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J Clin Invest. 1979;64(5):1245-1252. https://doi.org/10.1172/JCI109579.

Research Article

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Induction of Ouabain-resistant Mutation and Sister Chromatid Exchanges in Chinese Hamster Cells with Chemical Carcinogens Mediated by Human Pulmonary Macrophages

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ABSTRACT Pulmonary macrophages (PAM) metabolically activated benzo[*a*]pyrene [B(*a*)P] and its proximate carcinogenic metabolite, (±)trans 7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (7,8-diol), to ultimate mutagens that were detected in cocultivated Chinese hamster V79 cells. Increases in the frequency of ouabain-resistant (O^r) mutations and sister chromatid exchanges were found in V79 cells only when they were cocultivated with both PAM and the chemical procarcinogens. 7,8-Diol caused higher frequencies of both O^r mutations and sister chromatid exchanges than did the parent compound, B(*a*)P. When metabolically activated by PAM the mean O^r mutation frequency caused by B(*a*)P was 9 O^r mutants/10^6 surviving V79 cells per 10^6 PAM and a 10-fold interindividual variation (range, 2–21) was found. The mean O^r mutation frequency caused by 7,8-diol was 64 and a ninefold interindividual variation (range, 14–120) was found. In the absence of PAM, the O^r mutation frequency in V79 cells was one or less O^r mutant per 10^6 survivors. 7,8-Benzoflavone, an inhibitor of mixed function oxidases, reduced the frequencies of O^r mutations and of sister chromatid exchanges in V79 cells caused by 7,8-diol and B(*a*)P. As expected 7,8-benzoflavone did not influence the frequency of O^r mutations caused by one of the ultimate mutagens derived from B(*a*)P and 7,8-diol, (±)7β, 8α-dihydroxy-9α, 10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]-pyrene. These data are consistant with the hypothesis that PAM may play a role in the activation of environmental chemical procarcinogens.

INTRODUCTION

Benzo[*a*]pyrene [B(*a*)P][1] is found in the polluted atmosphere and in tobacco smoke. Approximately 1,300 tons of B(*a*)P are annually emitted into the air of the United States (1). B(*a*)P may be inhaled into the lung either directly or it may be combined with carriers such as airborne particulates, including those in the tobacco smoke (2, 3). Pulmonary macrophages (PAM) phagocytize inhaled foreign particulates that reach the peripheral airways, and generally transport them by the mucociliary system up the respiratory tract to be either expectorated or swallowed. During this journey, PAM may metabolize B(*a*)P and other carcinogens adsorbed on engulfed particulates and release both activated (proximate and ultimate carcinogens) and deactivated metabolites into the bronchial lumen, which is lined by epithelial cells (4).

Metabolism of B(*a*)P in pulmonary macrophages is of interest both because of potential interactions between PAM and bronchial epithelium in the activation of chemical carcinogens and because of the possible usefulness of PAM as an indicator of B(*a*)P metabolism

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Received for publication 17 January 1979 and in revised form 2 July 1979.

[1] *Abbreviations used in this paper*: AHH, aryl hydrocarbon hydroxylase; B(*a*)P, benzo[*a*]pyrene; B(*a*)P 7,8-diol, (±) trans 7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene; B(*a*)P-diol epoxide I, (±) 7β, 8α-dihydroxy-9α, 10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; O^r, ouabain resistant; PAM, pulmonary macrophage(s); SCE, sister chromatid exchange(s).

in the target tissue, i.e., bronchial epithelium. Human PAM obtained from lung tissues have been shown to contain aryl hydrocarbon hydroxylase (AHH) activity (5, 6). These mixed function oxidases metabolize B(*a*)P to form(s) that bind to cellular DNA. A sixfold inter-individual variation in AHH activity and a ninefold variation in binding levels of B(*a*)P to DNA were observed in PAM prepared from 13 individuals (7). A similar observation was reported by McLemore et al. (8) when they compared the levels of AHH in PAM, lung tissues and cultured mitogen-stimulated lymphocytes. They found also a good positive correlation of AHH level in these three tissues and cells from seven individual noncancer patients but no correlation among these three preparations from seven patients with lung cancer.

We recently found that human PAM can metabolize B(*a*)P to proximate and ultimate mutagens, and cause O^r mutations in Chinese hamster V79 cells (9). The O^r mutation frequency was dependent on the number of cocultivated PAM and on the concentration of either (±) 7β, 8α-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (7,8-diol) or B(*a*)P. Chinese hamster V79 cells do not effectively metabolize either B(*a*)P or 7,8-diol into ultimate mutagens (10). In the present report, these studies have been extended to evaluate the ability of PAM from 20 individuals to mediate mutations in V79 cells. In addition, the increase of sister chromatid exchange (SCE) frequency in V79 cells mediated with PAM was studied. The effects of 7,8-benzoflavone, an inhibitor of AHH, on ouabain-resistant (O^r) mutation frequency and on SCE frequency were also investigated.

METHODS

Chemicals. MNNG, B(*a*)P, 7,8-diol, and (±) 7β, 8α-dihydroxy-9α, 10α-epoxy-7,8,9,10-terahydrobenzo[*a*]pyrene [B(*a*)P diol epoxide I] were supplied by Chemical Carcinogen Repository, National Cancer Institute, Bethesda, Md. They were dissolved in dimethyl sulfoxide (Pierce Chemical Co., Rockford, Il.) before use. Ouabain and bromodeoxyuridine were purchased from Sigma Chemical Co., (St. Louis, Mo.) and Hoechst 33258 from American Hoeschst Corp., (Somerville, N. J.). Colcemid was obtained from Grand Island Biological Co., (Grand Island, N. Y.).

Cell cultures. PAM were isolated from surgical (patients 149, 149A, 152, 153, 157B, 161, 169B, 169C, 183A, 188A, 190, 201B, 201C, 204B, 207B, 207D, 213A) and autopsy specimens (patients 165, 182, 186A) (Table I) as described (7, 11). In brief, peripheral lung tissues were cut into four to 5-mm fragments. The fragments were trypsinized and centrifuged to separate PAM from the tissues. Isolated PAM were seeded on 100-mm dishes and washed 3 h later to remove unattached cells. More than 90% of the attached cells were PAM. The

TABLE I
Source of PAM

Patient No.	Age	Sex*	Diagnosis‡	Smoking history
	yr			
149	48	M	Adeno-squamous carcinoma	Smoker§
149A	57	M	Squamous cell carcinoma	Smoker
152	76	M	Adeno-squamous carcinoma	Unknown
153	55	F	Squamous cell carcinoma	Unknown
157B	62	M	Squamous cell carcinoma	Unknown
161	60	M	Adenocarcinoma	Smoker
165	16	M	Traumatic head injury	Unknown
169B	66	M	Adenocarcinoma	Smoker
169C	59	M	Squamous cell carcinoma	Smoker
182	28	F	Traumatic head injury	Smoker
183A	48	M	Poorly differentiated squamous cell carcinoma	Smoker
186A	51	M	Pulmonary arteriovenous aneurysm	Smoker
188A	51	M	Squamous cell carcinoma	Smoker
190	57	M	Squamous cell carcinoma	Nonsmoker
201B	45	M	Squamous cell carcinoma	Smoker
201C	71	M	Squamous cell carcinoma	Smoker
204B	41	M	Squamous cell carcinoma	Smoker
207B	55	M	Undifferentiated carcinoma	Smoker
207D	55	M	Squamous cell carcinoma	Smoker
213A	54	M	Squamous cell carcinoma	Smoker

* M, male; F, female.
‡ Carcinomas of the lung.
§ Smoker, person smoked more than half pack of cigarettes per day during the last 5 yr.

number of PAM per dish was counted on an inverted microscope equipped with an ocular reticule grid.

Chinese hamster V79 cells (12) were supplied by E. Huberman (Oak Ridge National Laboratory, Oak Ridge, Tenn.) and were grown in Dulbecco's modified Eagle's medium (catalog No. 196G; Grand Island Biological Co.) supplemented with 10% heat-inactivated fetal calf serum (Irvine Scientific Co., Irvine, Calif.), pencillin (100 U/ml), streptomycin (100 μg/ml), and glutamine (2 mM) (13). The cultures were dispersed with 0.05% trypsin/EDTA solution (Grand Island Biological Co.) and were subcultured twice a week at a dilution of 1:30 and incubated at 37°C in an atmosphere of 10% CO_2 in air.

Determination of O^r mutations and SCE in V79 cells. In the PAM-mediated mutagenesis assay, the PAM were cultured for 7–8 d before the addition of the V79 cells to allow AHH activity to reach basal levels (14) and then 5×10^5 V79 cells were added to each 100-mm culture dish containing $1-2 \times 10^6$ PAM. Within 4 h of cocultivation, the V79 cells were attached to the dishes and were then exposed to medium containing 1 μg/ml of either B(*a*)P (15) or 7,8-diol (0.5% dimethyl sulfoxide, final concentration). During the next 3 d the medium was replaced with fresh medium containing B(*a*)P or 7,8-diol every 24 h. 7,8-Benzoflavone was included in the medium of certain experimental groups during the period of treatment with B(*a*)P, 7,8-diol or B(*a*)P diol epoxide I to determine its effect on the frequencies of O^r mutation and of SCE. When V79 cells were treated with 0.05 μg/ml B(*a*)P diol epoxide I for 1 h, 7,8-benzoflavone (1 μg/ml) remained in the medium for 70 h for the purpose of comparison. 70 h after the treatment was initiated, the cocultivated cells were dispersed with 0.05% trypsin-EDTA solution. Chinese hamster V79 cells can be morphologically distinguished from the PAM and were counted with a hemocytometer. The V79 cells were then seeded at 100 cells/60-mm dish (5 dishes) for determination of cloning efficiency and at 10^5 cells/dish (20 dishes) for either measuring SCE or selecting O^r mutants as described (9, 13). SCE were measured in V79 cells by the technique described by Perry and Wolff (16, 17). One or two dishes of the cells in each group were treated with medium containing 10 μM bromodeoxyuridine 2–3 h after seeding and kept at 37°C in the dark for 28–30 h. Colecemid (0.1 μg/ml) was added for 2 h before the mitotic cells were collected by trypsinization.

To determine the variation caused by experiments conducted at different times, 0.4 μg/ml of *N*-methyl-*N*-nitro-*N*-nitrosoguanidine was included in medium of V79 cells at the beginning of the 70-h cocultivation. The cells were then dispersed for selection of O^r mutants as described above.

RESULTS

Chinese hamster V79 cells attached in the dishes which contained PAM (Fig. 1A). The V79 cells grew and replaced the cocultivated PAM in the dishes. At the end of cocultivation, most of the PAM were detached and over 80% of the surface area of the dishes was covered by V79 cells (Fig. 1B). During cocultivation, PAM metabolized either B(*a*)P or 7,8-diol to ultimate mutagens.

PAM isolated from 20 individuals were tested for their abilities to mediate O^r mutation in V79 cells. The O^r mutation frequency in V79 cells cocultivated with PAM in medium containing 1 μg/ml B(*a*)P from 16 individual cases studied to date is shown in Table II. The values range from 2 to 21 O^r mutants/10^6 surviving V79 cells per 10^6 cocultivated PAM with a mean value of 9. In six experiments, O^r mutation frequency were measured on duplicate or triplicate PAM dishes isolated from the same patient to determine the variation caused by methodology in the PAM-mediated mutation assay. The highest variation caused by experimental methods observed was ±30% of the mean value. As a positive control, selection of O^r mutants from *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (0.4 μg/ml)-treated cells were conducted in four separate experiments during a period of 15 mo to determine variations in the mutagenic responsiveness of the V79 cells at different times. The O^r mutations caused by *N*-methyl-*N*-nitro-*N*-nitrosoguanidine were within the range of 120–230 mutants/10^6 surviving V79 cells (mean±SD; 172±40) in the four experiments.

7,8-Diol-induced O^r mutation of V79 cells mediated by PAM is shown in Table III. 7,8-Diol caused 5–10 times more O^r mutation in V79 cells than did B(*a*)P. Again, there was approximately ninefold variation of capability of PAM to mediate O^r mutation with a mean value of 64 mutants/10^6 surviving V79 cells per 10^6 PAM among 11 individuals. The frequency of PAM-mediated O^r mutations caused by B(*a*)P were plotted against that caused by 7,8-diol in Fig. 2. A positive correlation was observed ($r = 0.80, P < 0.01$); (18) i.e., PAM that mediated a high frequency of O^r mutation by B(*a*)P also caused a high O^r mutation frequency by 7,8-diol.

When activated by PAM, B(*a*)P or 7,8-diol also increased the frequency of SCE in the cocultivated V79 cells. SCE in 7,8-diol treated V79 cells with or without PAM cocultivation are shown in Fig. 3. There are ≅8–11 exchanges per metaphase in control cells. In the absence of PAM, neither B(*a*)P nor 7,8-diol significantly alter the frequency in SCE in V79 cells. However, with metabolic activation by the cocultivated PAM, a twofold increase of the frequency of SCE by 7,8-diol and 60% increase by B(*a*)P were observed in the V79 cells (Table IV). Because both selection of O^r mutants and scoring of SCE were done in the cells that had been cocultivated and treated under the same conditions, the potential source of experimental variation in comparison should be minimized. 7,8-Diol produced 4–10 times more O^r mutants and 20–30% more SCE than did B(*a*)P. 7,8-Benzoflavone (1 μg/ml, an inhibitor of AHH (19), had no observable effect on the proliferation of V79 cells but almost completely inhibited the increase in the frequencies of O^r mutation and SCE caused by 7,8-diol and mediated by PAM (Table V). However, 7,8-benzoflavone did not change the O^r mutation frequency in V79 cells caused by a direct-acting ultimate carcinogen, B(*a*)P diol epoxide I (Table V).

DISCUSSION

PAM play an important role in host defense by virtue of their ability to phagocytize inhaled foreign material

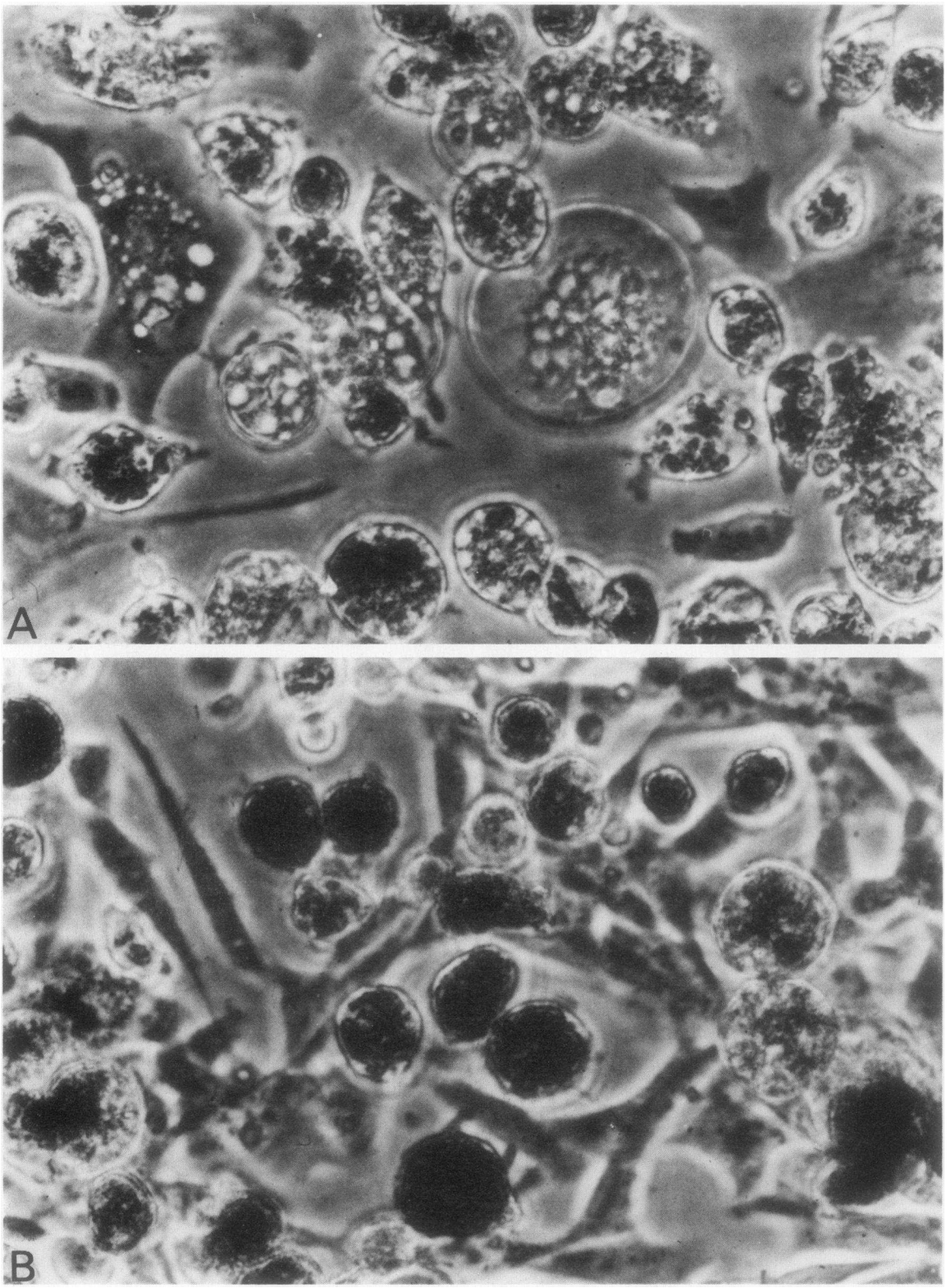

FIGURE 1 Cocultivation of PAM and V79 cells. Pictures were taken with a Leitz phase contrast microscope (X-200) at (A) 12 h and (B) 50 h, after cocultivation.

TABLE II

Interindividual Variation of O^r Mutation Frequency in V79 Cells Caused by B(a)P and Mediated by Human PAM

Source of PAM	O^r mutants/10^6 surviving V79 cells (survival %)*		O^r mutants/10^6 surviving V79 cells per 10^6 PAM
	Control‡	Experimental	
patient No.			
149	2 (70)	15 (65)	9 [8,9]§
152	<1 (68)	11 (60)	8 [6,9]
153	<1 (73)	6 (50)	4 [3,5]
157B	1 (78)	22 (76)	21 [20,22]
169B	<1 (60)	11 (80)	18
165	1 (95)	7 (90)	6
169C	<1 (80)	13 (79)	8
182	<1 (78)	10 (75)	6
183A	<1 (90)	8 (80)	6
186A	<1 (88)	22 (81)	12 [9,12,14]
188A	1 (82)	6 (79)	4 [4,4,5]
201B	<1 (71)	3 (50)	2
201C	<1 (81)	8 (75)	14
204B	<1 (70)	7 (50)	5
207D	<1 (72)	33 (55)	16
213A	<1 (93)	2 (89)	3
mean±SD	1.1±0.3	11.8±8.2‖	8.8±5.7

* Survival % was expressed as the cloning efficiency of the V79 cells treated with B(*a*)P in the presence (experimental) or absence (control) of PAM divided by that of V79 cells. The average cloning efficiency of V79 cells was ≅80%.

‡ The controls were V79 cells treated with B(*a*)P without PAM.

§ Mean value; numbers in brackets are values from experiments done in duplicate and triplicate.

‖ For difference from control, $P < 0.01$. All values <1 (no O^r mutants in 20 dishes) were calculated as being 1 and evaluated by the Student's *t* test.

TABLE III

Interindividual Variation of O^r Mutation Frequency in V79 Cells Caused by 7,8-Diol and Mediated by Human PAM

Source of PAM	O^r mutants/10^6 surviving V79 cells (survival %)*		O^r mutants/10^6 surviving V79 cells per 10^6 PAM
	Control‖	Experimental	
patient No.			
149	3 (90)	159 (56)	120‡ [115,126]
149A	1 (64)	99 (30)	76 [83,69]
157B	<1 (80)	112 (47)	124
161	1 (71)	38 (63)	105 [94,113]
165	<1 (90)	110 (49)	72
169C	<1 (99)	47 (89)	32
183A	<1 (80)	91 (65)	60
190	<1 (82)	34 (61)	14 [10,14,18]
204B	<1 (85)	80 (72)	56
207B	<1 (71)	13 (45)	15
213A	<1 (75)	25 (47)	28
mean±SD	1.2±0.6	73.5±45.5§	63.8±39.9

* Survival % was expressed as the cloning efficiency of the V79 cells treated with 7,8-diol in the presence (experimental) or absence (control) of PAM divided by that of V79 cells. The absolute cloning efficiency of V79 cells was ≅80%.

‡ Mean value, numbers in brackets are values from experiments done in duplicate and triplicate.

§ For difference from control, $P < 0.01$. All values <1 (no O^r mutants in 20 dishes) were calculated as being 1 and evaluated by the Student's *t* test.

‖ The control group was V79 cells treated with 7,8-diol without PAM. The experimental group was cells cocultivated with PAM and treated with 7,8-diol.

(20, 21). However, PAM can also activate procarcingens, e.g., B(*a*)P (cf. 7) that are known to be adsorbed to particulate material, to ultimate carcinogens. The activated metabolites of B(*a*)P produced by PAM are released into the culture medium and induce O^r mutation in cocultivated V79 cells (9). This fact indicated that in spite of their high reactivity and short life, the ultimate mutagens can pass the membrane barrier of PAM.

Induction of O^r mutation in V79 cells can also be demonstrated by treating PAM with B(*a*)P adsorbed on ferric oxide particles and then cocultivated with V79 cells suggesting a slowly release of active metabolites of B(*a*)P in the inhaled and phagocytized particles (22). PAM migrate into the alveolar spaces in response to inhaled foreign material including particulates in tobacco smoke. They carry the phagocytized particulates up the mucous ciliary transport system where PAM came in close contact with bronchial epithelium and may play in concert with respiratory epithelium in initiation of respiratory carcinomas by the activation of carcinogen. These facts were all consistent with our

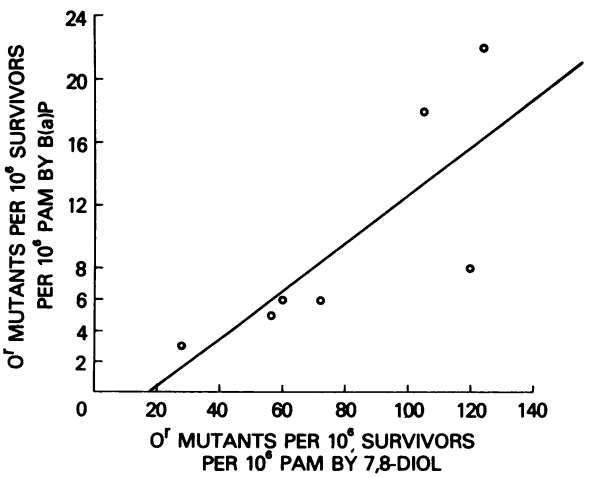

FIGURE 2 Comparison of O^r mutation in V79 cells by B(*a*)P and that by 7,8-diol-mediated with PAM from seven individuals.

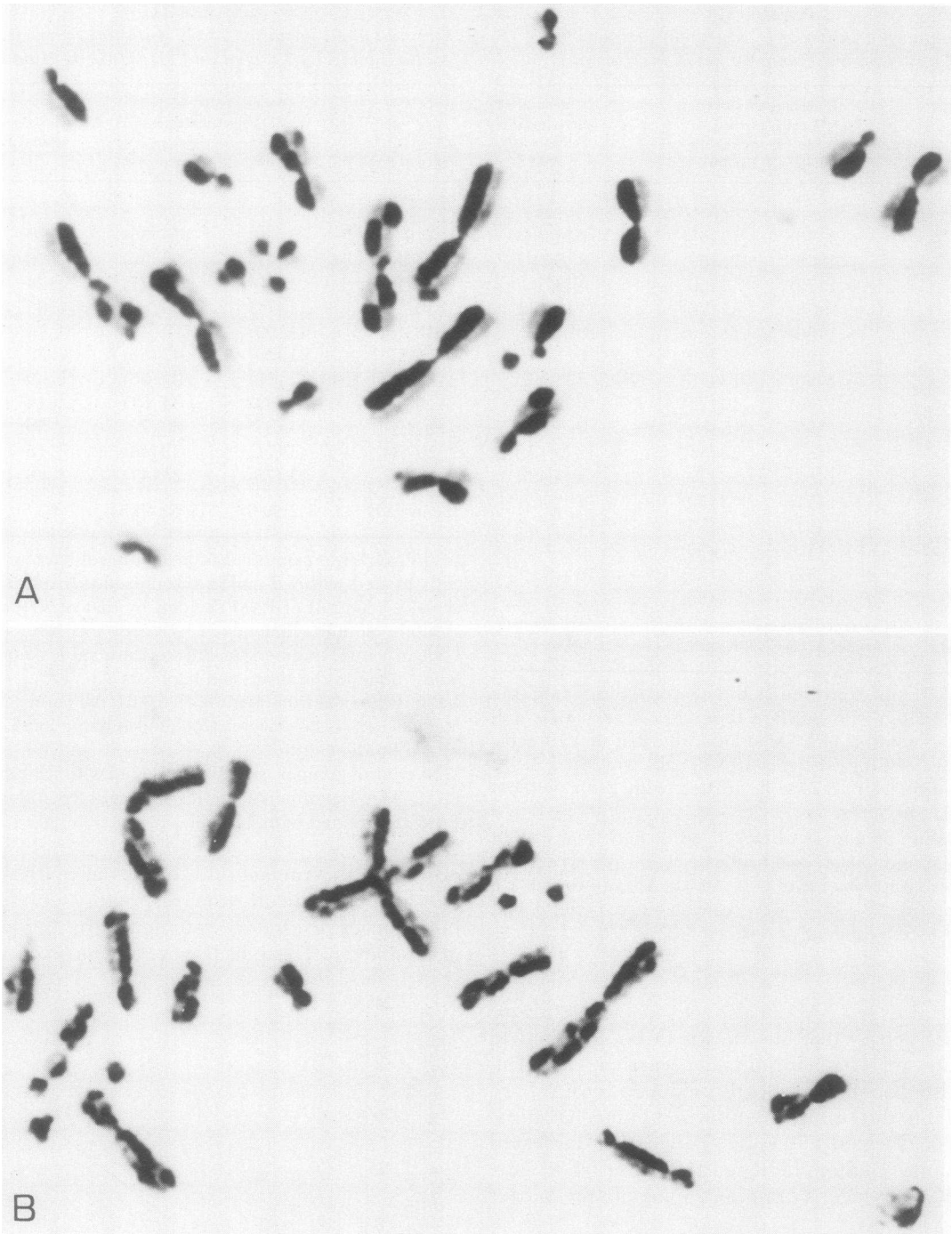

FIGURE 3 SCE in V79 cells treated with 7,8-diol for 70 h (A) without or (B) with the cocultivation of PAM.

hypothesis that PAM carry particulates with adsorbed carcinogens from tobacco smoke up to the respiratory tract and may interact with respiratory epithelium in the causation of respiratory cancer.

Our previous studies (9) have now been extended to test the capabilities of PAM preparations from lung tissues of 20 individuals to mediate O^r mutation. All samples of PAM could mediate O^r mutation and the mutation frequency of V79 cells caused by B(*a*)P was positively correlated with that caused by 7,8-diol in PAM preparations from different individuals. The inter-individual variations caused by B(*a*)P and by 7,8-diol

TABLE IV

*Mutagenesis and SCE in Chinese Hamster V79 Cells by B(a)P or 7,8-Diol and Mediated by Human PAM**

Agents	O^r mutants/10^6 surviving V79 cells	SCE/chromosome‡
Medium control	2	0.50±0.03
B(*a*)P control	<1	0.53±0.03
7,8-Diol control	<1	0.45±0.04
B(*a*)P and PAM	13	0.86±0.08§
7,8-Diol and PAM	47	0.99±0.13§

* PAM was prepared from lung tissue of patient 169C; 1.6 × 10^6 PAM/100-mm dish.

‡ Mean±SE SCE/chromosome.

§ The number of SCE is significantly higher than that of control (Student's *t* test, $P < 0.01$).

in the PAM-mediated mutation assay were approximately ninefold. The variation caused by experimental methodology was minimal. In addition, PAM had been cultivated for 7–8 d before use to minimize metabolic changes caused by exogenous factors, e.g., tobacco smoking and drug treatments, in the donors; chemical constitutents from cigarette tar were found to induce AHH activity in PAM (5, 23, 24).

Although PAM-mediated mutagenicity has not been compared previously among individuals, both AHH activity and B(*a*)P binding to DNA in PAM have been reported. A sixfold variation in AHH activity and a ninefold variation in binding levels of B(*a*)P binding to DNA were noted in PAM from 13 individuals (7).

In addition to the increase in O^r mutation frequency, an increase in the number of SCE by either B(*a*)P or 7,8-diol after their activation by PAM was observed in V79 cells. Perry and Evans (17) showed that the number of SCE per cell is a more sensitive indicator of exposure to mutagens than is the number of visible chromosomal aberrations. The relationship of mutation rate and frequency of SCE has been examined with three different types of DNA damaging agents by Carrano et al. (25). They found that in Chinese hamster ovary cells there is a linear relationship between the induction of SCE and of mutation. In our PAM-mediated assay system, 7,8-diol caused a higher number of both SCE and O^r mutations than did B(*a*)P. When the metabolic activation of 7,8-diol was inhibited by 7,8-benzoflavone, an inhibitor of AHH (19), the frequencies of SCE and of O^r mutations were near normal levels. Thus, our results are consistent with the notion that the number of SCE may be directly related to genetic damage and mutation rate (26).

TABLE V

*Effect of 7,8-Benzoflavone on Frequencies of Both Mutagenesis and SCE in V79 Cells Caused by Either B(a)P Diol Epoxide I or 7,8-Diol and Mediated by Human PAM**

Group‡	O^r mutants/10^6 surviving V79 cells	SCE/chromosome§
7,8-Diol control	1	0.51±0.03
7,8-Diol and PAM	91	1.01±0.08‖
7,8-Diol, PAM and 7,8-Benzoflavone	1	0.62±0.06‖
B(*a*)P Diol epoxide I¶	25	0.56±0.05
B(*a*)P Diol epoxide I and 7,8-benzoflavone	26	0.60±0.07

* PAM was prepared from tissue of patient 183A.

‡ Concentrations of 7,8-diol, 7,8-benzoflavone, and diol B(*a*)P epoxide I are 1, 1, and 0.05 μg/ml, respectively.

§ Mean±SE.

‖ 7,8-Benzoflavone significantly divert the effects caused by 7,8-diol Student's *t* test, $p < 0.01$).

¶ V79 cells were exposed to medium containing diol epoxide I for 1 h; the 1st h of the 70-h cocultivation.

When the increase in SCE (100% increase over control in 7,8-diol-treated group and 60% increase over control value in B(*a*)P-treated group) was compared to the frequency of O^r mutation (10- to 100-fold increase over control in the 7,8-diol-treated group and 2- to 10-fold increase in the B(*a*)P-treated group) it appears that O^r mutation frequency was a better indicator of PAM metabolic activation than was SCE frequency. In an experiment with a direct-acting ultimate mutagen (carcinogen), B(*a*)P diol epoxide I (27), a one- to twofold increase in SCE frequency was observed when bromodeoxyuridine was added 2 h after the exposure of V79 cells.[2] However, when bromodeoxyuridine was added 24 h after the exposure to B(*a*)P diol epoxide I, which has a half-life of <5 min, the increase in frequency of SCE was only 20–30% over the control value.[3] The SCE frequency of B(*a*)P diol epoxide I-treated group in Table V is near the control value because bromodeoxyuridine was added to V79 cells 72 h after the initiation of B(*a*)P diol epoxide I treatment. This finding suggests that SCE may be repaired if bromodeoxyuridine is not added to cells soon after the treatment and that the increased frequency of SCE in V79 cells by the active metabolites may be partly quenched by the cellular recovery during prolonged cocultivation period with PAM. This may be an explanation for a much greater enhancement of O^r mutation frequency than on SCE frequency in the PAM-mediated assay.

In conclusion, PAM enzymatically activated B(*a*)P and 7,8-diol, and released metabolites to the cocultivated

[2] Hsu, I. C., G. T. Bowden, and C. C. Harris. 1979. A comparison of cytotoxicity, ouabain-resistant mutation, sister chromatid exchanges, and nascent DNA synthesis in Chinese hamster cells treated with dihydrodiol epoxide derivatives of benzo-[*a*]pyrene. *Mutat. Res.* In press.

[3] Bowden, G. T., I. C. Hsu, and C. C. Harris. 1979. The effect of caffeine on cytotoxicity, mutagenesis, and sister chromatid exchanges in Chinese hamster V79 cells treated with dihydrodiol epoxide derivatives of benzo[*a*]pyrene. *Mutat. Res.* In press.

V79 cells which showed increases in the number of SCE and of O^r mutants. The ability of PAM to mediate O^r mutation varied among PAM preparations from different individuals. PAM may play a role in the etiology of lung diseases caused by environmental chemicals and may in part determine an individual's susceptibility to these chemicals by virtue of differential abilities to metabolize them to their active forms.

ACKNOWLEDGMENTS

The authors are grateful to Dr. U. Saffiiotti for his advice and encouragement. The technical assistance of G. Myers, F. Jackson, and D. Preston were helpful. We also thank L. De Leonibus and M. Bellman for their secretarial assistance.

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