Table 2 Effect of dose of B(a)P and 7,8-diol on O^r mutation frequency in V-79 cells mediated by PAM

Agent	Group*	Dose (μg ml $^{-1}$)	Cloning Efficiency (% control)	O^r mutants/ total dishes	O^r mutants/ 10^6 survivors	O^r mutants/ 10^6 survivors/ 10^6 PAM
Control		—	100	2/16	1	—
7,8-diol	C	1.0	97	6/13	3	—
	E	0.1	101	16/15	15	10
	E	0.5	91	71/15	70	46
	E	1.0	92	92/17	110	73
B(a)P	C	2.0	109	5/17	3	—
	E	0.2	87	3/14	2	2
	E	1.0	65	11/20	7	5
	E	2.0	60	7/12	7	5

*C, Control (V-79 cells incubated with either 7,8-diol or B(a)P and without human PAM); E, experimental (V-79 cells incubated with either 7,8-diol or B(a)P and with human PAM (patient 165). Cloning efficiency compared with that observed when V-79 cells were incubated without both chemical agent and PAM); the absolute cloning efficiency for V-79 cells cultivated without both PAM and chemical agents was 81.2%.

Geimsa for detection of O^r mutants. The O^r mutants have: the karyotype of V-79 cells; a stable phenotype for O^r after more than 20 cell divisions in medium without ouabain; and functional ATPase activity, as the rate of ^{86}Rb uptake in the presence of 1 mM ouabain is as expected (ref. 9 and Hsu *et al.*, unpublished).

The mutation frequencies for O^r in V-79 cells co-cultivated with PAM and B(a)P, B(e)P, or 7,8-diol are shown in Table 1. The mutation frequency is directly dependent on the number of PAM added to the V-79 cells in medium containing either B(a)P or 7,8-diol. B(e)P, the non-carcinogenic analogue of B(a)P, was not mutagenic. Cocultivation of V-79 cells with PAM but without B(a)P, B(e)P or 7,8-diol did not alter the O^r spontaneous mutation frequency of V-79 cells (less than two O^r mutants/10^6 surviving V-79 cells). An increase in concentration of either B(a)P or 7,8-diol enhanced O^r mutation frequencies (Table 2). Compared with B(a)P, 7,8-diol was a more potent promutagen. PAM also metabolised B(a)P to 7,8-diol which was released by PAM into the culture medium (Fig. 2)[3].

The clearance of inhaled particulates with adsorbed chemical carcinogens may be impaired by tobacco smoke, sulphur dioxide, anticholinergic drugs, chronic bronchitis and respiratory infections[10]. These particulates may also have a prolonged residence time in areas of squamous metaplasia because ciliary activity is interrupted in these areas. PAM phagocytose particulates and are also considered to play an important role as a host defence against inhaled foreign material[11]. The data presented here suggest that they can also metabolise B(a)P to proximate and ultimate mutagens, which are then released into the extracellular space. The proximate mutagenic and carcinogenic form of B(a)P, 7,8-diol (ref. 12), can enter into cultured bronchial explants from the medium and has been shown to be more effectively metabolised and bound to DNA in the bronchial mucosa than the 4,5-diol, 9-10-diol and the parent compound, B(a)P (ref. 13). The intimate contact between PAM and bronchial epithelium during clearance by the mucociliary transport of particulates containing chemical carcinogens within PAM suggests the possibility that PAM and bronchial epithelium may act in concert to metabolically activate chemical carcinogens during bronchogenic carcinogenesis.

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1. Cantrell, E. T., Warr, G. A. & Martin, R. R. *J. clin. Invest.* **52**, 1881-1884 (1973)
2. Dehnen, W. *Zentralbl. Bakteriol.* (Orig. B) **161**, 1-19 (1975).
3. Autrup, H. *et al. Lab. Invest.* (in press).
4. Huberman, E. & Sachs, L. *Int. J. Cancer* **13**, 326-333 (1974).
5. Chu, E. H. Y. & Malling, H. V. *Genetics* **61**, 1306-1312 (1968).
6. Huberman, E., Sachs, L., Yang, S. K. & Gelboin, H. V. *Proc. natn. Acad. Sci. U.S.A.* **73**, 607-611 (1976).
7. Harris, C. C. *et al. Cancer Res.* **37**, 3349-3356 (1977).
8. Selkirk, J. K., Gray, R. G., Roller, P. P. & Gelboin, H. V. *Cancer Res.* **34**, 3474-3480 (1974).
9. Mankowitz, R., Buchwald, M. & Barker, R. M. *Cell* **3**, 221-226 (1974).
10. Wanner, A. *Am. Rev. resp. Dis.*, **116**, 73-122 (1977).
11. Brain, J. D., Proctor, D. F. & Reid, L. M. (ed.) *Respiratory Defense Mechanism*, Part II (Marcel Dekker, New York, 1977).
12. Kapitulnik, J., Levin, W., Conney, A. H., Yagi, H. & Jerina, D. M. *Nature* **266**, 378-380 (1977).
13. Yang, S. K., Gelboin, H. V., Trump, B. F., Autrup, H & Harris, C. C. *Cancer Res.* **37**, 1210-1215 (1977).

Enhancement of carcinogenesis by prostaglandins

INCREASED amounts of prostaglandins, especially prostaglandin (PG) E_2 (PGE_2), have been detected in human cancer tissue as well as in cultured mouse fibrosarcoma cells[1]. Elevated prostaglandin synthetase activity has also been found in microsomal fractions of transformed cells from methylcholanthrene-treated mice[2]. In investigations of the role of PGE_2 and $PGF_{2\alpha}$ on skin tumours induced by 3-methylcholanthrene (MCA) in mice, I report here evidence that these prostaglandins can act as cocarcinogens.

The experiments were carried out on male albino Swiss mice weighing between 20 and 25 g. They were divided into six groups of 20 mice as follows: (1) mice which received only the diluent and served as controls; (2) mice treated topically with 0.2 ml of a 0.4% acetone solution of MCA in a marked region of the shaved dorsal skin, three times a week for 2 months; (3) mice treated with MCA as above and injected intramuscularly (i.m.) concomitantly with 10 μg of PGE_2 three times weekly for 2 months; (4) mice treated with MCA and injected i.m. concomitantly with 10 μg $PGF_{2\alpha}$ three times weekly for 2 months. Groups 5 and 6 were treated only with PGE_2 and $PGF_{2\alpha}$, respectively, as above. At the end of 2 months and 2 h before killing, 5 mice from each group received i.m. an injection of 10 μCi per g body weight ^{3}H-thymidine, for the study of DNA synthesis; another 5 mice from each group were injected i.m. with 10 μCi per g body wt ^{3}H-uridine, for the study of RNA

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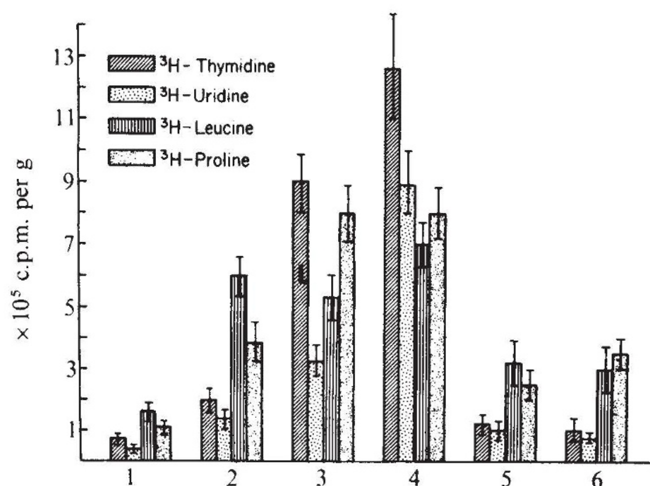

Fig. 1 Radioisotope incorporation at 2 h in the control mouse epidermis (1), following MCA (2), MCA + PGE_2 (3), MCA + $PGF_{2\alpha}$ (4), PGE_2 (5) and $PGF_{2\alpha}$ (6). The vertical bars at the top of each column represent the standard errors of the mean (mean ± s.e.).

synthesis; 5 other mice from each group received 10 μCi per g body wt ^{3}H-leucine, for the study of protein synthesis, and the last 5 mice from each experimental group were injected i.m. with 10 μCi per g body wt ^{3}H-proline, for the study of collagen synthesis. The period of 2 h before death was selected for the isotope studies, because previous experiments had shown that PGs exert their maximum effects on cell structure and metabolism in that time[3]. All radioisotopes were injected at the same time as PG and MCA administration. Radioactivity measurements were made using a nuclear liquid scintillation counting system (efficiency 40%), using (^{3}H) as internal standard; the skin specimens were trimmed, the subcutaneous fat and dermis were removed and the epidermis was homogenised with a Potter–Elvehjem tissue grinder and transferred to vials with scintillation fluid. Results were expressed as counts per minute (c.p.m.) and per g of homogenates (mean ± s.e.). For light microscopy and autoradiography, the specimens were fixed in Bouin's fluid, dehydrated and embedded in paraplast; sections 5 μm thick were stained with haematoxylin and eosin and examined under a light microscope. For autoradiography alone, the sections were covered with Ilford Nuclear Emulsion K_5 (diluted with distilled water 1:1) for 7 d, after which they were developed in D_{19}, fixed and washed, and stained with haematoxylin and eosin.

Multiple and large tumours, sometimes necrotic and haemorrhagic in the centre, occurred after 2 months in almost 90% of both the MCA plus $PGF_{2\alpha}$- and MCA plus PGE_2-treated mice at the treatment site. No tumours were observed in MCA-treated, PG-treated or in control mice (Table 1) and no toxic or other adverse reactions occurred. Investigations with radioisotopes revealed a marked increase (15–20-fold) of ^{3}H-thymidine, followed by ^{3}H-uridine, ^{3}H-proline and ^{3}H-leucine in the homogenised material from tumours of MCA plus $PGF_{2\alpha}$ and MCA plus PGE_2-treated mice, compared with those from MCA-treated, PG-treated or control epidermis (Fig. 1).

Histopathological examination showed a characteristic pattern of epidermal cells in control mouse skin (Fig. 2*a*). Epidermal cell hyperplasia with papillary projections protruding in the dermis, are visible in MCA-treated mice alone (Fig. 2*b*). Several invading epidermal masses in the dermis with a tendency to horn pearl formation are predominant in MCA plus $PGF_{2\alpha}$-treated mice. These are squamous cell carcinomas (Fig. 2*c*) and they occurred in almost 90% of MCA plus $PGF_{2\alpha}$-treated mice (see Table 1). At higher magnification, the tumour masses are composed of neoplastic squamous cells arranged in a palisading pattern. A mild dermal infiltration always accompanied the tumours. Few papillomas were seen in MCA plus PGE_2-treated mice and only a moderate epidermal hyperplasia occurred in PG-treated mice. Light microscopic autoradiography revealed an increased (5–6-fold) distribution of the heavily ^{3}H-thymidine labelled nuclei in the neoplastic cells following MCA plus $PGF_{2\alpha}$ administration, compared with that seen in controls or in only MCA-treated mice (Fig. 2*d*).

The present findings demonstrate that $PGF_{2\alpha}$ and PGE_2 enhanced the transformation of hyperplastic epidermal cells of MCA-treated mice to neoplastic cells and thus markedly shortened the latency period to tumour formation, in a manner similar to that of cocarcinogens[4]. The investigations using MCA application revealed that the tumours occurred only after 4 months, and mostly between 5 and 6 months; the PGs therefore decreased (or shortened) the latent period of tumour induction (2 months) by at least 65%. It is possible that PGs which are hormone-like substances, enhance tumour formation, as do other hormones such as oestrogens, prolactin and thyroid stimulating hormone (TSH) (ref. 5), all of which stimulate mammary and thyroid hyperplasia of mammary and thyroid tumours. These agents are not carcinogenic by themselves; PGs administered alone for the same period (groups 5 and 6) induced only a moderate epidermal cell hyperplasia, but their effects are similar to those of other cocarcinogens. It has been suggested that naturally occurring prostaglandin agonists include some cathartics (rhein, emodin) and the cocarcinogens anthralin and phorbol myristate acetate, and thus that PGs themselves might be cocarcinogens[6]. PG antagonists exhibit an anti-tumour activity. Human malignant breast tumours contain and synthesise more PG-like materials than normal breast tissue from the same patients or than do benign tumours[7]. In patients with breast carcinomas, osteolytic activity and bone metastases, a high PG activity ($PGF_{2\alpha}$, PGE_2) has been detected *in vitro*[8]. These findings suggest that $PGF_{2\alpha}$ and PGE_2 are important in the development of primary tumours and that they can influence the evolution of primary tumour metastases. $PGF_{2\alpha}$ added to Swiss mouse fibroblast cultures initiates DNA synthesis and cell proliferation. Insulin potentiates the effect of $PGF_{2\alpha}$ and DNA synthesis and cell division[9]. The present experiments also demonstrated that $PGF_{2\alpha}$ and PGE_2 markedly stimulate the DNA, RNA and protein synthesis in hyperplastic epidermal cells following MCA application. This could explain the enhancement of cutaneous carcinogenesis and shortening of the latent period. Electron microscopy has shown that during the latent period only preneoplastic changes resembling those of Bowen's disease are present in rat epidermis[10]. Thus, the present

Table 1 The incidence of skin tumours in mice following MCA and prostaglandin (PGE_2 and $PGF_{2\alpha}$) administration

Group	Treatment	Time (months)	No. of mice	Epithelial hyperplasia	Papillomas	Carcinomas	% of Tumours
1	Controls + diluent	2	20	0	0	0	
2	MCA + diluent	2	20	20	0	0	
3	MCA + PGE_2	2	20	2	2	16	90%†
4	MCA + $PGF_{2\alpha}$	2	20	0	1	19	100%†
5	PGE_2	2	20	18*	0	0	
6	$PGF_{2\alpha}$	2	20	18*	0	0	

The data presented are based on counts of tumours visible to the naked eye, as well as on diagnosis made by light microscopy.
*Moderate epidermal hyperplasia.
†Papillomas and carcinomas.

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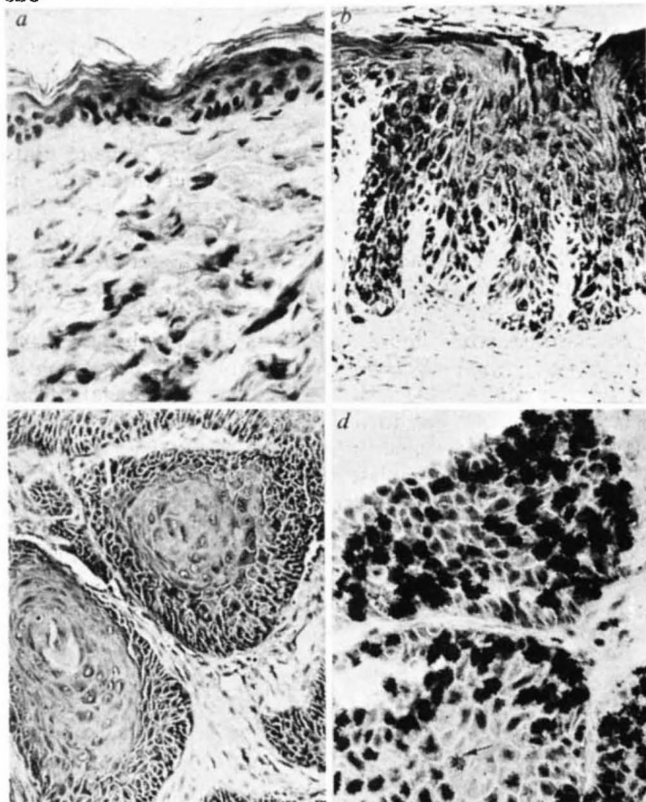

Fig. 2 *a*, Control mouse skin (haematoxylin and eosin, ×200); *b*, MCA-treated mouse skin. Epidermal cell hyperplasia with papillary projections in the dermis (haematoxylin and eosin, ×200); *c*, MCA + $PGF_{2\alpha}$-treated mouse. Large and invading tumour masses with a tendency to horn pearl formation are predominant (squamous cell carcinoma); mild dermal infiltration. (haematoxylin and eosin ×200); *d*, light microscopic autoradiogram of a MCA + $PGF_{2\alpha}$-treated mouse showing a heavy autoradiographic reaction of the neoplastic cells in a squamous cell carcinoma. Some dividing cells and their chromosomes are labelled with ^{3}H-thymidine (arrow) (haematoxylin and eosin ×400).

findings indicate that PGs can act as cocarcinogens and have important implications in cutaneous carcinogenesis.

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1. Jaffe, B. *Prostaglandins* **6**, 453–461 (1974).
2. Hong, S., Wheless, C. & Levine, L. *Prostaglandins* **13**, 271–279 (1977).
3. Lupulescu, A. *Prostaglandins* **10**, 573–579 (1975).
4. VanDuuren, B. in *Chemical Carcinogens* (ed. Searle, C) 24–51 (Am. Chem. Soc., Washington, D.C. 1976).
5. Furth, J. *Fedn Proc.* **20**, 865–873 (1961).
6. Smythies, J., Benington, F. & Morin, R. *Psychoneuroendocrinology* **1**, 123–130 (1975).
7. Bennett, A., Simpson, J., McDonald, A. & Stamford, I. *Lancet* **i**, 1218–1220 (1975).
8. Powles, T., Dowsett, M., Easty, G., Easty, D. & Neville, A. *Lancet* **i**, 608–610 (1976).
9. Jimenez de Asua, L., Clingan, D. & Rudland, P. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2724–2728 (1975).
10. Lupulescu, A. & Pinkus, H. *Oncology* **33**, 24–28 (1976).

Enhanced agglutination of all the erythrocytes when only half are trypsinised

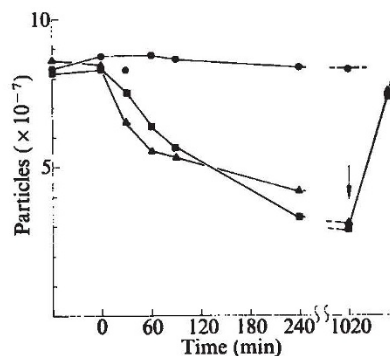
ENHANCED lectin-mediated agglutination following alteration of cells by transformation or protease treatment is well documented[1,2]. However, the mechanism underlying these observations remains obscure. Recent evidence suggests that enhanced agglutinability of erythrocytes treated with proteolytic enzymes, neuraminidase or both, may be the result of reduced surface charge and/or loss of sterically hindering peptides and glycopeptides[3]. We wondered whether the alteration in a part of the cell population would be sufficient to enhance the agglutinability of the entire cell population with concanavalin A (con A) and soybean agglutinin (SBA). We report here that the extent, and to a slightly lesser degree, the rate of lectin-mediated agglutination of untrypsinised erythrocytes mixed with equal volumes of trypsinised erythrocytes closely parallel the values obtained for trypsinised cells alone. We conclude that the proteolytic alteration of only some of the erythrocytes is sufficient to promote enhanced lectin-mediated agglutination of all cells when mixed with equal numbers of native cells. A similar phenomenon holds true for native cells when mixed with neuraminidase-treated cells.

Human erythrocytes were obtained from fresh blood. After removing serum and buffy coat, erythrocytes were washed four times with 10-fold volumes of phosphate-buffered saline (PBS), *p*H 7.4. Following trypsinisation (0.1 mg ml^{-1}, Sigma) of 4% (v/v) erythrocyte suspensions in PBS for 60 min at 37 °C, the cells were washed three times in PBS with or without 0.1 mg ml^{-1} soybean trypsin inhibitor (Sigma) in the first wash. Some cells were treated with trypsin in the presence of trypsin inhibitor in order to test for effects not attributed to proteolytic activity. Residual tryptic activity in the final washes of normal, trypsinised cells, and trypsinised cells washed with inhibitor, were twice tested by digestion of casein[4] and a synthetic substrate[5]. Cells were treated with neuraminidase (Fig. 3, *Vibrio cholerae*, Behringwerke and washed three times.

Con A was purchased from Miles-Yeda; SBA and peanut agglutinin were purified by affinity chromatography as described elsewhere[6,7]. The kinetics of agglutination[8] were followed on a Cytograf Model 6301 electronic cell counter (Bio/Physics, Mahopac). Briefly, 10 ml of a 2% erythrocyte suspension (~10^8 cells ml^{-1}) in solutions of lectin in PBS at room temperature were agitated in scintillation vials on a New Brunswick gyrotary shaker at 75 r.p.m. Mixed agglutination was usually accomplished by mixing equal volumes of 2% suspensions of trypsinised and untrypsinised erythrocytes in PBS and adding the lectin. Samples (50 µl) were diluted 1 : 200 with PBS and counted immediately at non-agglutinated levels of about 40,000 particles per 0.1 ml. Replicate readings on duplicate dilutions for each time point were averaged.

The results obtained using 50 µg con A per ml of 2% erythrocyte suspension are shown in Fig. 1. Untrypsinised cells and cells treated with trysin in the presence of soybean trypsin inhibitor (1 : 1 with trypsin) showed no agglutination. Cells treated with trypsin and then washed with or without trypsin inhibitor agglutinated within 15 min and reached equilibrium after 4–5 h. What is remarkable is that untrypsinised erythrocytes agglutinate when mixed with trypsinised cells as well as the trypsinised cells alone, even when the trypsinised cells were first washed with trypsin inhibitor. The agglutination was readily and specifically reversed by addition of 20 mM methyl α-D-mannopyranoside to the reaction mixture (arrow).

Fig. 1 The kinetics of erythrocyte agglutination using 50 µg con A per ml. ●, Untrypsinised erythrocytes; ■, trypsinised erythrocytes: ▲, mixed population of equal volumes of trypsinised and untrypsinised erythrocytes. After 17 h α-methyl-mannoside was added to a final concentration of 20 nM (arrow).



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