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ORNITHINE DECABOXYLASE ACTIVITY IN MALIGNANT TUMOURS

An Experimental and Clinical Study with reference to Cell Proliferation and Nutrition.

by

Thomas Westin

Göteborg 1990

D. 90. 131.

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An Experimental and Clinical Study with
reference to
Cell Proliferation and Nutrition.

AKADEMISK AVHANDLING

som för avläggande av doktorsexamen i medicinsk vetenskap med vederbörligt tillstånd av medicinska fakulteten, kommer att offentligen försvaras i föreläsningssal F3, Sahlgrenska sjukhuset, Göteborg, fredagen den 2 mars 1990, kl 13.00

av

Thomas Westin
leg.läkare

Avhandlingen baseras på följande arbeten:

I. Ornithine Decarboxylase Activity in Tumour Tissue in Response to Refeeding and its Dependency on Diet Components.
Westin, T., Edström, S. & Lundholm, K. Submitted Cancer Research.

II. Tumour Cytokinetic Effects of Acute Starvation versus Polyamine Depletion in Tumour-bearing Mice.
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ABSTRACT

WESTIN T: ORNITHINE DECARBOXYLASE IN MALIGNANT TUMOURS.
Departments of Otolaryngology and Surgery, University of Göteborg, Sahlgrenska hospital, S-413 45 Göteborg, SWEDEN.

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 , 3. 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 hours 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 G_0G_1 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 hours of starvation restored the energy charge of tumour tissue to pre-starvation levels and there was a general response of all variables but with different lag phases. ODC activity responded rapidly and reached higher than pre-starvation 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 G_2M 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.

Key words: cancer, polyamines, ODC, DFMO, cell proliferation, flow cytometry, cytokinetics, BrdUrd, DNA synthesis, nutrition, TPN, energy state, ^{31}P-NMR.
ISBN 91-7900-919-0

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An Experimental and Clinical Study with reference to Cell Proliferation and Nutrition.

by

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Göteborg 1990

To Eva, Gabriel and Olof.

Cover: Vinga lighthouse
by
Lars Gabrielson

Thomas Westin
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These papers are referred to by their Roman numerals in the text.

CONTENTS

ABBREVIATIONS

Frequently used abbreviations.

BrdUrd	Bromodeoxyuridine
DFMO	α-difluoromethylornithine
FITC	Fluorescein isothiocyanate
HPLC	High performance liquid chromatography
Kcal	Kilocalories
NMR	Nuclear magnetic resonance
ODC	Ornithine decarboxylase
RM	Relative movement
SAMD	S-adenosyl-methionine decarboxylase
TPN	Total parenteral nutrition
T_{pot}	Potential doubling time
T_S	DNA-synthesis time

INTRODUCTION

Progressive malnutrition due to cancer disease is well recognised and around 50 % of cancer patients will die with cachexia. This is certainly true for head and neck cancer patients, 20-30 % of who exhibit weight loss on admission for treatment [1-3]. Furthermore, oncological treatment with surgery, radiotherapy and chemotherapy adds to the catabolism of these cancer patients [4, 5]. Malnutrition in these patients adversely affects survival, the response rate to chemotherapy, subjective and objective tolerance to treatment and quality of life [1, 6-15]. It is recognised that nutritional support in malnourished patients reduces postoperative morbidity [15-21] and it is a common clinical observation that well-nourished patients have a better ability to sustain their oncological treatment [5]. However, it has not been possible to show any effects of nutritional support in cancer patients in terms of reduction of recurrence rate or increased survival [15, 22, 23]. It is often feared that nutritional treatment in a tumour-bearing host can activate tumour cell proliferation, as has been reported for a number of experimental tumour systems [24-28]. Such effects of nutritional support in human cancer patients are suggested in a recent report [29]. However, if tumour cell proliferation is possible to modulate with artificial nutrition in human cancer, this knowledge could possibly be used to choose and improve oncological treatment [30]. In this context, sensitive methods are needed to determine growth characteristics and metabolic changes of the tumour.

At present, the choice of optimal treatment for the individual cancer patient is based on a clinical examination, mainly considering the general condition of the patient and the tumour stage [31]. The biological aggressiveness of the tumour is determined by a routine morphological investigation where the cell type, differentiation, infiltration and degree

of inflammation are considered [13, 32-36]. At several institutions, measurements of DNA content have been performed in order to extend the biological characteristics of the tumour [37-49]. Such DNA determinations are accomplished after specific DNA staining. By using flow cytometric equipment, a considerable number of cells can be rapidly analysed. Since there is a progressive increase of cellular DNA content through the cell cycle, the relative number of cells in different phases of the cell cycle can be demonstrated (Figs 1 and 2, [50, 51]).

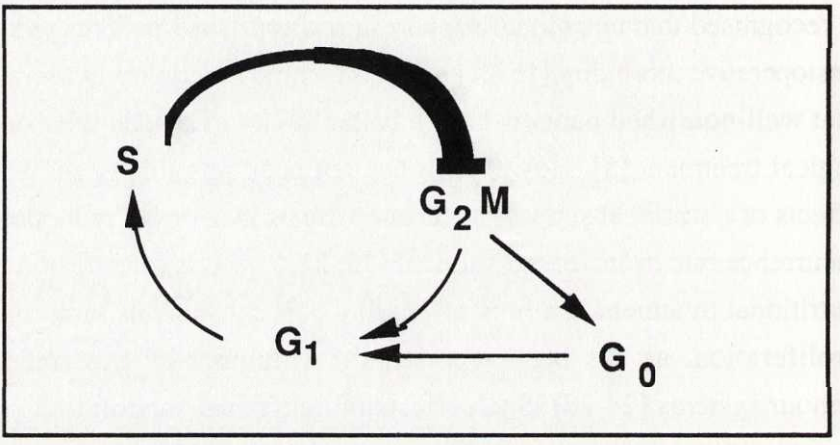

Figure 1: The cell cycle. The cells cycle through the G_1, S and G_2M phases which represent cells in the growth fraction. G_1 represents cells being "primed" for cell growth but not yet DNA synthesising, containing the same amount of DNA as cells in quiescence (G_0). During the S phase DNA synthesis occurs and the DNA content increases progressively until the cells have double the amount of DNA (G_2M). Then cell division occurs and the cells reenter the G_1 phase or pass into a quiescent state (G_0).

It has been suggested that the DNA content may have predictive value for survival in many human cancers and has been used in the evaluation of treatment for certain tumour diseases [37, 38, 46, 52-59]. DNA analysis and morphology are both methods providing a

"snapshot" of the tumour being of a static nature, however, and thus provides limited information of the dynamics of cell proliferation [60].

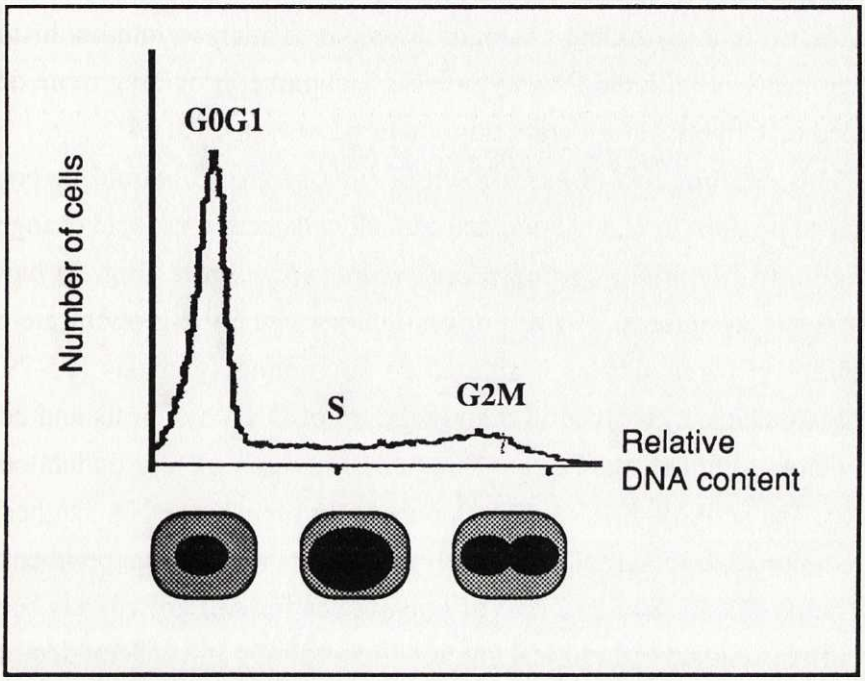

Figure 2: DNA-histogram. A DNA histogram can provide information on the relative DNA content of a tumour. This DNA content can then be put in relation to a known standard. Thus, ploidy and occurrence of different cell clones can be determined. The figure represents a flow cytometric DNA histogram from the MCG 101 sarcoma used for the experiments in papers I, II and III. Tumours from freely fed mice had around 60% of cells in the G_0G_1 phase, 30% in S phase and 10% in G_2M phase. The cells below the histogram illustrate the progressive increase in DNA content enabling the flow cytometric analysis to be performed after DNA staining.

Tumour volume and tumour weight reflect the net result of tumour growth [24, 61, 62]. Tumour growth rate, however, is dependent on the relative size of the growth fraction of tumour cells, the rate of cell cycle transfer and the cell loss [63-67]. Determination of the incorporation of various precursors into DNA has given further insight into the

cell kinetics of tumour cells [68-72]. This has been accomplished using tritiated thymidine and analysis with autoradiographic and scintillation techniques, which are time-consuming assays. Bromodeoxyuridine (BrdUrd) is a thymidine analogue possible to analyse immunohistochemically or with the flow cytometric technique, providing more detailed cell kinetic information on tumour growth [65, 73, 74].

The ideal method of assessment of tumour growth would be both easy to monitor in clinical practice and allow detection of rapid changes of cell proliferation. During recent years, polyamines (Fig. 3) have attracted the interest of many investigators, since the growth rate of rapidly growing tissues is related to polyamine synthesis [75-79]. Polyamines are involved in the regulation of DNA synthesis and cell division, although their exact function is not completely understood [80]. The activity of RNA polymerase and protein and DNA synthesis are reported to be stimulated by polyamines in a variety of experimental systems [75, 81-84]. The role of polyamines for cell growth has been confirmed in several clinical studies. Accordingly it has been demonstrated that urine polyamine levels correlate with the tumour burden of the host [85, 86] and elevated levels of polyamines have been found in sera from patients with advanced solid tumours [87]. Increased levels of polyamines have been demonstrated in tumour tissue from oral, breast and colorectal cancers [88-92]. Studies of polyamine levels in cerebrospinal fluid have been used to determine proliferative activity of certain brain tumours [93]. Furthermore, polyamine levels were increased in erythrocytes from a group of cancer patients receiving intravenous nutrition compared to a non-cancer control group [94].

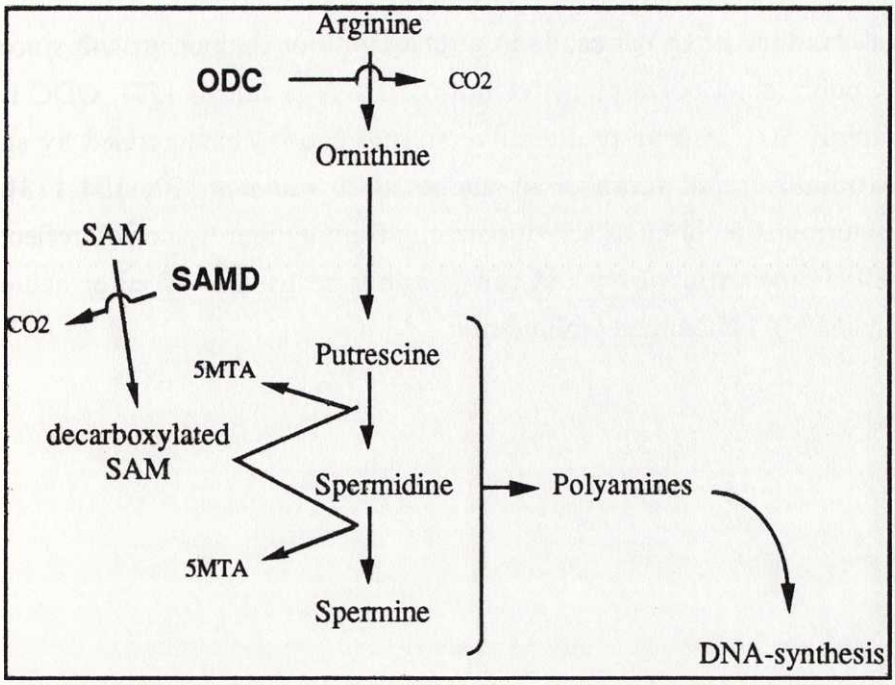

Figure 3.
A schematic illustration of the polyamine synthesis pathway. The polyamines putrescine, spermidine and spermine are present in all mammalian cells and are known to be essential for DNA synthesis in cell growth, although their exact function is unknown. The synthesis of putrescine is regulated by the availability of ornithine and the activity of ornithine decarboxylase (ODC; EC 4.1.1.17). The other polyamines are derived from putrescine by the action of spermidine synthase (EC 2.5.1.16) and spermine synthase (EC 2.5.1.22) which both require decarboxylated S-adenosyl-methionine, leaving an aminopropyl-group (5MTA = 5 methylthioadenosine). The second rate-limiting enzyme of this pathway is S-adenosyl-methionine decarboxylase (SAMD; EC 4.1.1.50)

Note the interaction steps of ODC and SAMD in the figure. The rate of polyamine synthesis can be determined by the activity of ornithine decarboxylase, which is assessed by the rate of CO_2 release under optimal *in-vitro* conditions.

Polyamine synthesis is dependent on the availability of ornithine and the activity of ornithine decarboxylase (ODC) [77, 95-97]. ODC-activity is generally increased in tumour tissue compared to normal tissues in both experimental [75, 76, 98] and human cancer [99-103]. Absence

of ornithine or ODC results in a retardation of tumour growth since tumours need polyamines for normal DNA synthesis [77]. ODC is rapidly activated by proliferative stimuli and is characterised by an extremely rapid turnover of about 10-20 minutes [77, 104-113]. Determination of ODC activity directly from tumour tissue will reflect polyamine metabolism and can possibly be used to monitor acute changes in tumour cell proliferation.

THE AIMS OF THE STUDY

The specific purpose of this study was;

- to evaluate whether refeeding after 24 h of starvation activates ornithine decarboxylase (ODC) in an experimental solid tumour and, if so, which component (carbohydrate, fat, protein) in the diet re-establishes the ODC activity (I).

- to study how ODC activity and starvation-refeeding influence tumour cytokinetics of the experimental tumour *in vivo* (II).

- to determine whether changes in ODC activity or starvation-refeeding modulate tumour growth by acute alterations of the energy state in the experimental tumour (III).

- to investigate whether nutritional support stimulates tumour cell proliferation in weight-loosing patients with head and neck cancer (IV,V).

MATERIAL

Patients

Altogether forty patients with various head and neck cancer tumours were examined (IV,V). Their clinical data and nutritional status are presented in Table 1. There were 30 men and 10 women with a mean age of 66 years (46-85). All patients had a recent history of ≥ 5 % weight-loss. The diagnoses were oral carcinoma 27, hypopharyngeal carcinoma 8, laryngeal carcinoma 2, maxillary carcinoma 2 and salivary gland carcinoma one. Most of the patients had advanced tumours (T4, n=24; T3, n=6; T2, n=10). All but four tumours were histologically classified as squamous cell carcinomas of which 15 were poorly differentiated and 21 were moderately or well differentiated. The remaining four tumours were one malignant fibrous histicytoma, one malignant non-Hodgkin lymphoma, one Hodgkin lymphoma and one anaplastic cancer. Patients with other diseases, such as diabetes or heart failure, were excluded from the study. None of the patients had received any kind of cytostatic treatment, radiotherapy, anti-inflammatory drug or hormonal substitution prior to the investigations.

Nutritional status was determined as follows: Body weight index was calculated as the actual weight divided by the reference weight for height [20, 114] Normal weight index was ≥ 0.80. Arm circumference, considered to be an anthropometric measure of proteins in the body and triceps skinfold considered to be an index of lipid stores were measured at the midpoint of the left arm. The arm muscle circumference (AMC) was then calculated and compared with a reference population [115]. Serum albumin was analysed using the hospital´s routine colour binding procedure. Low values were ≤ 33 g/l for women and ≤ 36 for men [20]. Total body potassium (TBK) was

No.	Sex	Age	Diagnosis	Diff.	Classification T	N	M	Weight loss %	Weight index	AMC cm	Albumin g/l	Total K^+ mmol
1	M	55	oral	poor	4	3	0	10	0.67*	21.4*	38	2544*
2	M	80	oral	mod	2	0	0	10	0.97	29.5	33*	3204*
3	M	63	oral	mod	2	0	0	5	0.73*	23.9	43	2911*
4	M	64	hypoph.	poor	4	0	0	10	0.63*	23.1	37	2925*
5	M	58	oral	Histioc.	4	0	0	13	0.91	28.6	37	4084
6	F	59	oral	poor	4	0	0	5	0.91	28.7	-	2697
7	M	57	hypoph.	high	4	1	0	20	0.60*	23.4	34*	2899*
8	M	84	hypoph.	mod	3	2	0	12	0.63*	21.1	43	2368*
9	F	65	submand.	mod	4	1	0	5	0.68*	21.1	37	1879*
10	F	59	oral	poor	4	1	0	5	0.60*	19.9*	32*	1623*
11	M	68	oral	mod	4	1	0	5	0.77*	24.6	39	2778*
12	M	65	oral	mod	4	1	0	5	0.77*	23.5	42	2897*
13	F	65	hypoph.	poor	4	1	0	8	0.74*	28.0	40	2344*
14	F	61	oral	poor	4	0	0	7	0.96	29.1	39	-
15	M	46	oral	Hodgkin	4	3	1	13	0.74*	25.1	39	3434
16	F	85	oral	high	2	0	0	5	0.68*	-	45	2116*
17	M	69	hypoph.	poor	4	1	0	5	0.81	25.9	45	3067*
18	M	64	oral	Anapl.	3	0	1	5	0.78*	23.1	37	2998*
19	M	60	oral	mod	2	0	0	5	1.05	26.3	42	-
20	M	61	oral	poor	4	2	0	14	0.82	26.8	37	3141*
21	M	67	oral	mod	3	3	0	5	0.84	28.9	40	3516
22	M	54	oral	mod	2	1	0	5	0.76*	-	41	-
23	M	81	oral	mod	4	2	0	5	0.88	29.5	40	3156*
24	M	67	hypoph.	high	4	0	0	5	0.68*	20.8*	-	-
25	M	69	maxill.	poor	4	1	0	15	0.95	27.1	34*	3678
26	M	61	oral	poor	4	1	0	5	0.77*	27.7	38	2448*
27	M	68	oral	poor	2	1	0	5	0.91	26.6	40	3629
28	F	71	oral	mod	3	0	0	5	0.77*	24.7	38	2161*
29	M	55	larynx	poor	2	1	0	5	0.94	-	46	4217
30	M	79	oral	Lymphoma	3	1	0	5	0.85	-	-	-
31	M	54	larynx	mod	4	1	0	5	0.89	-	-	-
32	M	74	oral	poor	4	0	0	5	0.95	28.5	31*	2840*
36	F	62	hypoph.	poor	4	0	0	20	0.48*	18.4*	40	1840*
38	M	75	oral	poor	3	0	0	8	0.78*	23.5	38	2880*
33	M	52	oral	mod	2	0	0	8	0.82	28.2	32*	2890*
34	M	46	maxill.	mod	4	0	0	8	0.73*	25.2	36*	3880
35	F	82	oral	mod	4	1	0	5	0.73*	22.0	39	2150*
37	M	67	hypoph.	mod	2	0	0	15	0.75*	21.0*	41	2560*
39	F	77	oral	mod	2	1	0	5	0.92	22.4	38	2260*
40	M	72	oral	mod	4	0	0	10	0.96	28.8	46	3510*

Table 1.
Clinical data and nutritional status of patients in this study. Patients no. 1-15 are the study group ("enteral nutrition") and no. 16-31 the control group in paper IV. Patients no. 32-40 are the study group ("intravenous nutrition") in paper V.
* = pathological value compared to a reference population.

determined measuring the natural 40K emission in a whole body counter as described in paper V [116].

Animal model

The tumour-bearing animal model used in this study (I,II,III) has been extensively used in our laboratory and is well characterised [117-122]. Three months old, weight stable (20-25 g), sex matched male and female mice (C57Bl/6J, Bomholtgård, Ry, Denmark) were used throughout. A poorly differentiated sarcoma (MCG 101), originally induced with methylcholanthrene, was used as an isogenic tumour graft. The tumour was transplanted subcutaneously in the flanks under aseptic conditions. The tumour does not penetrate the abdominal wall or metastasise. Its influence on the host metabolism has been evaluated in a series of reports [119, 123-125]. The tumour is palpable after 5-7 days and demonstrates an exponential growth rate until the tumour-bearing mice die with cachexia 15-17 days after tumour implantation [24, 62]. Anorexia is demonstrable from day 6-7 and the cachectic reactions of the host to the tumour are considered to be similar to those observed in clinical cancer [123, 126].

Most experiments were performed 8-10 days after tumour implantation, when the tumour size was approximately 1 gram. The animals were kept in individual cages with coarse sawdust bedding with free access to a Purina chow diet and tap water unless otherwise stated. The ambient temperature was 25-26°C and the mice were kept on a 12 h light/12 h darkness schedule. Body weight, tumour weight, food intake and water intake were registrated regularly throughout the experiments. The animals were killed by cervical dislocation. For every tumour-bearing animal studied, a corresponding control animal was killed simultaneously.

STUDY DESIGN

Clinical studies (IV,V)

All consecutive patients with head and neck cancer and a recent history of weight loss ≥ 5 % were invited to take part in the studies. Initial tumour biopsies were taken after an overnight fast. They were taken from apparently viable tumour tissue and were then subjected to histological examination and analysis of flow cytometric DNA content, ODC activity (IV,V) and Ki-67 reactivity (V). One tissue sample was also taken from the normal mucous membrane in the oral cavity for control measurements in the various assays. The patients in paper IV were randomised into a control and a study group. The study patients received enteral nutrition (Clinifeed Protein rich®, Roussel Nutrition, Stockholm, Sweden) by means of a nasogastric tube (IV). Harris-Benedict´s formula was used to estimate the patients resting energy expenditure [127]. An energy content corresponding to 150 % of resting energy expenditure was given as non protein calories (2675±59 and 2055±71 Kcal for men and women respectively) and proteins (0.8 to 1.0 g/kg/day) to the study group of patients. The study patients in paper V received the same energy and protein content as above but by means of artificial intravenous nutrition (TPN). The control group of patients were allowed to eat according to their spontaneous appetite. This means a caloric intake estimated to be less than 1000 Kcal/day and 0.5 g protein/kg/day even though the patients were hospitalised (IV, [3, 128]).

After 6-8 days of nutritional support, a second sample of tumour tissue was taken from all study and control patients as far as possible from the location of the first biopsy, in order to avoid tissue reactions from the the injury of the first biopsy.

Informed consent was obtained from all patients taking part in this study, which was approved by the Committee for Ethics of the Faculty of Medicine, University of Gothenburg, Sweden.

Experimental studies (I,II,III)

Starvation and Refeeding (I,II,III)

All animals were fed *ad libitum* with a Purina chow diet unless otherwise indicated. Starvation for 24 hours was used in order to study tumour growth retardation. Refeeding with Purina chow was instituted in several experiments after a period of 24 h starvation (I,II,III). In some experiments, various deficient diets were used for refeeding (I).

Inhibition of ODC activity (I,II,III)

α-difluoromethylornithine (DFMO) is a specific irreversible inhibitor of ODC [77, 82, 129-133]. It was kindly supplied by the Merrell Dow, Research Institute. Strasbourg, France. The effects on tumour growth were studied after inactivation of ODC with DFMO (I,II,III). DFMO was diluted with tap water (2%) and given from the 3rd day after tumour implantation until death. DFMO treatment did not affect water or food intake.

Insulin experiments (I)

Insulin experiments were performed to study whether insulin mediated the nutritional regulation of tumour ODC activity. Plasma insulin of the animals was measured by a double antibody technique (Novo) and plasma glucose was measured by the glucose oxidase method (kit from Boehringer, Mannheim) as described in detail elsewhere [134]. The plasma insulin response to refeeding was blocked by pretreatment with anti-insulin antibodies (I). These antibodies were shown to block the immune-recognising epitope on circulating mouse plasma insulin molecules, since insulin levels were undetectable in anti-insulin

tumour-bearing animals but not in sham-injected refed tumour-bearing controls. To ascertain that anti-insulin antagonised insulin´s biological activity, glycerol release in a bioassay system with incubated rat epididymal fat cells was used as an index of the plasma sample´s lipolytic activity [135].

Inhibition of protein synthesis (I)

Protein synthesis was inhibited to study whether changes in ODC activity were related to de novo synthesis of the enzyme protein. Cycloheximide is known to be an inhibitor of protein synthesis at the translational level [136, 137]. Tumour and hepatic protein synthesis of the animals was inhibited by intraperitoneal administration of 0.5 mg cycloheximide per g body weight. This dose was chosen since it was not lethal in any case. The possibility of a direct toxic influence of cycloheximide on the ODC assay could be excluded as shown by *in-vitro* experiments, since no direct influence on the enzyme could be demonstrated with different concentrations of cycloheximide in the incubation medium (I).

METHODOLOGICAL CONSIDERATIONS

In this study, ODC activity has been related to the findings of biochemical, flow cytometric and immuno-histochemical assays, as well as to the energy state of the tumour determined *in vivo* and *in vitro*.

Enzyme assays (I-V)

Homogenisation of tissue was performed in a sucrose buffer freshly made before each experiment. Centrifugation was performed at 40000xg for 2 hours in order to remove mitochondria, where enzymes can be located that may generate labelled CO_2 which are irrelevant for the ODC assay [138]. After centrifugation, the protein content of the supernatant was determined as described by Lowry [139]. The supernatant could then be stored at -70°C for one month before enzyme assay. The activity was shown to be stable during this time. There is a known diurnal variation of ODC activity in tissues [140, 141], probably due to variations of dietary intake. In order to avoid this diurnal variation of ODC activity, all experiments were started at 8 am in the morning and there were always control animals killed simultaneously with the study animals.

Ornithine decarboxylase (ODC)

ODC activity of the sample was determined in duplicate by measuring the release of $^{14}CO_2$ from an ornithine-saturated buffer containing ^{14}C-labelled DL-ornithine. The method used is a slight modification of the method described by Noguchi et al [142]. Scintillation counting was performed with an LKB Wallac 1215 Racbeta II Liquid scintillation counter, to give the released $^{14}CO_2$ /h. The tissue activity of ODC could then be expressed as nmoles CO_2 /h/mg protein.

S-adenosylmethionine-decarboxylase (SAMD)

SAMD activity was analysed with a procedure very similar to that described for ODC, using labelled S-adenosylmethionine ^{14}C (I). The method is described in detail by Pegg and Pösö [143].

Polyamine assay (I)

The polyamine content in tumour, liver, spleenic and intestinal tissues was determined with high performance liquid chromatography (HPLC) as described by others [144]. The polyamines were separated with a Nova-Pak C18 column using an automatic sample injector (WISP 710B, Waters Chromatography Division, Millipore Corporation, Milford, USA).

Protein synthesis (I)

Protein synthesis was estimated using the flooding technique originally described by Garlick [145] The flooding dose of ^{14}C-U-leucine was injected i.p. and the animals were killed 30 min. later. Tissue protein was extracted and radioactivity in proteins was determined as described elsewhere [146].

Flow cytometry (II,IV,V)

Flow cytometry was used to determine the relative size of the cell cycle compartments and for assay of labelled cells after incorporation of BrdUrd into DNA. Flow cytometry was also used to determine the relative size of the aneuploidic compartment in the human tumours studied.

For cytokinetic analysis of the experimental tumour, a single cell suspension was obtained by mincing the tumour tissue in a buffer solution containing collagenase (II,III). The trypan blue exclusion test

showed a cell viability in this solution of more than 90 % [147]. After denaturation of the double stranded DNA, the BrdUrd incorporation into DNA could be visualised by staining with fluorescein isothiocyanate (FITC)-conjugated BrdUrd. Furthermore, DNA content could be assessed after staining with propidium iodide. Measurements were performed with an MPV Flow cytometer (Leitz) attached to a Monroe 8888 computer system. Both fluorochromes were excited by the 395 - 495 nm interval light from a mercury source. Emission of propidium iodide and FITC-fluorescence was measured above 610 nm and in the interval 510 to 540 nm respectively.

A tumour sample of 100-200 mg was taken from 3 different locations of viable tumour for analysis of the human tumours (IV,V). The preparation was then processed according to Barlogie et al [148]. The sample was analysed with an ICP 11-PHYWE pulse cytophotometer (Göttingen, Germany) with human leucocytes as a standard. On average 20 000 - 30 000 cells from each sample were measured, with a coefficient of variation (CV) < 5%.

Ki-67 reactivity (V)

The monoclonal antibody Ki-67 is known to be directed against a proliferation-associated antigen present in the nuclei of proliferating cells [149, 150]. Thus, the growth fraction of tumours was determined from samples snap frozen in liquid nitrogen. Acetone and chloroform were used for fixation to preserve the antigen. After staining with Ki-67 using the immunoalkaline phosphatase anti-alkaline phosphatase (APAAP) technique as described previously [151, 152], tumour cells could be evaluated for nuclear staining (V). The percentage of stained tumour cells correlated significantly ($r=0.86$; $p<0.01$) with the number of aneuploidic cells in the replicative phases $S+G_2M$.

Thymidine incorporation (I)

The rate of DNA synthesis can be estimated from the rate of incorporation of labelled thymidine into DNA [69, 153]. The mice were injected intraperitoneally with a single dose of ^{14}C-thymidine one hour before death (I). Tumour tissue was immediately frozen in liquid nitrogen and placed in a -70°C freezer. Within one week DNA extraction was performed after homogenisation of the tumour tissue. DNA concentration was determined with calf thymus DNA as the standard [154]. The radioactivity of the sample was determined in triplicate by scintillation spectrometry. ^{14}C-labelled thymidine was chosen instead of tritiated in order to obtain stable scintillation counts. The specific radioactivity and hence the rate of thymidine incorporation into DNA/h, was calculated per mg DNA.

Bromodeoxyuridine incorporation (II,III)

Bromodeoxyuridine (BrdUrd) is a thymidine analogue and was used to measure DNA-synthesis in terms of BrdUrd incorporation into DNA. Cells incorporating BrdUrd into DNA are considered to be actively DNA-synthesising cells [60, 68, 72, 155, 156]. This technique can be combined with flow cytometric analysis of DNA content to obtain more detailed information on the cell cycle kinetics [65, 70, 71, 157, 158]. Tumour-bearing animals were injected i.p. with 0.1 mg BrdUrd/g body weight [159] as a single injection. Higher doses failed to label a larger percentage of cells suggesting that the chosen dose was sufficient to label all cells, capable of incorporating BrdUrd into DNA. This *in-vivo* labelling technique was more efficient and reliable than *in-vitro* labelling using an incubation medium containing BrdUrd. The *in-vitro* incubation gave a 40 % reduction in the yield of BrdUrd-labelled cells compared to *in-vivo* labelling. Furthermore, by using the trypan blue

exclusion test, we noted 30% non-viable cells in the cell suspension after *in-vitro* incubation and <10 % non-viable cells after *in-vivo* labelling. By means of tritiated BrdUrd, we found in separate experiments that only 4 % of of the tritiated BrdUrd remained in plasma after one hour. There was a positive correlation between the relative S-phase compartment size (flow cytometry) and immunocytochemically determined BrdUrd incorporation into tumour DNA (r = 0.81, not shown). There was no change in ODC activity or the relative size of the S-phase compartment, after repeated injections of BrdUrd performed in some experiments, suggesting that there was no cytotoxic effect of the BrdUrd dose.

Immunocytochemical staining for BrdUrd.

Monolayered cytospin preparations of tumour cells were made with a Shandon 2 cytocentrifuge. The slides were fixed in 70 % ethanol for 1h and allowed to dry. DNA was then denatured and cells in the S-phase were visualised after incubation with an anti-BrdUrd monoclonal antibody (Becton Dickinson Svenska AB, Hägersten, Sweden) and a peroxidase-conjugated secondary antibody (Dakopatts AB, Hägersten, Sweden) developed with diaminobenzidine as a chromogen. The percentage of labelled cells was determined by counting 500 cells (leucocytes omitted) under an ordinary light microscope. With this method, nuclei containing even small amounts of BrdUrd can be detected.

Staining for combined flow cytometric analysis of BrdUrd and cellular DNA content.

After ethanol fixation and DNA denaturation, the cells were incubated with an anti-BrdUrd monoclonal antibody. Then followed incubation with an FITC-conjugated secondary antibody. Cellular DNA was stained with propidium iodide. The amount of BrdUrd-labelled cells in

the different cell cycle compartments could be determined, since the staining procedure with propidium iodide for DNA content and the FITC-conjugated antibody for BrdUrd labelling were performed on the same sample, .

Cytokinetic evaluation (II)

The cell proliferation rate of the tumour cannot be deduced from tumour weight or volume measurements only, since the impact of cell loss and non-dividing cells must also be accounted for. Flow cytometry only provides a static picture of the distribution of DNA-content, illustrating the relative size of the cell cycle phases at the time of biopsy. Thus, flow cytometry and Ki-67 reactivity do not provide sufficient information to assess the cell cycle dynamics. By labelling the DNA-synthesising cells with BrdUrd and then using flow cytometry, it is possible to observe the labelled cells through the cell cycle and cell division, facilitating evaluation of the cytokinetics of tumour growth. All cytokinetic determinations are based on the assumption that the rate of cell cycle traverse is constant in all cell cycle phases.

Graphic estimation of DNA synthesis time.

In order to obtain the DNA synthesis time (T_S) of an exponentially growing cell culture, Sasaki suggested that serial labelling indices can be plotted against the corresponding times of incubation with a BrdUrd-containing medium [73]. The slope of the regression line obtained makes it possible to calculate T_S, assuming that the G_1 cells enter the S phase at a constant rate. This graphic method designed for assessment of growing cells in a cell culture was modified in the present study to enable estimation of T_S after *in-vivo* labelling with BrdUrd as described above.

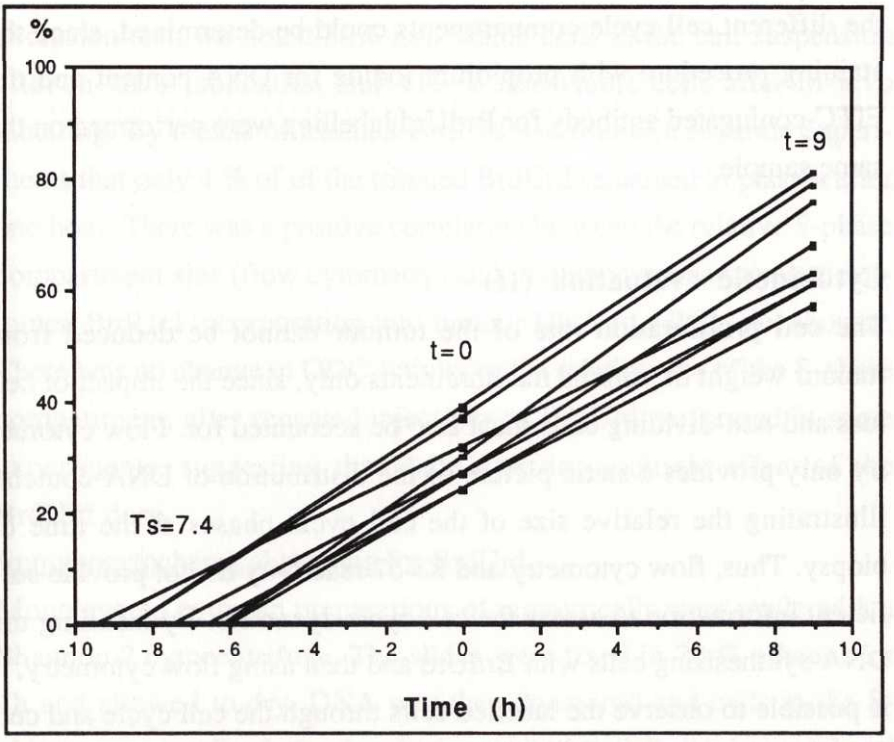

Figure 4.
Illustration of the graphic method described by Sasaki for obtaining DNA-synthesis time. A modification of this method was used for the estimations in table 2 (II) where the relative size of the S-phase compartment as determined by flow cytometry was plotted at time 0 hours. At time 9 hours after BrdUrd injection, the plotted value is the sum of the percentage of FITC fluorescence in G_0G_1 of cells having passed through the S phase after the BrdUrd injection i.p. (see formula I in paper II) plus the percentage of cells in the S phase as determined by flow cytometry. DNA-synthesis time is the negative intercept of the extrapolated regression line (7.4±0.5 h for freely fed animals). This graphic estimation was performed for 8 animals in each of the study groups and the results given in paper II represent the mean values.

The potential doubling time (T_{pot}) can also be determined graphically, as shown by Okada [160]. T_{pot} is defined as the time the tumour takes to double its cell content being capable of continuous proliferation.

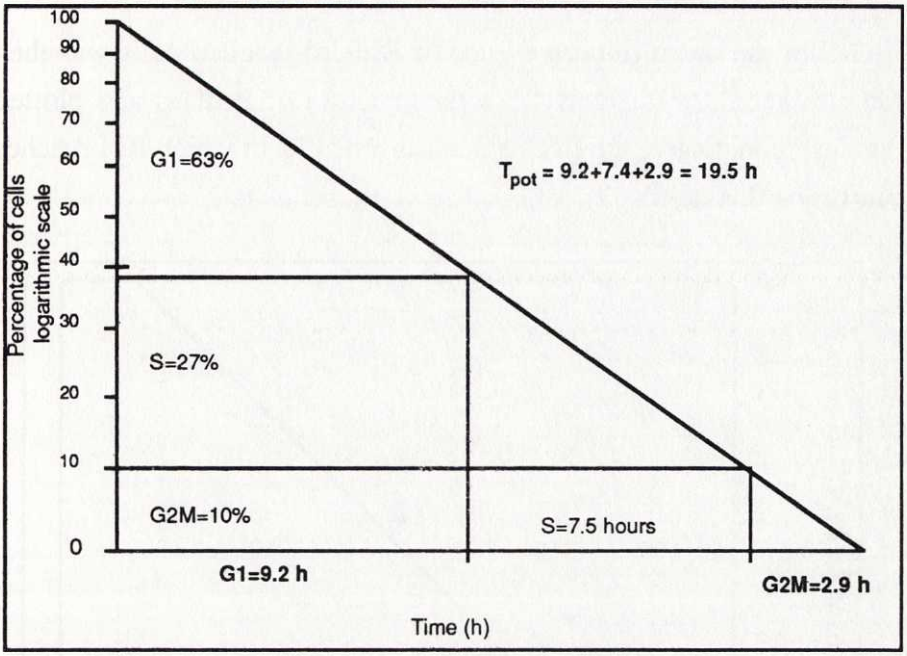

Figure 5

Illustration of the graphic method described by Okada for computing the parameters of the life cycle of cultured cells in the exponential growth phase. This figure shows the graphic estimation from a tumour from one of the animals of figure 4. The relative size of the cell cycle phases is known from flow cytometry. The duration of the S phase is also estimated (Ts=7.5 from Fig 4) giving the X-axis a time scale. The entire cell cycle time can then be estimated as shown in the figure.

Relative movement of BrdUrd-labelled cells.

An alternative method of determining the time of DNA synthesis (T_S) of the tumour from a single sample of BrdUrd-labelled cells was suggested by Begg [65]. The analysis is based on measurements of the relative movement (RM) of labelled cells in cell cycle transit as illustrated in chart 1 and described in paper II [65]. The calculation of cell cycle progression is based on the assumption that the entire DNA-synthesis time is 1.0 and that the mean value of fluorescing label is 0.5 at the time of injection of BrdUrd. In this study, the relative movement

(RM) of the mean fluorescesence of BrdUrd-labelled cells was then determined 1/2, 1, 2 and 4 h after injection of BrdUrd and plotted against time, to give the DNA synthesis time (T_S) in which RM reaches unity = 1.0 (Fig. 6).

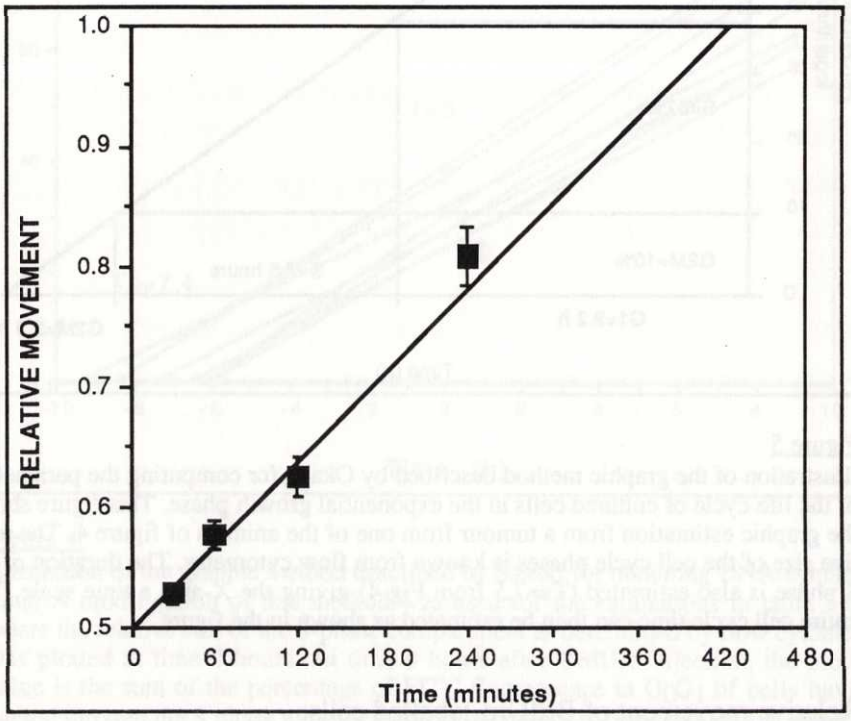

Figure 6.
Relative movement (RM) of BrdUrd-labelled cells. It is assumed that the mean fluorescence of the label is 0.5 at time zero (T_0), and that the entire duration of DNA synthesis is 1.0. The mean fluorescence of the label is plotted against time after BrdUrd injected at T_0. RM is calculated for each observation according to Begg [65] and a regression line is constructed. Each point represents the mean of 6 observations and bars represent SEM. Note the intercept of the extrapolated regression line around 420 minutes = 7 hours when RM reaches unity at 1.0. Thus the duration of DNA synthesis is estimated graphically to be T_S = 7 hours.

Since the initial value of RM at t=0 is 0.5 and RM at a time (t) is very near a linear function of time, a single measurement at some time (t)

can be used together with the initial value to obtain T_S [65, 74]. This assumption is supported by the results shown in figure 4. However, a non-linear plot must be expected in an expanding tumour cell population since cells in the S phase will then have a non-linear age distribution. This means that there will be more cells at the beginning of the S phase. Furthermore, the length of the G_2M phase relative to the S phase can affect the RM versus t plot, creating a curvature of the plot [65, 74]. The impact of these effects can be corrected for when estimating T_S from only one sample [65, 74]. However, in the experiment shown (Fig. 6) the regression line is based on 24 observations in all, so that no such correction was necessary.

Fractional new cell formation.

Repeated injection (pulses) of BrdUrd every second hour (0h, 2h and 4h) facilitates estimation of the fraction of new cells entering the S phase (FR_{new} from formula III in paper II), if the progression of cells through the S phase is constant, as assumed above. Immunocytochemical staining with peroxidase enables the labelling index to be determined after 1 h and 6 h respectively. The difference in labelling index equals the fractional new cell formation (II).

Another way to determine the fractional cell transit is by calculating the fraction of cells which have passed through the $S+G_2M$ phases through cell division and entered the G_0G_1 phase. This has been done by using the bivariate flow technique 9 hours after pulse labelling. The proportion of FITC-labelled cells per hour entering the G_0G_1 phase (FR_{FITC}) can then be estimated from the formula below (II):

$$FR_{FITC} = \frac{F_{G0G1}}{2 \times \left(\frac{F_{G0G1}}{2} + F_{SG2M} \right) \times t}$$

The fluorescence in G_0G_1 (F_{G0G1}) must be divided by 2 because these cells have undergone cell division. The rate at which BrdUrd-labelled cells enter G_0G_1 (FR_{FITC}) is thus expressed as the amount of fluorescence in G_0G_1 in relation to the total amount of fluorescence. The potential doubling time of the tumour can then be estimated as described in paper II (formula II).

Cell loss.

The DNA-synthesis time and the potential doubling time describe the dynamics of the cell cycle but the virtual tumour growth rate is also dependent on cell loss during cell replication [63, 64, 65, 67]. Begg suggested estimation of cell loss by relating T_{pot} to measurements of the volume doubling time of the tumour [65]. The volume doubling time of the tumour, however, needs to be measured during an observation period and serial volume registrations are required [61]. In the present study, an alternative measure of cell loss has been obtained by subtracting the fractional new cell formation (FR_{FITC}) as determined by the bivariate flow technique from the percentage increase of the peroxidase-stained cells (FR_{pex}). FR_{pex} was calculated by subtracting the labelling index after 1h from the corresponding index after 9h (II). This estimate of fractional cell loss is based on the assumption that the peroxidase technique only accounts for stained cells with a preserved nuclear structure, while the FITC technique cannot distinguish between viable and destructed cells as long as the fluorescing material has a nuclear content within the range of the DNA histogram (II; chart 1). When the potential doubling time is taken into account, the actual cell loss per cell division can be calculated as described in paper II (formula IV and V).

Energy state of tumour tissue (III)

The energy state of the tumour tissue was determined in order to examine whether changes in ODC activity and cell proliferation rate were correlated to changes in energy state. In recent years, the energy state of living tissue has been possible to establish *in vivo* by analysis of phosphorus metabolites using nuclear magnetic resonance spectroscopy, ^{31}P-NMR [161-169]. Since phosphorus-containing compounds play a key role in the energy metabolism of tissues, knowledge of their relative concentrations in high and low energy phosphorus metabolites give insight into the energy state of the tissues [163]. In the present study, Nuclear Magnetic Resonance (^{31}P-NMR) measurements were performed *in vivo* and they were then compared with *in-vitro* measurement using high performance liquid chromatography (HPLC).

Nuclear Magnetic Resonance (^{31}P-NMR)

In-vivo measurement of the energy state of tumour tissue by NMR-spectroscopy was performed on a Bruker Biospec, BMT 24/30. Anaesthetised mice were placed with the tumour in a Helmholz coil, 2 cm diameter. The mice remained in this position for around 10 minutes until the ^{31}P-NMR-spectra were obtained. The physical principles involved in obtaining an NMR-spectrum from a tissue are described elsewhere [170, 171] The relative amount of the metabolites phosphocreatinine (Pcr), inorganic phosphate (Pi), adenosinetriphosphate (ATP=βATP), phosphomonoesters (PME) and phosphodiesters (PDE) was calculated and expressed in relation to the total phosphorus area. The equipment used is presented in paper III and it is considered to have a good resolution for assay of the phosphorus compounds [172]. The great advantage of using ^{31}P-NMR-spectra for determination of changes in energy state of tissues is obviously the possibility of performing repeated measurements on the same individual. This technique

certainly has a promising future for assessment of changes in metabolism as a response to various treatments in human cancer [161, 165, 167, 173-175].

High Performance Liquid Chromatography

In-vitro measurements of the energy state of tumour tissue were performed with an HPLC manufactured by Pharmacia Fine Chemicals AB, Uppsala, Sweden [176]. Analysis of the energy metabolites such as adenosinetriphosphate (ATP), adenosinediphosphate (ADP), adenosinemonophosphate (AMP), inosine (In), hypoxantine (Hx), inosinemonophosphate (IMP), uric acid (Ua) phosphocreatinine (Pcr) and creatinine (Cr) could then be performed in μmoles/gram dry weight of tumour tissue. This procedure was performed to verify the results from the ^{31}P-NMR-spectra used for these experiments [177].

Energy State of tumour tissues

The energy state of tissues was assessed by the relative concentrations of high energy phosphates (ATP, PCr) and low energy phosphates (PME). The PCr/Pi ratio is often used as an index of the energy state or the metabolic respiratory activity of tissues [172]. The NMR technique and the HPLC technique are not directly comparable because NMR *in vivo* mainly determines cytosolic phosphorus compounds. We have therefore, chosen to report the data from both (III). Low energy phophates measured with HPLC are ADP,AMP,IMP and inosine.

The bioenergetic state was expressed as the energy charge of the tissue calculated according to Atkinson [163, 178, 179].

$$\text{Energy charge} = \frac{ATP + \frac{ADP}{2}}{ATP + ADP + AMP}$$

The energy charge is considered to be an equilibrium for energy production/utilisation and can be defined as the extent to which the adenylate system is filled with high energy phosphates. The ratio described varies between 0 and 1.0. If all the adenylate system is in the form of ATP, the energy charge will be 1.0. Ordinary living tissue in a steady state generally exhibits an energy charge of 0.85.

Statistics

Differences between two groups were compared by means of Student's t-test or Fischer´s exact test [180]. One factor factorial ANOVA with or without repeated measures was used for multiple group comparisons. Results are given as mean ± SE; a 95% confidence interval was used in ANOVA computations; $p<0.05$ was considered statistically significant. The rank sum test was used to test differences in survival in DFMO-treated animals compared to control animals.

RESULTS AND DISCUSSION

Food intake is one of the most powerful modulators of tumour growth in several experimental systems [24-28, 181-183], although not consistently so [184, 185]. Nutritional effects on tumour growth have been difficult to confirm in human cancer [23], although there was a recent report suggesting altered cell cycle kinetics after total parenteral nutrition (TPN) in head and neck cancer patients [29]. The risk of tumour growth enhancement is important to consider when deciding whether a cancer patient should receive artificial nutrition as part of the oncological treatment or as palliation treatment only [30, 186, 187]. The aim of this study was to identify growth-associated changes in tumour tissue in an experimental tumour model in response to nutritional modulation and to determine whether nutritional effects on tumour growth are demonstratable in human cancer.

ODC activity in tumour tissue has been determined and studied in relation to tumour growth (I-V). ODC has been demonstrated to be rate-limiting for the synthesis of polyamines, which play an important role in the regulation of DNA-synthesis and cell growth (Fig.3, [75-77, 78, 81-83, 95, 188]). Several studies have reported elevated polyamine levels in human tumours [88-92, 103, 189]. Furthermore, elevated polyaminelevels have been reported in urine, cerebrospinal fluid, plasma and erythrocytes of cancer patients and such observations may be consistent with rapid growth of malignant disease [85-87, 93, 94, 190-194].

We have determined ODC activity directly in tumour tissue from untreated and malnourished patients with head and neck cancer. A recent report demonstrates elevated polyamine levels in squamous cell carcinoma of the oral cavity and a variable ODC activity from the same

cancers [92], and the degree of differentiation in the tumours has been suggested to be related to polyamine content and ODC activity [102]. In the present study it was obvious that high levels of ODC activity were associated with poor differentiation and a correlation between ODC activity and the percentage of cells demonstrating aneuploidy (aberrant DNA content) was found (IV). Previous studies have shown that the presence of aneuploidy is an unfavourable predictor for the survival of cancer patients [37, 38, 46, 52-54, 57, 58]. We found that 70% of the patients demonstrated aneuploidic cell clones (IV and V, [37, 39, 54]). However, there was no significant increase in mortality within one year among patients demonstrating aneuploidy in their tumours, in contrast to cancer patients with high levels of ODC activity in tumour tissue, which had significantly shorter survival than others (IV). This suggests that high ODC activity in tumour tissue is associated with an aggressive cancer disease.

In order to obtain information about the tumour-cytokinetic effects in relation to nutritional modulation and ODC activity we used a well-known tumour model. This model consists of tumour-bearing mice with a poorly differentiated, transplantable subcutaneous sarcoma, which has been well characterised in our laboratory for several years [117-122]. The metabolic host reactions in response to the tumour are similar to those of cancer patients with progressive cachexia [123, 126]. The tumour comprised about 5% of the total body weight at the time of most of the experiments in this study. The cytokinetic data obtained from this rapidly growing experimental tumour model with a monoclonal appearance cannot be directly extrapolated to the clinical situation, since human cancer demonstrates a considerably slower growth rate and generally exhibits very heterogeneous cytokinetic characteristics [158, 195, 196]. Conclusions based on cytokinetic informa-

tion from experimental studies therefore need to be further evaluated in human cancer.

Starvation for 24 hours in our tumour-bearing mice reduced their body weight by 20% and caused an inhibition of the tumour growth rate. There was a significant retardation of net tumour growth (I) in terms of tumour weight measurements, which was associated with a pronounced fall in tumour ODC activity (I). The retardation of tumour growth was associated with a reduced thymidine and BrdUrd incorporation into DNA, as well as a prolonged doubling time for those cells which were involved in cell cycle passage (I,II). However the concomitant finding of an unchanged DNA-synthesis time suggested that fewer cells were traversing the $S+G_2M$ phases. This suggestion was supported by the flow cytometric studies demonstrating reduced relative size of the S phase cytocompartment obviously due to accumulation of tumour cells in the G_0G_1 phase (II).

Although the ODC activity in the tumour tissue decreased in response to starvation, the polyamine levels of tumour tissue were not reduced after starvation (I) This suggests a feedback mechanism by polyamines in the regulation of ODC activity [96]. The overall reduction in the number of cycling cells may reduce the demand for polyamines thus resulting in a down regulation of ODC activity. This implies that some other mechanism than tumour polyamine synthesis is involved in the prolongation of the potential doubling time after a period of starvation [27, 181].

The relative concentration of phophorus compounds in tumour tissue was analysed with ^{31}P-NMR-spectroscopy and the HPLC-technique as reported in paper III. From the analysis of phosphorus metabolites, the energy charge of tumour tissue could be estimated [163, 178]. The energy charge represents an equilibrium of the adeny-

late system (AMP,ADP and ATP) and is more than 0.85 in normal tissues. In tumour tissue, this ratio was significantly reduced, which suggests a different equilibrium of energy production/utilisation in tumour tissues than seen in normal tissues [197]. After starvation, the energy charge of tumour tissue was significantly reduced in comparison with freely fed animals (III). This suggests that the proposed accumulation of cells in G_0G_1 was caused by lack of energy rather than a reduction of ODC activity. The reduced energy charge of the tumour tissue may lead to an increase of cells entering the quiescent phase (G_0), thus reducing the growth fraction of the tumour [198] or alternatively slowing the traverse through the G_1-phase. Furthermore, the apparent reduction of energy charge is a plausible explanation for the increased cell loss noted after starvation (II; [198]). These findings support the concept that experimental tumour growth is highly dependent on substrate availability.

Retardation of tumour growth was also obvious when the tumour-bearing animals were treated with DFMO (I,II), which is a specific and irreversible inhibitor of ODC activity [77, 82, 129-133]. Such an inactivation of the enzyme was demonstrated in this study with a concomitant increase in SAMD activity, which is a well-recognised compensatory regulation following effective ODC inhibition (Fig. 3; [77, 199, 200]). DFMO treatment seemed to have influenced the tumour, without any obvious deleterious effects on the host since the animals demonstrated the same food and water intake as did untreated tumour-bearing control animals (I, [201]).

The retardation of tumour growth in DFMO-treated mice was characterised by a decreased labelling of DNA by BrdUrd and a prolonged DNA-synthesis time, concomitant with a prolonged potential doubling time. These findings, in combination with a relative accumulation of

cells in the $S+G_2M$ cytocompartment, suggest that DFMO treatment resulted in a decrease in tumour growth which primarily affected DNA synthesis and the proliferative phase prior to cell division [80, 132, 202-206]. This finding is in contrast to some other reports, which indicates that the role of polyamines may differ among different cell lines [207-210]. Furthermore, the results of our cytokinetic estimations suggested that DFMO treatment increased the fractional loss of cells along cell cycle progression compared with tumours from both starved and freely fed animals (II). This may indicate that polyamine depletion results in a deteriorated regulation of DNA synthesis and cell growth.

The energy charge of the tumour was not changed after DFMO treatment compared to freely fed animals. In fact, some of the high energy phosphates (PCr,ATP) were increased. This indicates that the decrease in DNA synthesis and the prolongation of the potential doubling time caused by ODC inhibition was not mediated by alterations in cellular energy-generating or requiring processes (III). ODC is therefore suggested to have a direct role in the induction or promotion of cell division.

Comparing the effects of polyamine depletion and starvation on tumour cytokinetics and energy state in tumour tissue, it is obvious that different mechanisms were operating in the retardation in tumour growth (I,II,III). Thus, polyamine depletion resulted in suppression of DNA synthesis and cell division with a preserved energy state, whereas starvation caused an accumulation of cells in G_0G_1 due to energy shortage in tumours from starved animals.

After starvation there was a reduction in tumour tissue of all the cytokinetic variables described above (S phase, thymidine, BrdUrd) and ODC activity(II). Refeeding of the tumour-bearing animals after a 24-hour period of starvation restored the energy charge in tumour tissue to

pre-starvation levels (III). There was a general response to refeeding with an activation of all variables but with different lag phases (II). Thus, ODC activity reached higher than pre-starvation values within one hour, while BrdUrd incorporation into DNA and the relative size of the S phase compartment were normalised after 4 and 8 hours respectively (II). The response of ODC activity to refeeding did not produce any changes in the polyamine levels in tumour tissue (I). The magnitude of the response of ODC activity was related to the composition of the food. Thus, oral intake of glucose demonstrated the strongest response in ODC activity compared to protein and lipid diets (I). However, intraperitoneal administration of glucose did not produce any response in ODC activity, although similar levels of glucose in serum were found to those after oral intake of glucose (I). There was also a difference in the response of plasma insulin to different substrates and a significant correlation between ODC activity and plasma insulin was shown ($r=0.82$, $p<0.01$; II). Thus, ODC activity seemed to reflect acute changes of the tumour metabolism in response to refeeding and this response was at least partly mediated by insulin stimulation [100, 211-217]. These findings support the suggestion that ODC precede DNA synthesis and is activated to stimulate tumour growth [84, 104, 105, 108-111, 218-220].

In this study, we have evaluated ODC activity as a marker for stimulation of tumour growth in cancer patients before and after artificial nutrition. The nutritional treatment was administered for 6-8 days either as total parenteral nutrition (V) or as formula diets supplied through a nasogastric tube (IV). These modes of nutrition are extensively used as adjuvant treatment or for palliative purposes in cancer patients [2, 5, 14, 21-23, 27, 186, 187, 221, 222]. We were not able to demonstrate any change in ODC activity after refeeding with artificial

nutrition (IV,V). There was no change in the size of the the growth fraction of the tumours after parenteral refeeding, evaluated by means of Ki-67 reactivity. However, an increase of the aneuploid compartment size was observed in tumours after enteral nutrition compared to the control group ($p<0.01$; IV). This alteration was not found in patients receiving parenteral nutrition (V). The clinical significance of this finding remains to be determined.

We conclude that tumour growth is highly dependent on food intake in our experimental tumour model and that there is little evidence in this study to support the fear that nutritional support will have any deleterious effects on tumour growth in cancer patients.

During the past twenty years, the treatment of certain specific malignant tumours has been successful in producing a better survival. Unfortunately, this is not the case for cancer disease in general and not for head and neck cancer, where survival statistics fail to improve in spite of advanced chemotherapeutic treatments in combination with traditional treatment [223, 224]. This fact is demonstrated in this study by the group of weight-loosing patients in paper IV, where only two of the 25 patients with an advanced tumour stage (T3-T4) have survived more than three years. These data emphasize the importance of identifying such weight-loosing cancer patients and regarding nutritional support as an essential part of the oncological palliation treatment [15]. Sensitive cytokinetic methods are needed to assess tumour biology on a molecular level, in order to adjust the oncological treatment in each individual case. In this context, determination of ODC activity or polyamines in tumour tissue may reflect changes in growth characteristics of cancer.

SUMMARY AND CONCLUSIONS

This study provides evidence that nutritional modulation of the host affects the growth rate of the MCG 101-sarcoma in mice. Starvation prolonged the potential doubling time of the tumour due to an accumulation of cells in the G_0G_1 phase in the presence of limited energy availability.

Refeeding restored tumour growth with an immediate activation of ornithine decarboxylase activity in tumour tissue. The increase in ODC activity preceded the increase in DNA synthesis and cell proliferation in response to refeeding. The activation of ODC was correlated to the carbohydrate content of the dietary intake and the levels of plasma insulin in the tumour-bearing animals.

Specific inhibition of ODC with DFMO reduced tumour growth by inhibition of DNA synthesis, causing an accumulation of cells in the $S+G_2$ phase and led to an increased fractional cell loss. However, the energy charge of such tumours was not affected by DFMO. This suggests that ODC has a direct role in the induction and promotion of cell division.

There was little evidence in this study to support the fear that nutritional support in cancer patients stimulates tumour cell proliferation.

Determination of ODC activity in tumour tissue may have prognostic significance for survival and can probably be to used to monitor rapid changes in DNA-synthesis.

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REFERENCES

1. **Brookes**, G. B. Nutritional Status - a Prognostic Indicator in Head and Neck Cancer. Otolaryngol Head Neck Surg. 93: 69-74, 1985.
2. **Copeland**, E. M., J. M. Daly and S. J. Dudrick. Nutritional Concepts in the Treatment of Head and Neck Malignancies. Head Neck Surg. 1: 350-363, 1979.
3. **Westin**, T., C. Trädhgård, K. Andersson, I. Warnold and S. Edström. Nutritional Assessment of Hospitalized Patients with Head and Neck Cancer. ORL J Otorhinolaryngol Relat Spec. 50: 193-198, 1988.
4. **Westin**, T., A. Jansson, C. Zenckert, T. Hällström and S. Edström. Mental Depression Is Associated With Malnutritional in Patients With Head and Neck Cancer. Arch Otolaryngol Head Neck Surg. 114: 1449-1453, 1988.
5. **Copeland**, E. M., J. M. Daly and S. J. Dudrik. Nutrition as an Adjunct to Cancer Treatment in the Adult. Cancer Res. 37: 2451-2456, 1977.
6. **Daly**, J., S. J. Dudrick and E. M. Copeland. Evaluation of Nutritional Indices as Prognostic Indicators in the Cancer Patient. Cancer. 43: 925-931, 1979.
7. **De Wys**, W., C. Begg, P. Lavin, P. Band, J. Bennet, J. Bertino, M. Cohen, H. Douglass, P. Engstrom, E. Ezdinli, J. Horton, G. Johnson, C. Moertel, M. Oken, C. Perlia, C. Rosenbaum, M. Silverstein, R. Skeel, R. Sponzo and D. Tourmey. Prognostic Effect of Weight Loss Prior to Chemotherapy in Cancer Patients. Am J Med. 69: 491-497, 1980.
8. **De Wys**, W. Management of Cancer Cachexia. Semin Oncol. 12: 452-460, 1985.
9. **Lawson**, D. H., A. Richmond, D. W. Nixon and D. Rudman. Metabolic Approaches to Cancer cachexia. Ann Rev Nutr. 2: 277-301, 1982.
10. **Nixon**, D. W., S. B. Heymsfield, A. E. Cohen, M. H. Kutner, J. Ansley, D. H. Lawson and D. Rudman. Protein-Calorie Undernutrition in Hospitalized Cancer Patients. Am J Med. 68: 683-689, 1980.
11. **van Eyes**, J. Nutrition and Cancer: physiological interrelationships. Ann Rev Nutr. 5: 435-461, 1985.
12. **Wesdorp**, R. I. C., R. Krause and M. F. Von Meyenfeldt. Cancer Cachexia and its Nutritional Implications. Br J Surg. 70: 352-355, 1983.
13. **Lanzotti**, V. J., D. R. Thomas, L. E. Boyle, T. L. Smith, E. A. Gehan and M. L. Samuels. Survival with Inoperable Lung Cancer: an Integration of Prognostic Variables Based on Simple Clinical Criteria. Cancer. 39: 303-317, 1977.
14. **Issel**, B. F., M. Valdivieso and H. A. Zaren. Protection Against Chemotherapy Toxicity by Iv Hyperalimentation. Cancer Treat Rep. 62: 1139-1143, 1978.
15. **Williams**, E. F. and M. M. Meguid. Nutritional Concepts and Considerations in Head and Neck Surgery. Nutr Head Neck Surg. 11: 393-399, 1989.
16. **Studley**, H. O. Percentage of Weight Loss. A Basic Indicator of Surgical Risk in Patients with Chronic Peptic Ulcer. JAMA. 106: 458-460, 1936.
17. **Buzby**, G. P., J. L. Mullen, D. C. Matthews, C. L. Hobbs and E. F. Rosato. Prognostic Nutritional Index in Gastrointestinal Surgery. Am J Surg. 139: 160-167, 1980.

18. **Mullen**, J. L. Implications of Malnutrition in the Surgical Patient. Arch Surg. 114: 121-125, 1979.
19. **Mullen**, J. L., G. P. Buzby, D. C. Matthews, B. F. Smale and E. F. Rosato. Reduction of Operative Morbidity and Mortality by Combined Preoiperative and Postoperative Nutritional Support. Ann Surg. 192: 604-613, 1980.
20. **Warnold**, I. and K. Lundholm. Clinical Significance of Preoperative Nutritional Status in 215 Noncancer Patients. Ann Surg. 3: 299-305, 1984.
21. **Klein**, S., J. Simes and G. L. Blackburn. Total Parenteral Nutrition and Cancer Clinical Trials. Cancer. 58: 1378-1386, 1986.
22. **Evans**, W. K., R. Makuch, G. H. Clamon, R. Feld, R. S. Weiner, E. Moran, R. Blum, F. A. Shepherd, K. G. Jeejeebhoy and W. D. DeWys. Limited Impact on Total Parenteral Nutrition on Nutritional Status During Treatment for Small Cell Lung Cancer. Cancer Res. 45: 3347-3353, 1985.
23. **Brennan**, M. F. Total Parenteral Nutrition in the Cancer Patient. N Engl J Med. 217: 375-382, 1981.
24. **Edén**, E., L. Lindmark, I. Karlberg and K. Lundholm. Role of Whole-Body Lipids and Nitrogen as Limiting Factors for Survival in Tumour-bearing Mice with Anorexia and Cachexia. Cancer Res. 43: 3707-3711, 1983.
25. **Popp**, M. B., S. D. Morrison and M. F. Brennan. Total Parenteral Nutrition in a Methylcholantthrene-Induced Rat Sarcoma Model. Cancer Treat Rep. 65: 137-143, 1981.
26. **Sauer**, L. A., W. O. Nagel, R. T. Dauchy, L. A. Miceli and J. E. Austin. Stimulation of Tumour Growth in Adult Rats in Vivo during an Acute Fast. Cancer Res. 46: 3469-3475, 1986.
27. **Stragand**, J., P. G. Braunscheiger, A. A. Pollice and L. M. Schiffer. Cell Kinetic Alterations in Murine Mammary Tumours Following Fasting and Refeeding. Eur J Cancer. 15: 281-286, 1978.
28. **Torosian**, M. H., K. C. Tsou, J. M. Daly, J. L. Mullen, T. P. Stein, E. E. Miller and G. P. Buzby. Alteration of Tumour Cell Kinetics by Pulse Total Parenteral Nutrition. Cancer. 53: 1409-1415, 1984.
29. **Baron**, P., W. Lawrence, W. Chan, F. White and W. Banks. Effects of Parenteral Nutrition on Cell Cycle Kinetics of Head and Neck Cancer. Arch Surg. 121: 1282-1286, 1986.
30. **Buzby**, G. P., J. L. Mullen, T. P. Stein, E. E. H. Miller C.L. and E. F. Rosato. Host-Tumour Interaction and Nutrient Supply. Cancer. 45: 2940-2948, 1980.
31. **Wolf**, G. T., R. W. Makuch and S. R. Baker. Predictive Factors for Tumour Response to Preoperative Chemotherapy in Patients With Head and Neck Squamous Carcinoma. Cancer. 54: 2869-2877, 1984.
32. **Ensley**, J. F., J. A. Kish, A. A. Weaver, J. R. Jacobs, M. Hassan, G. Cummings and M. Al-Sarraf. The Correlation of Specific Variables of Tumour Differentiation With Response Rate and Survival un Patients With Advanced Head and Neck Cancer Treated With Induction Chemotherapy. Cancer. 63: 1487-1492, 1988.
33. **Crissman**, J. D., W. Y. Liu, J. L. Gluckman and G. Cummings. Prognostic Value of Histopathologic Parameters in Squamous Cell Carcinoma of the Oropharynx. Cancer. 54: 2995-3001, 1984.

34. **Crissman**, J. D., T. F. Pajak, R. J. Zarbo, V. A. Marcial and M. Al-Sarraf. Improved Response and Survival to Combined Cisplatin and Radiation in Non-Keratinizing Squamous Cell Carcinomas of the Head and Neck. Cancer. 59: 1391-1397, 1987.
35. **Ensley**, J., J. Crissman, J. Kish, J. Jacobs, A. Weaver, J. Kinzie, G. Cummings and M. Al-Sarraf. The Impact of Conventional Morphologic Analysis on Response Rates and Survival in Patients With Advanced Head and Neck Cancers Treated Initially With Cisplatin-Containing Combination Chemotherapy. Cancer. 57: 711-717, 1986.
36. **Jakobsson**, P. Å., C.-M. Eneroth, D. Killander, G. Moberger and B. Mårtensson. Histologic Classification and Grading of Malignancy in Carcinoma of the Larynx. Acta RadiolTher Phys Biol. 12: 1-8, 1973.
37. **Barlogie**, B., M. N. Raber, J. Schumann, T. S. Johnson, B. Drewinko, D. E. Swartzendruber, W. Göhde, M. Andreeff and E. J. Freireich. Flow Cytometry in Clinical Cancer Research. Cancer Res. 43: 3982-3997, 1983.
38. **Ewers**, S. B., E. Langstrom, B. Baldetorp and D. Killander. Flow-cytometric DNA Analysis in Primary Breast Carcinomas and Clinopathological Correlations. Cytometry. 5: 408-419, 1984.
39. **Frankfurt**, O. S., H. K. Slocum, Y. M. Rustum, S. G. Arbuck, Z. P. Pavelic, N. Petrelli, R. P. Huben, E. J. Pontes and W. R. Greco. Flow Cytometric Analysis of DNA Aneuploidy in Primary and Metastatic Human Solid Tumours. Cytometry. 5: 71-80, 1984.
40. **Gray**, J. W. and P. Coffino. Cell Cycle Analysis by Flow Cytometry. Methods Enzymol. 58: 233-248, 1979.
41. **Laerum**, O. D. and T. Farsund. Clinical Application of Flow Cytometry: a Review. Cytometry. 2(1): 1-13, 1981.
42. **Melamed**, M. R., Z. Darzynkiewicz, F. Traganos and T. Sharpless. Cytology Automation by Flow Cytometry. Cancer Res. 37: 2806-2812, 1977.
43. **Göhde**, W. "Automation of Cytofluorometry by Use of the Impulsmicrophotometer." Fluorescence Techniques in Cell Biology. Thaer and Sernetz ed. 1973 Springer-Verlag Berlin. Heidelberg. 79-88.
44. **Nelson**, K. G., J. S. Haskill, S. Sloan, J. M. Siegfried, G. P. Siegal, L. Walton and D. G. Kaufman. Flow Cytometric Analysis of Human Uterine Sarcomas and Cell Lines. Cancer Res. 47: 2814-2820, 1987.
45. **van den Ingh**, H. F., G. Griffin and C. J. Cornelisse. Flow Cytometric Detection of Aneuploidy in Colorectal Adenomas. Cancer Res. 45: 3392-3397, 1985.
46. **von Roenn**, J. H., S. M. Kheir, J. M. Wolter and J. S. Coon. Significance of DNA Abnormalities in Primary Malignant Melanoma and Nevi, a Retrospective Flow Cytometric Study. Cancer Res. 46: 3192-3195, 1986.
47. **Wennerberg**, J. Tumour Cell Kinetic Studies in Heterotransplantated Squamous Cell Carcinoma of the Head and Neck. Thesis, 1984.
48. **Stål**, O. Static and Flow Cytometry for Tumour DNA Analysis. Thesis, 1989.
49. **Munck-Wikland**, E. Cancer of the Esophagus. Thesis, 1989.
50. **Stål**, O., C. Klintenberg, G. Franzén, B. Risberg, S. Arvidsson, K. Bjelkenkrantz, L. Skoog and B. Nordenskjöld. A Comparison of Static Cytoflurometry and Flow Cytometry for the Estimation of Ploidy and DNA Replication in Human Breast Cancer. Breast Cancer Res Treat. 7: 15-22, 1986.

51. **Vindeløv**, L. L., I. B. J. Christensen and N. I. Nissen. A Detergent-trypsin Method for the Preparation of Nuclei for Flowcytometric DNA Analysis. Cytometry. 3: 323-327, 1983.
52. **Atkin**, N. and R. Kay. Prognostic Significance of Modal DNA Value and Other Factors in Malignant Tumours Based on 1465 Cases. Br J Cancer. 40: 210-221, 1979.
53. **de Aretxabala**, X., Y. Yonemura, K. Sugiyama, T. Kamata, K. Konishi, K. Miva and I. Miyazaki. DNA Ploidy in Early Gastric Cancer and its Relationship to Prognosis. Br J Cancer. 58: 81-84, 1988.
54. **Goldsmith**, M. M., D. H. Cresson, L. A. Arnold, D. S. Postma, F. B. Askin and H. C. Pillsbury. Part 1. DNA Flow Cytometry as a Prognostic Indicator in Head and Neck Cancer. Arch Otolaryngol Head Neck Surg. 96: 307-318, 1987.
55. **Moberger**, B., G. Auer, G. Forslund and G. Moberger. The Prognostic Significance of DNA Measurements in Endometrial Carcinoma. Cytometry. 5: 430-436, 1984.
56. **Anniko**, M., B. Tribukait and J. Wersäll. Significance of High Percentages of S-Phase Cells in Human Pituitary Tumours. ORL J Otorhinolaryngol Relat Spec. 45: 177-186, 1983.
57. **Armitage**, N. C., R. A. Robins, D.F.Evans, D. R. Turner, R. W. Baldwin and J. D. Hardcastle. The Influence of Tumour Cell DNA Abnormalities on Survival in Colorectal Cancer. Br J Surg. 72: 828-830, 1985.
58. **Wooldridge**, T. R., H. L. Grierson, D. D. Weisenburger, J. O. Armitage, W. G. Sanger, M. M. Collins, J. L. Pierson, M. E. Pauza, R. Fordyce and D. T. Purtilo. Association of DNA Content and Proliferative Activity with Clinical Outcome in Patients with Diffuse Mixed Cell and Large Cell Non-Hodgkin´s Lymphoma. Cancer Res. 48: 6608-6613, 1988.
59. **McLaughlin**, P., B. M. Osborne, D. Johnston, P. Jennings, J. J. Butler, F. Cabanillas and B. Barlogie. Nucleic Acid Flow Cytometry in Large Cell Lymphoma. Cancer Res. 48: 6614-6619, 1988.
60. **Ronot**, X. and M. Adolphe. Update on the Concept of the Cell Cycle: the Contribution of Flow Cytrometry. Biol Cell. 58: 113-119, 1986.
61. **Morrison**, S. D. In Vivo Estimation of Size of Experimental Tumours. JNCL. 71: 407-408, 1983.
62. **Karlberg**, I., S. Edström, L. Ekman, S. Johansson, T.Scherstén and K. Lundholm. Metabolic Host Reaction in Response to the Proliferation of Nonmalignant Cells versus Malignant Cells in Vivo. Cancer Res. 41: 4154-4161, 1981.
63. **Baccino**, F. M., L. Tessitore and G. Bonelli. Control of Protein Degradation and Growth Phase in Normal and Neoplastic Cells. Toxicol Phatol. 12: 281-287, 1984.
64. **Begg**, A. C. Cell Loss from Several Types of Solid Murine Tumour : Comparison of ^{125}I-Iododeoxyuridine and Tritiated Thymidine Methods. Cell Tissue Kinet. 10: 409-427, 1977.
65. **Begg**, A. C., N. J. McNally, D. C. Shrieve and H. Kärcher. A Method to Measure the Duration of DNA Synthesis and the Potential Doubling Time From a Single Sample Cytometry. Cytometry. 6: 620-626, 1985.
66. **Quastler**, H. and F. G. Sherman. Cell Population Kinetics in the Intestinal Epithelium of the Mouse. Exp Cell Res. 17: 420-438, 1959.

67. **Steel**, G. G. Cell Loss from Experimental Tumours. Cell Tissue Kinet. 1: 193-207, 1968.
68. **Dean**, P. N., F. Dolbeare, H. Gratzner, G. C. Rice and J. W. Gray. Cell-cycle Analysis Using a Monoclonal Antibody to BrdUrd. Cell Tissue Kinet. 17: 427-436, 1984.
69. **Denekamp**, J. and R. F. Kallman. In Vitro and in Vivo Labelling of Animal Tumours with Tritiated Thymidine. Cell Tissue Kinet. 6: 217-227, 1973.
70. **Dolbeare**, F., H. Gratzner, M. G. Pallavicini and J. W. Gray. Flow Cytometric Measurement of Total DNA Content and Incorporated Bromodeoxyuridine. Proc Natl Acad Sci USA. 80: 5573-5577, 1983.
71. **Dolbeare**, F., W. Beisker, M. G. Pallavicini, M. Vanderlaan and J. W. Gray. Cytochemistry for Bromodeoxyuridine/DNA Analysis: Stoichiometry and Sensitivity. Cytometry. 6: 521-530, 1985.
72. **Gratzner**, H. G. Monoclonal Antibody to 5-Bromo- and 5-Iododeoxyuridine: A New Reagent for Detection of DNA Replication. Science. 218: 474-475, 1982.
73. **Sasaki**, K., T. Murakami and M. Takahashi. A Rapid and Simple Estimation of Cell Cycle Parameters by Continuous Labeling With Bromodeoxyuridine. Cytometry. 8: 526-528, 1987.
74. **White**, R. A. and M. L. Meistrich. A Comment on"A Method to Measure the Duration of DNA Synthesis and the Potential Doubling Time From a Single Sample". Cytometry. 7: 486-490, 1986.
75. **Jänne**, J., H. Pösö and A. Raina. Polyamines in Rapid Growth and Cancer. Biochim Biophys Acta. 473: 241-293, 1978.
76. **Pariza**, M. W., S. Yanagi, J. A. Gurr, D. E. Bushnell, H. P. Morris and V. R. Potter. Ornithine Decarboxylase Activity and DNA Synthesis in Morris Hepatomas 5123-C and 7800. Life Sci. 18: 39-48, 1975.
77. **Pegg**, A. E. Polyamine Metabolism and its Importance in Neoplastic Growth and as a Target for Chemotherapy. Cancer Res. 48: 759-774, 1988.
78. **Russell**, D. H. "Polyamines in Growth - Normal and Neoplastic." Russell ed. 1973 Raven Press, New York.
79. **Russel**, D. H. and C. C. Levy. Polyamine Accumulation and Biosynthesis in a Mouse L1210 Leukemia. Cancer Res. 31: 248-251, 1971.
80. **Heby**, O. and G. Andersson. "Polyamines and the Cell Cycle." Polyamines. In Biomedical Research. Gaugas ed. 1980 Wiley. New York. 17-34.
81. **Raina**, A. and J. Jänne. Physiology of the Natural Polyamines Putrescine, Spermidine and Spermine. Med Biol. 53: 121-147, 1975.
82. **Pegg**, A. E. and P. P. McCann. Polyamine Metabolism and Function. Am J Physiol. 243: 212-221, 1982.
83. **Blair**, D. G. R. Actvation of mammalian RNA Polymerases by Polyamines. Int J Biochem. 17: 23-30, 1985.
84. **Manen**, C.-A. and D. H. Russell. Regulation of RNA Polymerase I Activity by Ornithine Decarboxylase. 26: 2379-2384, 1977.
85. **Durie**, B. G. M., S. E. Salmon and D. H. Russell. Polyamines as Markers of Response and Disease Activity in Cancer Chemotherapy. Cancer Res. 37: 214-221, 1977.
86. **Horn**, Y., S. L. Beal, N. Walach, W. P. Lubich, L. Spigel and L. J. Marton. Relationship of Urinary Polyamines to Tumour Activity and Tumour Volume in Patients. Cancer Res. 44: 4675-4678, 1984.

87. **Nishioka**, K. and M. M. Romsdahl. Elevation of Putrescine and Spermidine in Sera of Patients with Solid Tumours . Clin Chim Acta. 57: 155-161, 1974.
88. **Kingsnorth**, A. N., H. M. Wallace, N. J. Bundred and J. M. J. Dixon. Polyamines in Breast Cancer. Br J Surg. 71: 352-356, 1984.
89. **Kingsnorth**, A. N., A. B. Lumsden and H. M. Wallace. Polyamines in Colorectal Cancer. Br J Surg. 71: 791-794, 1984.
90. **Romano**, M., L. Cecco and M. Cerra. Levels of Polyamines and Nucleic Acids in Human Breast Carcinoma. Tumouri. 67: 431-435, 1981.
91. **Luk**, G. D., T. K. Desai, C. N. Conteas, J. A. Moshier and A. L. Silverman. Biochemical Markers in Colorectal Cancer: Diagnostic and Therapeutic Implications. Gastroenterol Clin North Am. 17: 931-940, 1988.
92. **Dimery**, I. W., K. Nishioka, V. B. J. Grossie, D. M. Ota, S. P. Schantz, R. Byers, K. T. Robbins and W. K. Hong. Polyamine Metabolism in Carcinoma of the Oral Cavity Compared with Adjacent and Normal Oral Mucosa. Am J Surg. 154: 429-433, 1987.
93. **Marton**, L. J., M. S. Edwards, V. A. Levin, W. P. Lubich and C. B. Wilson. CSF Polyamines: A New and Important Means of Monitoring Patients with Medulloblastoma. Cancer. 47: 757-760, 1981.
94. **Ota**, D. M., K. Nishioka, M. Foulkes and B. Grossie. Nutritional Parameters Affecting Erythrocyte Polyamine Levels in Cancer Patients. J Clin Oncol. 2: 1157-1163, 1984.
95. **Canellakis**, E. S., D. Viceps-Madore, D. A. Kyriakidis and J. S. Heller. The Regulation and Function of Ornithine Decarboxylase and of the Polyamines. Curr Top Cell Regul. 15: 155-202, 1979.
96. **McCann**, P. P. Regulation of Ornithine Decarboxylase in Eukaryotes. 7: 109-124, 1980.
97. **Russell**, D. H. Ornithine Decarboxylase as a Biological and Pharmacological Tool. Pharmacology. 20: 117-129, 1980.
98. **Russel**, D. and S. H. Snyder. Amine Synthesis in Rapidly Growing Tissues: Ornithine Decarboxylase Activity in Regenerating Rat Liver, Chick Embryo, and Various Tumours. Biochemistry. 60: 1420-1427, 1968.
99. **Scalabrino**, G., D. Modena, M. E. Ferioli, M. Puerari and G. Luccarelli. Degrees of Malignancy in Human Primary Central Nervous System Tumours: Ornithine Decarboxylase Levels as Better Indicators Than Adenosylmethionine Decarboxylase Levels. JNCI. 68: 751-754., 1982.
100. **Narisava**, T., M. Takahashi, M. Niwa, H. Koyama, H. Kotanagi, N. Kusaka, Y. Yamazaki, O. Nagasawa, K. Koyama, A. Wakisaka and Y. Fukaura. Increased Mucosal Ornithine Decarboxylase Activity in Large Bowel With Multiple Tumours, Adenocarcinoma, and Adenoma. Cancer. 63: 1572-1576, 1989.
101. **Glikman**, P., I. Vegh, M. A. Pollina, A. H. Mosto and C. M. Levy. Ornithine Decarboxylase Activity, Prolactin Blood Levels, and Estradiol and Progesterone Receptors in Human Breast Cancer. Cancer. 60: 2237-2243, 1987.
102. **Berdinskikh**, N. K., S. P. Zaletok, L. I. Bobro and N. A. Ignatenko. Ornithine Decarboxylase Activity and Polyamine Content in Human Adenocarcinomas of the Stomach and Large Intestine. Eksp Onkol. 10: 39-41, 1988.

103. **Luk**, G. D., J. A. Moshier and M. N. Ehrinpreis. Ornithine Decarboxylase as a Marker for Colorectal Polyps and Cancer. Prog Clin Biol Res. 279: 227-239, 1988.
104. **Bull**, W. A., D. N. Nigro, W. A. Colembieski, J. D. Crissman and L. J. Marnett. In Vivo Stimulation of DNA Synthesis and Induction of Ornithine Decarboxylase in Rat Colon by Fatty Acid Hydroperoxides, Autoxidation Products of Unsaturated Fatty Acids. Cancer Res. 44: 4924-4928, 1984.
105. **Connor**, M. J. and N. J. Lowe. Induction of Ornithine Decarboxylase Activity and DNA Synthesis in Hairless Mouse Epidermis by Retinoids. Cancer Res. 43: 5174-5177, 1983.
106. **Haddox**, M. K., B. E. Magun and D. H. Rusell. Ornithine Decarboxylase Induction during G1 Progression of Normal and Rous Sarcoma Virus-transformed cells. Cancer Res. 40: 604-608, 1980.
107. **Hogan**, B. L. M., A. McIlhinney and S. Murden. Effect of Growth Conditions on the Activity of Ornithine Decarboxylase in Cultured Hepatoma Cells. J Cell Physiol. 83: 353-358, 1974.
108. **Marks**, F., S. Bertsch and G. Fürstenberger. Ornithine Decarboxylase Activity, Cell Proliferation, and Tumour Promotion in Mouse Epidermis in Vivo. Cancer Res. 39: 4183-4188, 1979.
109. **O'Brien**, T. G. The Induction of Ornithine Decarboxylase as an Early, Possibly Obligatory, Event in Mouse Skin Carcinogenesis. Cancer Res. 36: 2644-2653, 1976.
110. **O'Brien**, T. G. and L. Diamond. Ornithine Decarboxylase Induction and DNA Synthesis in Hamster Embryo Cell Cultures Treated with Tumourpromoting Phorbol Diesters. Cancer Res. 37: 3895-3900, 1977.
111. **Boutwell**, R. K., T. G. O´Brien, A. K. Verma, R. G. Weekes, L. M. DeYoung, C. L. Ashendel and E. G. Astrup. The Induction of Ornithine Decarboxylase Activity and its Control in Mouse Skin Epedermis. Adv Enzyme Regul. 17: 89-112, 1979.
112. **Jänne**, J. L., P. Alhonen-Hongisto, P. Seppanen and M. Siimes. Use of Polyamine Antimetabolites in Experimental Tumours and in Human Leukemia. Med Biol. 59: 448-457, 1981.
113. **Grillo**, M. A. Metabolism and Function of Polyamines. Int J Biochem. 17: 943-948, 1985.
114. **Bengtsson**, C., B. Hultén, B. Larsson, H. Noppa, B. Steen and I. Warnold. New Weight-height Tables in Swedish Middle Aged and Elderly Men and Women. Läkartidningen. 78: 3152-3154, 1981.
115. **Symreng**, T. Arm Anthropometry in Large Reference Population and in Surgical Patients. Hum Nutr Clin Nutr. 1: 211-219, 1982.
116. **Bruce**, Å., M. Andersson, B. Arvidsson and B. Isaksson. Body Composition. Prediction of Normal Body Potassium, Body Water and Body Fat in Adults on the Basis of Body Height, Body Weight and Age. Scand J Clin Lab Invest. 40: 461-473, 1980.
117. **Edström**, S. Glucose metabolism in cancer disease. A study with special reference to the development of cancer cachexia. Thesis, 1980.
118. **Ekman**, L. Protein metabolism in cancer. The role of the liver. Thesis, 1980.

119. **Lindmark**, L., S. Edström, L. Ekman, I. Karlberg and K. Lundholm. Energy Metabolism in Nongrowing Mice with Sarcoma. Cancer Res. 43: 3649-3654, 1983.
120. **Moldawer**, L. L. Interleukin 1, tumour necrosis factor-alpha (cachectin) and the pathogenesis of cancer cachexia. Thesis, 1986.
121. **Svaninger**, G. The role of hormone alterations in development of cancer cachexia. An experimental study with special reference to wasting of skeletal muscles. Thesis, 1987.
122. **Ternell**, M. Regulation of Protein Metabolism in Tumour-host Livers. Thesis, 1986.
123. **Lundholm**, K., S. Edström, L. Ekman, I. Karlberg, A.-C. Bylund and T. Scherstén. A comparative Study of the Influence of Malignant Tumour on Host Metabolism in Mice and Man. Cancer. 42: 453-461, 1978.
124. **Edström**, S., L. Reinholdtsen and K. Lundholm. Glucose Turnover in Adult non-growing Tumour-bearing Mice . Int J Biochem. 17: 649-652, 1985.
125. **Ternell**, M., C. Lönnroth and K. Lundholm. Nuclear RNA-polymerase Activity in Tumour-host Livers. Am J Physiol. 249: 461-469, 1985.
126. **Lundholm**, K., S. Edström, I. Karlberg, L. Ekman and T. Scherstén. Relationship of Food Intake, Body Composition, and Tumour Growth to Host Metabolism in Nongrowing Mice with Sarcoma. Cancer Res. 40: 2516-2522, 1980.
127. **Blackburn**, G. L., B. R. Biastrian, B. S. Maini and e. al. Nutritional and Metabolic Assessment of the Hospitalized Patient. JPEN. 1: 11-22, 1977.
128. De Wys, W. Changes in Taste Sensation and Feeding Behaviour in Cancer Patients: a review. J Hum Nutr. 32: 447-453, 1978.
129. **Pegg**, A. E. Recent Advances in the Biochemistry of Polyamines in Eukaryotes. Biochem J. 234: 249-262, 1986.
130. **Jänne**, J., E. Hölttä, A. Kallio and K. Käpyaho. Role of Polyamines and their Antimetabolites in Clinical Medicine. Spec Top Endocrinol Metabol. 5: 227-293, 1983.
131. **Kyriakidis**, D. A., F. Flamigni, J. W. Pawlak and E. S. Canellakis. Mode of Interaction of Ornithine Decarboxylase with Antizyme and alfa-Difluoromethylornithine. Biochem Pharmacol. 33: 1575-1578, 1984.
132. **Mamont**, P. S., P. Böhlen, P. P. McCann, P. Bey, F. Schuber and C. Tardif. α-Methyl-Ornithine, a Potent Competitive Inhibitor of Ornithine Decarboxylase, Blocks Proliferation of Rat Hepatoma Cells in Culture. Proc Natl Acad Sci USA. 73: 1626-1630, 1976.
133. **Sjoerdsma**, A. and P. J. Schechter. Chemotherapeutic Implications of Polyamine Biosyntesis Inhibition. Clin Pharmacol Ther. 35: 287-299, 1984.
134. **Svaninger**, G., C. Drott and K. Lundholm. Role of Insulin in Development of Cancer Cachexia in Nongrowing Sarcoma-bearing Mice: Special Reference to Muscle Wasting. JNCI. 78: 943-950, 1987.
135. **Lönnroth**, P., J. I. Davies, I. Lönnroth and U. Smith. The Interaction between the Adenylate Cyclase System and Insulin-stimulated Glucose Transport. Biochem J. 243: 789-795, 1987.
136. **Lehninger**, A. L. "Biochemistry." 1975 Worth Publishers Inc. New York. 946-947.

137. **Pestka**, S. Inhibitors of protein synthesis. Molecular mechanisms of protein biosynthesis. chapter 10: 468-553, 1977.
138. **Murphy**, B. J. and M. E. Brosnan. Subcellular Localization of Ornithine Decarboxylase in Liver of Control and Growth-Hormone-Treated Rats. Biochem J. 157: 33-39, 1976.
139. **Lowry**, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein Measurement with the Folin Phenol Reagent. J Biol Chem. 193: 265-275, 1951.
140. **Hayashi**, S., Y. Aramaki and T. Noguchi. Diurnal Change in Ornithine Decarboxylase Activity of Rat Liver. Biochem Biophys Res Commun. 46: 795-800, 1972.
141. **Noguchi**, T., Y. Aramaki, T. Kameji and S. Hayashi. Correlation between Circadian Rythms of Polyamine Synthesis and Cell Proliferation in Rat Liver. J Biochem. 85: 953-959, 1979.
142. **Noguchi**, T., A. Kashiwagi and T. Tanaka. A Factor Responsible for Increases in Ornithine Decarboxylase Activity in the Livers of Tumour-Bearing Mice. Cancer Res. 36: 4015-4022, 1976.
143. **Pegg**, A. E. and H. Pösö. [39] S-Adenosylmethionine Decarboxylase (Rat Liver). Methods Enzymol. 94: 234-239, 1983.
144. **Minchin**, R. F. and G. R. Hanau. Rapid and Simple Technique for the Quantitation of Polyamines in Biological Samples. J Liquid Head Neck Chromatogr. 7: 2605-2610, 1984.
145. **Garlick**, P. J., M. A. McNurlan and V. R. Preedy. A Rapid and Convenient Technique for Measuring the Rate of Protein Synthesis in Tissues by Injection of (3H)phenylalanine. Biochem J. 192: 719-723, 1980.
146. **Lundholm**, K., L. Ekman, S. Edström, I. Karlberg, R. Jagenburg and T. Scherstén. Protein Synthesis in Liver Tissue under the Influence of a Methylcholanthrene-induced Sarcoma in Mice. Cancer Res. 39: 4657-4661, 1979.
147. **Berry**, M. N. High-Yield Preparation of Morphologically Intact Isolated Parenchymal Cells from Rat Liver. Methods Enzymol. 32: 625-632, 1974.
148. **Barlogie**, B., W. Göhde, D. Johnston, L. Smallwood, J. Schumann and B. Drewinko. Determination of Ploidy and Proliferative Characteristics of Human Solid Tumours by Pulse Cytophotometry. Cancer Res. 38: 3333-3339, 1978.
149. **Gerdes**, J., W. Schwab, H. Lemke and H. Stein. Production of Mouse Monoclonal Antibody Reactive with a Human Nuclear Antigen Associated with Cell Proliferation. Int J Cancer. 312: 13-20, 1983.
150. **Gerdes**, J., H. Lemke, H. Baisch, H.-H. Wacker, W. Schwab and H. Stein. Cell Cycle Analysis of a Cell Proliferation-associated Human Nuclear Antigen Defined by the Monoclonal Antibody Ki-67. J Immunol. 133: 1710-1717, 1984.
151. **Cordell**, J., B. Falini, O. N. Erber, H. Stein and D. Y. Mason. Immunoenzymatic Labelling of Monoclonal Antibodies Using Immunecomplexes of Alkaline Phophatase and Monoclonal Anti-alkaline Phosphatase (APAAP Complexes). J Histocem Cytochem. 32: 219-229, 1984.
152. **Stein**, H., K. Gatter, H. Asbaler and D. Y. Mason. Methods in Laboratory Investigation. Use of Freeze-dried Paraffin-embedded Sections for Immunohistologic Staining with Monoclonal Antibodies. Lab Invest. 52: 676-683, 1985.
153. **Sasaki**, K. Measuement of Tritiated Thymidine Labeling Index by Incubation In Vitro of Surgically Removed Cervical Cancer. Gann. 68: 307-313, 1977.

154. **Ceriotti**, G. A Microchemical Determination of Desoxyribonucleic Acid. J Biol Chem. 198: 297-303, 1952.
155. **van Furth**, R. and T. L. van Zwet. Immunocytochemical Detection of 5-Bromo-2-Deoxyuridine Incorporation in Individual Cells. J Immunol Methods. 108: 45-51, 1988.
156. **Sasaki**, K., T. Murakami, T. Ogino, M. Takahasi and S. Kawasaki. Flow Cytometric Estimation of Cell Cycle Parameters Using a Monoclonal Antibody to Bromodeoxyuridine. Cytometry. 7: 391-395, 1986.
157. **Tsurusawa**, M., M. Niwa, N. Katano and T. Fujimoto. Flow Cytometric Analysis by Bromodeoxyuridine/DNA Assay of Cell Cycles Perturbation of Methotrexate-treated Mouse L1210 Leukemia Cells. Cancer Res. 48: 4288-4293, 1988.
158. **Riccardi**, A., M. Danova, G. Wilson, G. Ucci, P. Dörmer, G. Mazzini, S. Brugnatelli, M. Girino, N. J. McNally and E. Ascari. Cell Kinetics in Human Malignancies Studied with in Vivo Administration of Bromodeoxyuridine and Flow Cytometry. Cancer Res. 48: 6238-6345, 1988.
159. **Wilson**, G. D., N. J. McNally, E. Dunphy, H. Kärcher and R. Pfragner. The Labelling Index of Human and Mouse Tumours Assesed by Bromodeoxyuridine Staining In Vitro and In Vivo and Flow Cytometry. Cytometry. 6: 641-647, 1985.
160. **Okada**, S. A Simple Graphic Method of Computing the Parameters of the Life Cycle of Cultured Mammalian Cells in the Expotential Growth Phase. J Cell Biol. 34: 915-916, 1967.
161. **Daly**, P. F. and J. S. Cohen. Magnetic Resonance Spectroscopy of Tumours and Potential in Vivo Clinical Applications: A Review. Cancer Res. 49: 770-779, 1989.
162. **Glickson**, J. D., W. T. Evanochko, T. T. Sakai and T. C. Ng. "In Vivo NMR Spectroscopy of Tumours." NMR Spectroscopy of Cells and Organisms. Gupta ed. 1987 CEC Press Inc. Boca Raton FL. 99-134.
163. **Greiner**, J. V., S. J. Kopp and T. Glonek. Phosphorus Nuclear Magnetic Resonance and Ocular Metabolism. Surv Ophthalmol. 30: 189-202, 1985.
164. **Griffiths**, J. R. and R. A. Iles. NMR Studies of Tumours. Biosci Rep. 2: 719-725, 1982.
165. **Merchant**, T. E., L. W. Gierke, P. Meneses and T. Glonek. ^{31}P Magnetic Resonance Spectroscopic Profiles of Neoplastic. Cancer Res. 48: 5112-5118, 1988.
166. **Ng**, T. C., W. T. Evanochko, R. N. Hiramoto, V. K. Ghanta, M. B. Lilly, J. Lawson, T. H. Corbett, J. R. Durant and J. D. Glickson. ^{31}P NMR Spectroscopy of in Vivo Tumours. 49: 271-286, 1982.
167. **Ross**, B., V. Marshall, M. Smith, S. Bartlett and D. Freeman. Monitoring Response to Chemotherapy of Intact Human Tumours by ^{31}P Nuclear Magnetic Resonance. Lancet. 1: 641-646, 1984.
168. **Sostman**, H. D., I. M. Armitage and J. J. Fischer. "NMR in Cancer; I. High Resolution Spectroscopy of Tumours". Magn Reson Imaging. 2: 265-278, 1984.
169. **Wehrle**, J. P. and J. D. Glickson. ^{31}P NMR Spectroscopy of Tumours In Vivo Cancer. Cancer Biochem Biophys. 8(157-166): 1986.
170. **Koutcher**, J. A. and C. T. Burt. Principles of Nuclear Magnetic Resonance. J Nucl Med. 25: 101-111, 1984.
171. **Rosen**, B. R. and T. J. Brady. Principles of Nuclear Magnetic Resonance for Medical Application. Semin Nucl Med. 8: 308-318, 1983.

172. **Soussi**, B., J.-P. Idström, A.-C. Bylund-Fellenius and T. Scherstén. Dynamics of Skeletal Muscle Energetics during Ischemia and Reperfusion Assessed by *in vivo* ^{31}P NMR. NMR Biomed. : In press.
173. **Weiner**, M. W. NMR Spectroscopy for Clinical Medicine. Animal Models and Clinical Examples. Ann N Y Acad Sci. 508: 287-299, 1987.
174. **Oberhaensli**, R. D., D. Hilton-Jones, P. J. Bore, L. J. Hands, R. P. Rampling and G. K. Radda. Biochemical Investigation of Human Tumours in Vivo with Phosphorus-31 Magnetic Resonance Spectroscopy. Lancet. 2: 8-11, 1986.
175. **Degani**, H., A. Horowitz and Y. Itzchak. Breast Tumours: Evaluation with P-31 MR Spectroscopy. Radiology. 161: 53-55, 1986.
176. **Idström**, J.-P., B. Soussi, B. Wanag and A.-C. Bylund-Fellenius. Analysis of Purine Nucleotides in Muscle Tissue by HPLC. Scand J Clin Lab Invest. In press.
177. **Evanochko**, W. T., T. T. Sakai, T. C. Ng, N. R. Krishna, H. D. Kim, R. B. Zeidler, V. K. Ghanta, R. W. Brockman, L. M. Schiffer, P. G. Braunschweiger and J. D. Glickson. NMR Study of in vivo RIF-1 Tumours. Analysis of Perchloric Acid Extracts and Identification of ^{1}H, ^{31}P and ^{13}C Resonances. Biochim Biophys Acta. 805: 104-116, 1984.
178. **Lehninger**, A. L. "Biochemistry." 1975 Worth Publishers Inc. New York. 539-540.
179. **Atkinsson**, D. "Cellular Energy Metabolism and its Regulation." 1977 Academic Press.
180. **Randles**, R. H. and D. S. Wolfe. "Introduction to the Theory of Nonparametric Statistics." 1979 Wiley. New York.
181. **Allison**, D. C., K. K. Bose, S. Anderson, S. Curley and J. Robertson. Slowing of Cell Cycle Traverse for Cells in Exponential Monolayer Cultures Placed into Plateau-fed and Starved Medium. Cancer Res. 49: 1456-1464, 1989.
182. **Daly**, J. M., H. M. Reynolds, B. J. Rowlands, G. E. Baquero, S. J. Dudrick and E. M. Copeland. Nutritional Manipulation of Tumour-Bearing Animals: Effects on Body Weight, Serum Protein Levels, and Tumour Growth. Surg Forum. 29: 143-144, 1978.
183. **Steiger**, E., J. Oram-Smith, E. Miller, L. Kuo and H. M. Vars. Effects of Nutrition on Tumour Growth and Tolerance to Chemotherapy. J Surg Res. 18: 455-461, 1975.
184. **Goodgame**, J. T., S. F. Lowry and M. F. Brennan. Nutritional Manipulations and Tumour Growth II. The Effects of Intravenous Feeding. Am J Clin Nutr. 32: 2285-2294, 1979.
185. **Goodgame**, J. T., S. F. Lowry, J. J. Reilly, D. C. Jones and M. F. Brennan. Nutritional Manipulations and Tumour Growth I. The Effects of Starvation. Am J Clin Nutr. 32: 2277-2284, 1979.
186. **Levine**, A. S., M. F. Brennan, A. Ramu, R. I. Fisher, P. A. Pizzo and D. L. Glaubiger. Controlled Clinical Trials of Nutritional Intervention as an Adjunct to Chemotherapy with a Comment on Nutrition and Drug Resistance. Cancer Res. 42: 774-781, 1982.
187. **Silberman**, H. The Role of Preoperative Parenteral Nutrition in Cancer Patients. Cancer. 55: 254-257, 1985.
188. **Russell**, D. H. Clinical Relevance of Polyamines as Biochemical Markers of Tumour Kinetics. Clin Chem. 23(1): 22-27, 1977.

189. **Luk**, G. D. and R. A. J. Casero. Polyamines in Normal and Cancer Cells. Adv Enzyme Resul. 26: 91-105, 1987.
190. **Takami**, H. and K. Nishioka Raised. Polyamines in Erythrocytes from Melanoma-Bearing Mice and Patients with Solid Tumours. Br J Cancer. 41: 751-756, 1980.
191. **Grossie** Jr., V. B., K. Nishioka, D. M. Ota and R. G. Martin Sr. Relationship of Erythrocyte Polyamine Levels and Growth Rate of Transplantable Tumours in Rats. Cancer Res. 40: 3464-3468, 1986.
192. **Lipton**, A., L. M. Sheehan and G. F. Kessler. Urinary Polyamine Levels in Human Cancer. Cancer. 35: 464-468, 1975.
193. **Russel**, D. H. Increased Polyamine Concentration in the Urine of Human Cancer Patients. Nature (New Biol). 233: 144-145, 1971.
194. **Shideler**, C. E., M. E. Johns, R. W. Cantrell and e. al. Erythrocyte Polyamine Determinations in Patients with Head and Neck Cancer. Acta Otolaryngol. 107: 752-754, 1981.
195. **de Vere White**, R., A. D. Deitch and C. A. Olsson. Limitations of DNA Histogram Analysis by Flow Cytometry as a Method of Predicting Chemosensitivity in a Rat Renal Cancer Model. Cancer Res. 43: 604-610, 1983.
196. **Ljungberg**, B., R. Stenling and G. Roos. DNA Content in Renal Cell Carcinoma With Reference to Tumour Heterogeneity. Cancer. 56: 503-508, 1985.
197. **Warburg**, O. On The Origin of Cancer Cells. Science. 123: 309-314, 1956.
198. **Pardee**, A. B. A Restriction Point for Control of Normal Animal Cell Proliferation. Proc Natl Acad Sci USA. 71: 1286-1290, 1974.
199. **Mamont**, P., C. Danzin, J. Wagner, M. Siat, A.-M. Joder-Ohlenbusch and N. Claverie. Accumulation of Decarboxylated S-Adenosyl-L-Methionine in Mammalian Cells as a Consequence of the Inhibition of Putrescine Biosynthesis. Eur J Biochem. 123: 499-504, 1982.
200. **Shirahata**, A. and A. E. Pegg. Regulation of S-Adenosylmethionine Decarboxylase Activity in Rat Liver and Prostate. J Biol Chem. 260: 9583-9588, 1985.
201. **Löser**, C., U. R. Fölsch, P. Sahelijo-Krohn and W. Creutzfeldt. Ornithine Decarboxylase and Polyamines in Cholecystokinin-induced Pancreatic Growth in Rats: Effects of ∂-difluoromethylornithine and the CCK Receptor Antagonist L-364,718. Eur J Clin Invest. 19: 448-458, 1989.
202. **Heby**, O., J. W. Gray, P. A. Lindl, L. J. Marton and C. B. Wilson. Changes in L-Ornithine Decarboxylase Activity during the Cell Cycle. Biochem Biophys Res Commun. 71: 99-105, 1976.
203. **Anehus**, S., P. Pohjanpelto, B. Baldetorp, E. Långström and O. Heby. Polyamine Starvation Prolongs the S and G2 Phases of Polyamine-Dependent (Arginase-Deficient) CHO Cells. Mol Cell Biol. 4: 915-922, 1984.
204. **Harris**, W. B., B. V. Grossie, D. M. Ota, K. Nishioka, A. Jaffer, T. Chang and P. Domililia. Effect of Difluoromethylornithine on Host and Tumour Polyamine Metabolism during Total Parenteral Nutrtion. J Surg Res. 38: 592-598, 1985.
205. **Heby**, O., G. Andersson and J. W. Gray. Interference with S and G2 Phase Progression by Polyamine Synthesis Inhibitors. Exp Cell Res. 111: 461-464, 1978.

206. **Seidenfeld**, J., A. Block, K. Komar and F. Naujokas. Altered Cell Cycle Phase Distributions in Cultured Human Carcinoma Cells, Partially Depleted of Polyamines by Treatment with Difluoromethylornithine. Cancer Res. 46: 47-53, 1986.
207. **Sunkara**, P. S., M. B. Pargac, K. Nishioka and P. N. Rao. Differential Effects of Inhibition of Polyamine Biosynthesis on Cell Cycle Traverse and Structure of the Prematurely Condensed Chromosomes of Normal and Transformed Cells. J Cell Physiol. 98: 451-458, 1979.
208. **Harada**, J. J. and D. R. Morris. Cell Cycle Parameters of Chinese Hamster Ovary Cells during Exponential Polyamine Depleted Growth. Mol Cell Biol. 1: 594-599, 1981.
209. **Cress**, A. E. and E. W. Gerner. Hydroxyurea Inhibits ODC Induction, but not the G1 to S Phase Transition. Biochem Biophys Res Commun. 87: 773-780, 1979.
210. **Fuller**, D. J. M., E. W. Gerner and D. H. Russell. Polyamine Biosynthesis and Accumulation during the G1 to S Phase Transition. J Cell Physiol. 93: 81-88, 1977.
211. **Chideckel**, E. W. Insulin Does Not Play the Major Role in the Refeeding Stimulation of Hepatic Ornithine Decarboxylase Activity. Metabolism. 32: 106-107, 1983.
212. **Conover**, C. A., S. J. Rozovski and N. B. Ruderman. Regulation of Ornithine Decarboxylase in Skeletal Muscle. Evidence for the Involvement of an Insulin-dependent Serum Factor. Diabetes. 30: 678-684, 1981.
213. **Murakami**, Y., T. Noguchi and S. Hayashi. Role of Pancreatic Hormones in Dietary Induction of Ornithine Decarboxylase of Rat Liver. J Biochem. 90: 141-147, 1981.
214. **Rinehart** Jr., C. A. and E. S. Canellakis. Induction of Ornithine Decarboxylase Activity by Insulin and Growth Factors is Mediated by Amino Acids. Proc Natl Acad Sci USA. 82: 4365-4368, 1985.
215. **Rozovski**, S. J., C. A. Conover and N. B. Ruderman. Effect of Diabetes on the Induction of Ornithine Decarboxylase by Refeeding. Life Sci. 25: 553-560, 1979.
216. **Tabata**, K. and L. R. Johnson. Mechanism of Induction of Mucosal Ornithine Decarboxylase by Food. Am J Physiol. 25: 370-374, 1986.
217. **Iapalucci-Espinoza**, S., L. Haim-Muchnik, E. Robetto and M. T. Franze-Fernandez. Lack of Correlation between Liver RNA Polymerase I and Ornithine Decarboxylase Activities after Re-feeding of Protein-depleted Mice. Acta Physiol Pharmacol Latinoam. 34: 157-161, 1984.
218. **Alhonen-Hongisto**, L., A. Kallio, R. Sinervirta, O. A. Jänne, C. G. Gahmberg and J. Jänne. Tumourigenicity, Cell-Surface Glycoprotein changes and Ornithine Decarboxylase Gene Pattern in Ehrlich Ascites-Carcinoma Cells. Biochem J. 229: 711-715, 1985.
219. **Izumi**, K., Y. Hirao, L. Hopp and R. Oyasu. In Vitro Induction of Ornithine Decarboxylase in Urinary Bladder Carcinoma Cells. Cancer Res. 41: 405-409, 1981.
220. **Farwell**, D. C., J. B. Migues and E. J. Herbst. Ornithine Decarboxylase and Polyamines in Liver and Kidneys of Rats on Cyclical Regimen of Protein-Free and Protein-Containing Diets. Biochem J. 168: 49-56, 1977.

221. **Brennan**, M. F. and L. Ekman. Metabolic Consequemces of Nutritional Support of the Cancer Patient. Cancer. 54: 2627-2634, 1984.
222. **Nixon**, D. W. Hyperalimentation in the Undernourished Cancer Patient. Cancer Res. 42: 727-728, 1982.
223. **Bailar** III, J. C. and E. M. Smith. Progress Against Cancer ? N Engl J Med. 314(19): 1226-1232, 1986.
224. **Cairns**, J. The Treatment of Disease and the War Against Cancer. Sci Am. 253(5): 51-59, 1985.

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