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# Ornithine Decarboxylase Activity in Mouse Tumour Tissue in Response to Refeeding and Diet Components

**Thomas Westin, Helen Zachrisson, Staffan Edström and Kent Lundholm**

**Orthinine decarboxylase (ODC) and S-adenosylmethionine decarboxylase activity (SAMD) were measured in tumour tissue in mice during periods of starvation (24 h) and refeeding. Starvation led to a 60% reduction in tumour ODC activity. Refeeding normalised the activity within 4 h. Restitution in ODC activity, representing *de novo* enzyme synthesis, preceded DNA resynthesis. SAMD activity continued to fall along the increase in ODC activity during refeeding, while difluoro-methyl-ornithine (DFMO) caused a compensatory increase in SAMD activity as expected. A fall and regain in ODC activity was associated with inhibition and regrowth of the tumour. Starvation–refeeding was not related to any decrease in tumour polyamine concentrations, while systemic DFMO blockade was. Glucose stimulated ODC when refed orally, but not when given systemically. Tumour ODC activity was not decreased in refed mice by anti-insulin, a procedure that antagonised insulin's bioactivity. Exogenous insulin did not stimulate tumour ODC activity. Our results suggest that gastrointestinal metabolism of carbohydrates stimulates the release of a factor, which initiates both ODC activity and DNA synthesis in tumour cells. This factor was not insulin.**
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## INTRODUCTION

POLYAMINES AND ornithine decarboxylase (ODC) activity have received interest as potential markers and regulators in malignancy and cell proliferation [1]. We recently reported the presence of high activity of ornithine decarboxylase in human head and neck tumours, and high enzyme activity correlated to short survival in these patients [2]. It is well established that ODC is controlled by either dietary factors [3, 4] or hormones and growth factors [5–7] in both transformed [8] and non-transformed [9] cells. Therefore, it is possible that refeeding of cancer patients may stimulate polyamine synthesis and thereby initiate cell proliferation [2, 10], although it has been difficult to demonstrate this effect [10–12]. Tumour ODC activity and polyamine levels may be simple and sensitive parameters to monitor initiation of RNA and DNA synthesis in biopsy specimens from human tumours in response to nutrition and tumour treatment [13]. Therefore, the objectives of this study were to evaluate whether starvation–refeeding activates ODC activity

and subsequently influences polyamine concentration in experimental cancer *in vivo*, as reported for ODC activity in a variety of non-transformed cells *in vitro*; and if so, to evaluate which component (carbohydrate, fat, protein) in the diet re-establishes ODC activity and tumour growth.

## MATERIALS AND METHODS

*Experimental model*

All experiments were performed in 3-month-old, weight-stable tumour-bearing mice (C57B1/6J, 20–25 g). A low differentiated, rapidly growing tumour (MCG 101), originally induced by methylcholanthrene, was implanted subcutaneously in the flanks as an isogenic tumour graft with exponential growth rate corresponding to a doubling time of 57 h [14]. The tumour does not metastasise when transplanted subcutaneously and its influence on the host metabolism has been investigated [15–17]. All experiments were started 8–9 days following tumour implantation. The spontaneous survival time of the tumour-bearing animals is 15–17 days. All animals were kept in individual cages and always had free access to tap water. Spontaneous food intake and changes in carcass composition have been reported elsewhere [17]. Study and control mice were killed by cervical dislocation at the same time. The time course in tumour size was determined by weighing excised tumours from animals that were pair-killed at various time points.

*Refeeding*

All animals were fed *ad libitum* with a purine chow diet (Ewos maintenance food for rats and mice, Astra Ewos, Södertälje, Sweden) containing 22.6% crude protein, 4.9% fat, 7.0% minerals, 12.0% moisture, 4.2% fibre, 49.3% carbohydrates, 1.22% calcium, 0.90% phosphorus, 0.32% magnesium, 0.65% chlorine and trace elements (295 ppm iron, 44 ppm copper, 8 ppm cobalt, 133 ppm manganese, 164 ppm zinc, and 2.6 ppm iodine); fatty acids consisting of (in percentage of total fatty acids) 0.8% myristic acid, 19.6% palmitic acid, 8.1% stearic acid, 24.8% oleic acid, 41.3% linoleic acid and 1.1% others; and vitamin A, $D_3$, $K_3$, $B_1$, $B_2$, $B_6$, $B_{12}$, calciumpantothenate, niacin and cholinechloride in sufficient amounts. The food contained no antibiotics. The metabolizable energy was 13.0 MJ/kg (3.1 Mcal/kg).

Starvation started at 0800 h. Refeeding during 7 h was instituted at 0800 h the next morning following 24 h starvation. Different nutritional regimens were used for spontaneous refeeding: (1) peroral intake of purina chow, 3.1 kcal/ml; (2) a crystalline solution of aminoacids (Vamin 14, 0.35 kcal/ml, KabiVitrum, Stockholm); (3) a soybean lipid emulsion used for intravenous nutrition in humans (Intralipid, 2 kcal/ml, KabiVitrum); (4) water solution of D-glucose (30%, 0.8 kcal/ml); (5) a mixture of aminoacid solution, (Vamin) (2), lipids (Intralipid) (3) and glucose (4) in isovolumetric parts, 1.15 kcal/ml; (6) a bulk diet without any digestible components consisting of kauline (Astra, Södertälje, Sweden); and (7) a water solution containing 30% 3-O-methyl-glucose (Sigma), which is not phosphorylated following cellular uptake and thus is not metabolised. In additional experiments, "refeeding" was performed by intraperitoneal injection of D-glucose (30%, 10 μl/g body weight). Blood samples were immediately drawn by heart puncture for measurement of plasma glucose and insulin.

*ODC and SAMD*

ODC was determined according to Noguchi *et al.* [9] and S-adenosylmethionine decarboxylase (SAMD) according to Pegg and Pösö [18]. Non-necrotic tumour tissue, liver tissue, the entire small intestine, kidney and spleen were rapidly excised at sacrifice and washed with PBS. The specimens were homogenised in 0.25 mol/l sucrose containing 1 mmol dithiothreitol (20 ml/g wet weight). The homogenate was centrifuged at 40 000 *g* for 2 h at 4°C to remove mitochondria [11]. The supernatant was used for determination of enzyme activities and protein content by the Lowry method. Blanks consisted of all reagents without the enzyme. Enzyme activity was given as nmol $^{14}CO_2$ liberated per h/mg protein. Polyamines were determined by means of HPLC [19].

*DNA synthesis*

DNA synthesis was estimated as incorporation of [2-$^{14}$C] thymidine (0.1 3.7 kBq/g body weight; 0.8 μmol/ml) into tumour tissue DNA following intraperitoneal injection 1 h before death. Tumour tissue was immediately removed and frozen in liquid nitrogen. After thawing, 200 μg was diluted (1:20) in physiological NaCl. Perchloric acid (0.6 mol/l) was added to the ice-chilled homogenate (2.5 ml). The sediment was washed repeatedly and then dissolved in 2 ml of 0.3 mol/l KOH for 1 h at 37°C. Ice-chilled 1.2 mol/l perchloric acid (1.25 ml) was added. Precipitated DNA was dissolved in 2.5 ml 0.1 mol/l KOH for 15 min at 37°C. DNA concentration was determined with calf thymus DNA as the standard [20].

*Protein synthesis*

Protein synthesis was estimated as described by Garlick *et al.* [12]. A flooding dose of [$^{14}$C-U] leucine was injected intraperitoneally and the animals were killed 30 min later. Tissue protein was extracted and radioactivity in proteins was determined as described elsewhere [21]. The fractional protein synthesis was calculated in percentage per day.

*Administration of metabolic inhibitors, insulin and anti-insulin*

Cycloheximide experiments were performed to evaluate whether increase and decrease in ODC activity was related to *de novo* synthesis of the enzyme. A single dose of 1.0 mg cycloheximide/g animal resulted in a fatal outcome while 0.5 mg cycloheximide/g was not associated with any mortality. Protein synthesis and ODC activity were measured at 60 min and on following a single intraperitoneal injection of cycloheximide. Control animals received saline. Protein synthesis and ODC activity were determined in liver and tumour tissue. ODC activity was determined in liver and tumour tissue following the addition of cycloheximide *in vitro* at various concentrations (0–20 mg/ml incubation medium) to exclude the possibility of a direct influence of cycloheximide on the enzyme. Difluoromethylornithine (α-DFMO, Merrel-Dow) was supplied (2%) in the drinking water on day 3 until the end of experiments on day 11–12.

Anti-porcine insulin guinea pig serum (M8309 Novo, Malmö, Sweden) was given intraperitoneally (1:300; 100 μl) to tumour-bearing animals 4 h and immediately before the start of refeeding following 24 h starvation. Plasma insulin following anti-insulin showed no detectable levels of circulating insulin during at least 4 h in refed animals treated with the antiserum. Plasma samples

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from tumour-bearing mice, treated with and without anti-insulin *in vivo*, were tested for antilipolytic activity in a bioassay system with incubated rat epididymal fat cells [22]. Glycerol release into the incubation medium, taken as an index of lipolysis, was three times higher in anti-insulin treated tumour-bearing mice compared to sham-injected animals, which confirmed that anti-insulin antibodies used actually antagonised insulin's biological activity *in vivo*. Freely-fed tumour-bearing mice ($n$ = 20) were subjected to starvation for 24 h. Ten of these starved mice received an intraperitoneal injection of insulin (200 mU/g), while the remaining starved mice received saline and were refed by pellets. All 20 animals were then killed 4 h later for measurement of ODC activity in tumour tissue. Plasma samples were taken simultaneously for measurements of plasma glucose (glucose oxidase kit, Boehringer Mannheim) and insulin (double antibody radioimmunoassay Novo, Malmö, Sweden).

*Statistics*

Differences between the two groups were compared by Student's $t$ test. One-factor ANOVA was used among several groups, and intergroup differences were tested by a multiple range test. Time course changes were tested by one factor ANOVA for repeated measures. The rank sum test was used to test differences in survival in DFMO-treated animals compared with control animals.

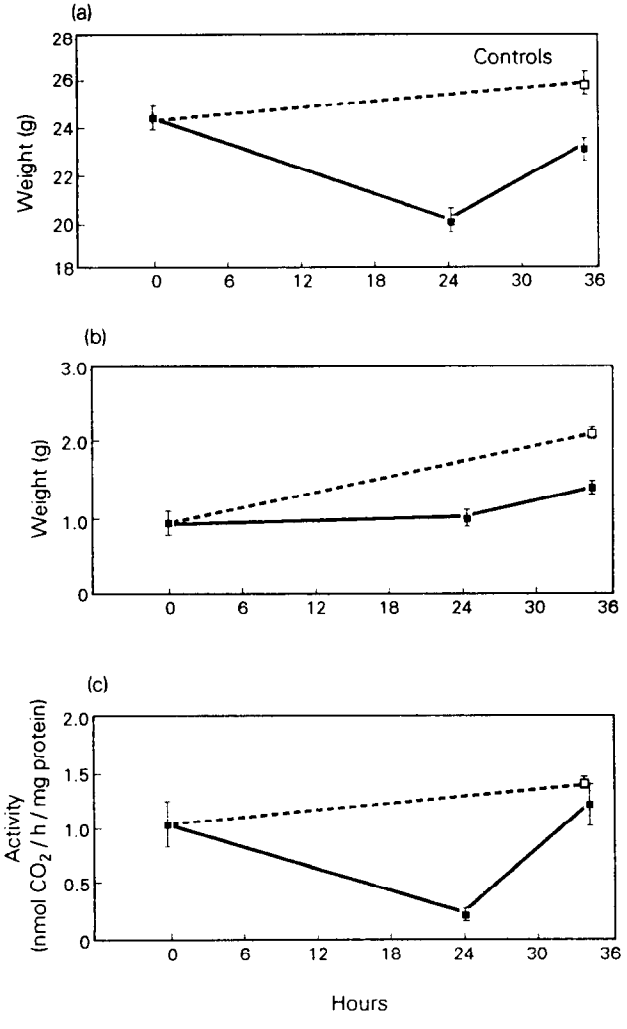


**Fig. 1. Changes in (a) animal weight, (b) tumour weight and (c) tumour ODC in mice that were freely eating (□) or starvation–refed (spontaneous) by pellets (■). 20 mice per group, mean (S.E.). Differences without overlapping bars were statistically significant by one-factor ANOVA for repeated measures.**

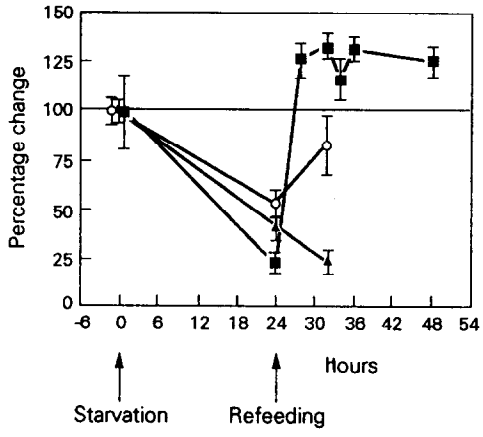


**Fig. 2. Changes in tumour ODC and SAMD in relation to thymidine incorporation into tumour tissue DNA during starvation–refeeding (spontaneous) by pellets. 10 mice per group. Overshoot in ODC activity above 100% was significant, $P < 0.01$. ■ = ODC, ○ = thymidine, ▲ = SAMD.**

## RESULTS

*Tumour growth*

24 h starvation of tumour-bearing animals on day 9 caused a significant decrease in carcass weight and inhibition of net tumour growth. A subsequent 10 h refeeding with chow diet caused a return of body weight and tumour growth (Fig. 1a, b). The regrowth of tumours did not reach a statistically significant difference among the dietary animal groups (results not shown).

*Enzyme activities and DNA synthesis*

ODC activity was 10-fold higher in tumour tissue [1.26 (S.E. 0.13) nmol/h per g protein] compared to liver, intestine, kidney and spleen tissue (<0.11 nmol/h per g protein) from freely-eating tumour-bearing animals. Starvation for 24 h caused a reduction in tumour ODC activity by more than 70%, while subsequent refeeding (normal pellets) normalised this activity within 12 h compared to continuously freely-fed mice (Fig. 1c).

*Table* 1. *Tumour ODC activity in relation to plasma glucose and insulin 2 h after start of refeeding**

| | ODC activity (nmol/h/mg protein) | Glucose (mmol/l) | Insulin (mU/l) |
|---|---|---|---|
| *Ad libitum* + NaCl intraperitoneally | 1.26[0.13](13)* | 9.4[0.4](10)* | 26[6](10)† |
| Starvation + NaCl intraperitoneally | 0.35[0.09](15)† | 5.4[0.4] (9)† | 9[1] (8)† |
| Glucose intraperitoneally | 0.47[0.12](12)* | 8.1[0.7](13)* | 17[1](13)† |
| Oral glucose | 1.42[0.31](14)* | 9.1[0.6](11)† | 35[5](11)* |
| Oral 3-O-methylgl. | 0.20[0.18] (8)† | 9.4[0.4] (8)* | 15[1] (5)† |
| Pellet refeeding | 1.23[0.14](16)* | 9.1[0.3] (9)† | 22[3] (9)* |

Mean [S.E.]. No. of animals in parentheses.
A common symbol (*, †) means not significantly different from each other by one-factor ANOVA ($P < 0.05$).

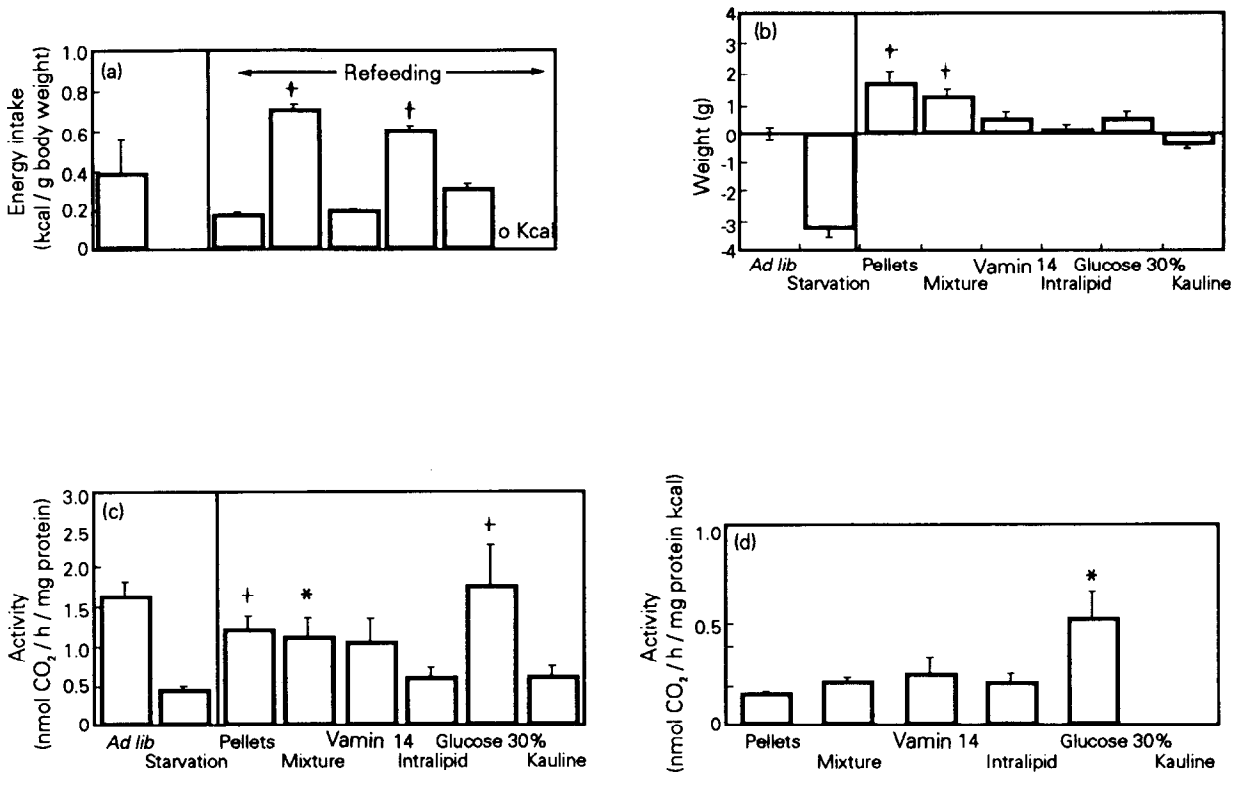


**Fig. 3. Tumour ODC activity and body weight gain in relation to dietary intake (Kcal/g body weight) in tumour-bearing mice that were starvation–refed (spontaneous) with different diet compositions (a–c). ODC activity normalised to energy content in (d). 10 mice per group. (a) Pellets, Vamin 14 and glucose 30% significantly different from mixture and intralipid. (b) Pellets and mixture significantly different from Vamin 14, intralipid, glucose 30% and kauline. (Weight changes from fasted values given for refed animals.) (c) Pellets, mixture and glucose 30% significantly different from kauline and intralipid. (d) Glucose 30% significantly different from other groups. *$P < 0.05$, †$P < 0.01$.**

In starved mice, tumour SAMD activity decreased similarly to ODC during starvation, but it showed a further decrease upon refeeding, while tumour ODC activity showed an overshoot of around 25% (Fig. 2). Oral intake of glucose alone normalised tumour ODC activity, while glucose given intraperitoneal in a similar amount had no such effect in starved animals. Oral intake of 3-O-methylglucose had no stimulatory effect on tumour ODC activity in starved mice (Table 1). A liquid mixture of D-glucose, fat (Intralipid) and crystalline aminoacids stimulated tumour ODC activity when given orally (Fig. 3a–d). The fat solution alone (Intralipid) and the bulk diet were the only components that did not stimulate tumour ODC activity at all following starvation, although the effect of aminoacids alone did not reach the level of statistical significance (Fig. 3c). When ODC and SAMD activity were compared to thymidine incorporation into DNA, the relative declines in ODC and SAMD activity were similar, but the fall in thymidine incorporation into DNA seemed to be somewhat less pronounced.

Refeeding induced incease in tumour ODC activity was probably due to *de novo* synthesis of enzyme protein, since a single intraperitoneal dose of cycloheximide (1 mg/g) caused a drop in tumour ODC activity and in tumour and hepatic protein synthesis for more than 8 h. A lower dose of cycloheximide (0.5 mg/g) caused a transient decrease in tumour ODC activity and protein synthesis in tumour and liver tissue with 100% survival of freely-fed animals, while starved animal had difficulties to tolerate even this dose. However, in some surviving refed mice, it could be demonstrated that cycloheximide also prevented the stimulation of tumour ODC activity during refeeding. The spontaneous normalisation of ODC activity following cycloheximide injection was rapid compared to tumour and hepatic protein synthesis. High concentrations of cycloheximide had no effect on ODC activity *in vitro*.

There was a positive correlation between plasma insulin and tumour ODC activity when observations were pooled from

*Table 2. Tumour ODC activity in starved (24 h) tumour-bearing mice receiving 100 µl anti-insulin intraperitoneally 4 h before and immediately before refeeding with ordinary pellets*

| | Starved | Refed by pellets | |
|---|---|---|---|
| | Saline | Without antiserum | With antiserum |
| Tumour ODC activity (nmol/h/mg protein) | 0.54[0.11](15) | 1.13[0.2](16)* | 0.89[0.24](7) |
| Plasma glucose (mmol/l) | 4.6 [0.3](6) | 8.9 [0.5](16)* | 9.6 [0.5] (7)* |
| Plasma insulin (mU/l) | 12 [0.3](6) | 32 [2](16)* | 0 |

*Significantly different from tumour-bearing animals injected intraperitoneally with saline, analysed by one-factor ANOVA ($P < 0.05$). Food intake following refeeding was 0.95 [0.1] g in mice without anti-insulin and 0.89 [0.1] g in animals with anti-insulin. Both groups increased body weight by 1.2 [0.1] g.

animals in different experimental groups (Fig. 4). Elimination of circulating insulin by an antiserum did not significantly attentuate tumour ODC activity following oral refeeding by pellets, although the value was numerically lower [1.13 (S.E. 0.20) vs. 0.89 (0.24), Table 2]. Therefore, additional experiments with exogenous insulin loads were carried out on starved tumour-bearing mice (Table 3). In such animals, high plasma insulin was concomitant with hypoglycaemia, but ODC activity was not stimulated at all. In fact, ODC activity in tumour tissue remained at levels generally seen in starved tumour-bearing mice.

*Polyamines*

Starvation did not decrease concentrations of any polyamine (Table 4). In contrast, spermine was increased in all investigated tissues during starvation and was normalised upon refeeding. DFMO blockade rapidly decreased both putresceine and spermidine concentrations in both tumour and spleen tissue (Table 4).

*Systemic DFMO administration*

DFMO (2%) in the drinking water caused a significant decrease in net tumour growth associated with a pronounced inhibition of tumour ODC activity and an increase in tumour SAMD activity (not shown). However, neither the overall tumour protein synthesis nor the tumour protein concentration was altered.

DFMO decreased the tissue concentrations of putresceine and spermidine while spermine concentration was unchanged. Water and food intake and carcass body weight in tumour-bearing animals given DFMO showed no alteration, which confirms a low toxicity of systemic DFMO administration [23]. DFMO had no effect on either plasma glucose or insulin in tumour-bearing or control animals (results not shown).

## DISCUSSION

Polyamines represent both direct and permissive factors to control cell proliferation [1]. Inhibition of ODC activity causing a fall in tissue polyamine concentrations may lead to attenuation of cell proliferation, as confirmed in both tumour and spleen tissue in the present study. Such inhibition can be compensated for by exogenous supply of polyamines as confirmed in cell culture experiments [1]. Classical hormones and dietary components including growth factors may influence upon intracellular processes by the control of ODC activity even in cells that are not dividing such as skeletal and heart muscle. Consequently, ODC activity does not always reflect cell proliferation and may then reflect DNA repair and RNA synthesis not associated with cell division. ODC activity may also display large changes within hours following acute phase response stimulation [24]. Alterations in ODC activity do generally reflect *de novo* synthesis of the enzyme, in which both translational and transcriptional control may be involved. Based on this background we have evaluated ODC activity and polyamine metabolism as possible markers of cell proliferation and as being a possible target for DNA/RNA synthesis in experimental and clinical malignant tumours related to nutrition and diet intake.

Our study confirms that ODC activity was 10-fold higher in experimental tumours than in proliferating intestinal mucosal cells. Tumour ODC activity responded to nutritional alterations

*Table 3. ODC in tumour tissue from starved and insulin-treated mice*

| | Tumour ODC activity (nmol/h/mg prot) | Plasma glucose (mmol/l) | Plasma insulin (mU/l) |
|---|---|---|---|
| Starved + refed ($n$ = 10) | 0.97(0.09)* | 5.3(1.3)* | 98(1) |
| Starved + insulin ($n$ = 10) | 0.36(0.09) | 0.5(0.2) | 87(8) |

Mean (S.E.). *$P$ < 0.01 compared to starved + insulin.

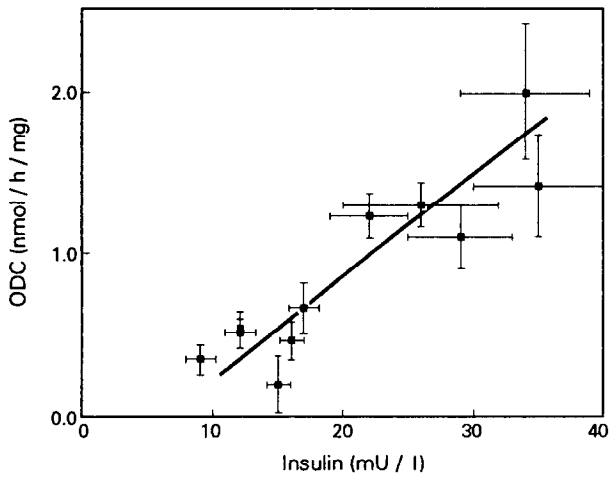


**Fig. 4. Plasma insulin and tumour ODC activity with observations from various experimental groups. Observations from individual animals was not used since insulin and ODC activity was not always measured on same animals ($r$ = 0.90, $P$ < 0.0001). At least 8 mice per each group.**

*Table 4. Organ content of polyamines in starved and refed (pellets) tumour–bearing mice*

| Organ | Animal:s (8 per group) | Putrescine | Spermidine | Spermine |
|---|---|---|---|---|
| Liver | FF | 22 (2) | 1158(51) | 1088 (52) |
| | S | 36 (8) | 1275(97) | 1577 (69)* |
| | RF | 27 (2) | 1066(54) | 1085 (42) |
| Intestine | FF | <5 | 1054(79) | 721 (29) |
| | S | <5 | 1277(85) | 1311(485)* |
| | RF | <5 | 1132(16) | 883(119) |
| Spleen | FF | 118 (2) | 1398(60) | 832 (23) |
| | S | 114(10) | 1593(85) | 923 (16)* |
| | RF | 119(12) | 1396(55) | 824(16) |
| Tumour | FF | 412(32) | 1890(55) | 623 (22) |
| | S | 449(48) | 2060(78) | 723 (36)* |
| | RF | 457(18) | 1844(40) | 592 (24) |
| Tumour | FF | 293(27)† | 1809(96)† | 759(50) |
| | FF+DFMO | <1 | 147 (8) | 766(68) |
| Spleen | FF | 198(13)† | 2005(50)† | 1302(65) |
| | FF+DFMO | 48 (5) | 1161(33) | 1444(42) |

*Mean (S.E.), nmol/g. Significantly different from FF and RF analysed with one-factor ANOVA and a subsequent multiple range test.
†Significantly different from FF+DFMO.
FF = freely-fed; S = starved; RF = refed tumour-bearing mice.

in the host as did also ODC activity in liver and skeletal muscles (unpublished results and ref. 6). Starvation caused a prompt decrease in tumour ODC activity, which was normalised and even supranormal within hours of refeeding. The increase in SAMD following inhibition of ODC activity may reflect a decrease in polyamine synthesis [25]. However, the decrease in tumour ODC activity and subsequent inhibition of DNA synthesis was not associated with a fall in tumour content of polyamines. In contrast, spermine increased during starvation and was then normalised during refeeding. Inhibition of ODC-activity by either the specific enzyme-inhibitor DFMO or by acute starvation was translated into a decrease in net tumour growth. This decline in ODC activity by DFMO was, in contrast to starvation, rapidly translated into a fall in tissue concentrations of putresceine and spermidine, while spermine concentrations remained unchanged. Our results, therefore, demonstrate that starvation-induced tumour growth inhibition, concomitantly with a decrease in both ODC-activity and DNA-synthesis, was not dependent on a decline in tissue polyamines. Such a decrease in ODC activity was likely an effect of decreased *de novo* synthesis based on experiments with systemic cycloheximide blockade. Cycloheximide, which blocks protein synthesis at the translational level, did not alter ODC activity directly judged from experiments with addition of cycloheximidine to the enzyme assay.

Alterations in hepatic ODC activity in response to refeeding are dependent on dietary proteins which may be explained by the appearance of certain aminoacids in the portal circulation [4]. Non-metabolised aminoacids, such as α-amino butyric acid seemed to play a permissive role for the induction of the enzyme [7, 26]. In addition to aminoacids, insulin and glucagon may also represent permissive effects. Our refeeding experiment showed that mixtures of substrates, either in the form of pellets or liquid mixtures, stimulated both body and tumour weight gain. Thus, our "artificial" meal with the combination of aminoacids, soybean lipids and glucose supported anabolic reactions with resynthesis of lipids and proteins in normal and tumour tissue, although, as expected, ordinary pellets had the highest nutrition efficiency. Individual substrates did not induce anabolic reaction in the host but oral glucose stimulated ODC in tumour tissue. This effect was not directly related to glucose itself, since intraperitoneal injection of glucose caused a considerably lower or insignificant effect. Ingestion of oral bulk or the non-metabolised 3-O-methyl-glucose did also not stimulate tumour ODC activity. This fact makes it unlikely that any mechanical or osmotic factor in the gastric or intestinal mucosa stimulated unspecifically the release of factor(s) with ODC-inducing effect. The "glucose associated" effect to induce tumour ODC activity was positively correlated to plasma insulin. However, the lack of a significant fall in tumour ODC activity in experiments with anti-insulin suggested that insulin was either permissive or a covariate. The role of insulin to stimulate ODC activity has been suggested by others in diabetes [27]. Such experiments suggested the presence of an insulin dependent serum factor for the control of ODC activity in skeletal muscles during feeding [6], while others have concluded that insulin did not play the major role in refeeding stimulation of hepatic ODC activity [28]. Our present experiments with both anti-insulin and exogenous insulin loads do not confirm that insulin served as a factor behind stimulation of tumour ODC activity in response to refeeding.

In conclusion, our results demonstrated that oral glucose intake initiated DNA synthesis in tumour cells by a mechanism which was not directly related to glucose or insulin. Our results are compatible with the suggestion that gastrointestinal metabolism of carbohydrates stimulates the release of a factor (hormone, growth factor) which initiates both ODC activity and DNA synthesis in tumour cells. Whether the induction or activation of ODC was directly related to initiation of DNA synthesis was not determined. The effect was not mediated by a classic decline and restitution of tissue polyamine concentrations.


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# Early Morphological Detection of Estramustine Cytotoxicity Measured as Alteration in Cell Size and Shape by a New Technique of Microperifusion

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**The present study describes a new microscopic perifusion technique for detecting momentary alterations in cell volume and shape. The method has been applied for evaluating early signs of cytotoxicity following chemotherapeutic treatments. The effects of estramustine phosphate (EMP) have been evaluated. EMP is a complex between oestradiol-17β and the alkylating agent nor-nitrogen mustard and has recently demonstrated a marked cytotoxicity against malignant glioma cells. The results showed a concentration-dependent increase in cell size and a concomitant decrease in shape factor following EMP-treatment of glioma cells. These changes correlated with cytotoxicity evaluated as cell proliferation and cell membrane alterations shown by $^{86}Rb$ fluxes and ultrastructural visible membrane damage. The colon cancer line HT-29 displayed no reactions at all following EMP treatment. It is suggested that acute alterations in cell morphology and shape display a strong correlation to the cytotoxicity of EMP encountered by traditional cell culture systems. The findings are discussed with respect to cell membrane disturbances caused by EMP and its potential role as an early test of cytoxicity.**
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## INTRODUCTION

AN INTACT cell volume is known to be of critical importance for the preservation of cell functions including growth and proliferation [1]. Extensive studies on cell volume regulation in the last few years indicate that a wide variety of cells share common regulatory capacities, although a pronounced diversity exists between different cell types in the nature of the ion transport systems involved. Predictive tumour sensitivity tests have received increasing attention and several different predictive cellular and animal systems have been developed with varying degree of success [2]. There is, thus, still a strong need for more effective predictive tests of drug sensitivity in clinical oncology.

It is obvious that drugs affecting different parts of the cytoskeleton as microtubules can have a major impact on the cellular volume and shape. Estramustine phosphate (EMP), used in the treatment of advanced prostatic carcinoma [3] and recently shown to exert considerable cytotoxic effects on several glioma cell lines [4, 5], is a conjugate between oestradiol-17β and the alkylating agent nor-nitrogen mustard. EMP has been shown to induce mitotic arrest [4, 6] and inhibited monocyte phagocytosis [7] by an interaction with the microtubule function.

In the present study, we describe a new microscopic technique which gives the ability to correlate early alterations in cell size and shape with the cytotoxicity of anticancer drugs. It is shown that acute changes in cell morphology strictly correlate to the cytotoxicity of EMP encountered in traditional cell culture systems.

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