and inhibited that in all type II fibres of the gastrocnemius muscle (figure 4).

This study has shown clearly that reciprocity of the ATPase reaction as displayed by type I fibres of the gastrocnemius muscle was absent in red fibres of the pectoralis major. The present results have substantiated that unlike the typical type I fibres, red fibres of pigeon pectoralis display high ATPase reaction, as do the type II red and type II white muscle fibres. Since the speed of contraction of a given muscle is directly proportional to its myosin ATPase content[10-13], and the myofibrillar ATPase of slow muscle is alkali-labile[15-17], it is generally assumed that individual muscle fibres with low ATPase reaction, histochemically, are slowtwitch; conversely muscle fibres showing high ATPase reaction are fast-twitch[14].

The avian[18] or mammalian[10,14,19,21] type I muscle fibres of mixed muscles or slow-twitch muscles[19] exhibit the characteristically activated ATPase reaction following acidic preincubation. The red fibres of pigeon pectoralis do not share this important property. By virtue of the presence of high ATPase and its acid lability, greater number of mitochondria, high SDH[2], and focal en plaque pattern of nerve endings[22], the red fibres of pigeon pectoralis are possibly type II red, i.e. not slow-twitch but fatigue resistant. There may be a spectrum[20] of fastness in the different sub-populations[10,18,21] of this particular variety of muscle fibres. Thus labelling red fibres of the pectoralis muscle as type I or slow is not justified[3-7].

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Effect of prostaglandins on skin tumorigenesis

A. Lupulescu

Wayne State University, Medical Research Bldg., 550 East Canfield, Detroit (Michigan 48201, USA), 28 November 1977

Summary. Concomitant administration of prostaglandins E_2 (PGE_2) and $F_2\alpha$($PGF_2\alpha$) with a carcinogen, 3-methylcholanthrene (MCA) to mice for 2 months markedly enhanced the occurrence of squamous cell carcinomas. Only epidermal cell hyperplasia occurred in mice treated with MCA alone by that time. Radioactivity measurements and electron microscopic autoradiography revealed that prostaglandins stimulate DNA, RNA and protein synthesis in neoplastic cells. These findings indicate that PGE_2 and $PGF_2\alpha$ can act as cocarcinogens on skin tumorigenesis.

Recently increased amounts of prostaglandins, especially PGE_2 were detected in human cancers as well as in cultured mouse fibrosarcoma cells[1]. Also an elevated prostaglandin synthetase activity was found in microsomal fractions of transformed cells from methylcholanthrene-treated mice[2]. However, the role of prostaglandins in carcinogenesis is still undetermined. The present report deals with the effect of prostaglandins E_2 (PGE_2) and $F_2\alpha$ ($PGF_2\alpha$) on the chemically induced skin tumors by 3-methylcholanthrene (MCA) in mice.

Material and methods. The experiments were carried out on male albino Swiss mice weighing 20–25 g and which were divided in 6 groups, 20 mice of each as follows: I) Mice which received only the diluent and served as controls; II) Mice topically treated with 0.4% acetone solution of MCA, each mouse received 0.2 ml by pippeting in a marked region of his shaved dorsal skin, 3× weekly for 2 months. III) Mice treated with MCA as above and concomitantly injected i.m. with 10 µg of PGE_2 3× weekly for 2 months. IV) Mice concomitantly treated with MCA and injected i.m. with $PGF_2\alpha$, a dose of 10 µg, 3× weekly for 2 months. Groups V and VI were treated only with PGE_2 and $PGF_2\alpha$ respectively as above. At the end of 2 months and 2 h prior to the sacrifice, 5 mice from each group received i.m. 10 µCi/g b.wt of ^{3}H-thymidine for the study of DNA synthesis; another 5 mice from each group were injected i.m. with 10 µCi/g b. wt of ^{3}H-uridine for the study of RNA synthesis; other 5 mice from each group received 10 µCi/g b.wt of ^{3}H-leucine for the study of protein synthesis and another 5 mice from each experimental group received i.m. 10 µCi/g b.wt of ^{3}H-proline for the study of collagen synthesis. We selected 2 h for the isotope studies, because we found in previous experiments that prostaglandins (PG) exert their maximum effects on cell metabolism in that time[3]. Radioactivity measurements were performed with Nuclear Liquid Scintillation Counting System, efficiency 40% using (^{3}H) as internal standard; the skin specimens were trimmed, the s.c. fat and dermis were removed and the epidermis was homogenized with a Potter-Elvehjem tissue grinder and transferred in vials with scintillation fluid. Results were expressed as cpm and per g of homogenates (mean±SE). For light microscopy and autoradiography, the specimens were fixed in Bouin's fluid, dehydrated and embedded in paraplast; the 5-µm thin sections were stained with hematoxylin and eosin and examined under light microscope. For EM autoradiography, small specimens from tumors were diced, fixed in 2.5% phosphate glutaraldehyde, then postfixed in 1% phosphate buffered 0_s0_4, dehydrated and embedded in a mixture of eponaraldite. Thin sections were covered with Ilford Nuclear Emulsion L_4 using a wire loop procedure[4] for 6–8 weeks, then developed in Microdol-X, fixed, washed and stained with uranyl acetate and lead citrate.

Observations and discussion. Multiple and large tumors, sometimes necrotic and hemorrhagic in the center, occurred after 2 months in almost 90% in the MCA and $PGF_2\alpha$ or

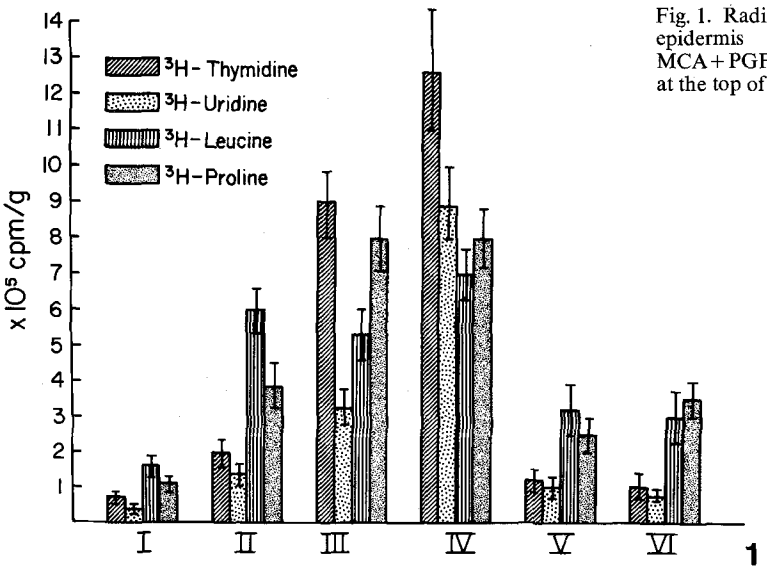
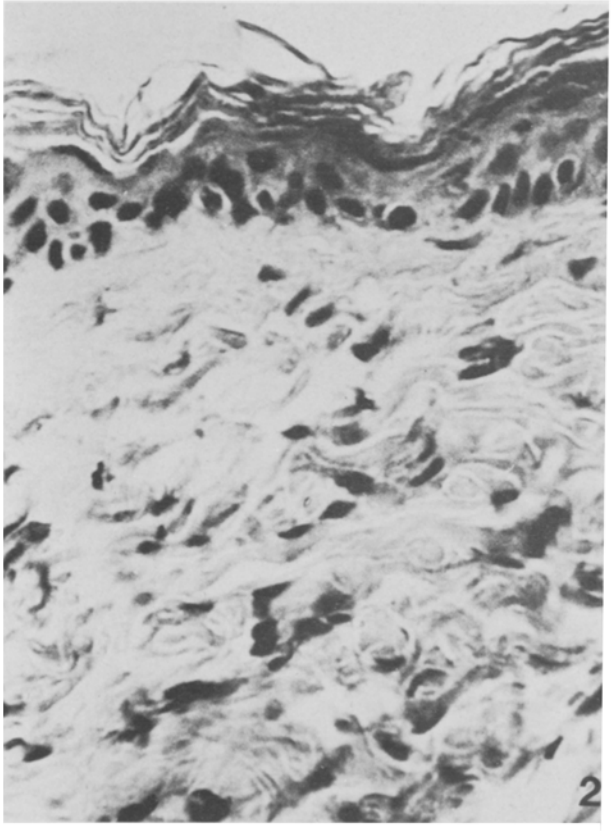


Fig. 1. Radioisotope incorporation at 2 h in the control mouse epidermis (I), following MCA (II), MCA + PGE_2 (III), MCA + $PGF_2\alpha$ (IV), PGE_2 (V) and $PGF_2\alpha$ (VI). The vertical bars at the top of each column represent means ± SE.

PGE_2-treated mice at the site of treatment. No tumors were observed in MCA-treated alone, PG-treated or in control mice (table) 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 homogenized material from tumors of MCA-$PGF_2\alpha$ and PGE_2-treated mice as compared to those of MCA-treated alone, PG-treated or controls (figure 1).

The histopathological examination showed a characteristic pattern of epidermal cells in control mouse skin (figure 2). Epidermal cell hyperplasia with papillary projections protruding in the dermis, are visible in MCA-treated mice alone (figure 3). Several invading epidermal masses in the dermis with a tendency to horn pearl formation are predominant in MCA-$PGF_2\alpha$ or PGE_2-treated mice. These are squamous cell carcinomas (figure 4) and occurred in almost 90% of PG and MCA-treated mice (table). A mild dermal infiltration always accompanied the tumors. Few papillomas were seen in MCA and PG-treated mice and only a moderate epidermal hyperplasia occurred in PG-treated mice. Electron microscopic autoradiography revealed an



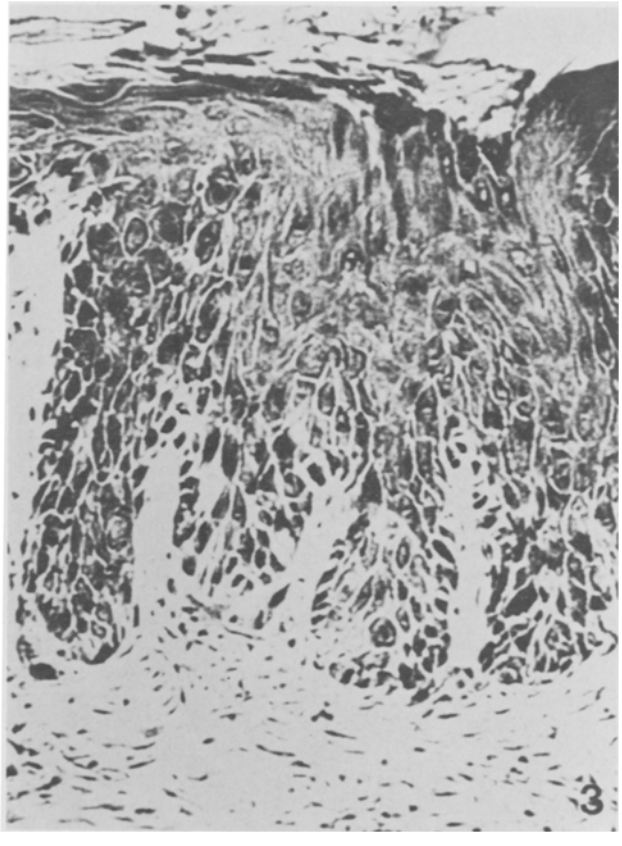
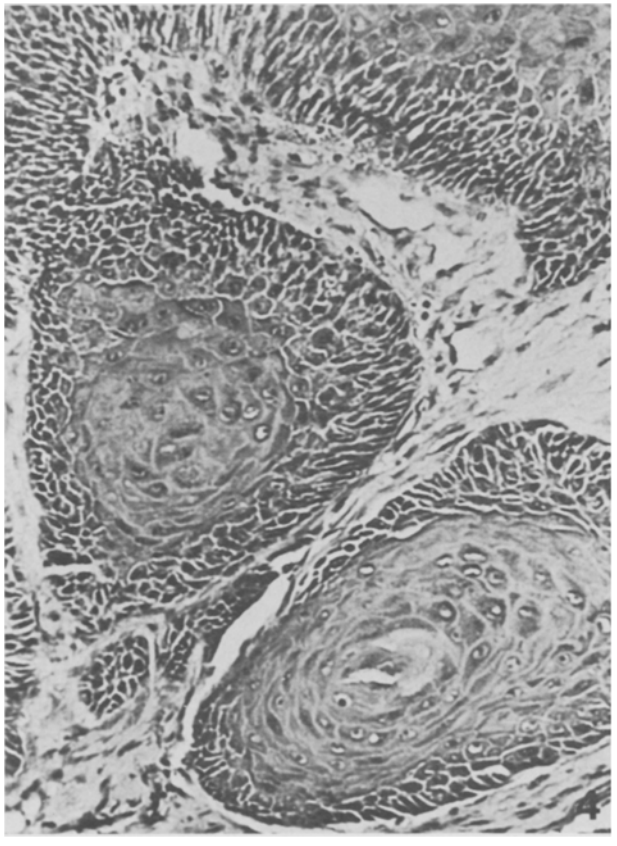


Fig. 2. Control mouse skin. Hematoxylin and eosin. × 200.

Fig. 3. MCA-treated mouse skin. Epidermal hyperplasia with papillary projections in the dermis. Hematoxylin and eosin. × 200.



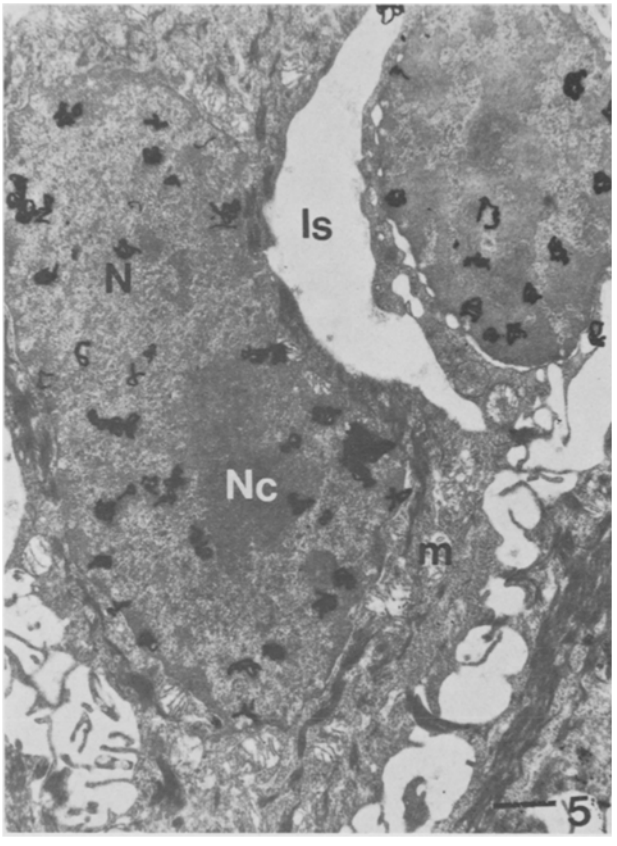


Fig.4. MCA + $PGF_2\alpha$-treated mouse. Large and invading tumoral masses with a tendency to horn pearl formation are predominant (squamous cell carcinoma); mild dermal infiltration. Hematoxylin and eosin. × 200.
Fig.5. Electron microscopic autoradiogram of neoplastic cells (squamous cell carcinoma) from MCA + $PGF_2\alpha$-treated mouse showing a heavy ^{3}H-thymidine incorporation over the enlarged nuclei (N), near the nucleolus (Nc); no reaction is seen over mitochondria (m) or intercellular space (Is). × 8.400.

The incidence of skin tumors in mice following MCA and prostaglandins (PGE_2 and $PGF_2\alpha$) administration

Group	Treatment	Time	No. of mice	Epithelial hyperplasia	Papillomas	Carcinomas	% of tumors
I	Controls + Diluent	2 months	20	0	0	0	
II	MCA + Diluent	2 months	20	20	0	0	
III	MCA + PGE_2	2 months	20	2	2	16	90
IV	MCA + $PGF_2\alpha$	2 months	20	0	1	19	100
V	PGE_2	2 months	20	18*	0	0	
VI	$PGF_2\alpha$	2 months	20	18*	0	0	

The data presented in this table are based on counts of tumors visible to the naked eye as well as of diagnosis made by the light microscopy.
* Moderate epidermal hyperplasia.

increased (5–6 fold) distribution of ^{3}H-thymidine over the enlarged nuclei in the neoplastic cells following MCA and $PGF_2\alpha$ administration as compared to that seen in controls, or that in MCA-treated alone (figure 5).

The present findings demonstrated that prostaglandins $F_2\alpha$ and PGE_2 markedly enhanced the onset and development of skin tumors in MCA-treated mice by shortening the latency period of tumor formation, similarly to that of cocarcinogens[5]. It is also possible that prostaglandins which are hormone-like substances, enhance the tumor formation similarly to other hormones, such as estrogens, prolactin, thyroid stimulating hormone (TSH)[6], or epidermal growth factor (EGF)[7] which stimulate mammary, thyroid or skin hyperplasia to mammary, thyroid or skin tumors. These agents are not carcinogenic by themselves; prostaglandins administered alone for the same period of time (groups V, VI) induced only a moderate epidermal hyperplasia, but their effect are similar to that of other cocarcinogens. It was also found that $PGF_2\alpha$ added to Swiss mouse fibroblast cultures initiates DNA synthesis and cell division[8]. 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 and this can explain the enhancement of cutaneous carcinogenesis and shortening of the latent period. With the electron microscope was shown that during the latent period (latency) only preneoplastic changes resembling those of Bowen's disease are present in rat epidermis[9]. Thus the present findings indicate that prostaglandins may act as cocarcinogens and have important implications in cutaneous carcinogenesis.

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Hepatoma induction in the rat by the subcutaneous administration of powdered 3′-methyl-p-dimethylaminoazobenzene

C. Carruthers, A. Baumler and S. Pauly

Orchard Park Laboratories, Roswell Park Memorial Institute, 666 Elm Street, Buffalo (New York 14263, USA), 31 October 1977

Summary. A relatively safe and simple procedure was developed for the induction of hepatomas in the rat by the s.c. administration of powdered 3′-methyl-p-dimethylaminoazobenzene.

Hepatomas in rat liver are induced by the incorporation of azocarcinogens into semisynthetic diets[1-3]. Since the azocarcinogens were placed on the restricted list in the United States by the Federal Government[4], special facilities are required for the production of diets containing carcinogens. For investigators who do not have special facilities, a relatively simple and safe procedure is needed for the induction of liver tumors in the rat by azocarcinogens. This paper briefly describes such a method.

Material and methods. Inbred male Fischer rats (Charles River Breeding Laboratories, Wilmington, Massachusetts) 6–8 months old and weighing about 300 g were used. The azocarcinogen, 3′-methyl-p-dimethylaminoazobenzene (3′-Me-DAB) in powder form was purchased from Eastman Organic Chemicals, Rochester, N.Y.

The rats were anesthetized with ether and a 5–6 cm incision was made in the abdominal skin in a manner that the peritoneum was not damaged. The skin on one side of the incision was lifted and about 100 mg of powdered 3′-Me-DAB was spread evenly under the skin flap. Then the other side of the skin incision was raised and the rest of the 200 mg sample of azocarcinogen was evenly deposited under the skin flap. The skin was then sutured with metal wound clips (Clays Adams, Parsippony, N.Y.). This procedure for the administration of 3′-Me-DAB was carried out every 30 days for a period of 3–7 months unless liver tumors developed. After the administration of 300–600 mg of this azocarcinogen, the rats were examined for abdominal swelling, and if presence of tumors was suspected, laparotomy was carried out under ether anesthesia. If hepatomas were present, the liver was perfused under ether anesthesia with cold borate buffer, pH 8. The tumors and/or adjacent liver were excised and quickly frozen on the quick-freeze attachment of an International Cryostat, model CT1. Portions of the tumor and liver were fixed in formalin, embedded in paraffin, sectioned, and stained with haematoxylin and eosin. If the liver did not show gross pathological changes after laparotomy, the skin was sutured with metal wound clips and the s.c. administration of 3′-Me-DAB was continued after a period of 1–2 weeks.

Results and discussion. The rats tolerated the s.c. application of powdered 3′-Me-DAB very well, and none of this azocarcinogen was visible at the end of each treatment period. In 4 experiment, 67–82% of rats developed liver tumors following the s.c. disposition of 3′-Me-DAB (table). This tumor incidence is somewhat less than that produced by incorporation of azocarcinogens into the diet[2]. The tumors produced by the s.c. administration of 3′-Me-DAB were multiple and mixtures of hepatocellular carcinomas and cholangiocarcinomas such as have been found by the dietary administration of azocarcinogens[1,3,5-7]. The incidence of tumors produced by the s.c. administration of powdered 3′-Me-DAB only once a month averaged 71% whereas that obtained by the weekly s.c. injection of 3′-Me-DAB dissolved in cottonseed oil averaged 47%[7] and that of p-dimethylaminoazobenzene dissolved in olive oil averaged 31%[1] or lower[3]. Therefore, the s.c. procedure for the induction of liver tumors should be useful for investigators who do not have facilities for incorporation of 3′-Me-DAB into the diet. However, for personal safety an isolated laboratory might be needed for the monthly administration to rats of the powdered and potent azocarcinogen, 3′-Me-DAB.

Induction of liver tumors in rats by the s.c. administration of powdered 3′-Me-DAB

Experiment No.	No. of rats	No. of rats with tumors	Dosage of 3′-Me-DAB mg	Time of tumor appearance month	Incidence of tumors (%)
1	6	4	600–1400	3–7	67
2	7	5	800–1200	4–6	71
3	6	4	1200	6	67
4	6	5	600–1200	3–6	82

The induction of hepatomas by the s.c. application of 3′-Me-DAB avoids the problems caused by 1 scattering by rats of carcinogen-treated food out of their cages; 2 leaving disposal of bedding (and carcinogens) to ill-trained animal caretakers; and 3 leaving carcinogens unattended in cages after feeding.

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