NCI/EPA COLLABORATIVE PROGRAM PROJECT
FISCAL YEAR 1980

I. TITLE

Human Epithelial Cell Metabolic Activation Systems for Use with Human Cell Mutagenesis (R80556310-02)*

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II. ABSTRACT

The need to develop ever more adequate test systems which can reliably determine the possible toxic, mutagenic, teratogenic and/or carcinogenic effects of exposure of the human population to chemicals in the environment is becoming more and more evident. It is, of course, not possible to carry out such testing in humans, and therefore, information on risk estimates has to be extrapolated to man from results obtained in test systems which utilize microorganisms, mammalian cells in culture, or animals. Mammalial cells in culture have increased in importance in biological testing because they retain many of the characteristics of the target cells at risk and, yet, can be grown into very large populations and analyzed and characterized by a wide range of biochemical and genetic techniques that are not possible with whole animals. We have developed a quantitative system for measuring the cytotoxic and mutagenic effect of radiation and direct-acting chemicals in diploid human skin fibroblasts in culture and have shown that these effects are directly related to the capacity of human cells to repair damage to DNA. Since the human population is far more likely to be exposed to parent compounds than to direct-acting model compounds, we are extending the capability of the diploid human fibroblast cell mutagenesis system to include parent compounds, or mixtures of chemicals, which require metabolic activation by coupling it with cell-mediated activation. Cell lines derived from human tumors are being utilized as feeder layers to provide metabolic activation of carcinogenic agents. To find cells capable of metabolizing the various test chemicals, we prescreen our series of tumor cell lines for evidence that the chemical causes interference with DNA synthesis. (The compound under investigation is administered to the candidate metabolizing cells over a wide range of concentrations. After 48 hrs. of incubation, the amount of semiconservative DNA synthesis is measured by incorporation of radioactively-labeled thymidine and compared with that of untreated control cells.) When a cell line appears to activate a particular chemical to a form which interferes with DNA synthesis, it is then examined for ability to act as a metabolizing layer for target cells. To enhance the sensitivity, the target cells in this co-cultivation system are usually excision repair defective XP cells which are examined for evidence of cell killing (loss of colony formation). We have found that normal fibroblasts may be used as target cells once conditions are optimized. Using this prescreen, we have identified cells capable of activating a wide range of agents, including polycyclic aromatic hydrocarbons, aflatoxins, aromatic amides and amines, nitrosamines, nitrogen-containing polycyclics, etc. We are now investigating the usefulness of these cells lines: (a) to quantitate the cytotoxic effect of environmental agents by measuring cells' loss of ability to clone, (b) to quantitate the induction of mutations using several markers, and (c) using radioactive parent compounds to determine the number and kinds of adducts formed on DNA by covalent attachment of chemical residues. The activation systems developed are not limited in application to the human fibroblast mutation assay and can be expected to have more general applicability.

*Project officer, Dr. Michael D. Waters, Environmental Protection Agency.

III. INTRODUCTION

It is now realized that most chemical carcinogens require metabolic activation before they can exert their carcinogenic effect. James and Elizabeth Miller were the first workers who clearly realized the importance of metabolic activation and its near universal application to chemical carcinogens in their studies of the 1960's (1). This led to a realization that assays of the mutagenic or carcinogenic potential of compounds using bacteria, fungi, or mammalian or human cells in culture required a source of metabolic activation of the carcinogens if the cells being used were not able to metabolize the compounds themselves as was most frequently the case. This led Malling to develop the use of liver homogenates to provide the metabolic activation for such in vitro systems (2). These liver homogenates have been widely used and adapted as in the Ames test with Salmonella and may consist of microsomal preparations or of S-9 preparations of liver homogenates (3). Once the need for metabolic activation was realized, a parallel development also took place in mammalian cell culture where researchers made use of feeder layers of metabolizing cells such as primary liver hepatocytes which could metabolize many of the carcinogens that one wished to examine (4-6). In practice, the target cells which one wishes to mutagenize or transform into cancer cells are placed in contact with the feeder layer cells and carcinogen is added for 24 or 48 hours. The cells are then trypsinized and replated for determination of the mutagenic or transformation response. The studies with feeder layers have generally been qualitative. That is, the authors have shown a plus or minus response but have not usually shown a dose response or other more elaborate kinetic analysis of the interaction of the carcinogen with the cells.

The value of using activation systems derived from liver or other cell homogenates has been questioned since high concentrations of benzo(a)pyrene (BP), e.g., 1,320 nmol per mg of microsomal protein, produced extensive DNA adducts, but only a small percentage of these represented the principal cellular DNA adduct formed from BP, viz., the N_2-guanine adduct formed by the anti 7,8-diol 9,10-epoxide of BP (7). In contrast, low concentrations of BP, e.g., 15 nmol per mg of protein, produced the diol epoxide DNA adduct of BP exclusively (8). A recent report by Santella, et. al. (9) indicates that with appropriate induction of microsomal enzymes, an S-9 fraction can also be shown to catalyze the binding of BP to DNA exclusively through the diol epoxide pathway when a low substrate concentration (12.5 nmol per mg of protein) is used. The maximum extent of DNA modification in these latter studies was 8.1 per 10^9 nucleosides. Using diploid human fibroblasts which are totally lacking in excision repair of BP adducts from DNA we have determined that 250-fold greater levels of DNA binding are necessary to see significant induction of mutations to thioguanine resistance. Even higher initial levels of bound adducts are required to observe mutations in normally-excising human fibroblasts. The only practical way to obtain greater binding with S-9 fractions is to use higher concentrations of BP since the S-9 fraction itself is toxic. Unfortunately, as noted above, this higher concentration can be expected to generate the wrong adducts. Similar problems have been noted with dimethylbenz(a)anthracene (DMBA) metabolism by Bigger, et. al. (10) when the rat liver microsomes were used for carcinogen metabolism. At a high concentration of DMBA, K region DNA adducts were formed while at low concentrations, bay region adducts were formed. Most signifcantly, they found that when intact mouse cells were used for metabolism, no such qualitative differences were found in the DNA adducts formed over a 40 fold dose range. Since the K region as well as the bay region adducts of BP and DMBA are mutagenic (11), it seems likely that

many of the reports of mutagenicity of these compounds in short term tests which make use of S-9 fractions or microsomal protein for metabolic activation are the result of formation of K region adducts. Thus, it appears that the assays used have given the "correct" answers but for the wrong reasons. It should, therefore, be clear that at least for metabolism of polycyclic aromatic hydrocarbons, intact cells are to be preferred to microsomal or S-9 preparations. Detailed comparative studies of metabolism of other compounds have not yet been carried out, so the problem may well extend to other classes of compounds.

We have extensively studied the effect of active derivatives of carcinogenic agents on human fibroblasts derived from normal individuals as well as repair deficient fibroblasts derived from xeroderma pigmentosum patients (11-15). Because such fibroblasts have either no or extremely low levels of metabolic activating activity for carcinogenic agents, it is necessary to utilize direct acting carcinogens with such cells to see their effect. However, to make broader use of this assay, as well as to explore the effect of agents that require metabolic activation or of unknown chemical agents (such as in complex mixtures), we have been studying the ability of feeder layers derived from various cell types to provide metabolic activation for this cell system. Because we were working with human fibroblasts as target cells, it seemed ideal to select human cells for metabolic activation. Primary epithelial cells derived from various tissue of man would seem likely to provide the ideal system for metabolic activation. However, there are a number of problems connected with obtaining and standardizing any such epithelial cell system. First, it is difficult to obtain pure populations of epithelial cells in culture since fibroblasts tend to overgrow the epithelial cell cultures. Second, it is extremely likely that epithelial cells derived from various human donors (and even from the same donor at different times) will show different levels of metabolizing ability for a particular carcinogen. This will make it extremely difficult to standardize any assay based on the use of primary epithelial cells. Third, epithelial cells from organs such as liver which might be extremely useful for metabolizing carcinogens are not readily available for use, show extreme variability between various donors, and are not easily adapted to cell culture. In addition, working with human liver material poses the health threat of hepatitis infection. To get around all the limitations of metabolic activation systems for cells in culture discussed above, we have chosen to use tumor cell lines derived from various human tissues. These human cell lines maintain the ability to activate various carcinogens and, have an infinite lifespan in culture. These properties suggested they would be extremely useful as feeder layers.

IV. OVERALL OBJECTIVE

The objectives of the research are to develop procedures that will allow for more adequate in vitro testing of environmental chemicals and to shed light on the mechanisms involved in mutagenic processes and related events. The use of diploid human cells in culture for environmental research is very useful first of all, because of the relevance to man of results obtained with such cells and secondly, because the existence of DNA repair deficient human cells derived from xeroderma pigmentosum (XP) patients which have been characterized and shown to be unable to remove many different types of carcinogen residues covalently bound to DNA (11,12) allows one to determine the potency of various chemicals without the interference of excision processes operating during the period when the target cells are being incubated with the chemical. Furthermore, comparing the effects of various agents in excision repair-proficient and deficient human target cells allows one to study the biological effects of DNA excision repair on the mutagenic process.

V. MAJOR FINDINGS AND PROGRESS

A. Development of a Short Term Assay for Identifying Human Epithelial Cell Lines Capable of Metabolizing Parent Carcinogens into Reactive Forms:

In our initial studies of human cell-mediated metabolism, we made use of benzo(a)pyrene BP, as our model compound. One of the reasons for choosing BP is that there is a simple rapid technique for measuring its metabolism, viz., conversion of tritiated BP into the water soluble product. We used this assay to screen 17 cell lines and found several which could metabolize BP. When these were identified we assayed them for ability to serve as a metabolizing layer capable of activating not only BP but a whole series of polycyclic hydrocarbons into forms which are cytotoxic to repair deficient diploid human skin fibroblasts.

However, not all carcinogens which require metabolic activation for activity can easily be obtained in tritiated form nor is metabolism of a compound to a water-soluble form necessarily an indication of activation to a form which can bind to DNA. Therefore, we modified a DNA synthesis inhibition assay developed by Painter (17) which uses the inhibition of incorporation of ^{3}H-thymidine into DNA as an indication of metabolic activation of carcinogen and resulting DNA damage.

In this method, 5×10^{4} actively growing cells to be assayed for ability to metabolize a carcinogen are plated per 16mm well (Costar 3524 Tissue Culture Cluster) in 1 ml medium. Approximately 24 hr later, these cells are treated with carcinogen by adding 1 ml of carcinogen solution to the 1 ml of medium already present in the well. After 48 hr incubation, the treatment is removed and replaced with 1 ml of medium containing ^{3}H-Tdr (5uCi/ml). After a two hour labeling period, the medium is removed and the cells are rinsed with PBS. The cells are removed in 1 ml of trypsin-EDTA, diluted to 4 ml with phosphate buffered saline (PBS), rinsed with PBS two times on a glass membrane filter, and then rinsed two times with 10% chilled TCA. The filters are then removed, treated in 1 ml 0.5N HCl for 60 minutes at 90^{o} and counted in a liquid scintillation counter. The data are calculated and presented as percentage of the amount incorporated by the untreated control cells.

B. Metabolizing Strains Identified:

1. Interference with DNA Synthesis:

Using the DNA synthesis inhibition assay described above, many human carcinoma cell lines were examined for their ability to metabolically activate several classes of carcinogens to forms which would interact with cellular DNA and thus inhibit DNA synthesis. Two cell lines which we had already found to be capable of metabolizing benzo(a)pyrene were examined in this assay as positive controls. Figure 1 shows the results of this experiment. Both cell lines show inhibition of DNA synthesis, 703 to a greater extent than 835. This inhibition data agrees with the level of metabolism of BP to water-soluble compounds found in these cells, i.e. 703 is greater than 835. Figure 2 shows the results of exposing PC-3, 703, XP and normal human fibroblasts (NF) to dimethylnitrosamine (DMN) and diethylnitrosamine (DEN) and measuring the inhibition of thymidine incorporation.

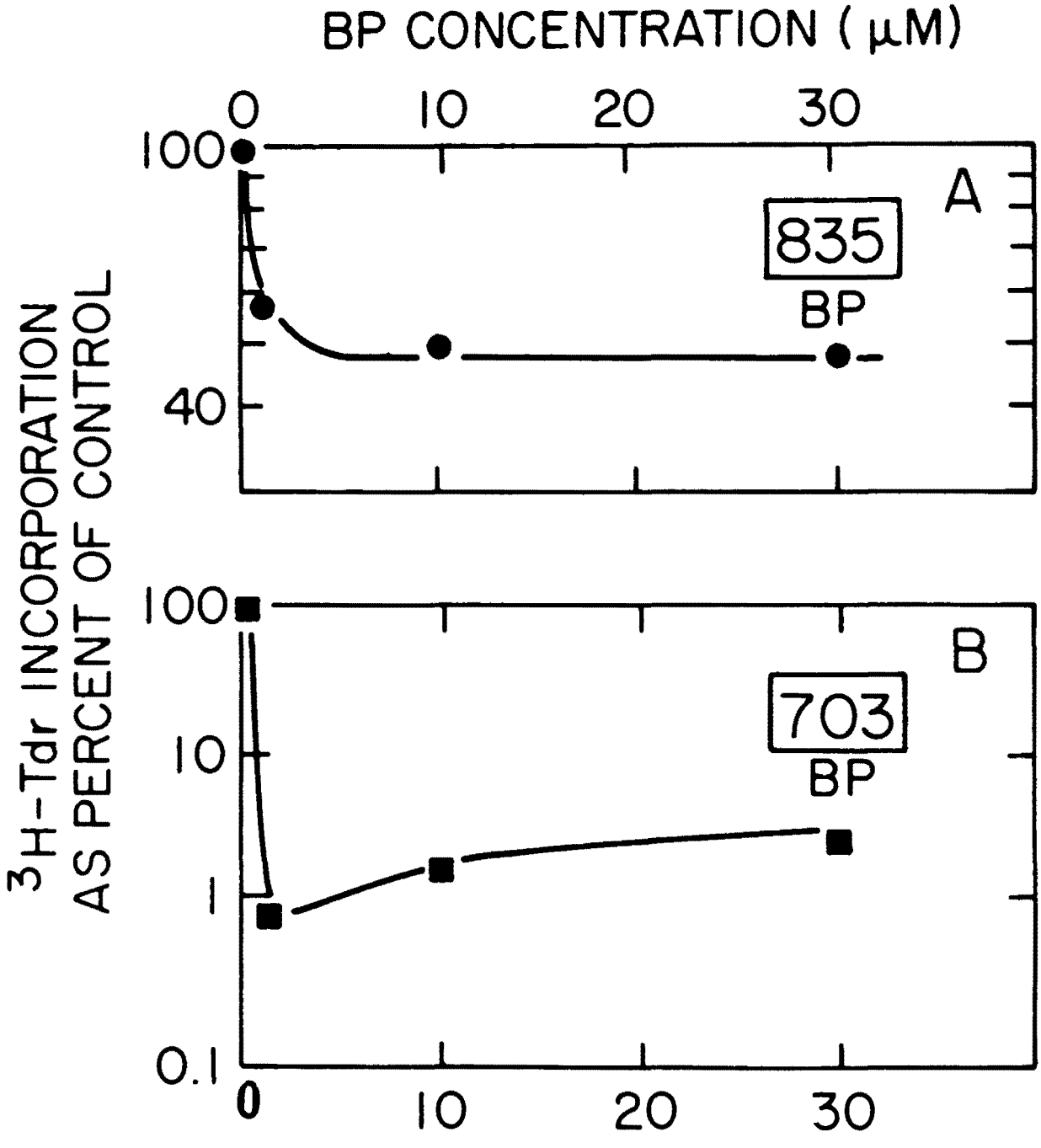

Figure 1. Inhibition of tritiated thymidine incorporation in tumor lines 835 and 703 after a 48 hour pre-treatment with BP at various concentrations.

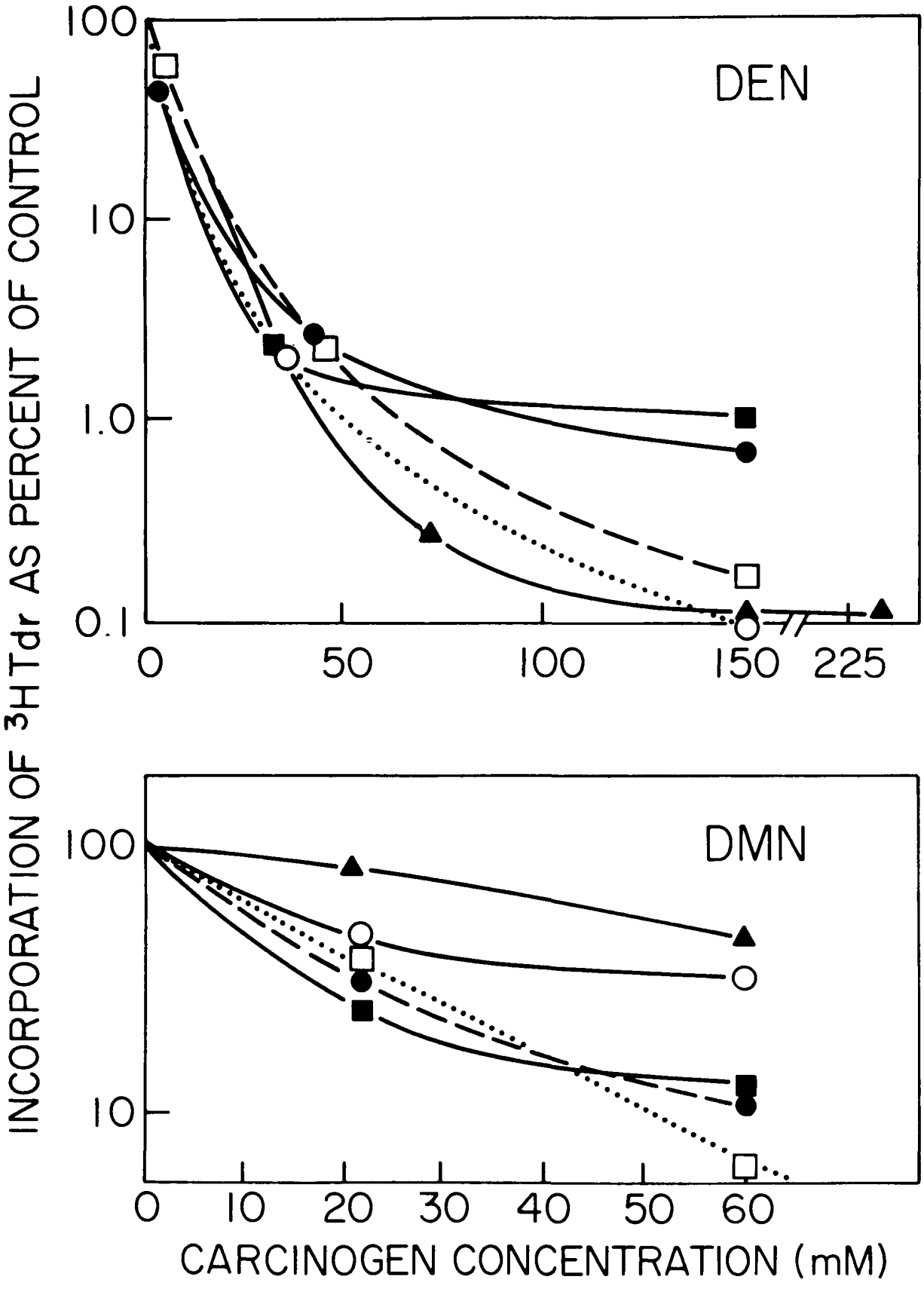

Figure 2. Inhibition of tritiated thymidine incorporation in tumor lines ▫ 562, ▲ PC-3, and ○ 703, and in ■ XP and ● normal human fibroblasts after a 48 hour treatment with various doses of DEN or DMN.

2. Direct Cell Killing:

To further examine the biological consequences of the metabolic activation of the carcinogens discussed above, we examined the cytotoxicity of the compounds directly on the metabolizing cells themselves by treating the cells with carcinogens at the indicated concentrations for 48 hr at 37° in Eagles Minimum Essential Medium containing 10% fetal bovine serum and antibiotics. At the end of the exposure period, the cells were trypsinized, replated at cloning density, and cultured for approximately two weeks until macroscopic clones developed. The results of exposing 703, 835 and 549 cells to BP are shown in Figure 3. It is obvious that 703 cells shows the greatest toxicity per dose. these results were the results obtained above with 703 and 835 cells. The results of exposing 703 cells (polycyclic hydrocarbon metabolizing cells) to aflatoxin B_1 are shown in Figure 4. The aflatoxin B_1 appears to be very cytotoxic to these cells.

3. Cell-mediated Cytotoxicity in Xeroderma Pigmentosum Cells:

Since it is always possible that direct cytotoxicity assays are not as sensitive as they could be due to DNA repair in the carcinoma cell lines, we used the cell-mediated assay previously developed in this laboratory in which lethally irradiated metabolizing cells are co-cultivated with target xeroderma pigmentosum cells (XP) in the presence of the carcinogen requiring activation for a period of 48 hr at 37°. At the end of the exposure period the cells are trypsinized, replated, and the XP cells allowed to clone. The percent survival of the cloning ability of XP cells was determiend by dividing the cloning efficiency of co-cultivated XP cells in the presence of carcinogen by the cloning efficiency of co-cultivated XP cells in the absence of carcinogen, multiplied by 100.

Optimal conditions for co-cultivation were previously established using BP as the model carcinogen. The effect of increasing the number of metabolizing cells and changing the concentration of BP was investigated. The cytotoxicity of BP in the XP target cells was found to increase with increasing number of metabolizing cells and BP concentration. In addition, the number of induced mutations to thioguanine resistance in the XP target cells also increased with increasing BP concentration in the presence of metabolizing cells. Using the cell-mediated cytotoxicity assay system developed for BP other carcinogens and metabolizing cells were examined. (Figure 5-8)

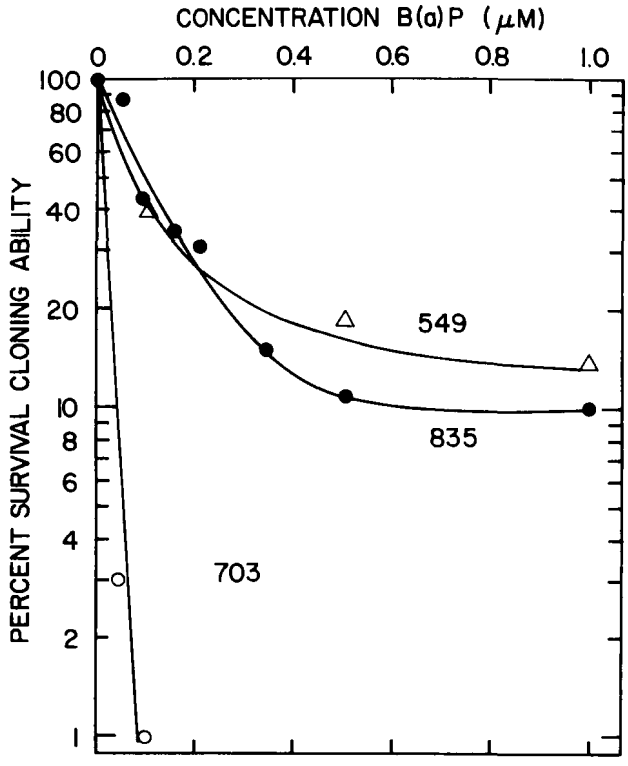

Figure 3. Direct cytotoxic action of BP on 549, 835 and 703 tumor cells. The cells were incubated for 48 hours with BP at various concentrations and then assayed for cloning ability.

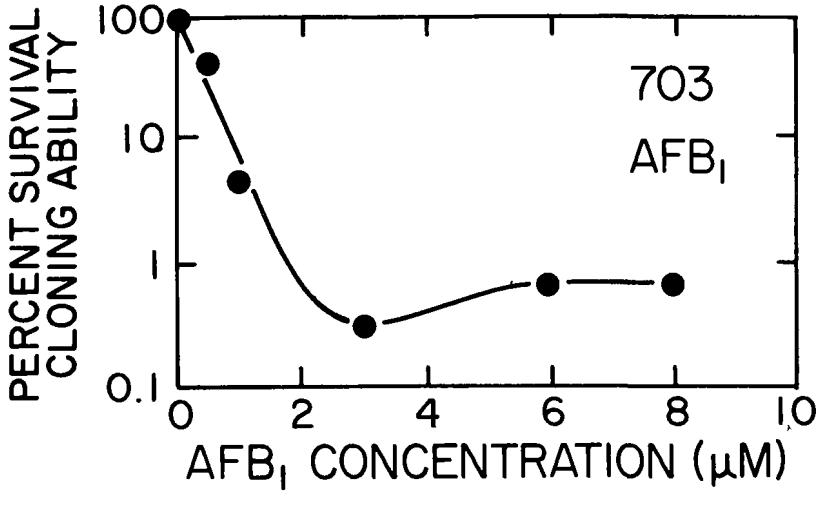

Figure 4. Direct cytotoxic action of AFB_1 on 703 tumor cells. The cells were incubated for 48 hours with AFB_1 at various concentrations and then assayed for cloning ability.

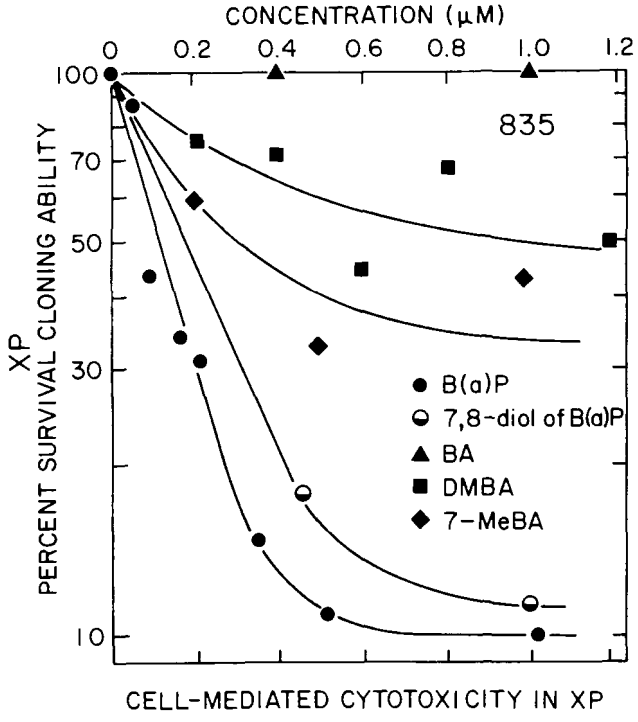

Figure 5. Cell-mediated cytotoxicity of XP cells coincubated for 48 hours with lethally-irradiated 835 tumor cells in the presence of various concentrations of polycyclic aromatic hydrocarbons or their derivatives. After the 48 hour coincubation, the cells were trypsinized and the XP cells assayed for cloning ability.

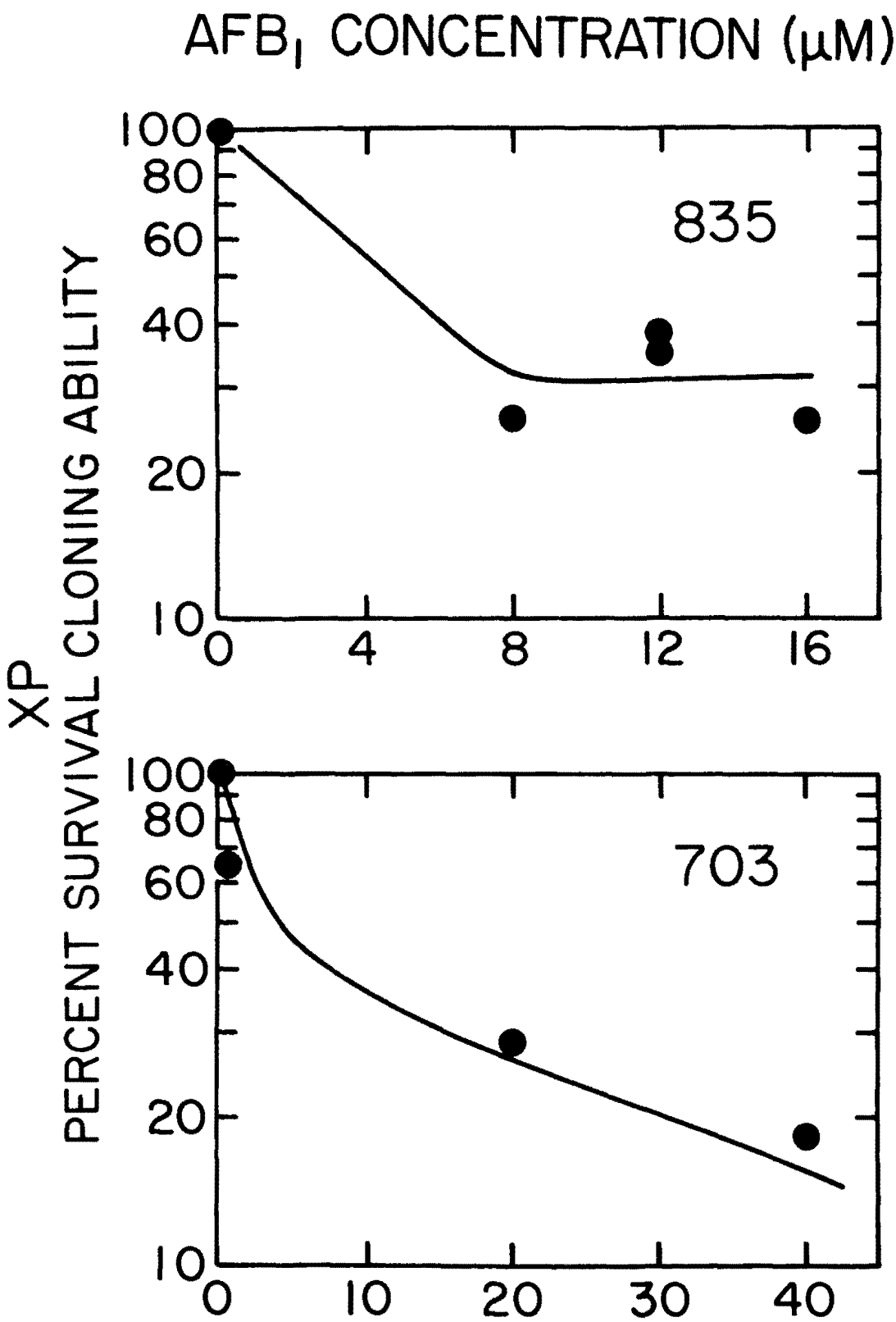

Figure 6. Cell-mediated cytotoxicity of XP cells coincubated for 48 hours with lethally-irradiated 835 or 703 tumor cells in the presence of various doses of aflatoxin B_1. After the 48 hour coincubation, the cells were trypsinized and XP cells assayed for cloning ability.

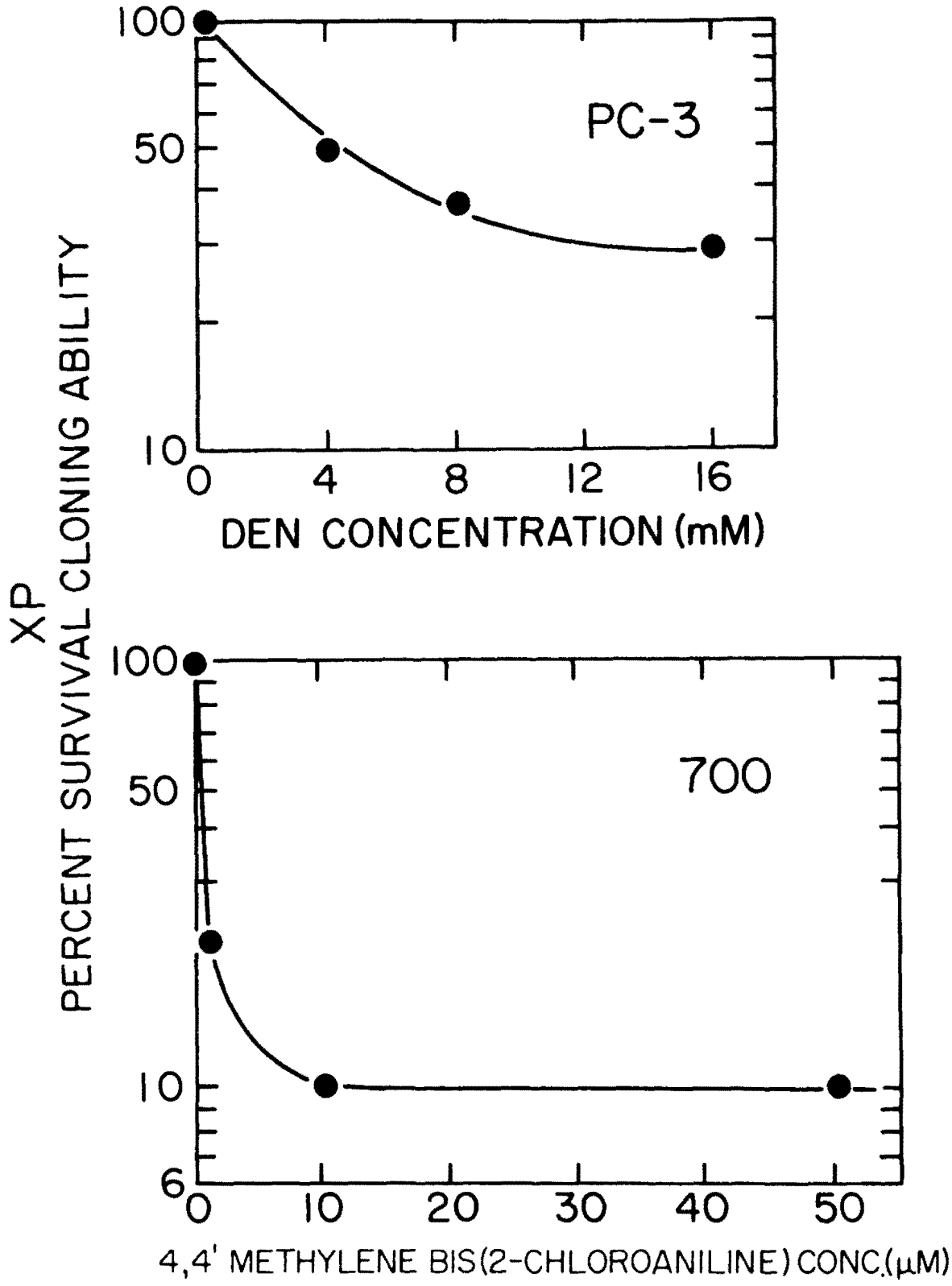

Figure 7. Cell-mediated cytotoxicity of XP cells coincubated for 48 hours with lethally-irradiated PC-3 or 700 tumor cells in the presence of various concentrations of DEN or 4.4' methylene bis (2-chloroaniline). After 48 hours of coincubation, the cells were trypsinized and the XP cells assayed for cloning ability.

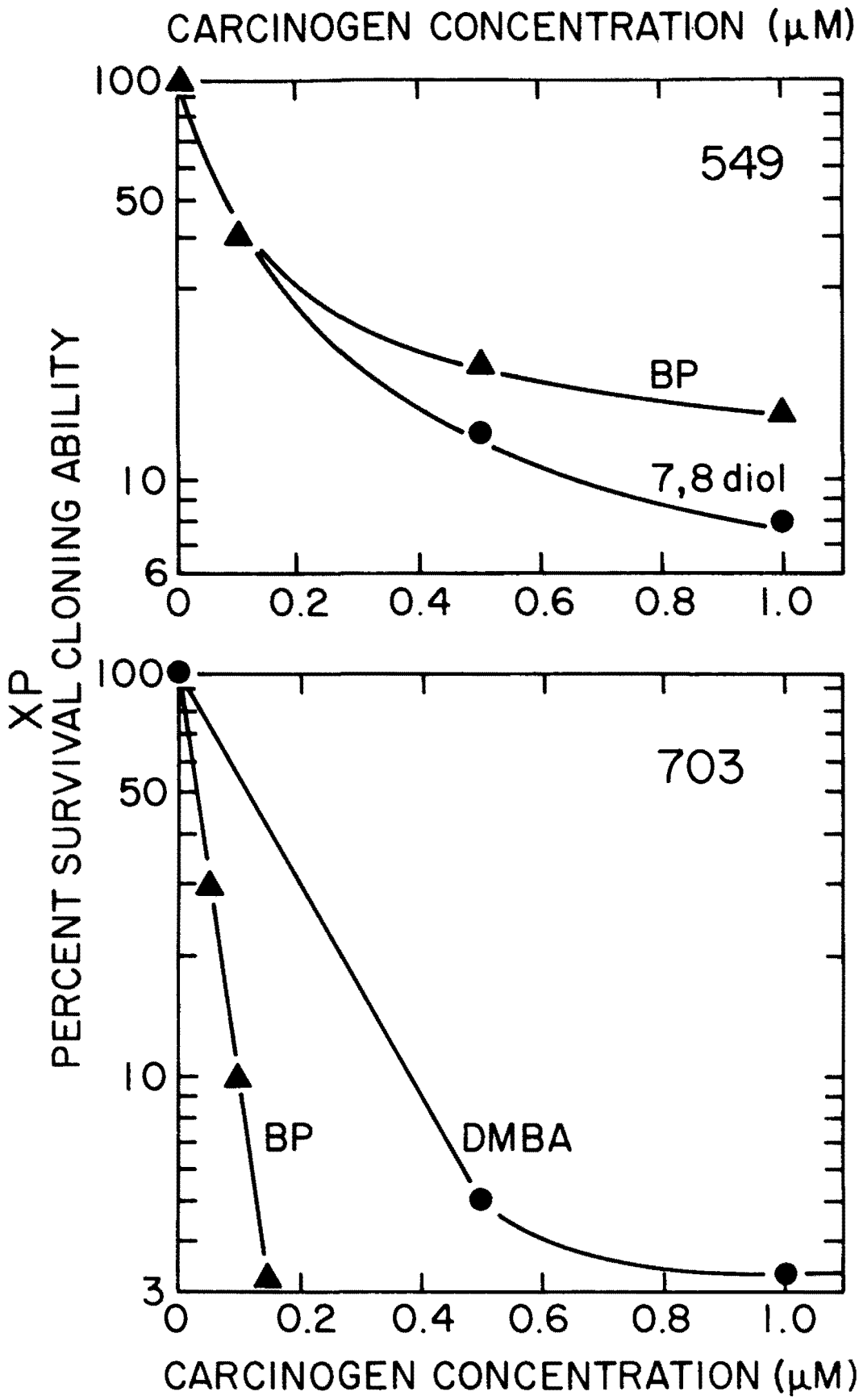

Figure 8. Cell-mediated cytotoxicity of XP cells coincubated for 48 hours with lethally-irradiated 549 or 703 tumor cells in the presence of various concentrations of polycyclic aromatic hydrocarbons or their derivatives. After 48 hours coincubation, the cells were trypsinized and the XP cells assayed for survival.

4. Cell-mediated Mutagenicity in Xeroderma Pigmentosum Target Cells:

We have tested the system for use as source of metabolic activation of two parent polycyclic aromatic hydrocarbons viz., benzo(a)pyrene and dimethylbenz(a)anthracene. To assay the co-cultivated target cells for the frequency of BP-induced mutations to 6-thioguanine resistance, cells were trypsinized and counted as above and plated into 250 ml flasks to allow for expression of mutations at cell densities which allowed surviving target cells to replicate without reaching confluence. (Sufficient numbers of flasks were employed for each determination to insure a minimum of 10^6 surviving target cells at the beginning fo the expression period.) The cells were trypsinized, pooled, and replated into flasks one or more times to insure logarithmic growth. The total length of the expression period was adjusted for each experimental determination to allow the cells to undergo at least 4.5 population doublings before selection was begun, i.e., 7 to 10 days (15).

At the end of the expression period, the cells were trypsinized, pooled, and 1-2 x 10^6 cells plated into selective medium at a density of 500 cells/sq cm (180 x 60mm dishes per point). To determine their cloning efficiency at the time of selection, these cells were further diluted and plated at cloning densities into medium identical to selective medium, but lacking 6-thioguanine. A reconstruction experiment with $HPRT^-$ Lesch Nyhan cells (18) accompanying each determination indicated that the efficiency of recovery of 6-thioguanine resistant colonies in the experiments reported here was 85%. Selection was continued for 14 to 18 day one refeeding. The frequency of mutations to 6-thioguanine resistance was calculated from the probability of a mutant per dish using the P(0) method (13). The results are shown in Figure 9.

It will be seen that DMBA is more mutagenic than BP when these are compared as a function of the concentration administered. This is also true as a function of the cytotoxic effect of the hydrocarbons. (Compare these data with those of Fig. 8 for the percent survival of cells exposed to DMBA and in Fig. 11 for that of cells exposed to BP for 48 hrs.)

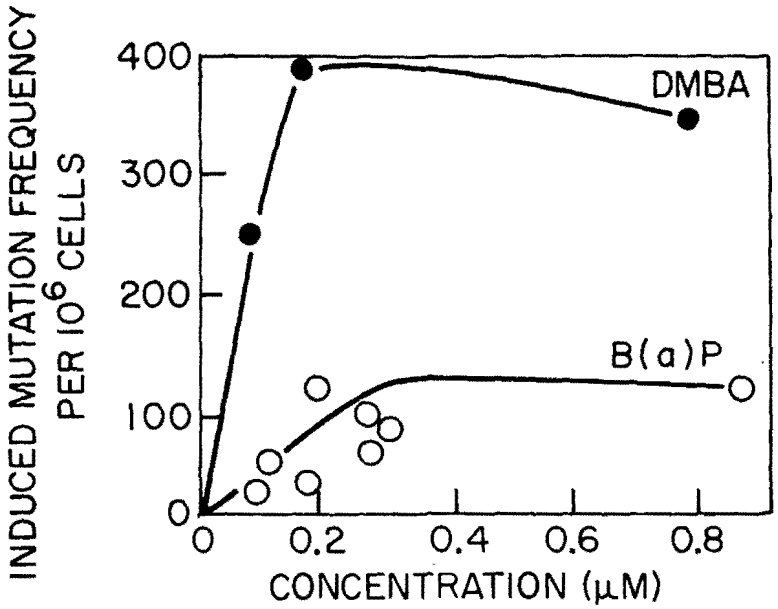

Figure 9. Induced mutation frequency of XP cells per 10^6 cells. XP cells were coincubated for 48 hours with lethally-irradiated 703 tumor cells in the presence of various doses of BP or DMBA and then assayed for 6-thioguanine resistance as described in the text.

5. High Pressure Liquid Chromatography Analysis of B(a)P-DNA Adducts Produced in Co-cultivated Human Cells:

Since previous data had shown that BP induces an increase in the frequency of mutations to thioguanine resistance in target human diploid fibroblasts co-cultivated with human tumor cells capable of metabolizing BP, this suggested an interaction of BP metabolites with cellular DNA. To investigate the extent and nature of this interaction, we determined the number of BP-DNA adducts formed during a 48 hr exposure of co-cultivated target cells and metabolizing cells to tritiated BP per 10^7 moles of DNA nucleotides were found to be, respectively, 1.4, 4.1, and 12. These results confirm that metabolites of BP were binding to the cellular DNA and showed a direct relationship between the concentration of BP in the medium and the number of BP-DNA adducts formed.

To identify these BP-DNA adducts, we analyzed those formed at the highest concentration using high pressure liquid chromatography (HPLC). Figure 10 shows the HPLC elution profile obtained. The major peak, 82% of the radioactivity associated with the adducts, co-chromatographed with the optical standard-adduct formed between deoxyguanosine (dG) and anti BPDE. This cell-mediated BP-DNA adduct also co-chromatographed with the adduct formed in normal diploid human skin fibroblasts exposed for 2 hr to anti BPDE (12). The minor radioactive peak in Figure 12 co-chromatographed with the optical standard formed between dG and syn BPDE. These results are consistent with reports on BP-DNA adducts formed in human explant tissue from lung (19), colon (20), and bronchus (21) as well as cells derived from human lung carcinoma, A549, in which the N^2-dG-anti BPDE adduct was the major adduct observed.

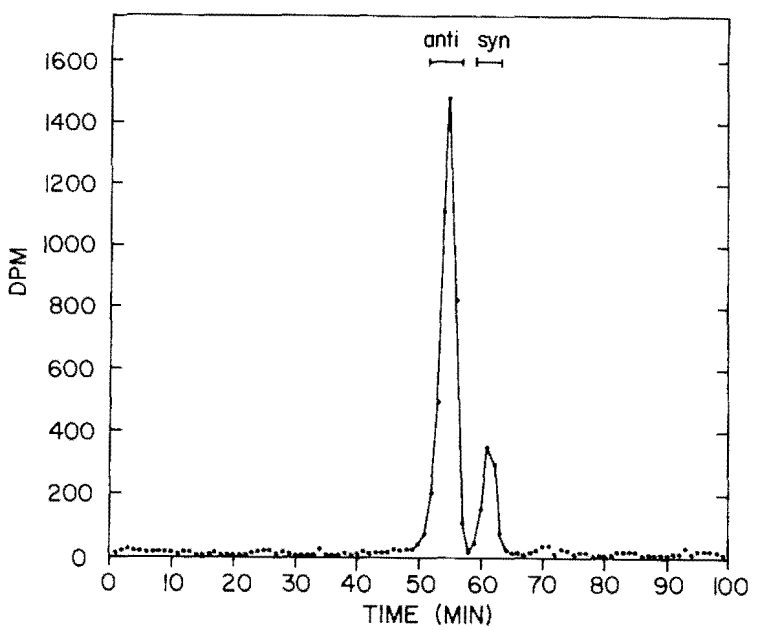

Figure 10. HPLC profile of the DNA nucleoside adducts formed in 835 cells coincubated with XP cells. The major peak is the N^2-dG-anti BPDE adduct and the minor peak is the N^2dG-syn BPDE adduct.

C. Time-Dependent Dose Response of XP Cells to BP Using Cell-Mediated Metabolic Activation:

To further characterize our assay system to changing doses of BP for varying times of exposure, logarithmically growing XP cells were plated at 200,000 per 30mm diameter culture dish. After 24 hours, X-irradiated transformed epithelial cells were plated at a final attached cell density equal to that of the XP cells. The cultures were exposed to increasing concentration of BP for 24 or 48 hours and then replicate sets of treated cells were trypsinized and replated at cloning densities. In order to determine the number of XP cells in co-cultivated treatment dishes accurately, a dish containing XP cells alone without epithelial cells was counted. As shown in Figure 11, the percent survival of XP cells decreased with increasing concentration of BP and in addition, the 48 hr incubation resulted in greater cytotoxicity than the 24 hr.

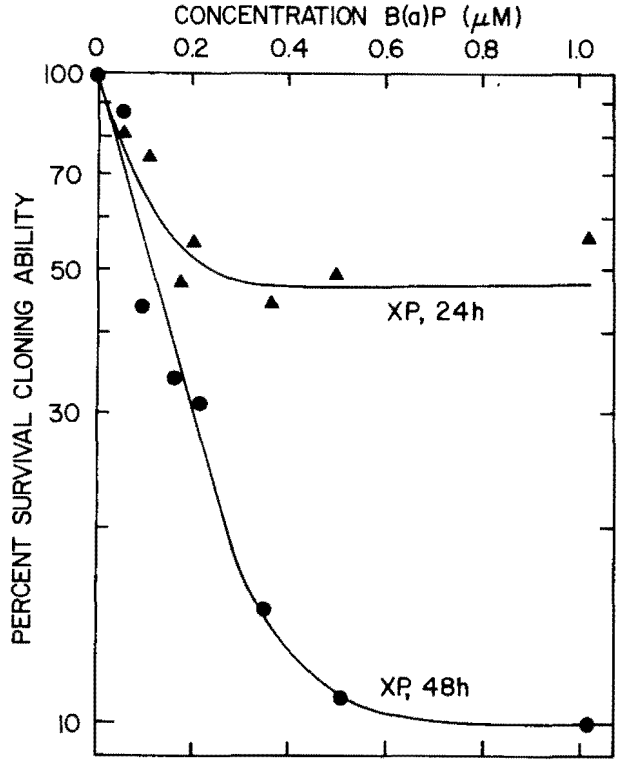

Figure 11. Cell-mediated cytotoxicity of XP cells incubated for 24 or 48 hours with lethally-irradiated 835 tumor cells in the presence of various concentrations of BP. After the coincubation, the cells were trypsinized and the XP cells assayed for cloning ability.

VII. SIGNIFICANCE

In vitro assays of mutagenicity and transformation are used as short term tests to determine the potential danger of unknown agents as well as to carry on mechanistic studies regarding the mechanisms of mutagenesis and transformation. In both types of studies, one is frequently faced with the need to utilize agents which require metabolic activation. It is clear from published studies that the incubation of target cells with preparations from liver homogenates such as S-9 fractions or microsomal preparations, results in the production of potentially mutagenic and transforming adducts in the DNA of the cells, but that these may be different from those actually produced in the DNA of the cells used to make the homogenate. Thus, the conclusion that a particular compound is potentially mutagenic may be the correct one, but for the wrong reasons, i.e., the wrong DNA adduct was produced. It is obvious that any reliance on short term assays which makes use of the metabolizing ability of cell homogenates may be held up to question precisely because of this problem. This poses potential problems for regulating agencies as well as for those interested in mechanistic studies. The use of cells able to metabolize carcinogens as feeder layers allows one to produce the expected DNA adducts in target cells which are the same as those produced in vivo and will therefore be useful for many types of studies.

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Dr. Morris, EPA: Certainly within the regulatory sphere we are interested in batteries of tests and so on for predictors and do you envision then that as we proceed, certainly in the rule-making exercise for Section 4 of TSCA we might be involved in not only selecting the cell type for the mutation or transformation, whatever assay but we are, also, maybe wanting to hone in on the specificity of the cell line for metabolizing? Is that the direction we are going in?

Dr. Waters, EPA: Yes, I think that is definitely true, especially as we progress to higher levels of testing. I think what I have said in terms of the specificity of metabolism should not be taken as an indictment of an Ames test and its S-9 preparation because I still believe that this is a very good detection system. However, as we attempt to extend results from detection systems to confirmatory in vitro bioassays, and to the level of the in vivo bioassay we must be much more concerned about the specificity of the metabolism. We must be concerned about it if we are to have really relevant confirmatory bioassays. So, I would see the kind of work that is being performed under this grant at Michigan State University as being indicative of the type of testing that ought to be carried out in the confirmatory phase of assessment of compounds. Does that answer your question?

Dr. Morris, EPA: Yes.

Dr. Kraybill, NCI: I am Dr. Kraybill, NCI. I am still not quite clear then because I feel that using the S-9 fraction here, you are getting the result, but the relevancy of that result to an in vivo system; after all, that is what we are interested in, what happens in man, how do you look then upon the Ames system with the S-9 fraction here, as an indicator or--

Dr. Waters, EPA: Yes, as an indicator system.

Dr. Kraybill, NCI: Just simply that?

Dr. Waters, EPA: Right, and in point of fact, it is the correlation that has been developed between Ames test results for many compounds and results of whole animal carcinogenesis bioassays that is the real strength of that assay. I think that these correlations argue very strongly for the use of this kind of test as a detection system. However, when the question of specificity of metabolism arises, as we progress from the detection level to the confirmatory level of testing we should be concerned about the formation of specific metabolic products in the intact animal and possibly in man. I think that is the reason that we need to be doing this kind of research--to solidify our confidence in the kind of metabolism carried out in confirmatory bioassays.

Dr. Chandler, NIOSH: Jerry Chandler, NIOSH. Mike, there are two facts that we have to keep in mind, I suppose. One is that many of the reactive intermediates that are generated by microsomal oxidase systems are extremely short-lived and may not be as long as the BAP half life or reactive intermediate. Secondly, when you use two cell systems, one is an activation system. You are requiring that the compound must diffuse out of the one cell across the other membrane into another cell. Do you think this is going to be generally applicable?

Dr. Waters, EPA: The data that has been obtained up to now by a number of investigators, Huberman and Sacks in Israel, Weibel in Germany, indicate that despite our concerns, the ones that you mentioned, that a compound has to be metabolized in one cell, get out of that cell and into another to show its effect, despite those concerns, in many cases, with a number of compounds that require metabolic activation, the systems are working. I believe it must not be terribly difficult for this cell-cell interaction to occur. The key element may be the proximity of the two cell types. In fact, it appears that they must be in close proximity to one another. I don't know how far one can separate the activation cell on the one hand and the indicator cell on the other. We have performed some experiments in our laboratory where we have interposed dialysis membranes, and it is still possible under those circumstances, in the case of compounds, for the metabolites to get out and to enter the indicator cells. So I don't yet know what the limits are, but I do think, that based on the results obtained thus far, that it does seem feasible to use these kinds of systems. Also, it is indicative, I think, of an important concern that we probably should have, and that is that metabolism that occurs in one cell type in an intact organism may be highly influential on another cell type. That is something we need to keep in mind.

Dr. Hegyeli, NCI: Hegyeli, NCI. Mike, I see from your description of the procedure that these cell lines, the feeder cell lines are derived from human tumors. Did you ever try to use primary cells?

Dr. Waters, EPA: This is, of course, not our own work but that of Drs. McCormick and Maher. We have used primary cells in our laboratory, yes, and they have as well but not as a part of this particular program to date. We have used primary hepatocytes, and we are using primary bladder cells and primary lung cells. As a specific example, Dr. Robert Langenbach in our laboratory has worked with a series of nitrosamine analogs. He finds that using whole cell hepatocyte activation, the correlation that is obtained for mutagenic activity in V-79 cells and carcinogenic activity in whole animal systems is much better than that obtained if mutagenic activity in an Ames test using S-9 activation is compared with carcinogenic activity in the intact animal. Please, let me say again, I am not indicting the Ames test. I am simply indicating that for certain kinds of chemicals whole cell metabolism may be more relevant to the intact animal, and I agree with you that primary cell

metabolism may even be better than that observed in many cell lines. However, primary cells in culture do have some significant disadvantages. They are much harder to reproducibly prepare and control. If we can select cell lines that can carry out a broad range of metabolic activities, then I think they should be, in theory, preferable to the primary cells because of the difficulty of having to recover cells from the intact animal for each experiment.

Dr. Herberman, NCI: Herberman, NCI. Is there some concern that different cell lines might metabolize the same agent somewhat differently and that if you use as the primary screen the direct cytotoxicity that that might not reflect the metabolites that you would be interested in for mutagenic or carcinogenic effects?

Dr. Waters, EPA: Yes, the Painter screen was used primarily because of its rapidity, and they were able to examine a series of 19 different cell lines very quickly. However, before they would recommend the use of these cell lines for screening purposes it would be essential to confirm that the metabolic activation capability possessed by these lines is indicative of the type of metabolism that we are looking for in vivo. So, this is, in fact, what they have done in one part of the study. Did I answer the first part of your question? Would you repeat that? Maybe I did not quite catch all of it.

Dr. Herberman, NCI: The question was whether the primary screen that was being used was the best one or whether it would be better to go directly to a mutagenic or carcinogenic one?

Dr. Waters, EPA: I think it would, but of course, it takes a lot longer to do that, and they are doing it secondarily, and as long as it is done before you propose using those cells, I think it is probably okay.

Dr. Morris, EPA: Thank you, Mike.

NIOSH

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