limitations, the effects of aristolochic acid in onion root tips were also studied. In the root tip cells the nuclei showed condensation, breakages, laggards and bridge formation in anaphase under the influence of aristolochic acid[8]. Only the examination under high magnification of housefly testicular cells would reveal whether the cytopathological manifestations are the same as in onion root tips, but the possibilities of finding parallel symptoms can not be ignored altogether. Chromosomal aberrations like breakages, acentric fragments, fused chromosomes etc. are also induced by tepa in *Culex pipiens fatigans*[9].

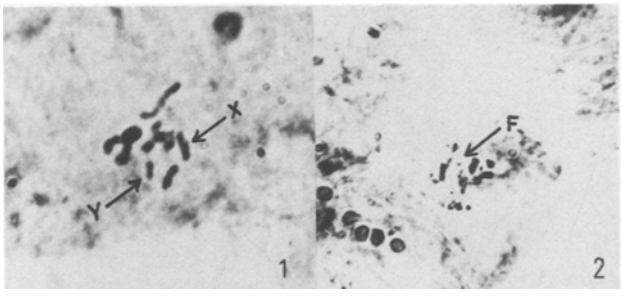

Fig. 1. Diakinesis in the normal spermatogonial cell, showing 2n=12 [5×II+1 X+1 Y] chromosomes. Fig. 2. Metaphase in treated spermatogonial cell showing high fragmentation (F) of chromosomes. ×2500.

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Heavy incorporation of ^{3}H-prostaglandin $F_{2\alpha}$ in the neoplastic cells as revealed by autoradiographic studies

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Summary. A marked uptake (9-fold) of the ^{3}H-PGF$_{2\alpha}$ was found specifically over heterochromatin in the nuclei of neoplastic cells. Lower but significant uptakes of ^{3}H-PGF$_{2\alpha}$ were also found in the nuclei of control epidermal cells, which indicate the presence of nuclear receptors in the epidermal neoplastic cells.

Prostaglandins are hormone-like substances which exert various cellular and metabolic effects in mammalian cell systems. Recently it was found that prostaglandins $F_{2\alpha}$ and E_2, but mostly $F_{2\alpha}$ significantly enhanced the tumor development (squamous cell carcinoma) following 3-methylcholanthrene and thus can act as cocarcinogens[1]. However, their mechanism of action in the cells are not yet determined. Autoradiography is a valuable method for the study of different hormone localization, and particularly their cytoplasmic or nuclear receptors. It was shown that ^{3}H-estradiol, ^{3}H-testosterone or ^{125}I-insulin are mostly located in the cytoplasm and thus have cytoplasmic receptors[2,3]. Only macroautoradiography was previously used for the study of gross anatomical distribution of the ^{3}H-tritiated prostaglandin $F_{2\alpha}$(^{3}H-PGF$_{2\alpha}$) throughout the whole body of the pregnant mouse and showed high concentrations of radioactive material in the kidney, liver and connective tissue of dermis[4]. To detect the cellular receptors of prostaglandins, as well as their mechanism of action in carcinogenesis, we studied the intracellular localization of ^{3}H-PGF$_{2\alpha}$ in the control and neoplastic epidermal cells using light and electron microscope autoradiography.

Materials and methods. Adult male Swiss mice, 2 months old, weighing 25 g, were used in this study. Some of them developed squamous cell carcinoma following a topical application of 0.4% 3-methylcholanthrene in acetone solution, 3 times weekly for 6 months. At the end of the experiment and 2 h before sacrifice, 5 control mice and 5 mice with squamous cell carcinoma were injected i.m. with 2 µCi per g b.wt of ^{3}H-prostaglandin $F_{2\alpha}$ (New England Nuclear, sp. act. 120.0 Ci/mM and a radiochemical purity greater than 98%). We selected 2 h for the autoradiographic studies because in previous experiments we found that PGF$_{2\alpha}$ exerted its maximum effect on cell metabolism in that time[5]. For light microscopic autoradiography specimens were removed from tumors as well as from control skin and fixed for 24 h in Bouin solution, dehydrated and embedded in paraplast; the 5-µm sections were stained with hematoxylin and eosin and covered with Ilford K_5 nuclear emulsion and exposed at 4 °C for 2 months, then developed in Kodak D_{19}, fixed and washed. Autoradiograms were used for counting and quantitative estimation of grain distribution. The results are expressed as percentage of labeled nuclei (mean ± SE) from 3000 consecutive cells in experimental groups. For electron microscope autoradiography, small specimens were fixed in 2.5% phosphate buffered glutaraldehyde for 24 h, dehydrated and embedded in a mixture of epon:araldite. Thin sections (600 Å) were mounted on grids, covered with Ilford L_4 nuclear emulsion using a wire loop procedure[6], exposed at 4 °C for 4 months, then developed in Micro-

Quantitative analysis of autoradiograms of [^{3}H]-prostaglandin $F_{2\alpha}$ in mouse control epidermis and neoplastic cells

Experimental groups	No. of animals	No. of labeled nuclei/total No. of nuclei*	% of labeled cells (mean ± SE)
Control epidermal nuclei	5	155/3000	5.17 ± 0.43
Neoplastic nuclei (squamous cell carcinoma)	5	1440/3000	48** ± 2.18

* Autoradiograms were examined at ×400 (objective 40; occular ×10). ** Statistically significant ($p < 0.001$) from respective controls.

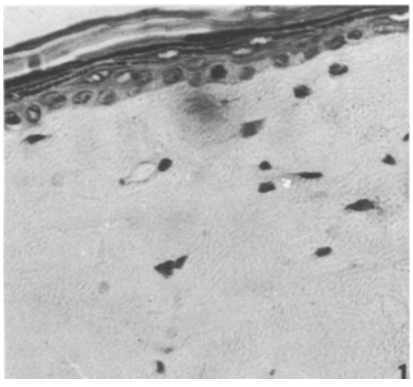
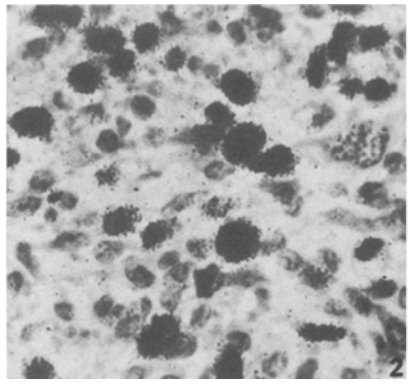
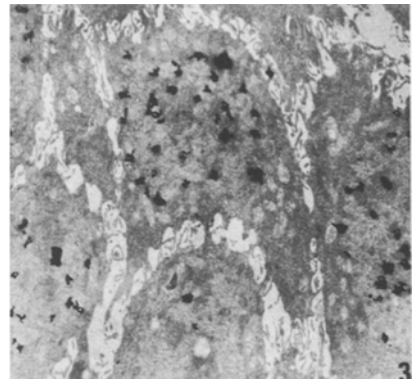

Fig. 1. Light microscope autoradiogram showing the incorporation of ^{3}H-PGF$_{2\alpha}$ in the cell nuclei of mouse control epidermis. × 200. Fig. 2. Light microscope autoradiogram showing a heavy incorporation of ^{3}H-PGF$_{2\alpha}$ only into the nuclei of neoplastic cells of a mouse with squamous cell carcinoma. Mitotic cells (arrow) can be frequently seen. × 200. Fig. 3. Electron microscopic autoradiogram showing a heyvy and specific incorporation of ^{3}H-PGF$_{2\alpha}$ as several developed grains only over the nuclear chromatin (dense chromatin, Dc); of neoplastic cells. No grains are visible over mitochondria (M), endoplasmic reticulum or intercellular spaces (Is). × 4000.

dol X, fixed and washed. Autoradiograms were examined under HS-8 electron microscope.

Observations and discussion. Light microscopic autoradiograms revealed a marked uptake of ^{3}H-PGF$_{2\alpha}$ in the nuclei of the neoplastic cells, as compared to that of mouse control epidermal cells (figures 1 and 2). Frequently mitotic figures with a heavy autoradiographic reaction and an intense reaction is also visible in the multinucleated cells. The autoradiographic reaction is mostly located over nuclei and their nucleoli. No reaction is visible over the cytoplasm. Quantitative analysis revealed significant differences ($p < 0.001$) between control and neoplastic cells (table). Electron microscopic autoradiograms also revealed a heavy concentration of ^{3}H-PGF$_{2\alpha}$ in the neoplastic cells. Several enlarged nuclei of neoplastic cells, heavily incorporated the ^{3}H-PGF$_{2\alpha}$ as developed grains, mostly in dense chromatin (heterochromatin) and at the periphery of the nucleolus (figure 3). No ^{3}H material was found in the loose chromatin (euchromatin) or in the cytoplasmic organelles (mitochondria, endoplasmic reticulum).

The role of PGF$_{2\alpha}$ and PGE$_2$ on the cell growth and proliferation, namely of neoplastic cells has been of considerable interest in recent years[7-9]. However, the mechanism by which prostaglandins exert their effects is still a matter of speculation. In the present studies, we have shown that ^{3}H-PGF$_{2\alpha}$ is strongly bound to the macromolecules of nuclear chromatin of cancerous cells of mouse squamous cell carcinoma. The ^{3}H-PGF$_{2\alpha}$ binding is 9-fold greater than that found in the nuclei of control mouse epidermis, although concentrations of ^{3}H-PGF$_{2\alpha}$ in control epidermal nuclei are still significant. Electron microscopic autoradiograms revealed that ^{3}H is specifically bound to nuclear chromatin, especially dense chromatin (heterochromatin). It is therefore possible that prostaglandins exert their physiological effects, by interfering with DNA synthesis. To our knowledge there are no previous publications which studied the intracellular binding of ^{3}H-PGF$_{2\alpha}$ in cancerous cells as compared to that of control cells from the same tissue. This marked increase of ^{3}H-PGF$_{2\alpha}$ in the neoplastic cells can explain the enhancement of carcinogenesis induced by prostaglandin F$_{2\alpha}$ and indicate the presence of nuclear receptor(s).

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The in vitro effect of aprotinin upon spleen cells from normal and tumour-bearing mice exposed to PPD and tumour cells[1]

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Summary. Aprotinin (Trasylol) is shown to enhance the response of spleen cells from normal and tumour bearing mice to PPD and tumour cells. This enhancement is greater in the tumour-bearing mice.

Protease inhibitors have been shown to inhibit both tumour growth and invasiveness in animal systems[2-5]. It has recently been suggested that these agents may produce their effects by stimulating the hosts immune system[6,7], although they may have other effects.

The effect on the immune system has been further investigated in the studies reported here by assessing the effect of protease inhibitor aprotinin (Trasylol) upon the in vitro activity of spleen cells against the antigen PPD (purified protein derivative of mycobacterium tuberculosis), and