upon the formation and distribution of the polyanions on the cell surface. Some observations[17] reporting alterations of the glycoproteins of the glomerular basement membrane induced by methylprednisolone, are consistent with the last hypothesis.

The production of podocytes in anomalous location reported here, might be useful for future experimental studies of some unresolved questions on the biology of the podocytes, such as their possible participation in the formation and renovation of the glomerular basement membrane.

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Epidermal growth factor stimulates proliferation of rat hepatoma cells producing α-fetoprotein

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Summary. Epidermal growth factor stimulated both [^{3}H]thymidine uptake and proliferation of rat AH66 hepatoma cells. However, the increase in cell number was not accompanied by a proportional increase in the levels of α-fetoprotein of the culture media. The effects of EGF on the cell proliferation were antagonized by N^6,$O^{2'}$-dibutyryl cAMP.

Epidermal growth factor (EGF) is a polypeptide with a molecular weight of about 6000, which has been isolated from the submaxillary gland of male mice[1], and recently from human urine[2]. EGF is capable of stimulating DNA synthesis and proliferation in epidermal cells[3] and various fibroblastic cells[4,5]. It has also been reported to stimulate the secretion of hCG by human choriocarcinoma cells, and is suggested to be a trophic modulator of hormonal secretion[6]. There seems, however, to be no report up to now about the EGF action on tumor cells producing onco-fetal proteins. We studied the effect of EGF on the transplantable rat hepatoma cells secreting α-fetoprotein (AFP)[7]. The observation suggests a role for EGF for the 1st time as a modulator of proliferation and AFP production in hepatoma cells.

Materials and methods. EGF was purified from the submaxillary glands of male mice by the method of Savage et al.[1]. Rat AH66 hepatoma cells (5×10^4) were cultured in plastic culture dishes (25 mm, Falcon) containing 2 ml of Ham's F12 medium supplemented with 0.2% fetal calf serum. Various concentrations of EGF, bovine insulin (Sigma) and/or N^6,$O^{2'}$-dibutyryl cyclic AMP(Bt_2cAMP, Sigma) were added into the low serum medium and the cells were cultured for varying times at 37 °C in a 5% CO_2-95% air mixture. Cells were counted with a hemocytometer, and the AFP concentration of the culture media was determined by radioimmunoassay[8]. To measure [^{3}H]thymidine uptake, 2 μCi of [^{3}H]thymidine (5 Ci/mmole, Radiochemical Center, Amersham, England) was added to 2 ml of culture media (final, 1 μCi/ml) on the 3rd day, and the culture was continued for further 6 h. The amount of [^{3}H]thymidine incorporated into the acid-precipitate of cell lysate was measured as described previously[9].

Results. The proliferation of AH66 cells cultured in Ham's F12 medium + 0.2% fetal calf serum was almost negligible. The addition of more than 0.5 ng/ml of EGF into the low serum medium caused a significant increase in [^{3}H]thymidine incorporation and cell proliferation after 3 days (figure). The presence of more than 5 ng/ml of EGF resulted in maximum stimulation of cell proliferation (figure). The manifestation of the EGF effect appeared to require a relatively long period of culture, since regardless of the presence of EGF no significant increase in the cell number was detected after 24 h in culture. On the contrary, the amount of AFP in the culture medium did not increase in proportion to the increase in cell number (figure). AFP synthesized and secreted into the medium during the 3 days of culture of AH66 cells with 5 ng/ml of EGF was rather smaller (107 ± 11 ng/ml) than that of the control (153 ± 18 ng/ml). From these data it was suggested that the amount of AFP produced by AH66 cells was smaller in those cells cultured with EGF. Similar effects on cell proliferation and AFP production were observed when the low serum medium was supplemented with insulin (50 ng/ml). Bt_2cAMP (2 mM), which inhibited the prolifera-

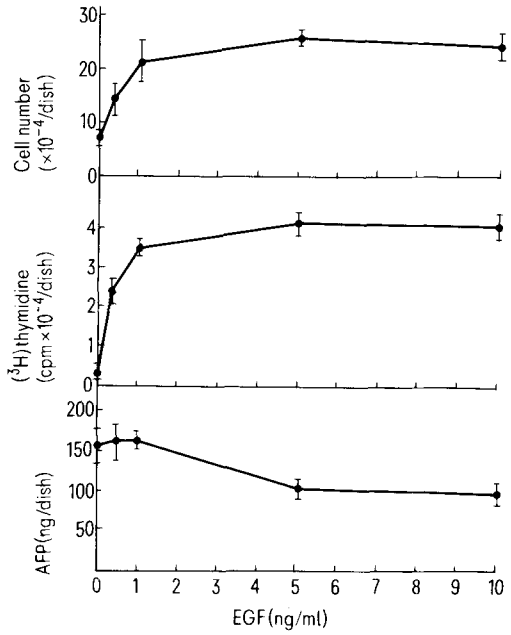
Effects of EGF, insulin and Bt_2cAMP on the proliferation and AFP production of AH66 hepatoma cells in culture

Treatment	Cells	AFP	
	$\times10^{-4}$/dish	ng/dish	ng/10^5 cells
None	7.3 ± 0.76	184 ± 35	250 ± 29
EGF	22.8 ± 2.8	174 ± 12	77.2 ± 12
Insulin	18.4 ± 3.8	201 ± 19	112 ± 28
EGF + insulin	17.6 ± 3.0	146 ± 14	83.8 ± 5.9
Bt_2cAMP	6.5 ± 1.7	300 ± 112	451 ± 89
EGF + Bt_2cAMP	11.3 ± 1.2	353 ± 30	312 ± 42.5

5×10^4 of AH66 cells were cultured for 3 days with EGF (10 ng/ml), insulin (50 ng/ml) and/or Bt_2cAMP (2 mM). The number of cells and the amount of AFP in the media were determined as described in materials and methods. Each point is the mean ± SD of triplicates.

tion and stimulated AFP production of AH66 cells, antagonized EGF action on the hepatoma cells (table).

Discussion. EGF stimulated both [^{3}H]thymidine uptake and multiplication in AH66 cells, as reported for various other cells[3-5]. In the past EGF was reported to stimulate [^{3}H]thymidine incorporation without subsequent mitoses in the hepatocyte culture[10]. However, this dissociation seems to be due to the fact that the culture conditions and other factors necessory for normal hepatocyte division in vitro have not yet been fully defined[10]. Our results showed that the number of AH66 cells in cultures treated with EGF for 3 days was about 3 times greater than that of cells in cultures without EGF. If AFP production per cell is the same regardless of the presence of EGF, the medium after 3 days of culture of AH66 cells with EGF would be expected to contain at least 30 to 50% more AFP than the control culture. Our results showed that the amount of AFP produced by AH66 cells cultured with EGF was not significantly greater than that without EGF, suggesting the cells actively proliferating in the presence of EGF were synthesizing a smaller amount of AFP than the resting cells. However, we still do not know whether this is due to a direct effect of EGF on AFP production or secretion[11] or to a secondary effect resulting from the stimulation of cell proliferation by EGF[7,9].



Effects of EGF on the proliferation, [^{3}H]thymidine uptake and AFP production of AH66 cells. 5×10^4 of AH66 cells were cultured for 3 days in the low serum medium supplemented with various amounts of EGF. Cell number, [^{3}H]thymidine uptake and AFP concentration of the culture media were determined as described in materials and methods. Each point is the mean ± SD of triplicates.

The action of EGF on the proliferation of AH66 cells appeared to be similar to that of insulin (table). In the past it was shown that the receptors for EGF were distinct from the cell receptor for insulin and that EGF and insulin seemed to act additively[12]. However, since AH66 cells proliferating in response to EGF seem to be insensitive to insulin action (table), it seems not to be clear whether the 2 hormones act additively on hepatoma cells. EGF action on human fibroblasts is modulated by cholera toxin, theophyllin and Bt_2cAMP[13]. In AH66 cells, as well, the stimulation of cell proliferation by EGF was completely inhibited by Bt_2cAMP (table). Further details of the mechanism of EGF action remain to be studied.

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Elimination and metabolism of dimethylnitrosamine (DMN) by *Xenopus laevis* and other amphibians[1]

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Summary. The elimination of (^{14}C)-DMN after i.p. injection into *Xenopus* was measured, as was the metabolism in vitro of (^{14}C)-DMN by liver from *Xenopus* and 9 other amphibian species. In view of its rapid elimination from the body and low rate of metabolism by *Xenopus* liver in vitro, DMN is unlikely to be toxic or carcinogenic in *Xenopus*.

Nitrosamines have been shown to be toxic and carcinogenic in a wide range of vertebrate species ranging from fish[4, 5] to primates[6, 7]. The nitrosamines are not themselves active, but they can be dealkylated in some tissues; the resulting metabolite can alkylate cellular components including nucleic acids and proteins[8]. Species and tissue susceptibility to damage and tumour formation are to a certain extent correlated with the ability to metabolize nitrosamines.

Montesano et al.[9] found that the rate of metabolism of dimethylnitrosamine (DMN) by *Triturus helveticus* liver slices in vitro was comparable with that of rat kidney slices, and concluded that DMN would be expected to have toxic and/or carcinogenic effects on the liver of that species. However, they also found that injected DMN was very rapidly eliminated from the newt body into the surrounding water. Ingram[10] found that a single DMN injection had