Abstracts of Theses from the Scandinavian Countries

Abstracts of Scandinavian theses on oncologic subjects are published under this heading. The full theses are as a rule published by the universities or as supplements to different journals. They can usually be obtained after contact with the author.

Ornithine decarboxylase activity in malignant tumours—An experimental and clinical study with reference to cell proliferation and nutrition

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Ornithine decarboxylase (ODC) is a rate-limiting enzyme for the synthesis of polyamines. Polyamines regulate DNA synthesis by a mechanism which is not fully understood. High levels of polyamines and ODC activity are associated with rapid cell growth, particularly in tumour tissues. The aim of this study was to evaluate ODC activity as a possible marker for rapid alterations in tumour growth and to determine whether this method could be used to establish whether nutritional support in cancer patients can stimulate tumour cell proliferation.

Weight-loosing head and neck cancer patients and tumour-bearing mice (MCG 101 C57/BL) were studied during different feeding regimens. The ODC activity in tumour tissue was investigated in relation to 1) histopathological differentiation, 2) DNA content, 2) thymidine and bromodeoxyuridine (BrdUrd) incorporation into DNA and 4) Ki-67 reactivity. The energy state of tumour tissue was determined in vivo with Nuclear Magnetic Resonance-spectroscopy (^{31}P-NMR) and in vitro with high performance liquid chromatography (HPLC).

After 24 h of starvation, a significant reduction of tumour growth was demonstrated in the experimental tumour, along with a reduction of ODC activity, an accumulation of cells in the G0G1 phase and a reduction of cells incorporating thymidine or BrdUrd into DNA. The energy charge of the tumour tissue was reduced compared to freely fed animals. Refeeding after 24 h of starvation restored the energy charge of tumour tissue to prestarvation levels and there was a general response of all variables but with different lag phases. ODC activity responded rapidly and reached higher than prestarvation values within 1 h. The magnitude of this response to refeeding was related to the carbohydrate content of the food and to the levels of plasma insulin.

After specific inhibition of ODC with difluoromethylornithine (DFMO) a prolonged potential doubling time of the tumour was demonstrated. There was a prolonged DNA-synthesis time, causing an accumulation of cells in the G2M phase and an increased fractional cell loss. The energy charge of tumour tissue was not reduced.

Tumour biopsies from head and neck cancer patients demonstrated aneuploidy in 70% of the patients and a growth fraction of around 55% of tumour cells. High ODC activity in tumour tissue was demonstrated mainly among poorly differentiated tumours and ODC activity was correlated to the proportion of aneuploidic cells in the tumour. High ODC activity may indicate a poor short-term survival (one year).

'Enteral nutrition' to cachectic cancer patients increased the proportion of aneuploidic cells in tumours compared to spontaneous feeding. 'Parenteral nutrition' did not produce any cytokinetic effects in the tumour.

It was concluded that experimental tumour growth is highly dependent on the host feeding. However, there was little evidence to support the fear that nutritional support in cancer patients stimulates tumour cell proliferation. ODC is suggested to have a direct role in the induction and promotion of cell division. Determination of ODC activity may have prognostic significance for survival and can probably be used to monitor rapid changes in DNA synthesis.

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Prognostic factors in breast cancer—A long-term survival study with special reference to nuclear DNA content

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During the years from 1945 to 1965 four hundred and thirty-nine women with histologically verified BC and with sufficient clinicopathologic data were diagnosed in the city of Turku, South-Western Finland. These patients have been followed up for at least 22 years (median 28 years) or until death. In this material, several clinicopathologic and DNA flow cytometric parameters were evaluated retrospectively, and their impact on long-term survival investigated.

The presence of axillary node metastases was the most important prognostic factor indicating poor survival both in invasive unilateral cancer of any histologic type ($n = 342$), and in invasive ductal carcinoma ($n = 222$). A large primary tumor size, poor histologic differentiation grade, poorly circumscribed tumor margin, severe tumor necrosis, high number of mitoses, and slight or absent tubule formation also adversely influenced survival in multivariate analyses.

DNA aneuploidy, a large DNA index (DI), and a large S-phase fraction (SPF) were significant prognostic factors indicating poor survival in univariate analyses, especially among patients with node negative cancer and among postmenopausal patients, but they were closely related to many other clinicopathologic factors associated with differentiation and spread of the tumors, and only the SPF was an independent factor in multivariate analyses. Also the 'combined DNA factor C' had independent prognostic value in axillary node negative ductal carcinomas.

After the 10th follow-up year advanced age at the time of diagnosis, the occurrence of a new contralateral BC and a large primary tumor size (T3–4) were significant prognostic factors in predicting late mortality from BC, but axillary nodal status, histologic grade, tumor margin circumscription and extent of necrosis had lost their prognostic value both in uni- and multivariate analyses. A large DNA index (> 1.2) had marginal value ($p = 0.06$) as prognostic factor indicating poor survival in a univariate analysis after the 10th year of follow-up, but DNA ploidy and the SPF did not.

The 30-year corrected survival for the whole series was 34%, and for operable patients 38%. More than 70% of all BC mortality occurred during the first 5 years of following-up, and only 1.2% after the 20th year. During the study period, the age-adjusted incidence increased from 31 to 42/100 000 women, the 30-year corrected survival increased from 31% to 41% ($p = 0.02$) and the percentage of poorly differentiated (Gr III) cancers decreased from 44% to 28% ($p = 0.009$). No significant changes with time could be observed in any other prognostic variables nor therapy.

Mucinous carcinoma (MC) of the breast was studied as a model of the special histologic types. MCs differ in many respect from BCs with unselected histology. Patients with MC were significantly older, and if MCs are divided into pure (PMC) and mixed (MMC) forms, patients with PMC had significantly fewer axillary

node metastases, and their long-term survival was significantly better ($p < 0.001$). Only 4% of PMCs had an aneuploid stemline measured by DNA FCM as compared with 58% of MMCs and 68% of cancers of any histologic type ($p < 0.001$).

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Low grade non-Hodgkin's lymphomas—Diagnostic and prognostic studies

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Malignant non-Hodgkin's lymphomas (NHL) comprise a heterogenous group of diseases regarding clinical picture, survival and suitable mode of treatment. It is therefore important to discriminate between the various histopathological subgroups and, within the subgroups, to identify prognostic factors in order to facilitate the choice of treatment modality in each individual case.

In a population based study (the county of Uppsala 1969–1987), where the National Cancer Register was supplemented with hospital registers, the incidence rates and the proportions of the different NHL subgroups were calculated. The age standardized incidence rate was 15/100 000/year, with a small, but statistically significant, annual increase. The registration deficit in the National Cancer Register for all malignant lymphomas (NHL and Hodgkin's disease = HD) was 7%. The largest registration problem was the overregistration of HD (often misinterpreted NHL) before 1980. Low grade NHL comprises two-thirds of all NHL, the largest subgroup being lymphocytic lymphoma.

The morphological discrimination between B-CLL lymphoma and immunocytoma (IC) is difficult but important. The addition of immunohistochemical staining for cytoplasmic Ig changed the morphological diagnosis of IC in 46% of the cases - either to B-CLL or to a high grade NHL. The discrimination between B-CLL and IC might further be facilitated by the monoclonal antibody FMC7, which was positive in 14/16 IC cases, but only in 1/14 B-CLL.

The Kiel classification of NHL comprises two main groups—high grade and low grade malignant. In the present studies, it was shown that low grade NHL can be divided into one 'truly low grade' and one 'intermediate' grade group regarding estimated overall survival and symptom-free survival. The overall survival was, in a multivariate analysis, best predicted by the presence or absence of initial symptoms. Additional independent information was given by S-thymidine kinase (S-TK), S-haptoglobin and histopathological subgroup. Within the initially asymptomatic cases, the symptom-free survival was predicted by histopathological subgroup. In 'intermediate grade' NHL, S-TK determinations allowed the identification of patients who will become symptomatic within a short time (6–8 months) after diagnosis.

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Lodgement of tumour cells—Experimental studies on some factors influencing the survival of tumour cells after arrest in the microvasculature

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The lodgement of circulating tumour cells, i.e. the arrest and survival of the tumour cells in distant organs, is a prerequisite for metastasis formation. Many factors, ranging from biochemical to mechanical, have been implicated as determinants of the arrest and survival of tumour cells in the microvasculature and, also, of metastatic organ selection. The aims of the present study, using a methylcholantrene-induced rat fibrosarcoma and isotope technique, were: to study the involvement of serotonin (5-HT) in the lodgement process and the effects of 5-HT_2 receptor and calcium channel blockade; to study the effect of hepatic artery and portal vein occlusion on tumour cell lodgement in the liver; to compare tumour cell lodgement in liver and muscle and, finally, to study how tumour cell survival is influenced by different rates of deformation into the microvasculature.

Intraportal tumour cell infusion led to a significant increase in caval vein 5-HT levels, with a maximum 15 min after infusion. 5-HT_2 receptor blockade with ketanserin and R 56413 as well as calcium channel blocking with verapamil all reduced hepatic lodgement of intraportally injected tumour cells. Ketanserin and verapamil showed about equal reduction but were less efficient than R 56413, which reduced lodgement by approximately 30%.

Permanent occlusion of the left hepatic artery, made immediately before intraportal tumour cell infusion, reduced lodgement and metastasis in the left liver lobes. In contrast, portal clamping for 5 min immediately after intraportal tumour cell infusion significantly increased hepatic lodgement.

The proportion of tumour cells arrested in the rat leg muscle 5 min after local arterial infusion did not differ from that found earlier in the liver, but later (3 h) in the lodgement process more tumour cells had died in muscle than in the liver. Vital microscopy showed that the initial arrest of tumour cells in the muscle microvasculature was similar to that seen earlier in the liver, i.e. it typically consisted of a very abrupt retardation and trapping of the cells. After 2 h, 72% of the arrested and initially living cells had died, but this rate of death was not different from that of cells only kept in physiological medium in test tubes at the same temperature (35°C).

When tumour cells were subjected to varying deformation rates (based on different cell velocities of 0.25 and 2.5 mm/s but with similar and very small pressure gradients) in 5 μm Nuclepore filters, no significant difference in survival, as determined repeatedly up to 2 h, was noted. Scanning electron microscopy neither revealed any qualitative differences between tumour cells deformed at high or low flow velocities.

To summarize, the present investigation shows: that 5-HT is released, probably from platelets, in response to intraportal fibrosarcoma cell infusion and that lodgement can be reduced by 5-HT_2 receptor and calcium channel blocking; that arterial occlusion of the liver reduces lodgement and metastasis, whereas portal clamping increases lodgement; that circulating tumour cells are arrested in a similar way in muscle and in the liver but the tumour cells then die more rapidly in muscle—this may be due to mechanical trauma as related to the degree of deformation in the narrow microvessels but it does not seem to be related to the rate of tumour cell deformation.

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Perturbation of Ca^{2+} homeostasis and inhibition of growth in a leukemic T cell line by unsaturated fatty acids

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The effects of unsaturated fatty acids (UFAs) on a leukemic T cell line (JURKAT) were investigated. One aspect was to

characterize the mechanisms involved in the perturbation of Ca^{2+} homeostasis in JURKAT cells by exogenous free UFAs. Another aspect was to examine how UFAs can affect JURKAT cell growth.

Addition of free *w*-3 and *w*-6 UFAs to JURKAT cells was found to induce a transient increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]i$) followed by a prolonged sustained phase that returned to basal level after 10–15 min. The initial transient increase in $[Ca^{2+}]i$ appears to be due to a direct effect of these fatty acids on the Ins(1, 4, 5)P_3-sensitive Ca^{2+} pool, and can be dissociated from the hydrolysis of phosphoinositides and the influx of extracellular Ca^{2+}. Increasing the number of carbon atoms and double bonds in the hydrocarbon chain, increases the efficiency of these UFAs in mobilizing free Ca^{2+} from the Ins(1, 4, 5)P_3-sensitive Ca^{2+} pool. The lack of Ca^{2+} influx during UFAs-induced $[Ca^{2+}]i$ increase was found to be due to inhibition of Ca^{2+} influx into the cells by the presence of these fatty acids. The UFAs were more potent than $NiCl_2$ in blocking receptor mediated Ca^{2+} influx and the effect could be reversed by adding fatty acid free bovine serum albumin. Using the unique properties of the UFAs, α-linolenic acid (one of the active UFAs) was used to study the Ca^{2+} influx mechanism in JURKAT cells. The results indicate that Ca^{2+} influx in JURKAT cells is activated by the emptying of the Ins(1, 4, 5)P_3-sensitive Ca^{2+} pool. The rate of influx appears to correlate closely with the extent of the emptying of the intracellular Ca^{2+} pool, suggesting that extracellular Ca^{2+} first enters and refills the pool before being released into the cytosol.

JURKAT cell growth was suppressed when the cells were grown in medium supplemented with *w*-6 and *w*-3 UFAs. In the presence of *w*-9 UFA, cell growth was normal. The suppressive effects of both *w*-6 and *w*-3 UFAs was not due to eicosanoid production, and lipid peroxidation was only partly involved, since α-tocopherol only partially reversed the inhibitory effects. In cell cultures where α-tocopherol was absent, the ether-phospholipid (plasmalogens) levels in JURKAT cells were reduced. When JURKAT cells were grown in medium supplemented with low concentrations of UFAs and α-tocopherol, the fatty acyl composition of the cellular membrane phospholipids were extensively modified. The changes in fatty acyl composition of membrane phospholipids did not affect the presentation of the T cell receptor complex or the affinity of CD3 complex for anti-CD3 antibodies. However, in *w*-3 and *w*-6 polyunsaturated but not *w*-9 monounsaturated fatty acid modified cells, the receptor-mediated $[Ca^{2+}]i$ increase was suppressed. A decrease in *w*-9 monounsaturated fatty acyl content in cellular membrane lipids was associated with the suppressed receptor-mediated $[Ca^{2+}]i$ increase in JURKAT cells. The low $[Ca^{2+}]i$ after cell activation, presumably due to lack of Ca^{2+} influx, could disrupt Ca^{2+} dependent processes which are required for cell growth.

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Prostacyclin and thromboxane A_2 in human cancers of the breast, uterus and ovary

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The present study was undertaken to investigate the production of antiaggregatory and vasodilator agent PGI_2, and its physiological antagonist TxA_2 in cancers of the breast, endometrium, cervix and ovary and in some benign gynecological tumors. The correlation between the prostanoid production and the clinical parameters and behavior of the cancers was studied, as well as the effect of cytostatics in vivo and that of MPA in vitro on PGI_2 and TxA_2 production.

Breast cancer superfused in vitro released 3.2 times more 6-keto-$PGF_{1\alpha}$ and 6.3 times more TxB_2 than did mastopathic breast tissue. The presence of estradiol or progesterone receptors did not affect the production of PGI_2 and TxA_2 by breast cancer. Furthermore, MPA added to the superfusion medium had no effect on prostanoid production by cancer tissue.

The urinary excretion of 6-keto-$PGF_{1\alpha}$ was normal in patients with endometrial or cervical cancer, as well as in patients with leiomyomas or benign ovarian cysts. In contrast, patients with ovarian cancer excreted increased amounts of 6-keto-$PGF_{1\alpha}$, 2,3-dinor-6-keto-$PGF_{1\alpha}$, TxB_2 and 2,3-dinor-TxB_2.

Endometrial cancer tissue incubated in vitro released normal amounts of 6-keto-$PGF_{1\alpha}$, but the release of TxB_2 was increased 5.6-fold. In leiomyomas the release of TxB_2 was reduced.

Ovarian cancer tissue released increased amounts of 6-keto-$PGF_{1\alpha}$ (11.6-fold rise) and TxB_2 (30-fold rise) into the incubation medium. Anaplastic cancer tissue tended to produce more TxB_2 than the well-differentiated tissue. Ovarian cancer produced significantly more of both prostanoids than did ovarian metastases originating from cancers of the breast, fallopian tube and colon. The production of TxB_2 by benign ovarian cysts was only slightly increased.

Therapy with a combination of cisplatin, 4'epi-adriamycin and cyclophosphamide caused a 50–120% rise in the urinary excretion of 6-keto-$PGF_{1\alpha}$, 2,3-dinor-6-keto-$PGF_{1\alpha}$, TxB_2 and 2,3-dinor-TxB_2 during the first 9 h after infusion, but in the subsequent 10 h their output was 25–45% below the initial values, and remained low for at least two weeks. After repeated courses the excretion of prostanoids was almost normal in patients responding favorably to treatment, whereas the excretion of TxA_2 metabolites remained elevated in patients with a progressive disease.

It is concluded that cancers of the breast and ovary produce elevated amounts of PGI_2 and TxA_2, whereas endometrial cancer is associated only with increased production of TxA_2. The ratio of 6-keto-$PGF_{1\alpha}$ to TxB_2 is lower in these malignancies than that in healthy tissues or in benign tumors arising from the same tissues. Cytostatics (cisplatin, 4'epi-adriamycin and cyclophosphamide) decreased the excretion of 6-keto-$PGF_{1\alpha}$, 2,3-dinor-6-keto-$PGF_{1\alpha}$, TxB_2 and 2,3-dinor-TxB_2 in patients with ovarian cancer concomitantly with a regression of the disease. Thus, PGI_2 and TxA_2 may be involved in the development, the clinical behavior and response to the treatment of these cancers.

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