lar elements were well preserved and observation was permissible (figure 6).

Discussion. In his study of the mechanism of precipitation caused by tannic acid fixation, Futaesaku et al.[3] assumes that the precipitate of protein-TA-heavy metal complex stays in the tissue and that TA-fixation gives the high electron density of the region of protein selectivity. Singer and Nicolson[16] propose the fluid mosaic model of membrane structure. According to this model, surface proteins are free to diffuse in a lipid matrix and thus to assume a random or homogenous distribution over the cell surface. If the above hypotheses are correct, then the precipitate of protein-TA-heavy metal deposit on the cell membrane as observed in the present study can probably be used as one of the indicators of the localization of cell membrane protein components.

On the other hand, Futaesaku et al.[3] observed that, on the small intestine, the substance of the cell coat was stained strongly by tannic acid. A similar result was also observed by the author (unpublished result). It is of course possible that the protein-TA-heavy metal complex, as observed by Futaesaku et al. and as seen in the present investigation, only indicates the presence of mucoprotein molecules in the cell coat rather than on the cell membrane itself. It is rather premature at this moment to conclude which of the above possibilities is true. From the present investigation, TA seems to be a nonspecific stain for surface proteins. It may well be that both possibilities are true. The exact mechanism of the precipitate caused by TA fixation needs further investigation.

Our preliminary result also indicates an increase in microvilli and cytoplasmic bleb formation as a result of tannic acid fixation of EA cells in suspension. Tannic acid has also been used to demonstrate the subunit structure of microtubules[17,18]. Cytoskeletal elements are shown interacting indirectly with the plasma membrane or with each other through cross-links or bridging structures[18]. It is likely that, during tannic acid fixation, some of the microtubular system is altered which in turn leads to microvilli and cytoplasmic bleb formation. The precise mechanism by which tannic acid binds proteins and brings about changes in the cell surface configuration remains to be established.

Regardless of the mechanism of protein-TA-heavy metal precipitations on the cell surface, tannic acid obviously acts as a ligand for osmium tetroxide. In view of this, tannic acid can further be used as a ligand for osmium tetroxide in the study of cell surface structures in scanning electron microscopy.

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Determination of proliferative compartments in human tumors

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Summary. Extracorporeal normothermic perfusion with radioactive thymidine allows a cytological, histological and structural analysis of proliferative compartments of human kidney carcinomas in whole-tumor autoradiograms.

From experimental animals it is known that tumor growth is a result of antagonistic intratumoral dynamics which have been described in terms of cell kinetics[2], growth fraction[3] and cell loss[4]. In humans, a direct view on the internal growth pattern of solid tumors is difficult to obtain. Tumor biopsis incubated in tritiated thymidine for labelling cells in DNA synthesis[5-7], do not reflect the proliferative structure of the whole tumor. Systemic application to the patient of radioactive DNA precursors and subsequent serial resections from tumor tissue for the determination of cell cycle parameters[8] should, for ethical reasons, be confined to extreme cases. A new method has therefore been developed to study the intratumoral growth pattern in human tumors by means of postoperative perfusion. Kidney carcinomas are favourable objects for this approach. A single renal artery supplies blood to both, kidney and carcinoma. The idea is to perfuse, immediately after operative resection, the tumor-bearing organ through this artery with a physiological medium containing radioactive DNA precursors and thus to label cells in DNA synthesis.

Materials and methods. The apparatus used for kidney tumor perfusion consists of a chamber to incubate the tumor-bearing organ at 37.5 °C in blood compatible with the tumor-patient's blood group, with additions of dextran solution to lower the hematocrit down to about 25-30%, heparin to prevent blood clotting, and $NaHCO_3$ to adjust a physiological pH. The perfusate is circulated by an occlusive roller pump (Stöckert, Munich, FRG) with an electonically regulated pressure and a variable flow rate of up to 425 ml/min into the renal artery. Physiological P_{O2} values are obtained by a membrane oxygenator (5M0321, Travenol Laboratories, Morton Grove, Ill., USA). The venous outflow is recirculated. During the perfusion, tritiated (1.3 μCi/ml, sp. act. 5 C/mmole) or ^{14}C-labelled thymi-

dine (0.17 µCi/ml, sp. act. 60 mCi/mmole, both from Radiochemical Centre, Amersham, England) is added to the perfusate. The proliferative compartments are determined in whole-tumor autoradiograms prepared with K 2 emulsion (Ilford, Essex, England, exposure time 14 days).

Results and discussion. Clear and granular cells are the 2 main cell types in human adenocarcinomas of the kidney[9]. The autoradiogram reveals that these cells are endowed with different proliferative activity. After 120 min perfusion with labelled thymidine, in selected areas of 9 tumors granular cells showed a mean labelling index of 3.21%±0.47, clear cells a value of 0.65%±0.25 (mean±SEM). Besides cellular differentiation, the histological structure determines proliferative activity in the tumor. In solid, trabecular or tubular areas, proliferating cells are preferentially found near the supporting stroma. This is most impressive in papillary projections or cord-like parts of the tumors with an axial vessel. A critical factor for cell proliferation and cell loss in experimental tumors is the gradient of oxygen and metabolites with increasing distance of the supporting blood vessel[10]. The same holds true for human kidney carcinomas. The labelling index exhibits a significant decrease from the vessel to the periphery of solid tumor cords to reach zero at about 200 µm (figure 1). These results stress the importance of the vascular structure for human tumor growth. A tumor will fail to increase in size if incapable of inducing its own vascular supply[11].

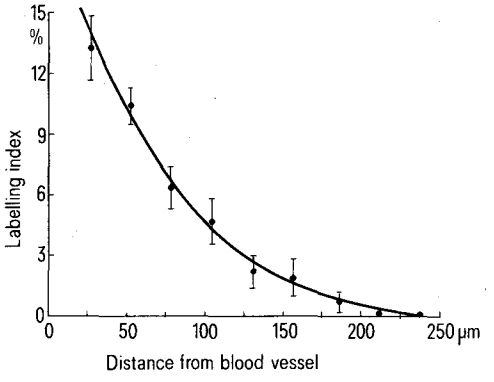
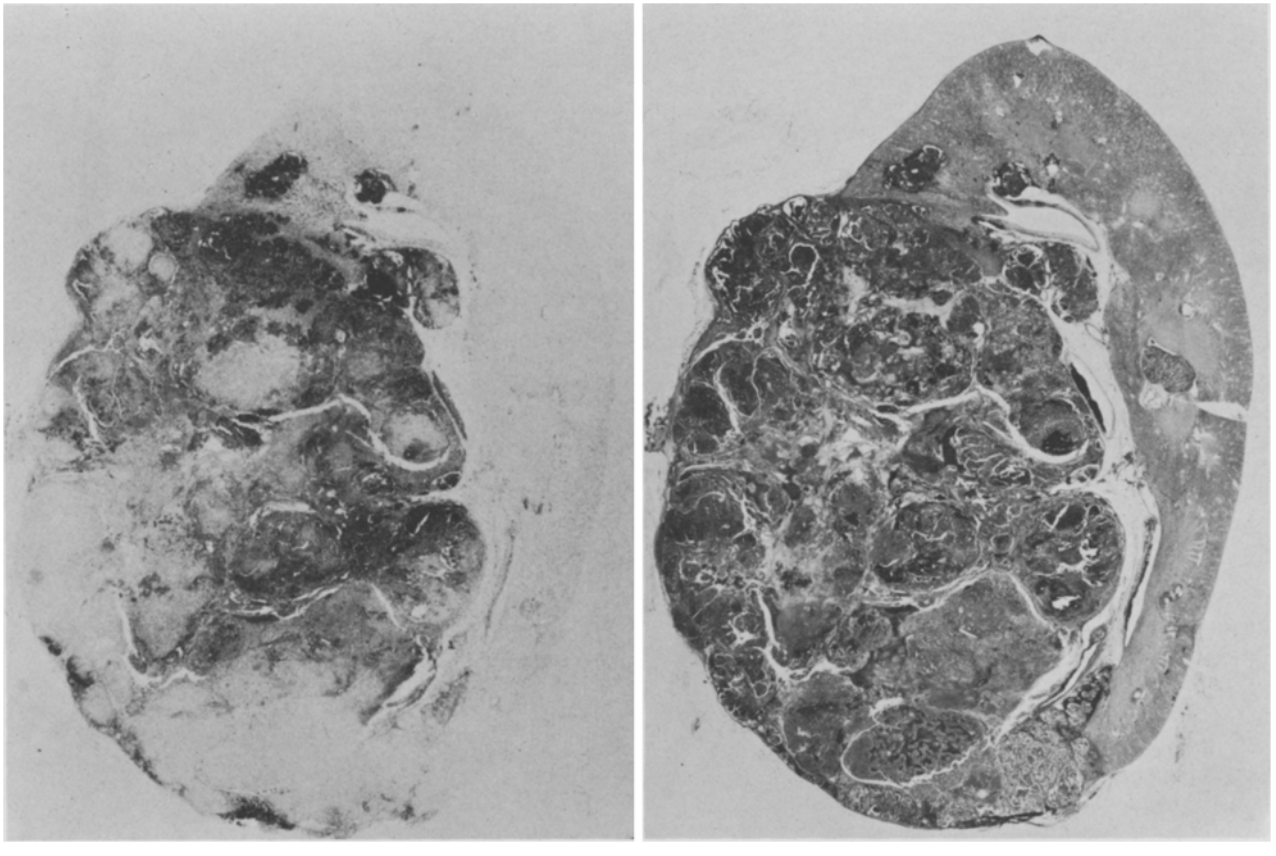


Fig. 1. Labelling index of tumor cells at different distance from the supporting blood vessel. Renal adenocarcinomas were perfused for 45 min with ^{3}H-thymidine and, after an isotope-free interval of 30 min, with ^{14}C-thymidine for 45 min. The percentage of all labelled cells was determined in 9 subunits of tumor cell cords surrounding an axial vessel. Mean±SEM from 10 determinations.

A map of the internal distribution of growing populations in a solid human tumor can be obtained by exposing whole-tumor sections from ^{14}C-thymidine perfused tumor-bearing kidneys with photographic film. The unstained film shows the distribution of proliferating compartments in the tumor (figure 2, a). The density is proportional to uptake of ^{14}C-thymidine and to the relative frequency of DNA synthesizing cells. By comparing the H&E-stained section (figure 2, b) with congruent areas of the film, it can be concluded that labelling is most intense in parts of the tumor adjacent to the normal kidney. But even in distant



a b

Fig. 2. *a* Map of the proliferative compartments of a human adenocarcinoma of the kidney. The tumor-bearing organ was perfused with ^{14}C-thymidine for 45 min in a perfusion medium as outlined in the text. A histological whole-tumor section (5 µm) was exposed for 57 days with X-ray film (Curix RP 1, Agfa-Gevaert, Munich, FRG). Dark areas of the unstained film correspond to zones of ^{14}C-thymidine incorporation and give a relative measure of the distribution of DNA synthesizing cells in the tumor, kidney and intrarenal metastases. *b* H&E-stained histological section of the tumor exposed to the film shown in *a*.

peripheral subcapsular tumor regions, spots of high labelling are seen. It deserves special attention that the tumor does not grow exclusively at its periphery invading the normal tissue, though apparently very actively just here, but a considerable proliferative compartment is found in the interior of the tumor mass. This part may be of minor importance for the increment of tumor size because of rapid cell loss. However, this proliferating compartment could be critical for metastatic spread via blood vessels.

This map gives an impression of the heterogeneity of human tumor growth. It stresses the need for direct studies of human tumors as the rules of proliferation in small animal tumors are obviously not without limitations applicable to voluminous masses of human tumors. The new method of tumor perfusion with radioactive DNA precursors might be helpful for understanding the strategy of human tumor growth.

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The control of onset of melanoblast differentiation in *Xenopus* larvae[1]

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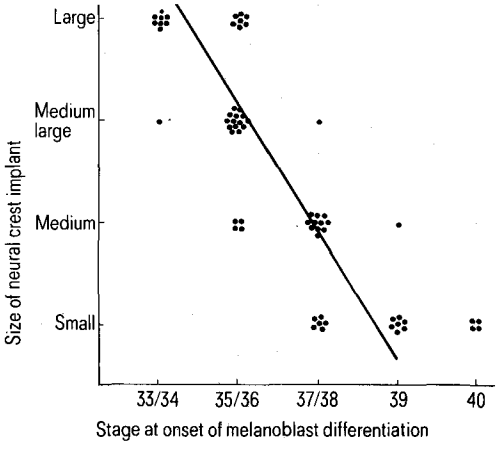
Summary. Studies on the time of first appearance of melanophores in uniformly-sized vesicles of hypomeric tissues containing different masses of neural crest tissues suggest that regional differences in the time of first appearance of trunk melanophores in *Xenopus* are regulated by regional variation in the population density of melanoblasts.

Xenopus larvae show regional differences in pigmentation and in the time of first appearance of melanophores. Trunk melanophores first appear at stage 33/34 (staging according to Nieuwkoop and Faber[3]), taking the form of a dense band of pigmentation in the hypomeric mesoderm immediately ventral to the somites. At stage 35/36 a 2nd band of melanophores forms on the dorsal surface of the neural tube followed by the appearance at stage 37/38 of melanophores sparsely distributed along the dorsal ridges of the somites. During this time and until stage 41 the hypomeric band of melanophores spreads ventrally and less densely in the lateral mesoderm. In general the most ventral tissues of the hypomere do not become pigmented. The present study describes an investigation of the mechanism determining the time of first appearance of melanophores. Previous studies[4,5] by the present author have demonstrated that various tissues of the trunk have similar capacities for supporting melanoblast differentiation, such capacities arising several stages prior to those at which melanoblasts normally differentiate. Hence a regional variation in the time of first appearance of melanophores cannot be explained by a successive development of local environmental factors favourable for melanoblast differentiation. Regional variation in the time of onset of melanoblast differentiation does however appear to be related to the final distribution of trunk melanophores. Heavily pigmented tissues, e.g. upper hypomeric mesoderm and neural tube were observed to exhibit melanophores at earlier stages than tissues such as the more ventral hypomere and somites which are only sparsely pigmented. MacMillan[5] has shown that the distribution of trunk melanophores is determined by a hierarchy of melanoblast-tissue affinities which influence melanoblast migration. Melanoblast-tissue affinity is reflected by the numbers of melanoblasts colonising tissues. Hence the timing of melanoblast differentiation in different regions of the trunk may depend upon regional differences in the population density of melanoblasts. To test this hypothesis the effect of different melanoblast population densities on the time of onset of melanoblast differentiation was investigated by recording the time at which melanophores first appeared in uniformly-sized vesicles of hypomeric tissues containing different masses of implanted neural crest tissues. Melanoblasts migrate from implant into vesicular tissues; the resulting population density of melanophores in vesicular tissues was considered to be directly related to the initial size of the neural crest implant.

Methods. Eggs of *Xenopus laevis* were obtained by standard methods[6] and allowed to develop until stage 22. A portion of neural crest was excised from the anterior trunk region of a stage 22 embryo and divided into 4 unequally-sized portions; the latter, arbitrarily defined as small, medium, medium-large and large, were cultured separately in vesicles derived from similar-sized sheets of lateral hypomeric epidermis and subadjacent mesoderm also obtained from stage 22 embryos. The techniques used in preparing and



Curve relating size of neural crest implant to time of onset of melanoblast differentiation. Dots correspond to individual vesicles.