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# The Use of Adult Rat Liver Cultures in the Detection of the Genotoxicity of Various Polycyclic Aromatic Hydrocarbons

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The hepatocyte primary culture (HPC)–DNA repair test and the adult rat liver epithelial cell (ARL)–hypoxanthine-guanine phosphoribosyl transferase (HGPRT) mutagenesis assay are two in vitro short-term tests that possess intrinsic capability for xenobiotic biotransformation. Both assays detected the genotoxicity of a variety of carcinogenic polycyclic aromatic hydrocarbons. Thus, these two tests, which embody intact cellular metabolism, are useful for the evaluation of this class of carcinogens and provide results that strengthen those obtained in tests dependent upon subcellular metabolism.

**Key words: hepatocyte, polycyclic aromatic hydrocarbons, DNA repair, HGPRT mutagenesis**

## INTRODUCTION

Major advances have been made in the development of in vitro short-term tests for the detection of genotoxic effects of chemicals. However, no single test system has yet demonstrated the capacity to detect all chemicals capable of producing the biological effects which may result from genotoxicity. Thus, it has been recognized that a battery of short-term tests is essential for the testing of chemicals [Bridges, 1976; de Serres, 1979; Flamm, 1974; Weisburger and Williams, 1978]. Two elements that determine the usefulness of any short-term test in a battery [Williams, 1980] are: 1) the metabolic parameters of the test, and 2) the nature of the end point. The metabolic parameters should either extend the capability of the battery for detection of activation-dependent genotoxic chemicals or should provide metabolism representative of the in vivo situation. The end point should be reliable, of clear biologic significance, and different from that of other tests in the battery.

Among screening tests, the Ames Salmonella–microsome bacterial mutagenesis test [Ames et al, 1973], because of its sensitivity and extensive data base, must be part of a screening battery. Since this test is dependent upon mammalian subcellular enzyme preparations for xenobiotic biotransformation, the additional test systems to be included in a battery should provide metabolic parameters that extend those obtained in this test.

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Additionally, proposed tests should also provide end points that supplement the information provided by bacterial mutagenesis.

Two in vitro short-term tests developed in this laboratory are the hepatocyte primary culture (HPC)–DNA repair test [Williams, 1976, 1977] and the adult rat liver epithelial cell (ARL)–hypoxanthine-guanine phosphoribosyl transferase (HGPRT) mutagenesis assay [Tong and Williams, 1978, 1980]. In both tests, metabolism is performed by intact liver cells, either freshly isolated hepatocytes in primary culture or long-term lines of epithelial cells, respectively, without reliance on added enzyme preparations. The end point of the HPC–DNA repair test is autoradiographic unscheduled DNA synthesis, a specific response to DNA damage. The ARL–HGPRT mutagenesis assay measures the production of HGPRT-deficient mutants recovered by their resistance to a toxic purine analog such as 6-thioguanine. Results on a wide variety of compounds in these tests have been reported [Tong et al, 1980; Williams, 1980]. In this report, we describe the application of these tests to detection of polycyclic aromatic hydrocarbons (PAHs), a class of compounds requiring metabolic activation and whose effects in liver-derived systems is of particular interest because of their lack of significant carcinogenicity for the liver under most conditions.

## MATERIALS AND METHODS

### Protocol for the Hepatocyte Primary Culture–DNA Repair Test

**Chemicals.** Benz(a)anthracene and benzo(a)pyrene were obtained from Sigma (St. Louis, Mo). Pyrene was obtained from Pfaltz and Bauer (Stamford, Conn.). 7,12-Dimethylbenz(a)anthracene and 3-methylcholanthrene were obtained from Eastman Organics (Rochester, NY). Benzo(e)pyrene was obtained from the NCI Chemical Repository. Chrysene, 5-methylchrysene, 1,4-dimethylphenanthrene, and 1-methylphenanthrene were kindly furnished by Dr. S. Amine, Dr. E. LaVoie, and Dr. D. Hoffmann of this Institute.

**Initiation of hepatocyte primary cultures.** Rat hepatocyte primary cultures (HPCs) are initiated according to the methods of Williams et al [1977], wherein the livers of adult rats are dissociated following collagenase perfusion. This perfusion technique regularly yields 90% viable suspensions of $200 \times 10^6$ cells per 100 g of body weight, making it suitable for HPC–DNA repair studies.

**Hepatocyte primary culture–DNA repair assay.** The HPC–DNA repair test is performed according to a defined protocol [Williams, 1978, 1980]. Two hours after inoculation of freshly prepared hepatocytes obtained by the perfusion previously described, HPCs are washed with 2 ml of Williams Medium E (WME). Triplicate coverslips of HPCs are then exposed to the test compound and 10 $\mu$Ci/ml of tritiated thymidine ([$^3$H]Tdr). Tritiated thymidine–WME solution is prepared by diluting 1 mCi/ml of tritiated methyl thymidine to 40–60 $\mu$Ci/mM in sterile aqueous solution (NEN) in WME. After 18–20 hr of exposure to the test compound and [$^3$H]TdR–WME, the cultures are washed and the nuclei swelled in Na citrate for 10–15 min and fixed in three 30-min changes of ethanol–glacial acetic acid (3:1), air-dried, and mounted cell surface up on glass slides with Permount (Fisher). Mounted coverslips are allowed 2 days to dry. Autoradiographs are prepared by dipping slides in NTB-2 emulsion (Eastman Kodak), dried from 1 to 4 hr, and stored for 6 days in the cold. They are developed in D19 (Eastman Kodak). Results are quantified by determining the net increase in grains per nucleus induced by a chemical carcinogen. Grain counts are performed on an Artek Model 880 electronic counter with microscopic attachment.

Net nuclear grain counts of repair synthesis are calculated by subtracting the cytoplasmic area count from the nuclear area count.

### Protocol for the Adult Rat Liver Epithelial Cell—Hypoxanthine-Guanine Phosphoribosyl Transferase Mutagenesis Assay

The ARL–HGPRT mutagenesis assay is conducted according to procedures previously described [Tong and Williams, 1980].

**Cell lines.** Rat liver epithelial cell lines [Williams, 1976a; Williams and Gunn, 1974] are initiated from the hepatocyte primary cultures described earlier. To minimize possible variations in responses to mutagens by an individual cell line due to aging in culture, they were generally frozen down in liquid nitrogen when they were not being used. They were routinely revived a few days before the start of any experiments and discarded at the completion. Line ARL 18 is frozen down around the 25th passage.

**Exposure conditions.** Cells in log phase at a density between $0.25 \times 10^5$ and $0.5 \times 10^5/cm^2$ are the usual population at the time of exposure. In any one experiment, the various experimental or control flasks have the same number of cells seeded at the same time prior to mutagen exposure. For mutagens–carcinogens exposure, cells are washed twice with WME and then exposed to different concentrations for 72 hr at 37°C in WME supplemented with 10% calf serum (WMES). Control cultures are given WMES alone or with 0.1% dimethyl sulfoxide (DMSO). After exposure, the cells are then washed twice with WME and refed with WMES.

**Mutant expression period.** Studies on the mutant expression time of ARL 18 (unpublished observations) indicated that the induced mutant incidences stabilized around 16 to 17 days postmutagen exposure and remained at that level for the additional 14 days the cultures were monitored. Studies in this line as well as in other ARL lines [Tong and Williams, 1978, 1980 and unpublished observations] indicated that the induced mutant expression time is characteristic of the individual lines and is independent of the type of mutagens or concentrations. Therefore, for ARL 18, following exposure, the exposed population and the controls are maintained with intermittent subculturing for a minimum of 18 days for mutant expression before they are seeded for 6-thioguanine-resistant ($TG^r$) mutant selection. During the mutant expression period, the cells are maintained in Falcon or Corning T-75 cell culture flasks and replated whenever they reach 75% confluency. For the control population at a seeding density of $2 \times 10^4$ cells/cm$^2$, that would be about every 4 days.

**Mutant selection.** Reconstruction experiments had indicated that there is a slight metabolic cooperation effect when the population is selected at $10^4$ cells/cm$^2$ [Tong and Williams, 1978]. However, the consistency of results obtained between selection flasks seeded at this density prompted us to adopt this density. In addition, at this seeding density, the number of selection flasks required is much reduced and the experiments are rendered more manageable. For mutant selection, cells are seeded at $10^4$ cells/cm$^2$ in WMES in 25-cm$^2$ cell culture flasks. Twenty-four hours later, WMES containing 20 $\mu$g/ml of 6-thioguanine (TG) (Sigma Chemical Co) is applied and refed every 4 days thereafter. TG is dissolved in WME with the use of an ultrasonic vibrator and stock solutions of serum-free medium containing TG are stored under refrigeration for no longer than 2 weeks. Calf serum is added to the medium containing TG only on the day of feeding. After 14 days, colonies are fixed with Formalin and stained with Giemsa for counting.

The colony-forming efficiency of these cultures in nonselective media is determined

by seeding 20 cells/cm$^2$ in WMES in 25-cm$^2$ cell culture flasks. At the end of 7–9 days, colonies are fixed and stained as above.

## RESULTS

### Hepatocyte Primary Culture–DNA Repair Test

Selected carcinogenic and noncarcinogenic analogs of polycyclic aromatic hydrocarbons were examined for genotoxicity in the HPC–DNA repair test using rat hepatocytes. Dose-dependent increases in DNA repair synthesis were observed in hepatocytes after exposure to the carcinogenic PAHs (Table I and Fig. 1). The noncarcinogenic analogs pyrene,

**TABLE I. Survey of Polycyclic Aromatic Hydrocarbons in the HPC–DNA Repair Test**

| Compound | Carcinogenicity | DNA repair | |
|---|---|---|---|
| | | Dose (M) | Grains/nucleus[a] |
| 5-Methylchrysene | + | $10^{-3}$ | Toxic |
| | | $5 \times 10^{-4}$ | 15.9 ± 4.7 |
| | | $10^{-4}$ | 11.9 ± 1.2 |
| | | $5 \times 10^{-5}$ | 4.1 |
| Chrysene | – | $10^{-4}$ | 0.1 ± 0.1 |
| | | $5 \times 10^{-5}$ | 0.4 ± 0.4 |
| | | $10^{-5}$ | 0.1 ± 0.2 |
| 1,4-Dimethylphenanthrene | + | $10^{-4}$ | 55.5 ± 3.1 |
| | | $5 \times 10^{-5}$ | 46.1 ± 23.4 |
| | | $10^{-5}$ | 5.5 ± 4.8 |
| 1-Methylphenanthrene | – | $5 \times 10^{-4}$ | Toxic |
| | | $10^{-4}$ | 4.8 ± 0.6 |
| | | $5 \times 10^{-5}$ | 2.8 ± 0.8 |
| | | $10^{-5}$ | 0 |
| 3-Methylcholanthrene | + | $10^{-3}$ | 77.8 ± 12.5 |
| | | $5 \times 10^{-4}$ | 69.4 ± 15.2 |
| | | $10^{-4}$ | 58.6 ± 9.2 |
| 7,12-Dimethylbenz(a)anthracene | + | $10^{-3}$ | Toxic |
| | | $5 \times 10^{-4}$ | 58.7 ± 10.1 |
| | | $10^{-4}$ | 38.9 ± 4.9 |
| | | $5 \times 10^{-5}$ | 22.0 ± 2.1 |
| Benz(a)anthracene | + | $10^{-3}$ | Toxic |
| | | $5 \times 10^{-4}$ | 17.2 ± 6.0 |
| | | $10^{-4}$ | 14.8 ± 2.6 |
| | | $5 \times 10^{-5}$ | 0.6 |
| Anthracene | – | $10^{-3}$ | 1.9 ± 1.4 |
| | | $5 \times 10^{-4}$ | 1.5 ± 0.6 |
| | | $10^{-4}$ | 0.3 ± 0.2 |
| Benzo(a)pyrene | + | $10^{-3}$ | 65.6 ± 17.8 |
| | | $5 \times 10^{-4}$ | 47.7 ± 3.7 |
| | | $10^{-4}$ | 45.1 ± 3.7 |
| Benzo(e)pyrene | – | $10^{-3}$ | 1.1 ± 0.4 |
| | | $5 \times 10^{-4}$ | 0.5 ± 0.5 |
| | | $10^{-4}$ | 1.2 ± 0.9 |
| Pyrene | – | $5 \times 10^{-4}$ | Toxic |
| | | $10^{-4}$ | 1.4 ± 0.5 |
| | | $5 \times 10^{-5}$ | 0.6 ± 0.5 |
| | | $10^{-5}$ | 0.5 ± 0.5 |
| 0.1% DMSO control | | | 0.1 ± 0.1 |

[a]Mean ± standard deviation of triplicate coverslips exposed to the test agent plus [$^3$H]TdR for 20 hr.

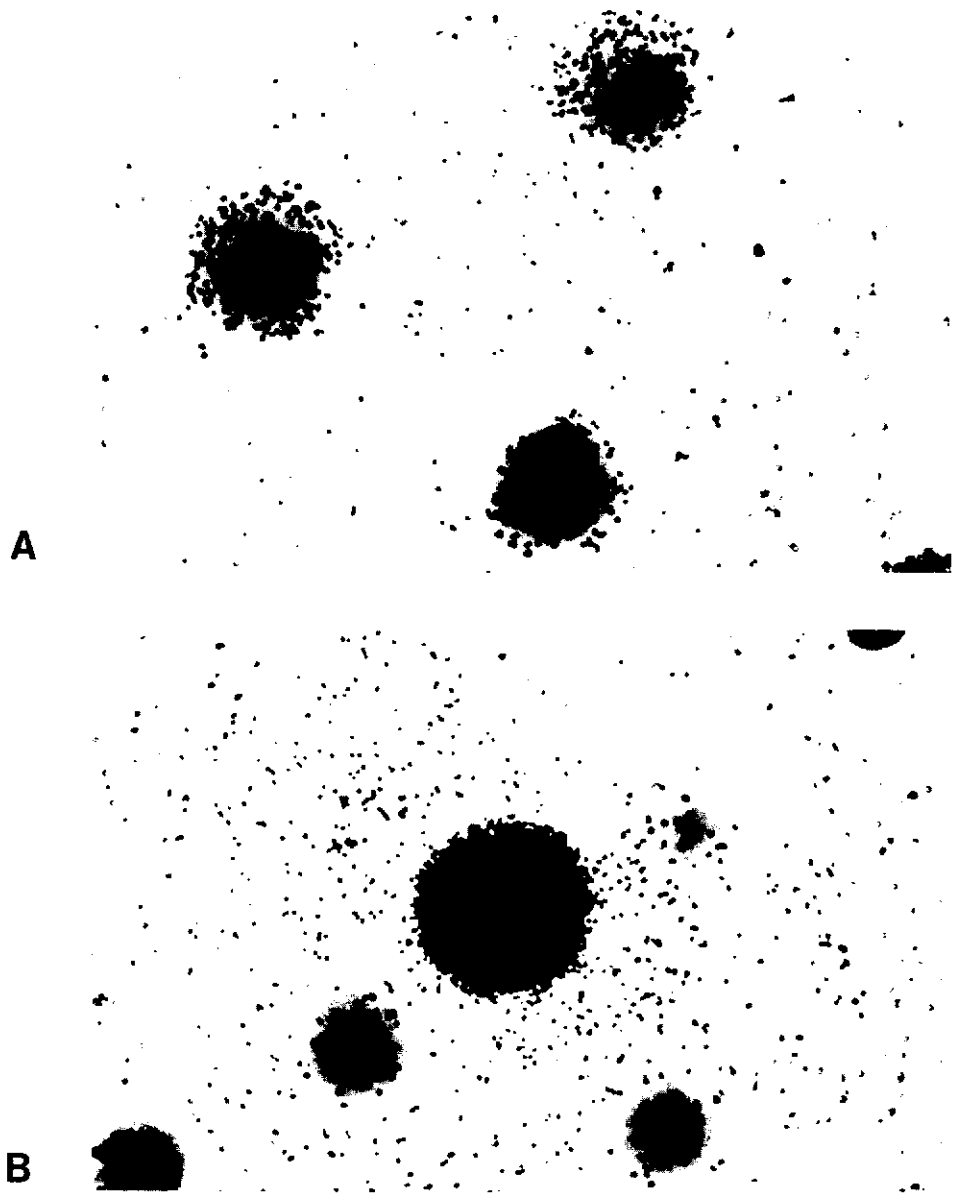


Fig. 1. Autoradiographs of HPCs prepared and exposed as described in the text. The nuclei are stained with hematoxylin (1452×). (A) HPCs exposed to benzo(a)pyrene at $10^{-4}$ M showing nuclei engaging in DNA repair synthesis. (B) Untreated control HPCs showing a heavily labeled nucleus in S-phase with several cells in resting phase. The incidence of S-phase cells in HPCs is less than 0.1%.

benzo(e)pyrene, chrysene, 1-methylphenanthrene, and anthracene were consistently negative in this assay. Benz(a)anthracene, which is a weak initiating agent for mouse skin [IARC, 1973], was also positive in this assay.

### ARL–HGPRT Mutagenesis Assays

The characterization of purine analog-resistant mutants isolated from line ARL 6 has previously been reported [Tong and Williams, 1978, 1980; Williams et al, 1978b]. Line ARL 18 has also been shown in preliminary studies to be a suitable target cell line for the ARL–HGPRT assay exhibiting sensitivity to a wide range of compounds including the PAHs [Tong and Williams, 1979; Tong et al, 1980]. A stable spontaneous $TG^r$ mutant incidence in ARL 18 was observed starting at 10 $\mu$g of TG/ml (Fig. 2). $TG^r$ mutants isolated and maintained in nonselective medium for a minimum of 12 weeks were examined for the stability of their phenotype. As shown in Tables II and III, the mutants were phenotypically stable, displayed the appropriate sensitivity in HAT medium, and had only a residual HGPRTase activity.

Exposure of ARL 18 to benzo(a)pyrene (B(a)P) resulted in a significant increase in the $TG^r$ mutant incidence (Table IV). Exposure to identical concentrations of benzo(e)-pyrene and pyrene, however, did not result in any significant increase in the $TG^r$ mutant incidence above the parallel run nonexposed control. Exposure of ARL 18 to $10^{-5}$ M B(a)P was repeated in six separate assays; each assay consistently resulted in a significant increase in $TG^r$ mutant incidence over parallel run control.

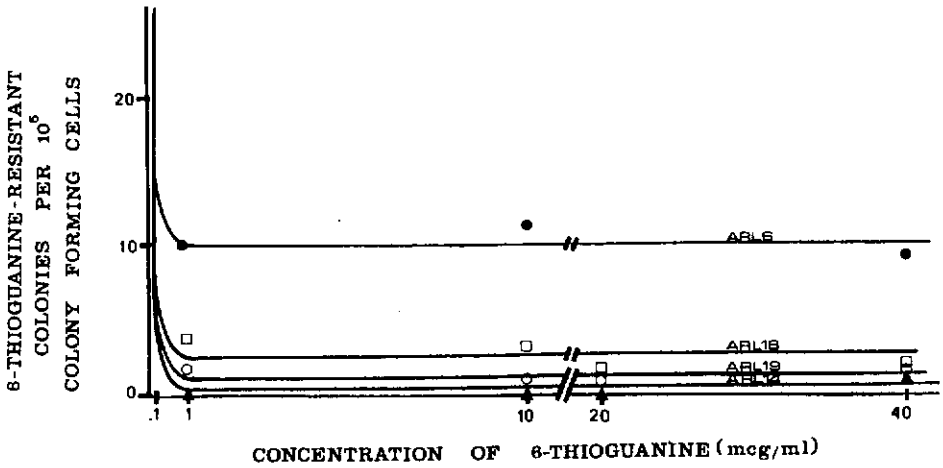


Fig. 2. Effect of the concentration of 6-thioguanine on the recovery of spontaneous 6-thioguanine-resistant mutants.

**TABLE II. Growth Ability in Selective Media of an Isolated Mutant Clone After a 12-Week Maintenance in Nonselective Media**

| Line | Colony-forming efficiency (%)[a] | | | |
|---|---|---|---|---|
| | WMES | +TG | +AG | +HAT |
| ARL 18 parental | 30.2 | 0 | 0 | 28.5 |
| ARL 18-TG$^r$-clone 1 | 18.5 | 19.1 | 17.9 | 0 |
| ARL 18-TG$^r$-clone 2 | 25.5 | 24.3 | 26.1 | 0 |

[a]Seeding density for colony-forming efficiency (CFE) was 20 cells/cm$^2$ or 500 cells/25-cm$^2$ flasks in Williams Medium E supplemented with 10% calf serum (WMES). Twenty-four hours later, the media were changed to WMES, WMES + 60 μg/ml of AG, WMES + 20 μg/ml of TG, and WMES + $10^{-4}$ M hypoxanthine, $10^{-4}$ M aminopterin, $10^{-5}$ M thymidine. The media were changed every 2 days, and the colonies that developed by 12–14 days were fixed with Formalin and stained with Giemsa for counting.

**TABLE III. Hypoxanthine-guanine Phosphoribosyl Transferase Activity in Normal and Resistant Clones**

| Line[a] | Hypoxanthine phosphoribosylated[b] (%) | Normal (%) |
|---|---|---|
| ARL 18 parental | 27 | 100 |
| ARL 18 TG$^r$-clone 1 | 3 | 11 |
| ARL 18 TG$^r$-clone 2 | 1 | 4 |

[a]TG$^r$ stands for 6-thioguanine-resistant clones.
[b]The 800 cell sonicates were incubated with [$^3$H]hypoxanthine for 3 hr, and the portion converted to inosine and inosine monophosphate was determined by paper chromatography.

**TABLE IV. ARL 18 6-Thioguanine-Resistant Mutagenesis**

| Exposure | | CFE (%) | TG$^r$ mutants | |
|---|---|---|---|---|
| | | | Per flask | Per $10^6$ CFC |
| Benzo(a)pyrene | $10^{-4}$ M | 21.0 | 32.0 ± 4.7 | 610 ± 90 |
| | $10^{-5}$ M | 28.2 | 23.5 ± 2.0 | 335 ± 28 |
| Benzo(e)pyrene | $10^{-4}$ M | 23.3 | 0.5 ± 0.8 | 9 ± 14 |
| | $10^{-5}$ M | 26.6 | 0 | 0 |
| Pyrene | $10^{-4}$ M | 25.8 | 0.3 ± 0.5 | 5 ± 8 |
| | $10^{-5}$ M | 31.6 | 0.2 ± 0.4 | 2 ± 5 |
| 7,12-Dimethylbenz(a)anthracene | $10^{-4}$ M | 21.7 | 32.4 ± 4.5 | 597 ± 58 |
| | $10^{-5}$ M | 34.8 | 44.7 ± 5.0 | 513 ± 58 |
| | $10^{-6}$ M | 33.6 | 10.8 ± 3.3 | 129 ± 39 |
| Benz(a)anthracene | $10^{-4}$ M | 24.4 | 0.7 ± 0.8 | 11 ± 13 |
| | $10^{-5}$ M | 22.1 | 0.3 ± 0.5 | 6 ± 9 |
| DMSO control | 0.1% | 30.9 | 0.3 ± 0.8 | 4 ± 11 |
| | 0.1% | 35.0 | 0.5 ± 0.6 | 6 ± 6 |

**TABLE V. Comparison of PAH Results Obtained in Rat Liver Culture Systems With In Vivo Carcinogenicity and Salmonella Mutagenicity**

| Compound | Rat liver culture systems | | Carcinogenicity | Salmonella–microsome mutagenesis |
|---|---|---|---|---|
| | HPC–DNA repair | ARL–HGPRT mutagenesis | | |
| 5-Methylchrysene | + | NT | + | +[b] |
| Chrysene | – | NT | – | +[b] |
| 1,4-Dimethylphenanthrene | + | NT | + | +[b] |
| 1-Methylphenanthrene | – | NT | – | +[b] |
| 3-Methylcholanthrene | + | NT | + | +[a] |
| 7,12-Dimethylbenzanthracene | + | + | + | +[a] |
| Benz(a)anthracene | + | – | + | +[a] |
| Anthracene | – | NT | – | –[b] |
| Benzo(a)pyrene | + | + | + | +[a,b] |
| Benzo(e)pyrene | – | – | – | +[a,b] |
| Pyrene | – | – | – | – |

[a]Hollstein et al [1979].
[b]LaVoie et al [1979] and personal communication.

Exposure of ARL 18 to another polycyclic aromatic hydrocarbon 7,12-dimethylbenz(a)anthracene (DMBA) also resulted in significant increase in $TG^r$ mutant incidence (Table IV). The weakly carcinogenic analog benz(a)anthracene, however, was not mutagenic in ARL 18 in two of the three times it was assayed. Exposure of ARL 18 to $10^{-5}$ M DMBA was repeated in three separate assays; each assay consistently resulted in a significant increase in the $TG^r$ mutant incidence over the parallel run control.

A summary of the results of the two tests and comparison with reported carcinogenicity and bacterial mutagenicity is presented in Table V.

## DISCUSSION

Cultured mammalian cells have been used extensively for the study of the genotoxicity of polycyclic aromatic hydrocarbons [Clive et al, 1979; Hsie et al, 1979; Kuroki et al, 1979]. Of the studies reported, however, none of the target cell lines employed, with the exception of embryonic lines [Barrett et al, 1978; Langenbach et al, 1978], have demonstrated a consistent and significant capacity to biotransform PAHs to their genotoxic metabolites. This deficiency is compensated for by the addition of subcellular enzyme preparations [Clive et al, 1979; Hsie et al, 1979; Krahn and Heidelberger, 1977], as in bacterial mutagenesis assays [Ames et al, 1973; Nagao et al, 1978]. As such, no metabolic parameter in addition to that which is part of the bacterial tests is provided by these mammalian systems. This is particularly noteworthy because the use of enzyme preparations for the activation of chemicals, such as N-substituted aryl compounds [Takeishi et al, 1979; Williams, 1981], nitrosamines [Rao et al, 1979], and polycyclic aromatic hydrocarbons [LaVoie, 1979] to mutagenic metabolites have yielded results that do not correlate with the in vivo carcinogenicity of the compounds. These discrepancies appear to be due to an imbalanced metabolism performed by subcellular fractions [Billings et al, 1977; Selkirk, 1977; Schmeltz et al, 1978; Casciano, 1979]. To provide a more realistic assessment of potential genotoxicity, we have examined the responsiveness of adult liver cell

systems to several important classes of environmental carcinogens.

In the present study, the HPC–DNA repair test consistently demonstrated the genotoxicity of carcinogenic representatives of polycyclic aromatic hydrocarbons while the ARL–HGPRT mutagenesis assay responded to all tested carcinogens except benz(a)anthracene. The specificity of the two assays is also well demonstrated by the inactivity of the noncarcinogens benzo(e)pyrene and pyrene.

The specificity of the HPC–DNA repair test was further demonstrated by the inactivity of the noncarcinogens anthracene, chrysene, and 1-methylphenanthrene, the latter two of which, although not carcinogenic, have been positive for bacterial mutagenicity [LaVoie et al, 1979]. The failure of the weak carcinogen benz(a)anthracene [IARC, 1973] to elicit a positive response in the ARL–HGPRT system is under further study. Benz(a)anthracene gave a dose-dependent positive response in the HPC–DNA repair test in this series of testings, although it was previously reported not to elicit a positive response [Williams, 1977]. These conflicting results, which may be a reflection of the weak carcinogenicity of benz(a)anthracene, are being further examined using cesium chloride density gradient centrifugation analysis which confirmed the genotoxicity of N-4-fluorenylacetamide [McQueen and Williams, 1981], another chemical that gave mixed results in the HPC–DNA repair test [Williams, 1977].

Thus, both hepatocyte cultures and established liver epithelial lines were found to convert members of the PAHs into their genotoxic metabolites. This supports the previous demonstrations that HPCs can mediate mutagenesis at the HGPRT locus in an established rat liver epithelial line [San and Williams, 1977] and recently, in a human cell line [Tong et al, 1981]. These findings, as well as others [Bermudez et al, 1980; Green et al, 1977; Probst et al, 1980] differ from the observations of Langenbach et al [1978] who reported that rat hepatocytes did not activate benzo(a)pyrene to a mutagenic metabolite and suggested that this reflected the tissue specificity for the action of PAH. Rather, our findings correlate with metabolism studies [Schmeltz et al, 1978] and clearly indicate that rat liver cell cultures do retain their intrinsic metabolic capability for biotransformation of PAH.

In conclusion, the HPC–DNA repair test and the ARL–HGPRT assay which embody intact cellular metabolism provide sensitive assays for the detection of activation-dependent genotoxic chemicals. Thus, these two assays greatly strengthen results obtained in a test dependent upon subcellular metabolism.

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## REFERENCES

Ames BN, Durston WE, Yamasaki E, Lee FD (1973): Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proc Natl Acad Sci USA 70:2281–2285.

Barrett JC, Bias NE, Ts'o POP (1978): A mammalian cellular system for the concomitant study of neoplastic transformation and somatic mutation. Mutat Res 5:121–136.

Bermudez E, Tillery D, Couch DB, Butterworth BE (1980): Use of primary rat hepatocytes (PRH) to achieve metabolic activation of promutagens in the Chinese hamster ovary/hypoxanthine guanine phosphoribosyl transferase (CHO/HGPRT) mutagenesis assay. Proc Am Assoc Cancer Res 21:39.

Billings RE, McMahon RE, Ashmore J, Wagle SR (1977): The metabolism of drugs in isolated rat hepatocytes, a comparison with in vivo drug metabolism and drug metabolism in subcellular liver fractions. Drug Met Dispos 5:518–526.

Bridges BA (1976): Use of three-tier protocol for evaluation of long-term toxic hazards, particularly mutagenicity and carcinogenicity. In Montesano R, Bartsch H, Tomatis L (eds): "Screening Tests in Chemical Carcinogenesis." IARC Scientific Publication No. 12. IARC Lyon, France, pp 529–559.

Casciano DA (1979): Use of isolated rodent hepatocytes to evaluate potential premutagens and procarcinogens. In Hsie AW, O'Neill IP, McElheny VK (eds): "Banbury Report 2, Mammalian Cell Mutagenesis – The Maturation of Test Systems." New York: Cold Spring Harbor Laboratory, pp 125–132.

Clive D, Johnson KO, Spector JFS, Batson AG, Brown MMM (1979): Validation and characterization of the L5178Y/TK$^{+/-}$ mouse lymphoma mutagen assay system. Mutat Res 59:61–108.

DeSerres FJ (1979): Problems associated with the application of short-term tests for mutagenicity in mass-screening programs. Environ Mutagenesis 1:203–208.

Flamm WG (1974): A tier system approach to mutagen testing. Mutat Res 26:329–333.

Green MHL, Bridges BA, Rogers AM, Horspool G, Muriel WJ, Bridges JW, Fry JR (1977): Mutagen screening by a simplified bacterial fluctuation test: Use of microsomal preparations and whole liver cells from metabolic activation. Mutat Res 48:287–294.

Hollstein M, McCann J, Angelosanto FA, Nichols WW (1979): Short-term tests for carcinogens and mutagens. Mutat Res 65:133–226.

Hsie AW, Couch JP, O'Neill JR, San Sabastian PA, Brimer R, Machanoff JC, Li AP, Fusco JC, Forbes N, Hsie MH (1979a): Utilization of a quantitative mammalian cell mutation system, CHO/HGPRT in experimental mutagenesis and genetic toxicology. In Butterworth B (ed): "Critical Reviews in Toxicology, Strategies for Short-Term Testing for Mutagens/Carcinogens." Florida: CRC Press, pp 39–54.

IARC Monographs on the evaluation of carcinogenic risk of the chemical to man (1973): Certain polycyclic aromatic hydrocarbons and heterocyclic compounds (IARC, Lyon) vol 3, pp 45–68.

Krahn DF, Heidelberger C (1977): Liver homogenate-mediated mutagenesis in Chinese hamster V79 cells by polycyclic aromatic hydrocarbons and aflatoxins. Mutat Res 46:27–44.

Kuroki T, Malaveille C, Drevon C, Piccoli C, Macleud M, Selkirk JK (1979): Critical importance of microsome concentration in mutagenesis assay with V79 Chinese hamster cells. Mutat Res 63:259–272.

Langenbach R, Freed HF, Raveh D, Huberman E (1978): Cell specificity in metabolic activation of aflatoxin $B_1$ and benzo(a)pyrene to mutagens for mammalian cells. Nature (London) 276:277–280.

LaVoie E, Bedenko V, Hirota N, Hecht SS, Hoffmann D (1979): A comparison of the mutagenicity, tumor-initiating activity and complete carcinogenicity of polynuclear aromatic hydrocarbons. In Jones PW, Leber P (eds): "Polynuclear Aromatic Hydrocarbons." Michigan: Ann Arbor Sciences Publishers, Inc., pp 705–721.

McQueen CA, Williams GM (1981): Characterization of DNA repair elicited by carcinogens and drugs in the hepatocyte primary culture/DNA test. J Toxicol Environ Health (in press).

Nagao M, Sugimura T, Matsushima T (1978): Environmental mutagens and carcinogens. Ann Rev Genet 12:117–159.

Probst GS, Hill LE, Bewsey BJ (1980): Comparison of three in vitro assays for carcinogen-induced DNA damage. J Toxicol Environ Health 6:333–349.

Rao TK, Young JA, Lijinsky W, Epler JL (1979): Mutagenicity of aliphatic nitrosamines in Salmonella typhimurium. Mutat Res 66:1–7.

San RH, Williams GM (1977): Rat hepatocyte primary cell culture mediated mutagenesis of adult rat liver epithelial cells by procarcinogens. Proc Soc Exp Biol Med 156:534–538.

Schmeltz I, Tosk J, Williams GM (1978): Comparison of the metabolic profiles of benzo(a)pyrene obtained from primary cell cultures and subcellular fractions derived from normal and methylcholanthrene-induced rat liver. Cancer Lett 5:81–89.

Selkirk JK (1977): Divergence of metabolic activation systems for short-term mutagenesis assays. Nature (London) 270–604–605.

Takeishi K, Okuno-Kaneda S, Seno T (1979): Mutagenic activity of 2-acetylaminofluorene by guinea-pig liver homogenates: Essential involvement of cytochrome P-450 mixed-function oxidases. Mutat Res 62:425–437.

Tong C, Fazio M, Telang S, Williams GM (1980): The detection of genotoxic chemicals in the adult rat liver epithelial cell/hypoxanthine-guanine phosphoribosyl transferase. (ARL/HGPRT) mutagenesis assay. Ann NY Acad Sci 349:407–408.

Tong C, Fazio M, Williams GM (1981): Rat hepatocyte-mediated mutagenesis of human fibroblast by polycyclic aromatic hydrocarbons, but not organochlorine pesticides. Proc Soc Exp Biol Med (in press).

Tong C, Williams GM (1978): Induction of purine analog-resistant mutants in adult rat liver epithelial lines by metabolic activation-dependent and -independent carcinogens. Mutat Res 58:339–352.

Tong C, Williams GM (1979): Variation in response in the adult rat liver epithelial cell/hypoxanthine-guanine phosphoribosyl transferase (ARL/HGPRT) mutation assay with different metabolic activation-dependent carcinogens. Environ Mut 1:148.

Tong C, Williams GM (1980): Definition of conditions for the detection of genotoxic chemicals in the adult rat liver epithelial cell/hypoxanthine-guanine phosphoribosyl transferase (ARL/HGPRT) mutagenesis assay. Mutat Res 74:1–9.

Weisburger JH, Williams GM (1978): Decision point approach to carcinogen testing. In Asher IM, Zervos C (eds): "Proceedings of the Second FDA Office of Science Summer Symposium-Structural Correlates of Carcinogenesis and Mutagenesis, A Guide to Testing Priorities?" Maryland: The Office of Science, FDA, pp 45–52.

Williams GM (1976): Carcinogen-induced DNA repair in primary rat liver cell cultures: A possible screen for chemical carcinogens. Cancer Lett 1:231–236.

Williams GM (1976a): Primary and long-term culture of adult rat liver epithelial cells. Methods Cell Biol 14:357–364.

Williams GM (1977): Detection of chemical carcinogens by unscheduled DNA synthesis in rat primary cell cultures. Cancer Res 37:1845–1851.

Williams GM (1978): Further improvements in the hepatocyte primary culture DNA repair test for carcinogens: Detection of carcinogenic biphenyl derivatives. Cancer Lett 4:69–75.

Williams GM (1980): The detection of chemical mutagens-carcinogens by DNA repair and mutagenesis in liver cultures. In DeSerres F, Hollaender A (eds): "Chemical Mutagens, Vol 6." New York: Plenum Press, pp 61–79.

Williams GM (1981): Mammalian culture systems for the study of genetic effects of N-substituted aryl compounds. In Horgeirsson ST, Weisburger EK (eds): NCI Monograph 58 (in press).

Williams GM, Bermudez E, Scaramuzzino D (1977): Rat hepatocyte primary cell cultures. III. Improved dissociation and attachment techniques and the enhancement of survival by culture medium. In Vitro 13:809–817.

Williams GM, Bermudez E, San RHC, Goldblatt PJ, Laspia MG (1978): Rat hepatocyte primary cultures. IV. Maintenance in defined medium and the role of production of plasminogen activator and other proteases. In Vitro 14:824–837.

Williams GM, Gunn JM (1974): Long-term cell cultures of adult rat liver epithelial cells. Exp Cell Res 89:139–142.

Williams GM, Tong C, Berman JJ (1978): Characterization of analog resistance and purine metabolism of adult rat liver epithelial cell 8-azaguanine-resistant mutants. Mutat Res 49:103–115.