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# Polyamine metabolism in primary human colon adenocarcinoma cells (SW480) and their lymph node metastatic derivatives (SW620)

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**Summary.** The natural polyamines are multifunctional constituents of all eucaryotic cells. The objective of this work was to compare aspects of polyamine metabolism in two related cell lines with the idea to investigate whether metabolic differences can be attributed to functional differences of the cells. The human colon carcinoma-derived cell lines SW480 and SW620 were chosen as models. SW480 cells were isolated from the primary tumour, SW620 cells from a lymph node of the same patient. SW620 cells grow faster, and the key regulatory enzymes of polyamine biosynthesis (ODC and AdoMetDC) are more active in the metastatic cells. Moreover, their ability to accumulate polyamines from the environment is more important than of SW480 cells. Likewise polyamine concentrations were markedly higher in SW620 cells, although they are much smaller than SW480 cells, and have a particularly small cytoplasmic space. Both cell lines show a striking diminution of ODC and AdoMetDC activities and changes in the polyamine patterns at the transition from exponential to non-exponential growth – most probably as a consequence of high cell density. Depletion of putrescine and spermidine due to inactivation of ODC by DFMO causes accumulation of cells in G1, and a proportional decrease of S-phase cells in both cell lines. Based on morphologic and other criteria SW480 and SW620 cells were typified as poorly differentiated. In agreement with their low grade of differentiation they exhibit a low alkaline phosphatase activity. However, the time-dependent decrease of alkaline phosphatase is not typical of differentiation patterns of other adenocarcinoma-derived cell lines or of normal enterocytes. The high capacity of de novo polyamine biosynthesis and of polyamine uptake is presumably a prerequisite for the rapid growth and invasiveness. The fact that these properties were more accentuated in the case of SW620 cells and paralleled enhanced metastatic properties indicate relationships between basic parameters of polyamine metabolism and malignancy.

**Keywords:** Polyamines – Metabolism – Uptake – Human colon carcinoma cells – SW480 – SW620

**Abbreviations:** AdoMetDC S-adenosylmethionine decarboxylase; dAdoMet decarboxylation product of S-adenosylmethionine; DFMO (D,L)-2-(difluoromethyl)ornithine; ODC ornithine decarboxylase; PAO polyamine oxidase (FAD dependent)

## 1. Introduction

The idea to compare polyamine metabolism in cells of different malignancy is not new. Williams-Ashman and his colleagues reported presumably the first example in 1973 by showing a positive correlation between the malignancy of rat hepatomas and the activity of ornithine decarboxylase (ODC) activity (Williamas-Ashamn et al., 1973). Since then different aspects of polyamine metabolism have been studied in a great variety of cells, but systematic comparisons of polyamine metabolism in closely related cell lines with well characterised properties are still rare. With the present work we try to fill in a gap. As model we chose two cell lines deriving from the same patient with a grade III adenocarcinoma of the colon. SW480 cells were isolated from the primary tumour, SW620 cells from a lymph node (Leibovitz et al., 1976). In the course of the years SW480 and SW620 cells have been well characterised and compared with regard to cellular, biochemical and pharmacological properties. Among others their karyotype (Gagos et al., 1995; Melcher et al., 2000), growth properties and growth factor requirements (Maybaum et al., 1991; Turowsky et al., 1994; Huschtscha et al., 1991), invasiveness

and metastatic activity (Vermeulen et al., 1995; De Vries et al., 1995; Zirvi et al., 1991), migration (Kubens and Zanaker, 1998), sensitivity to some anticancer drugs (Drewinski et al., 1985; Trujillo and Yang, 1985; Warrington et al., 1994), transforming growth factor alpha and beta expression (Coffey et al., 1987), synthesis and release of carcinoembryonic antigen (Shi et al., 1983), L-arginine transport (Cendan et al., 1996), and nitric oxide synthase gene expression (Jenkins et al., 1994) have been studied in some detail. Although the cell lines are not homogenous – two subpopulations which differ in malignancy have been isolated from the SW480 line (Tomita et al., 1992) – they seem nevertheless suited for the exploration of biochemical properties, which are important in tumour progression and metastatic activity. Since the natural polyamines putrescine, spermidine, and spermine are basic elements of cell metabolism and function (Cohen, 1998), we decided to compare major parameters of polyamine metabolism and related properties of the two cell lines, presuming that functional differences will be reflected in differences of polyamine metabolism.

## 2. Materials and methods

### *2.1. Chemicals*

If not stated otherwise, chemicals were from Merck (Darmstadt, Germany) or Sigma Chemical Co. (St. Louis, MO). Cell culture media were from Gibco (Life Technologies SARL, Cergy-Pontoise, France). [1-$^{14}$C]L-ornithine (specific activity 55 mCi/mmol; 2.03 GBq), [1-$^{14}$C]S-adenosyl-L-methionine (specific activity 60 mCi/mmol; 2.22 GBq), and [$^{14}$C]putrescine.2HCl (spec. activity 118 mCi/mmol; 4.37 GBq) were from Amersham (Orsay, France). $N^1$,$N^{12}$-diacetylspermine dihydrochloride was synthesised as described earlier (Bolkenius, Seiler 1981).

### *2.2. Cells and cell culture*

SW480 and SW620 cells were obtained from the European Collection of Cell Cultures (Sophia Antipolis, France). First passages were done in Leibovitz medium. Then the Leibovitz medium was gradually substituted by Dulbeccos's modified Eagle medium (DMEM), containing 25 mM glucose and 2 mM L-glutamine, 10% horse serum, 100 U/ml penicillin, and 100 $\mu$g/ml streptomycin. Incubations were carried out at 37°C in a humidified atmosphere of 5% $CO_2$. The culture medium was changed every 48 h. In experiments horse serum was reduced to 3%, and the medium was supplemented with transferrin (5 $\mu$g/ml), selenium (5 ng/ml) and insulin (10 $\mu$g/ml) (ITS-defined medium; Gibco). (The use of horse serum avoids oxidative deamination of spermidine and spermine by serum amine oxidase).

### *2.3. Cell growth rate*

Cell growth rates were determined after trypsinisation (0.5% trypsin in 2.6 mM EDTA) by counting (using a haemocytometer), and in 96-well culture clusters (Corning Inc., Corning, N.Y.), (initial cell density 5,000 cm$^{-2}$) by determination of cell protein using staining with sulforhodamine B (Skehan et al., 1990). At low cell numbers absorbance was measured at 564 nm, the absorption maximum of sulforhodamine B, at high cell numbers at 490 nm. The relationship between protein content per well and absorbance was linear over a very wide range.

### *2.4. RNA, DNA, proteins*

RNA, DNA, and proteins were separated from the same sample by a modified Schmidt-Thannhauser procedure (Seiler and Schmidt-Glenewinkel, 1975). Cells grown in 10 ml culture dishes (initial cell density 5,000 cm$^{-2}$), were washed with PBS, harvested by scraping, and homogenised in 3 ml 0.2 N perchloric acid. After centrifugation (20 min; 800×g) the pellet was incubated for 60 min at 37°C with 1.8 ml 0.3 M KOH. Then 0.2 ml of 70 percent perchloric acid was added, in order to precipitate DNA and proteins. RNA was determined in the supernatant at 260 nm, using the Gene Quant spectrophotometer (Amersham, Pharmacia Biotech Europe (Saclay, France). The precipitate was suspended in 1.1 ml of 1.5 percent perchloric acid and heated for 20 min at 90 ± 1°C. To the chilled samples 70 $\mu$l of 70 percent perchloric acid was added, and the proteins were precipitated by centrifugation. Of the supernatant 1 ml was mixed with 20 $\mu$l 70 percent perchloric acid and then brought to a volume of 2 ml with diphenylamine reagent (0.5 g diphenylamine, 0.5 ml conc. sulphuric acid, 25 ml glacial acetic acid plus 1.25 ml of 2 percent acetaldehyde in water). The samples were stored for colour development for 3 days at +3°C. Absorbance was measured at 600 nm. As standard served calf thymus DNA (25–800 $\mu$g), which was treated as the cell pellets. Samples with an absorbance >1.00 were diluted with glacial acetic acid. Proteins were dissolved in 0.5 ml 0.1 N NaOH, and determined according to Lowry et al. (1951). Bovine serum albumin was used as standard.

### *2.5. Determination of enzyme activities and polyamines*

Cells were seeded at a density of 5,000 cm$^{-2}$ in 10 ml Petri dishes and grown under the above mentioned conditions. At certain intervals the cells were washed with phosphate-buffered saline (PBS), and harvested by scraping. The cell pellets were stored frozen at −80°C until analyses were carried out.

#### 2.5.1. Ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (AdoMetDC) activities

Cells were homogenised by sonication in 100 mM Tris-HCl buffer pH 7.4, containing 1 mM EDTA, 1 mM dithiothreitol, 0.5 $\mu$M leupeptin, and 0.5 mM phenylmethylsulfonyl fluoride. Enzyme activities were determined in the high speed supernatant (60 min; 90,000 × g) by $^{14}CO_2$ trapping methods (Holm et al., 1988), using [1-$^{14}$C]L-ornithine, respectively [1-$^{14}$C]S-adenosyl-L-methionine as substrates. For selective inhibition of the enzyme ODC blanks contained 10 mM 2-(difluoromethyl)ornithine (DFMO). AdoMetDC blanks contained the assay mix without homogenate.

#### 2.5.2. Alkaline phosphatase

Alkaline phosphatase activity was determined in cell extracts by the method of Garen and Levinthal (1960).

#### 2.5.3. Polyamine oxidase (PAO)

The assay of PAO activity relied on the determination of hydrogen peroxide by horseradish peroxidase-catalysed formation of quinone from phenol, and reaction of the quinone with aminoantipyrine. The

assay conditions were the same as described previously. $N^1$,$N^{12}$-diacetylspermine at 2.5 mM was used as substrate (Seiler et al., 2000).

### 2.6. Determination of polyamines

Polyamine concentrations were determined as follows (Seiler et al., 1990): Cells were homogenised in 1 ml 0.2 N perchloric acid. After standing over night at 3°C, proteins were precipitated by centrifugation. Reverse-phase ion pair HPLC directly separated aliquots (0.2 ml) of the cell extracts. The column eluate was reacted with *o*-phthalaldehyde/2-mercaptoethanol reagent. The primary amino groups form fluorescent 1-alkylthio-2-alkylisoindole derivatives, which were determined by monitoring fluorescence intensity ($\lambda$(excitation) = 325 nm; $\lambda$(emission) = 455 nm).

### 2.7. Flow cytometry

After seeding ($8 \cdot 10^5$ cells per 10 ml Petri dish) cells were grown for 24 h, then they were exposed to 1 mM DFMO for up to 72 h (with medium change every 48 h). Floating cells and attached cells were collected and fixed with methanol + PBS = 9 + 1, and stored at −20°C until flow cytometry was carried out as described by Crissman et al. (1975). The propidium iodide-stained cells were analysed with a FACScan flow cytometer (Becton Dickinson, San Jose CA, USA). Cell cycle phase distribution, percentage of cell debris, and hypodiploid DNA cells were measured according to Nicoletti et al. (1991). Fluorescence emission of 20,000 cells was analysed using the CellFit software (Becton Dickinson).

### 2.8. Determination of putrescine uptake

The method followed the description of Morgan (1998). The commercial solution of [$^{14}$C]putrescine.2HCl (spec. activity 118 mCi/mmol; 4.37 GBq) was diluted with Hank's buffered salt solution to give 50 $\mu$M, 25 $\mu$M, 12.5 $\mu$M, 6.25 $\mu$M, 3.125 $\mu$M and 1.56 $\mu$M solutions. Both cell lines were grown on the same 96-well culture clusters. After 2, 4 and 7 days in culture putrescine uptake was determined from two culture clusters for each time point. The cell-containing wells were washed with 200 $\mu$l portions of Hank's solution (pre-warmed to 37°C), and after addition of 50 $\mu$l radioactive putrescine solutions, the plates were incubated for 10 min at 37°C. Uptake was stopped by addition of 150 $\mu$l ice-cold PBS, and cooling on ice. After 5 washings with ice-cold PBS proteins were determined using Bradford reagent (BioRad, Hercules CA, USA). After scanning the plates at 620 nm, the contents of the wells was mixed with 100 $\mu$l formic acid and after 30 min transferred into plastic scintillation counting vials, and the wells were rinsed with 200 $\mu$l water. Radioactivity was determined by $\beta$-counting after mixing with a scintillation cocktail (Ultima Gold, Packard Instrument BU, Chemical Operations, Groningen, The Netherlands). Extracellular putrescine was determined by incubating an analogous plate for 10 min on ice.

### 2.9. Statistics and calculations

If not stated otherwise, data were given as means ± S.D. Statistically significant differences ($p < 0.05$) between groups were established by Student's t-test. Doubling times of cells were determined graphically from the slope of the linear part (exponential growth) of semi-log plots of the growth curves. For the calculation of $K_M$, and $V_{max}$ from Michaelis-Menten plots, and of $EC_{50}$ values by a four parameter logistic the Erithacus software, GraFit (version 3.0) (Leatherbarrow, 1992) was used.

## 3. Results

### 3.1. Growth rate

The commercial SW480 cell line consists mainly of elongated cells with a structured cytoplasm, but contains also spherical cells. In contrast SW620 cells are small and spherical; their nucleus fills out almost the entire cell volume. Both cell lines grow in clusters or islands, and are epithelium like (Leibovitz et al., 1976). Cell growth rate and doubling times were determined of cells growing in DMEM medium with 3% horse serum either by counting (Fig. 1), or by protein determination (initial cell density 5,000 cm$^{-2}$). Both methods gave identical results. Under our culture conditions cell growth was exponential until 6 days after seeding, as was evident from semilogarithmic plots of the growth curves. At this time cell density was about $(1.3 \pm 0.4) \cdot 10^5$ cells/cm$^2$ for the primary tumour cells, and about $(3.1 \pm 0.2) \cdot 10^5$ cells/cm$^2$ for their metastatic derivatives. The doubling time of SW480 cells was 25 ± 1 h, that of SW620 cells 23 ± 1 h.

In spite of a small cytoplasmic space, the metastatic cells have a significantly higher RNA content than SW480 cells, as is demonstrated by the RNA/DNA-ratio (Table 1). At high cell density (day 10 after seeding) RNA/DNA-ratios decreased (compared with day 4) in both cell lines, but the protein content increased.

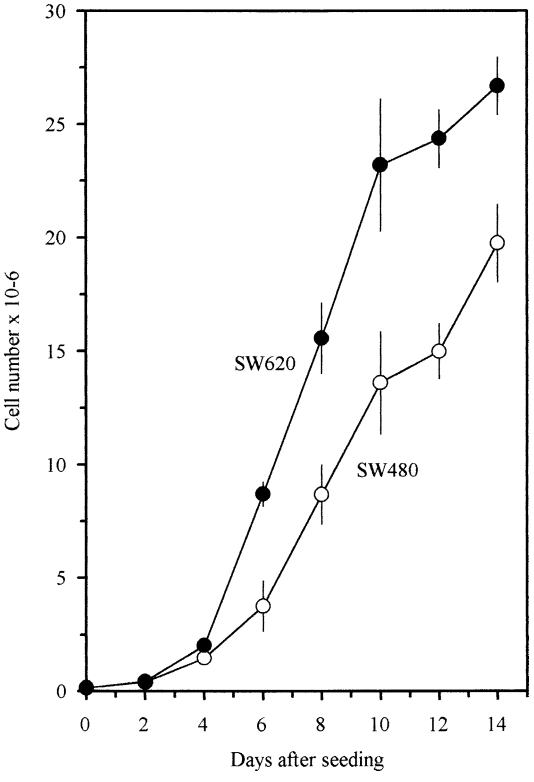


**Fig. 1.** Growth of SW480 and SW620 cells under standard culture conditions. Cell numbers were determined after trypsinisation by counting. Cells were seeded at a density of $5000 \cdot \text{cm}^{-2}$ in 5 ml culture dishes, and were grown under standard conditions in supplemented DMEM medium in the presence of 3 percent horse serum (For details see the Materials and methods section). Mean values of three culture dishes per time point ± SEM

**Table 1.** RNA/DNA and protein/DNA ratios of SW480 and SW620

| Cell type | Time after seeding days | RNA/DNA $\mu g/\mu g$ | Protein/DNA $mg/\mu g$ |
|---|---|---|---|
| SW480 | 4 | 2.1 ± 0.2 | 0.20 ± 0.01 |
| | 10 | 1.8 ± 0.2 | 0.25 ± 0.04 |
| SW620 | 4 | 2.5 ± 0.1* | 0.22 ± 0.01 |
| | 10 | 2.4 ± 0.2* | 0.27 ± 0.04 |

Mean values ± S.D. (n = 3); the asterisk indicates a statistically significant difference ($p < 0.056$) between SW480 and SW620 cells

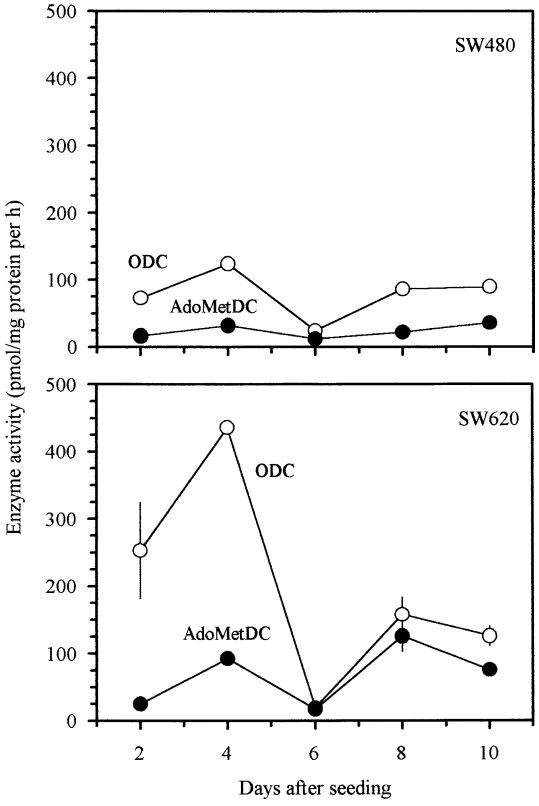


**Fig. 2.** Ornithine decarboxylase (*ODC*) and S-adenosylmethionine decarboxylase (*AdoMetDC*) activities in cultures of SW480 and SW620 cells as a function of time in culture. Cell culture conditions were the same as described in the legend of Fig. 1, except that cells were grown in 10 ml culture dishes; mean values ± S.D. (n = 6)

### *3.2. ODC and AdoMetDC activities*

The products of ODC and AdoMetDC, putrescine and decarboxylated S-adenosylmethionine (dAdoMet) are the precursors of spermidine and spermine. Both decarboxylases are highly regulated (Cohen, 1998).

The pattern of ODC and AdoMetDC activities during cell growth was comparable for both cell lines. However, the enzymes had considerably higher specific activities in SW620 than in SW480 cells (Fig. 2). ODC activity was always significantly higher than AdoMetDC activity, except on day 6, when both enzymes showed minimum values. The two maxima on days 4 and 8 after seeding, as well as the striking minimum on day 6 were repeatedly observed.

### *3.3. Alkaline phosphatase*

Alkaline phosphatase is a cytosolic enzyme, but it is highly abundant in brush borders of normal colonocytes. It is also considered as a differentiation marker of CaCo-2 cells (Pinto et al., 1983), another human colon adenocarcinoma-derived cell line. Its activity increases when CaCo-2 cells approach confluence. SW480 cells (Leibovitz et al., 1976) and CaCo-2 cells (Herold et al., 1993), in contrast with SW620 cells, develop brush borders. The comparison of the developmental pattern of alkaline phosphatase in SW480 and SW620 cell lines was, therefore, of interest.

Alkaline phosphatase activity was higher throughout the experimental period in SW480 than in SW620 cells (Fig. 3), but it was low in comparison with values found in CaCo-2 cells. While in confluent CaCo-2 cells an impressive increase of alkaline phosphatase activity was observed (Pinto et al., 1983), its activity in SW480 and SW620 cells declined gradually with the time in culture, except that SW480 cells had a peak activity on day 4 after seeding.

### *3.4. Polyamine oxidase (PAO)*

FAD-dependent PAO is a widely occurring tissue amine oxidase. Spermine, $N^1$-acetylspermidine, $N^1$-acetylspermine and $N^1,N^{12}$-diacetylspermine are natural substrates of this enzyme (Bolkenius and Seiler, 1981). Spermine and the $N^1$-acetyl derivatives of spermidine and spermine are also substrates of other amine oxidases. Therefore $N^1,N^{12}$-diaceylspermine was used as a selective substrate (Seiler, 1995). On day 3 after seeding the activity of PAO was in both cell lines 70–90 pmol/mg protein per h. This value is similar to that previously determined for CaCo-2 cells (Seiler et al., 2000). No significant increase of PAO activity with the time in culture was observed between days 4 and 10 after seeding.

### *3.5. Polyamines*

The differences in ODC and AdoMetDC activities of the two cell lines are reflected by correspondingly large differences in putrescine and spermidine concentrations (see Fig. 4 and Fig. 5). The striking minima of the ODC and AdoMetDC activities on day 6 after seeding (Fig. 2) are not reflected by correspondingly dramatic changes of the polyamine concentrations. However, the decrease of spermidine concentration on day 8 (Fig. 4 and Fig. 5) is presumably a consequence of the low decarboxylase activities of day 6. In any case, day 6 marks a profound change in polyamine metabolism: From this time point on the concentrations of $N^1$-acetylspermidine and $N^1$-acetylspermine increased abruptly with an especially high accumulation in SW620 cells. During this later phase of growth (i.e. at high cell density) SW480 and SW620 cells differed also with regard to their putrescine, spermidine and spermine ratios. While in SW480 cells putrescine and spermine concentrations increased until day 10 (with an intermediary drop of spermidine and spermine on day 8) (Fig. 4), in SW620 cells putrescine and spermine, but especially spermidine concentrations decreased gradually from day 6 on. This is also reflected by a maximum of total polyamine charge on day 6 in SW620 cells (Fig. 5). Owing to the increase of spermine, SW480 cells exhibited a maximum polyamine charge on day 10 (Fig. 4).

In both cell lines the spermidine/spermine – ratio decreased with increasing time in culture, i.e. with increasing cell density (Fig. 6). Again day 6 marks an abrupt change that is particularly striking in the case of SW620 cells. The higher spermidine/spermine – ratio of SW620 cells is paralleled by a higher rate of growth.

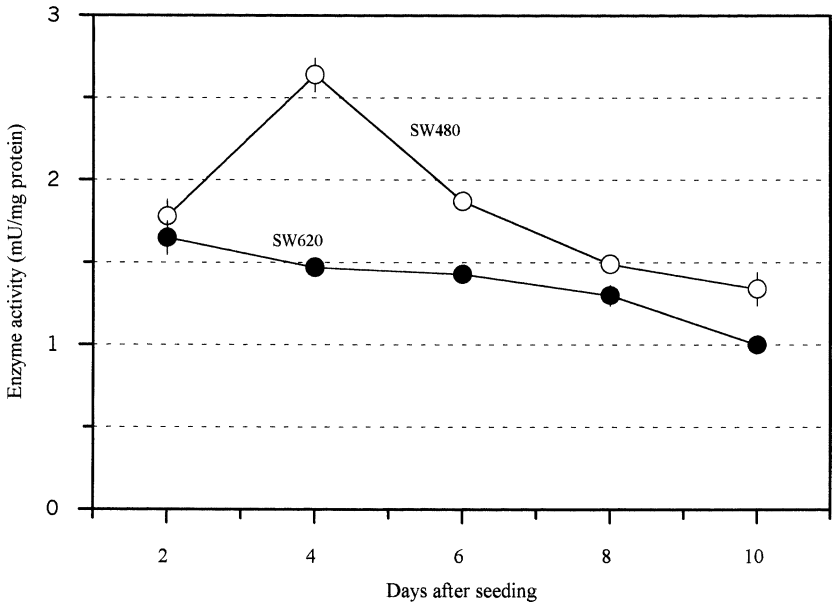


**Fig. 3.** Activity of alkaline phosphatase in homogenates of SW480 and SW620 cells as a function of time in culture. Culture conditions were the same as described in the legend of Fig. 2. Mean values ± S.E.M (n = 3)

### *3.6. Effect of polyamine depletion by DFMO on cell cycle phase distribution*

Exposure of cells to the ODC inactivator DFMO causes cell growth arrest due to depletion of putrescine and spermidine pools (Sunkara et al., 1987). If SW480 or SW620 cells were exposed to 1 mM DFMO, cell growth rate was reduced by 85 percent within 48 h. Determination of cell cycle phase distribution by FACS showed for both cell lines a gradual increase of G1-phase cells, and a concomitant decrease of the proportion of S-phase cells. The proportion of cells in G2/M was not changed significantly during 72 h of exposure to 1 mM DFMO (Fig. 7). Under the treatment conditions no evidence was found for enhanced apoptotic or non-apoptotic cell death.

### *3.7. Putrescine uptake*

Environmental polyamines are an important sources of many cells. They are taken up by specific transport (Seiler and Dezeure, 1990).

The affinity of putrescine to the transporter of SW620 cells was only marginally higher than to the transporter of SW480 cells (Table 2). However, in comparison with SW480 cells, the lymph node-derived metastatic cells had a considerably higher transport rate. With increasing time in culture the transport rate of both cell lines increased; between day 2 and 7 by a factor 3–3.5, as can be taken from the Michaelis-Menten type curves (Fig. 8).

## 4. Discussion

From numerous investigations on isolated aspects of polyamine metabolism the impression emerged that a higher growth rate of tumour cells correlates with increased polyamine concentrations (Jänne et al., 1978). Our work is in support of this claim. But not only the concentrations of the polyamines, but also the capacity of the more malignant SW620 cells to form polyamines *de novo* is higher. Based on our AdoMetDC activity determinations, SW620 cells have about a three-fold higher capacity of spermidine synthesis than SW480 cells.

In both cell lines the biosynthetic decarboxylases exhibited a striking minimum at the time when exponential growth changed to non-exponential growth. Although the cell density differed on day 6 considerably in terms of cell numbers (SW480: $1.3 \cdot 10^5 \cdot cm^{-2}$, SW620: $3.1 \cdot 10^5 \cdot cm^{-2}$), the larger SW480 cells occupy about the same area as the smaller SW620 cells. In view of the reproducibility of the minimum of ODC and AdoMetDC activities and their subsequent increase, SW480 and SW6290 cells appear particularly suitable for studying density dependent inhibition of growth ("contact inhibition"). There is little documentation at present on the molecular nature that underlies this phenomenon (Fagotto and Gumbiner, 1996; Aplin et al., 1999). ODC and AdoMetDC appear to be involved in the signalling of "contact inhibition". Evidence in favour of a role of the polyamines in different signalling pathways has recently been complied (Bachrach et al., 2001).

In addition to having a higher biosynthetic activity, SW620 cells accumulated polyamines faster than SW480 cells by a factor of 2.5–3, as became evident from the determinations of [$^{14}$C]putrescine uptake. Cells accumulate polyamines via active transmembrane transport. In some tumour cell types putrescine, spermidine and spermine share the same

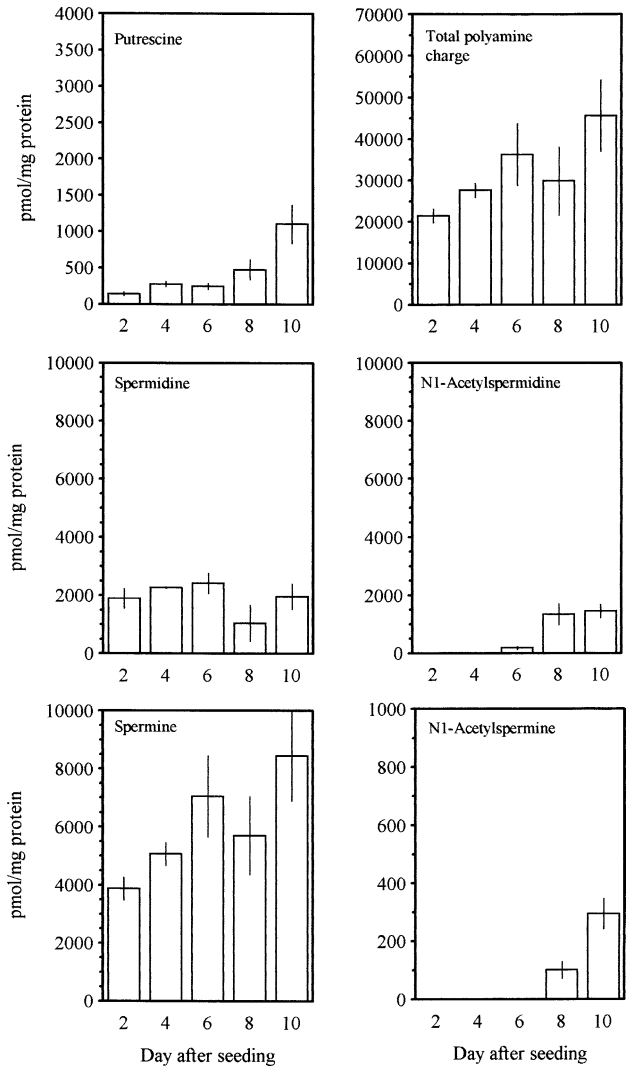


**Fig. 4.** Polyamine patterns of SW480 cells as a function of time in culture. Culture conditions were the same as described in the legend of Fig. 2. Mean values ± S.D. (n = 3)

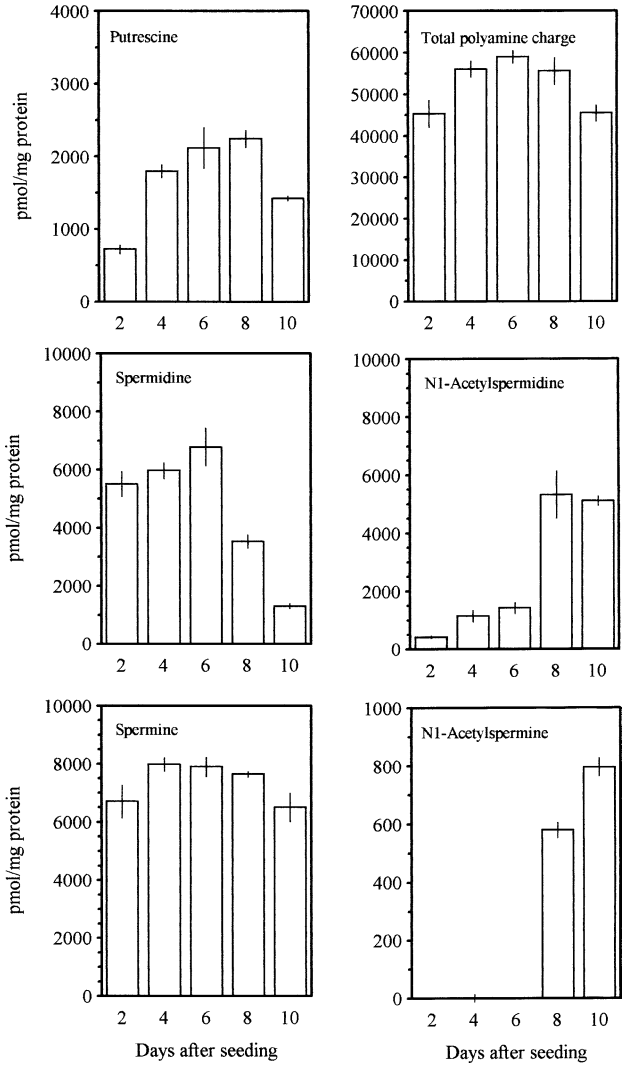


**Fig. 5.** Polyamine patterns of SW620 cells as a function of time in culture. Culture conditions were the same as described in the legend of Fig. 2. Mean values ± S.D. (n = 3)

transporter, others have more or less selective transporters for the individual amines (Seiler and Dezeure, 1990; Seiler et al., 1996). For SW480 and SW620 cells a detailed kinetic analysis of their polyamine transport system(s) is not yet available. However, the present data on putrescine transport demonstrate typical polyamine uptake characteristics. In both lines the maximum transport rate (Vmax) increased with increasing time in culture, without changing affinity of the transporter ($K_M$) for putrescine (Table 2). Evidently the number of transporter molecules increases with increasing cell density. This change may be interpreted as compensatory reaction to the increasing demand for polyamines by the high cell density and the decreasing polyamine pool in the

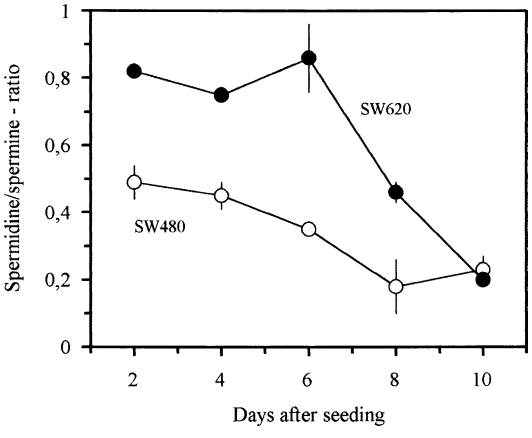


**Fig. 6.** Spermidine/spermine ratios of SW480 and SW620 cells, as calculated from the data of Fig. 4 and Fig. 5. The error bars indicate maximum deviation from the mean value (n = 3)

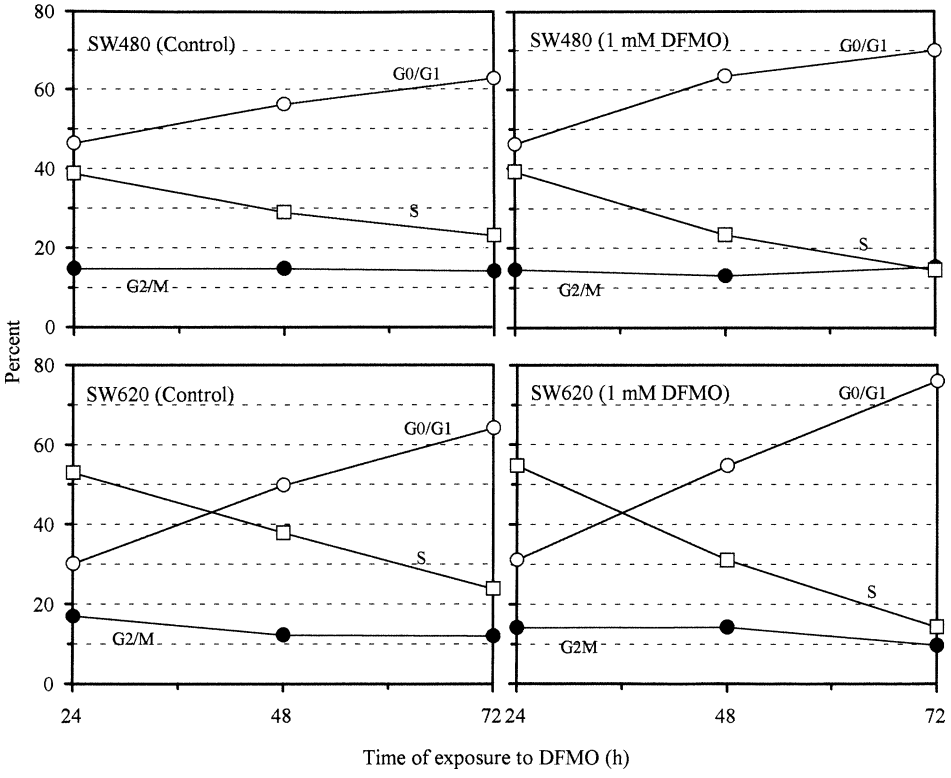


**Fig. 7.** Cell cycle phase distribution of SW480 and SW620 cells after exposure to 1 mM DFMO for up to 72 h. (For details see the Materials and methods section)

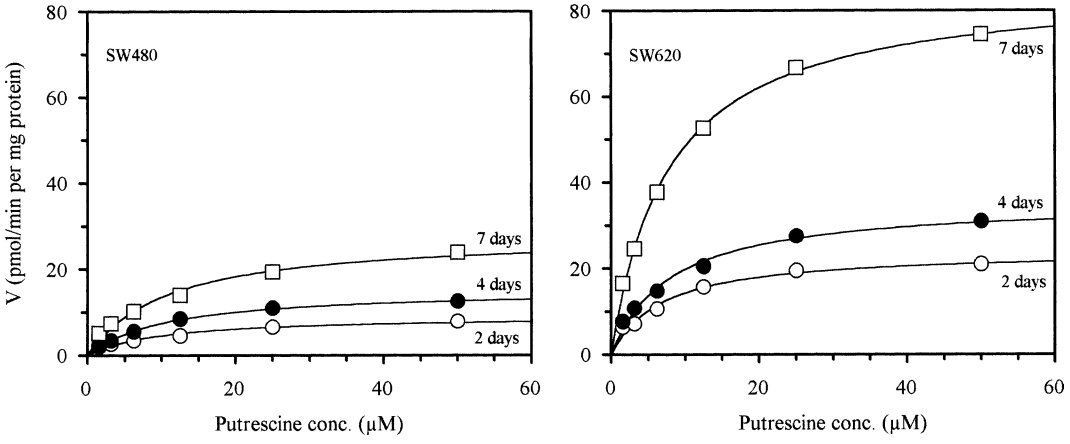


**Fig. 8.** Uptake of [$^{14}$C]putrescine by SW480 and SW620 cells; effect of time in culture. The Michaelis-Menten curves are representative for several uptake experiments. The kinetic parameters of the curves are shown in Table 2. (For experimental details see the Materials and methods section)

cellular environment. From the relationships between biosynthetic decarboxylase activities, polyamine patterns, and putrescine uptake characteristics one may conclude for both cell lines that *de novo* polyamine synthesis is more important than polyamine uptake during early exponential growth. Since biosynthetic activity and transport capacity of SW620 cells exceeded those of SW480 cells, the former appear better equipped for adaptation to changing compositions of their environment. This idea is supported by the fact that SW620 cells have a higher capacity than SW480 cells to accumulate arginine, the precursor of ornithine (Cendan et al., 1996).

The high spermidine/spermine-ratio (Fig. 6), together with the high putrescine levels of SW620 cells, indicate an excessive availability of putrescine for *de novo* spermidine formation during the rapid growth phase. The sharp decline of ODC and

**Table 2.** Uptake of putrescine by SW480 and SW620 cells. Kinetic parameters

| Time after seeding days | SW480 Vmax pmol/min per mg protein | $k_M$ $\mu$M | SW620 Vmax pmol/min per mg protein | $K_M$ $\mu$M |
|---|---|---|---|---|
| 2 | 9 ± 1 | 10 ± 3 | 24 ± 1 | 7 ± 1 |
| 4 | 15 ± 1 | 11 ± 1 | 36 ± 2 | 8 ± 1 |
| 7 | 28 ± 2 | 11 ± 2 | 86 ± 2 | 8 ± 1 |

The kinetic parameters were calculated from Michaelis-Menten type curves (Fig. 8) using the Erithacus software GraFit version 3 (Leatherbarrow, 1992)

AdoMetDC activities between days 4 and 6 after seeding (Fig. 2) followed by a nearly as abrupt decline of the spermidine/spermine-ratio indicates profound changes in polyamine metabolism, concomitantly with decreased proliferation rates. Comparable changes of polyamine metabolism at high cell density have to our knowledge not been described previously.

The changes in metabolic activities at the transition from exponential to non-exponential growth are also manifested by the intracellular accumulation of the acetyl-derivatives of spermidine and spermine (Fig. 4 and Fig. 5). The activity of spermidine $N^1$-acetyltransferase has not been determined at high cell density. In exponentially growing cells it was low, as is the case for most cells (data not shown). But even if one had observed an increase of the acetylase activity at high cell density, it would not have been possible to distinguish between a physiological and a pathological event, as will be discussed below. Taking into account the low PAO activity of the cell lines, it seems likely that the complete removal of the acetylderivatives is not possible, if acetylation rate is enhanced. Accumulation of $N^1$-acetylspermidine and $N^1$-acetylspermine within the cells, and the fact that the total polyamine-derived positive charge was not significantly different between exponentially growing cells and cells at high density indicates that acetylpolyamines are used for charge neutralisation. It will be of considerable interest to explore, whether the partial substitution of the parent compounds by their $N^1$-acetylderivatives as ligands of anionic binding sites is of physiological significance, or rather an artefact. Unfortunately it is not possible to rule out that the accumulation of $N^1$-acetylspermidine and $N^1$-acetylspermine is a consequence of non-physiological culture conditions. Evidently the culture conditions are not precisely the same at high and low cell density. Acidification of the culture medium (and the consequent decrease of the intracellular pH) and an inadequate $O_2$ supply may induce acetylation and affect a considerable number of other metabolic reactions. Moreover, a diminished $O_2$ supply of the tightly packed cells diminishes oxidative splitting of $N^1$-acetylspermidine and $N^1$-acetylspermine by PAO (and other $O_2$-dependent reactions). In accordance with this suggestion is the observation that rats with a solid tumour excrete enhanced amounts of $N^1$-acetylspermidine at the time when the blood supply (i.e. $O_2$-supply) is inadequate due to the size (and the low vascularisation) of the tumour, so that acetylpolyamines escape oxidative degradation (Seiler et al., 1981a).

Since SW620 cells have a very small cytoplasm (Leibovitz et al., 1976) but contain considerably more polyamines than SW480 cells, one has to ask the question, how are the polyamines stored? For the maintenance of charge neutrality a high polyamine content requires a proportionately high number of anionic polyamine binding sites. Within the cytoplasm ribosomal RNA is a major structure for polyamine binding (Watanabe et al., 1991). In addition, a considerable proportion of spermidine and spermine is localised within the cell nucleus (Sarhan and Seiler, 1989), and acidic groups of proteins are most probably also of importance for polyamine binding. If the polyamine binding structures are degraded, acetylation of spermidine and spermine is enhanced, and the polyamine content decreases proportionately with the loss of binding sites (Seiler et al., 1981b). As was pointed out above, the total RNA content was higher in SW620 than in SW480 cells (Table 1). The higher number of anionic binding sites due to the increase of these macromolecules explains most probably the ability of SW620 cells to store (in spite of a small size) a larger amount of polyamines than SW480 cells, without increasing the intrinsic osmotic pressure.

Depletion of putrescine and spermidine by exposure of cells to DFMO, the well known ODC inactivator, arrests different cell lines at different phases of the cell cycle (Sunkara et al., 1987). No explanation was available for these observations. Recently however Ray et al. (2001) reported that DFMO arrests IEC-6 cells, a non-transformed colonic cell line in G1. In contrast CaCo-2 cells, a human colon carcinoma-derived cell line with a mutated tumour suppression gene p53 is not arrested in a particular

phase by the same treatment. Since growth arrest of IEC-6 cells by DFMO was accompanied by elevation of p53 levels, it was concluded that progression through the cell cycle in IEC-6 cells is (among others) p53 dependent. Both, SW480 and SW620 cells exhibit the point mutation Arg-273 – His of the tumour suppressor gene p53 (Rodrigues et al., 1990). Nevertheless, both these cells arrested in G1. They may, therefore, serve as models in further investigations on the role of polyamines in the regulation of cell cycle traverse.

Based on morphology, cytogenetic studies and secretion of carcinoembryonic antigen (CEA) SW480 and SW620 cells were characterised as poorly differentiated cells (Leibovitz et al., 1976; Shi et al., 1983). Alkaline phosphatase activity is considered a suitable measure of colon cancer cell differentiation (see e.g. Turowsky et al., 1994; Pinto et al., 1983). Metastatic activity is usually correlated with a low degree of differentiation. Since SW620 cells have a higher invasive capacity than SW480 cells, both *in vitro* and *in vivo* (de Vries et al., 1995), it was not surprising that alkaline phosphatase activity was significantly lower throughout growth in SW620 cells (Fig. 3). However, the specific activity of this enzyme decreased gradually with increasing cell density in both cell lines, whereas it attains maximum values in confluent CaCo-2 cells (Pinto et al., 1983). In the light of our observation one may question the usefulness of alkaline phosphatase as a differentiation marker of colon cancer cells. Another potential explanation for our observations is that neither SW480 nor SW620 cells differentiate, even though SW480 cells form brush border membranes (similar to CaCo-2 cells) which, however, is not reflected by an increased activity of alkaline phosphatase.

As has already been mentioned, the idea that a direct relationship exists between polyamine metabolism and malignancy has presumably first been exemplified by Williams-Ashman et al. (1973) at the hand of a spectrum of Morris hepatomas. Our results are topical to this idea. They demonstrate that basic parameters of polyamine metabolism differ even in closely related cell lines, if these cells differ in physiological properties. Since the polyamines are ubiquitous multifunctional constituents of most cells (Cohen, 1998; Seiler and Douaud, 1998), it is usually not possible to pinpoint specific functions. However, comparative studies of related cell lines with different physiological or pathogenic properties, as exemplified in the present work, may allow one to identify new targets for investigations devoted to potential roles of the polyamines in specific cell functions, particularly of functions which are essential for the invasiveness and other characteristics of malignant cells.

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