carcinogenic or non-carcinogenic in animal studies. Mutagenicity in the CHO/HGPRT assay of 77 of these agents correlated with the documented animal carcinogenicity (IARC Monographs 1-10, USPHS Publication 149). The existence of a high correlation [77/84 (92%)] between mutagenicity and carcinogenicity speaks favorably for the utility of this assay in prescreening the carcinogenicity of chemical and physical agents. However, this result should be viewed with caution, since so far only limited classes of chemicals have been tested and some of the preliminary results remain to be confirmed. A possible false negative was formaldehyde, which has been shown to be either carcinogenic or non-carcinogenic depending on the way test animals are exposed to it. An apparent false positive was ICR-191, a potent mutagen for microorganisms and CHO and other mammalian cells, which has been shown to be non-carcinogenic in a recent study (Peck et al, 1976b) and the other, folpet, which is a weakly mutagenic in this system. Thus the assay appears to be applicable for monitoring the genetic toxicity of diverse chemical and physical agents.

Interactive effects between physical- and chemical-agents.

The demonstration that the mutagenicity of various physical- and chemical-agents can be quantified in the CHO/HGPRT system suggests that it should be possible to employ this system to study the interactive effects between these agents. We have shown that the near ultraviolet light (UVB) up to 300 J/m^2, and 8-methyoxypsoralen up to 20 μg/ml is neither cytotoxic nor mutagenic to the cells. However, the "dose"-dependent cytotoxicity and mutagenicity of each agent when the other is kept at a constant level has been shown recently

(Schenley and Hsie, unpublished). Similarly, the mutagenicity of B(a)P in the presence of UVB can be demonstrated independent of S_9-activation, as shown earlier by others in experiments similar to ours (Barnhart et al, 1978). It appears that the system is useful to study the photosensitizing as well as photoprotective agents. Similar experiments should be extended to interaction between two physical agents, two chemicals, and 1 physical- and 1 chemical-agent.

Applications to screenings for organic mixtures and environmental mutagens.

We have found that the CHO/HGPRT system can determine cytotoxicity and mutagenicity of a crude organic mixture, in this case, the acetone effluent which contains identifiable heterocyclic nitrogen compounds derived from the basic fraction of a liquified coal sample, is mutagenic with S_9-activation (Hsie et al, 1979). Du Pont Company has found it useful not only to screen for chemicals in the solid form, but also in gaseous state such as vinyl chloride (Barskey et al, 1979; Krahn, this conference). Other institutions such as Allied Chemical Corp., Carneige-Mellon Institute of Research, and Chemical Industry Institute of Toxicology have set up CHO/HGPRT as an integral part of their toxicological research, development and screening program.

A multiplex CHO genetic toxicology system: Simultaneous determination of cytotoxicity, mutation induction, chromosome aberration and sister chromatid exchange in mammalian cells.

We have so far shown that CHO cells are useful for studying the cytotoxicity and mutagenicity of ionizing- and non-ionizing agents and various classes of chemicals. The CHO cells and other hamster cells in culture were also found to be suitable for studying carcinogen-induced chromosome and chromatid aberrations and sister-chromatid exchanges (SCE).

We have studied the interrelationships among four distinct biological effects - cytotoxicity, mutation induction at the HGPRT locus, chromosome aberration and SCE - in CHO cells, utilizing carcinogenic/noncarcinogenic pairs of chemicals. These include the direct acting carcinogen, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and its noncarcinogenic analogue, N-methyl-N'-nitroguanidine (MNG), as well as the procarcinogens, benzo(a)pyrene [B(a)P], and dimethylnitrosamine (DMN), and their analogues, pyrene and dimethylamine (DMA), respectively. The procarcinogens were studied utilizing a rat liver S_9 protein system to mediate metabolic activation. The determination of SCE frequencies were performed using the BUdR-Hoechst-Giemsa method with a BUdR to thymidine ratio which gives a minimal spontaneous incidence. The carcinogenic agents showed induction of mutation, chromosome aberrations and SCEs, while the noncarcinogenic agents showed little activity. Cytotoxicity does not appear to correlate with activity with any of the pairs. On an equimolar basis, pyrene shows toxicity similar to B(a)P, DMA is less toxic than DMN, and MNG shows minimum toxicity. The frequency of SCEs can be quantified over a lower concentration range than is necessary for the determination of chromosome aberrations and mutation induction.

The successful development and validation of the multiplex CHO cell genetic toxicity system will be extremely valuable because this system will allow the simultaneous determination of four distinct biological effects. Recently, we have found that the CHO/HGPRT system is also useful for determination of mutagen-induced micronuclei formation, the so-called micronucleus test (Schmid, 1975) and endoduplication of chromosome (San Sebastian and Hsie, unpublished) and the HeLa DNA synthesis inhibition test (Painter, 1977) can be adapated to CHO cells in a preliminary study (Painter, personal communication). Thus, if fully developed and validated, the system can determine these two additional

endpoints of genetic toxicity.

From quantitative mutagenesis to mutagen screening.

The CHO/HGPRT system has been developed and defined for quantifying mutation induced by various physical- and chemical-agents at the HGPRT locus in CHO cells. Various genetic, biochemical and physiological evidence support the genetic basis of mutation induction in this system. Quantitative mutagenesis of direct-acting mutagens can be exemplified by the use of various alkylating agents, heterocyclic nitrogen mustards and platinum compounds. Quantification of mutagenicity of procarcinogens through the use of S_9-coupled assay is complicated by the difference of optimum activation condition as exhibited between B(a)P and DMN. The system can be expanded to determine mutagen-induced chromosome aberration, sister chromatid exchange, micronucleus formation and inhibition of DNA synthesis in addition to gene mutation and cytotoxicity. Although application to mutagen screening appears to be feasible, we have been engaged in the development and utilization of an "universal" metabolic activation system suitable for all major classes of industrial and environmental chemicals, a sensitive and reliable statistical method well-defined in consideration of specific intrinsic characteristics of the CHO/HGPRT protocol to clearly delineate mutagenicity vs non-mutagenicity, and an experimental design to determine compounds with low yet detectable mutagenicity.

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