Effect of Extracellular AMP on Cell Proliferation and Metabolism of Breast Cancer Cell Lines with High and Low Glycolytic Rates*

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Sybille Mazurek‡§, Andrea Michel¶, and Erich Eigenbrodt‡:

From the Institut for Biochemistry and Endocrinology, Veterinary Faculty, Justus-Liebig-University Giessen, Frankfurter Straße 100, 35392 Giessen and §ScheBo Tech GmbH, Bahnhofstrasse 6, 35435 Wettenberg, Federal Republic of Germany

In differentiated tissues, such as muscle and brain, increased adenosine monophosphate (AMP) levels stimulate glycolytic flux rates. In the breast cancer cell line MCF-7, which characteristically has a constantly high glycolytic flux rate, AMP induces a strong inhibition of glycolysis. The human breast cancer cell line MDA-MB-453, on the other hand, is characterized by a more differentiated metabolic phenotype. MDA-MB-453 cells have a lower glycolytic flux rate and higher pyruvate consumption than MCF-7 cells. In addition, they have an active glycerol 3-phosphate shuttle. AMP inhibits cell proliferation as well as NAD and NADH synthesis in both MCF-7 and MDA-MB-453 cells. However, in MDA-MB-453 cells glycolysis is slightly activated by AMP. This disparate response of glycolytic flux rate to AMP treatment is presumably caused by the fact that the reduced NAD and NADH levels in AMP-treated MDA-MB-453 cells reduce lactate dehydrogenase but not cytosolic glycerol-3-phosphate dehydrogenase reaction. Due to the different enzymatic complement in MCF-7 cells, proliferation is inhibited under glucose starvation, whereas MDA-MB-453 cells grow under these conditions. The inhibition of cell proliferation correlates with a reduction in glycolytic carbon flow to synthetic processes and a decrease in phosphotyrosine content of several proteins in both cell lines.

Both proliferating cells and tumor cells maintain a high glycolytic rate even under aerobic conditions, a process referred to as aerobic glycolysis. Observations on aerobic glycolysis in tumor cells prompted Warburg (1) to postulate an altered respiratory function leading to an increased glycolytic capacity and a high rate of lactate formation from glucose in the presence of oxygen. Data from former reports suggest that there are many factors contributing to the origin of aerobic glycolysis (2). The altered control of glycolysis by expression of certain isoenzymes is one important factor (2–12). Furthermore, the glycerol 3-phosphate shuttle and the malate-aspartate shuttle are altered in such a way that transport of cytosolic hydrogen into the mitochondria is reduced, requiring tumor cells to reoxidize NADH cytosolically by lactate dehydrogenase (13–15). Additionally, oxidation of pyruvate is reduced in favor of glutamine oxidation (16–25). Due to the expression of the mitochondrial, NAD-dependent malate decarboxylase, malate is converted to pyruvate and lactate (22–24). The conversion of glutamine to lactate is called, in analogy to glycolysis, glutaminolysis (25). In tumor cells the glycolytic capacity can be so great that all of the cell’s energy requirements are derived from glycolysis (2, 26). Therefore, high glycolytic activity ensures the survival and the migration of tumor cells in hypoxic areas (2, 26, 27). The main role of the glutaminolytic pathway is the generation of energy (2, 25). However, a high glycolytic rate is not always linked to cell proliferation or tumor formation. There are several cell lines that are able to grow in a medium with 5 mM galactose or with low glucose supply (0.5 mM) without producing lactate via glycolysis (19–21, 28–33). Investigations with labeled glucose and galactose have shown that the carbons of the two carbohydrates can either be used to synthesize nucleotides, phospholipids, and complex carbohydrates or can flow through pyruvate kinase to pyruvate and lactate for energy production (2, 19, 20, 29–33). Under glucose starvation, energy is not produced by glycolysis but by pyruvate oxidation or by conversion of glutamine to lactate (18–25). When those cells are replaced in a medium with a high glucose concentration (5 mM), all phosphometabolites above pyruvate kinase accumulate until the level of fructose 1,6-bisphosphate is high enough to activate pyruvate kinase (34–36). The mass of lactate is then derived from glucose. As a consequence, all intermediates of glycolysis between hexokinase and pyruvate kinase increase. By this mechanism the supply of phosphometabolites for synthetic processes is ensured, although pyruvate kinase is activated (2, 36). From these observations and the fact that growth factors and oncogene-dependent phosphorylation regulate glycolysis and phosphometabolite pools, one can assume that some phosphometabolites or synthetic products derived from the phosphometabolites, e.g., sugar phosphates, AMP, NAD, NADH and serine for sphinganine synthesis, regulate cell proliferation (2, 4, 6, 36–49). Indeed, by searching for such metabolic signals we found that extracellular AMP inhibits DNA synthesis in MCF-7 cells and stops cell proliferation. Extracellular AMP is split to adenosine by the ecto-5′-nucleotidase. Adenosine is transported into the cells via an adenosine translocator and phosphorylated by the cytosolic adenosine kinase to AMP (49–53). The increase in intracellular AMP inhibits P-ribose-P synthetase and reduces NAD and NADH synthesis (49–54). NADH levels drop so low that lactate dehydrogenase is no longer able to transfer the hydrogen from NADH to pyruvate. As a consequence, glycolysis is inhibited at the level of the NADH producing glyceraldehyde-3-phosphate dehydrogenase reaction (49). The metabolic behavior of MCF-7 cells is in complete contrast to differentiated tissues and cells where the increase of AMP under hypoxic conditions drastically activates 6-phosphofructo-1-kinase and the glycolytic flux rate (55–59).

In order to investigate the mechanisms by which AMP stimulates glycolysis in differentiated cells and inhibits glycolysis in tumor cells, we decided to study another human breast cancer cell line MDA-MB-453, which has a more differentiated...
metabolic phenotype (e.g. low aerobic glycolytic flux rate, high pyruvate consumption). In addition, we found that MDA-MB-453 cells grow well in a medium with a low glucose concentration and with galactose, whereas MCF-7 cells are unable to grow under these nutrient conditions.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**

**Tumor Cells**—MCF-7 cells were obtained from Prof. Dr. K. Goerttler, Institute for Experimental Pathology, German Cancer Research Center, Heidelberg, Germany. MDA-MB-453 cells were from the German Collection of Microorganisms and Cell Cultures in Braunschweig, Germany.

**Materials and Culture Conditions**—For cell culture, the basic medium was used glucose-free Dulbecco’s minimal essential medium, supplemented with 100 units of penicillin/ml, 100 μg of streptomycin/ml, 2 mM glutamine, and 4 mM pyruvate (all from Biochrom, Berlin, Germany). For MCF-7 cells 20% (v/v) fetal calf serum (FCS) from Biochrom, Berlin, Germany, were added to the basic medium. For MDA-MB-453 cells the basic medium was supplemented with 10% (v/v) FCS and 10 mM HEPES, pH 7.0. Glucose and galactose from Sigma were added to the media of both cell lines as described under “Results.” MCF-7 cells were cultured at 37°C in a 5% CO2 environment. MDA-MB-453 cells were cultured in a CO2-free atmosphere. For the proliferative experiments 4-cm diameter dishes (MDA-MB-453 cells) and 25-cm² flasks (MDA-MB-453 cells) were used (both from Nunc, Wiesbaden, Germany). For the intracellular measurements MCF-7 cells were cultured on 14-cm diameter dishes, and experiments were started with 1 million cells/dish. MDA-MB-453 cells were cultured in 83-cm² flasks, and experiments were started with 2 million cells/flask. MCF-7 cells were passaged every 4 days and MDA-MB-453 cells every 5–6 days. AMP was derived from Boehringer Mannheim, Germany, and was added to the media at a final concentration of 3 mM.

For the proliferation rate and glycolytic and glutaminolytic flux measurements, AMP was added to the medium at the beginning of each passage. For the intracellular measurements AMP was added to the media on the 2nd day of the culture period. After 2 (MCF-7 cells) or 3 days (MDA-MB-453 cells), the AMP-treated cells arrested at a cell density of 5 million cells/dish, whereas control cells without AMP treatment continued to proliferate, reaching a density of 10–15 million cells/dish (MCF-7 cells) or 15–20 million cells/flask (MDA-MB-453 cells).

**Proliferation Rate and Glycolytic and Glutaminolytic Flux Measurements**

Every 24 h cell culture supernatants were collected and immediately frozen in liquid nitrogen. The cells were removed from the plates with trypsin/EDTA from Biochrom, Berlin, Germany, and counted in a hemocytometer. The frozen supernatants were heated for 15 min at 80°C and were subsequently centrifuged at 8000 × g for 10 min (49). Glucose, lactate, pyruvate, glutamine, and glutamate concentrations were determined enzymatically (60). The NADH concentration was calculated via the equation \[ [\text{NADH}] = 1.11 \times 10^{-4} \times [\text{NAD}] / [\text{ lactate}] / [\text{pyruvate}] \] (47). The protein content in the pellet was determined using the commercially available Bio-Rad test-kit (Bio-Rad, Munich, Germany).

**Determination of Intracellular Metabolite Concentrations**

For the extraction of intracellular lactate, pyruvate, and NAD, cells were treated at 80°C in aqua bidest for 15 min as described previously (49). The concentrations of lactate, pyruvate, and NAD were measured enzymatically (60). The NADH concentration was calculated via the equation \[ [\text{NADH}] = 1.11 \times 10^{-4} \times [\text{NAD}] / [\text{lactate}] / [\text{pyruvate}] \] (47). The protein content in the pellet was determined using the commercially available Bio-Rad test-kit (Bio-Rad, Munich, Germany).

**Determination of Intracellular Enzyme Activities**

For the extraction of the intracellular enzymes, a homogenization buffer, pH 7.4, containing 20 mM KH2PO4/K2HPO4, 1 mM mercaptoethanol, 1 mM EDTA/Na2, 2 mM ε-amino-n-caproic acid, and 0.2 mM phenylmethylsulfonyl fluoride was used. For the measurements of malate dehydrogenase a homogenization buffer containing 10 mM Tris, 1 mM NaF, and 1 mM mercaptoethanol, pH 7.4, was used. The extractions and the measurements of enzyme activities were carried out as described in Ref. 49. Protein concentrations were measured by the Biuret method using a commercially available test kit from Boehringer Mannheim, Germany. The pyruvate kinase subunits (dimer and tetramer) were separated by gel filtration as described in Ref. 36.

**Isoelectric Focusing**

Cells were extracted with a homogenization buffer containing 10 mM Tris, 1 mM NaF, and 1 mM mercaptoethanol, pH 7.4. Isoelectric focusing was carried out with a linear gradient of glycine (50% to 0% (v/v)) and amphotiles (pI 3.5–10.5) as described previously (61).

**Immunological Detection of Phosphotyrosine**

After separation on a 10% SDS-polyacrylamide gel, the proteins were transferred onto a nitrocellulose membrane by electroblotting. For the detection of phosphotyrosine a peroxidase-conjugated monoclonal anti-phosphotyrosine antibody from ICN (Costa Mesa, CA) was used. Immunostaining without anti-phosphotyrosine antibody resulted in no detectable reaction (61).

**Statistical Analysis**

For the glycolytic and glutaminolytic flux measurements as well as for the specific enzyme activities, statistical analysis was performed by a one- or two-factor (co)variance analysis with the “statistical program package BMDP,” whereby metabolite conversions or enzyme activities were compared versus cell densities (62). Possible effects of cell density were taken into consideration. In all other analyses Student’s t test was employed.

**RESULTS**

**Effect of Glucose, Galactose, and AMP on the Proliferation of MCF-7 and MDA-MB-453 Cells**—MCF-7 and MDA-MB-453 cells were cultured in media with different glucose and galactose concentrations. The basic medium was glucose-free Dulbecco’s minimal essential medium. After addition of fetal calf serum a final glucose concentration of 0.5 mM was obtained. Galactose was not detectable. In order to achieve other glucose and galactose concentrations, corresponding carbohydrates were added to the medium. To obtain a glucose-free medium fetal calf serum was dialyzed in a dialysis bag three times (for 8 h each) against 40 volumes of phosphate-buffered saline. To ensure that no vital necessary factors were lost during dialysis, the glucose-free medium was supplemented with 0.5 mM glucose, and cell proliferation was checked. There was no difference between the proliferation rate of MDA-MB-453 cells cultured in the glucose-supplemented medium (0.5 mM glucose) and that of the cells held in medium with 0.5 mM glucose from undialyzed fetal calf serum (data not shown). For cell stock breeding MCF-7 cells were cultured in the basic DMEM supplemented with 5 mM glucose. MDA-MB-453 cells were cultured in the basic DMEM supplemented with 5 mM galactose and 0.5 mM glucose from FCS (compare “Experimental Procedures”). Henceforth, these cells will be referred to as “proliferating” MCF-7 or MDA-MB-453 cells. For the described experiments with other glucose and galactose concentrations, cells were cultured for one passage in the new medium, and the cells of the second passage were used for the measurements. The effect of AMP on metabolites and enzymes was always determined according to Bergmeyer (60). For galactose measurement a commercially available test kit from Boehringer Mannheim, Germany, was employed.

**Figure 1, A and B**, shows the effect of AMP and different glucose and galactose concentrations on the cell proliferation rate of MCF-7 and MDA-MB-453 cells. In MCF-7 cells the highest proliferation rate was reached at a glucose concentration of 5
mM in the medium (Fig. 1A). Reduction of the glucose concentration to 0.5 mM led to an inhibition of cell proliferation to less than half the maximal rate. In glucose-free DMEM supplemented with 5 mM galactose, the cells became adherent but ceased to proliferate. The addition of AMP to the culture medium (DMEM with 5 mM glucose) totally inhibited cell proliferation. This inhibition was reversible. After degradation of AMP in the medium or after reculture in AMP-free medium, cell proliferation began again and reached normal values (49).

MDA-MB-453 cells demonstrated a totally different association between proliferation rate and availability of glucose and galactose in the medium. MDA-MB-453 cells grew best in a medium containing 5 mM galactose (Fig. 1B). In the absence of galactose, cell proliferation was inhibited in MDA-MB-453 cells. If glucose was also removed cell proliferation was totally arrested. The cells did not become confluent. In the presence of glucose in the medium, the cell proliferation rate was only about half the rate in galactose containing medium. An increase of the glucose concentration from 0.5 to 5 mM had no effect on the proliferation rate. As in MCF-7 cells, incubation of MDA-MB-453 cells with AMP led to a total inhibition of cell proliferation. After reculture of the AMP-treated MDA-MB-453 cells in AMP-free medium, cell proliferation once again reached normal rates (data not shown).

Effect of Glucose, Galactose, and AMP on the Glycolytic and Glutaminolytic Flux in MCF-7 and MDA-MB-453 Cells—For flux measurements, two different forms of calculations were chosen. The first calculation is in nmol/(h·dish) and describes the direct correlation between the consumption of a specific carbon source (glucose, galactose, glutamine, or pyruvate) and lactate or glutamate production. The second form of calculation in nmol/(h·10^5 cells) describes the consumption or production of a certain metabolite by each cell.

In MCF-7 cells the measurements of the glycolytic flux in nmol/(h·dish) showed a close linkage between glucose consumption and lactate production, independent of the glucose concen-
tration in the medium (0.5 mM or 5 mM) (Fig. 2A and Table I). The slope of the regression line with 5 mM glucose was 1.7, with a correlation coefficient of 0.932. This value approaches the ideal maximal value of 2 for the ratio of lactate production: glucose consumption. In glycolysis 1 mol of glucose is converted into 2 mol of lactate; therefore, a ratio between lactate production and glucose consumption of nearly 2 indicates that all lactate produced must be derived from glucose. A slope of 1.7 means that 85% of the glucose consumed was converted to lactate. Therefore, in MCF-7 cells 37 nmol of glucose consumed were converted to lactate, and 7 nmol were used for synthetic processes (calculated with data from Table III). The intercept of the regression line reflects the lactate production without glucose consumption (Fig. 2 and Tables I and II). This lactate can derive from glutamine and/or pyruvate consumption (Tables I and II). In MCF-7 cells with 5 mM glucose 91 nmol of lactate/(h-dish) were derived from sources other than glucose (Table I). If cultured in a medium with 5 mM glucose, there was a significant correlation between glutamine consumption and lactate production (Table I) but not between pyruvate consumption and lactate production (data not shown). Therefore, when no glucose was consumed by MCF-7 cells, the mass of lactate derived from glutamine. Furthermore, glutamine consumption increased with glucose consumption when the cells were cultured in 5 mM glucose (slope = 0.08; intercept = 6.2 nmol/(h-dish); r = 0.777; n = 33). A reduction of the glucose concentration to 0.5 mM led to an inhibition of cell proliferation (Fig. 1A) and a reduction of the total glucose conversion (Table III), but the strong linkage between glucose consumption and lactate production was not influenced at low glucose concentrations (Fig. 2A and Table I). The slope of the regression line was 1.8 with a regression coefficient of 0.642. The intercept of the regression line dropped from 91 nmol/(h-dish) in medium with 5 mM glucose to 25 nmol/(h-dish) in medium with 0.5 mM glucose (Table I). Galactose was not converted in measurable amounts in MCF-7 cells. The AMP-induced proliferation stop was correlated with disruption of the strong linkage between glucose consumption and lactate production in MCF-7 cells (Table I). The slope of the regression line was 0.4 with a correlation coefficient of 0.136. The intercept of the regression line increased to 0.26 with a correlation coefficient of −0.136. The intercept of the regression line with 121 nmol/(h-dish) indicates that more lactate was derived from carbon sources other than glucose (Table I).

In contrast to MCF-7 cells, in MDA-MB-453 cells glucose consumption and lactate production were not closely linked (Fig. 2B and Table II). The slope of the regression line was 0.7 for both glucose concentrations tested (0.5 and 5 mM). Therefore, 35% of the glucose consumed was converted to lactate. This reveals that in a medium with 5 mM glucose, about 4 nmol of glucose consumed were converted to lactate, whereas 7 nmol of glucose were used for synthetic processes (calculated with data from Table I). In MCF-7 cells there existed a correlation between pyruvate consumption and lactate production in MDA-MB-453 cells when cultured in a medium with 5 mM glucose (Table II). When all pyruvate is converted to lactate the slope of the regression line reaches the ideal maximal value of 1. For MDA-MB-453 cells, the estimated value of the regression line was 0.4; therefore, 40% of the pyruvate consumed was converted to lactate. No correlation was observed between glutamine consumption and lactate production or between glucose consumption and glutamine consumption in MDA-MB-453 cells (data not shown). MDA-MB-453 cells need galactose for an optimal proliferation rate (Fig. 1B). Nevertheless, glucose was converted first when 5 mM galactose and 0.5 mM glucose were available in the medium. Galactose consumption was only measurable when no glucose was present in the medium. Under these conditions there existed a correlation between galactose consumption and lactate production with a regression line slope of 0.25 (r = 0.679, n = 17). Therefore 13% of the galactose consumed was converted to lactate, and 87% was used for synthetic processes. In AMP-arrested MDA-MB-453 cells, the slope of the regression line (lactate production plotted versus glucose consumption) is enhanced from 0.7 (= 35%) to 1.3 (= 65%) with a correlation coefficient of 0.862 (Table II). The increased slope and the reduced intercept (108 nmol/(h-dish)) in proliferating cells and 60 nmol/(h-dish) in AMP-arrested cells) might indicate that AMP-arrested MDA-MB-453 cells are more glycolytic than the untreated proliferating cells (Table II).

The calculation in nmol/(h·10^6 cells) revealed a highly significant correlation between pyruvate consumption and cell density for both cell lines under optimal proliferation conditions (5 mM glucose for MCF-7 cells and 0.5 mM glucose with 5 mM galactose for MDA-MB-453 cells). In MCF-7 cells the curve follows the equation \[ y = 9.88e^{-0.26x} \] (49). In MDA-MB-453 cells the curve follows the equation \[ y = 24.81e^{-0.42x} \] (49). In both cell lines pyruvate consumption declined exponentially with cell density. At high cell densities pyruvate consumption approached zero values. The dependence of cell density on glutamine consumption and glutamate production was not significant in either cell line. Glucose consumption, galactose consumption, and lactate production all declined with cell density in MDA-MB-453 cells (slope for glucose consumption = −0.6, slope for galactose consumption = −0.17, and slope for...
lactate production was reduced under these conditions (p < 0.001). Glucose consumption was the same in the two cell lines. Glutamate consumption as well as glutamate production were enhanced in AMP-arrested MDA-MB-453 cells (p < 0.001). Glutamine consumption was not significantly different between AMP-arrested and proliferating cells (p < 0.001), whereas glutamate production was not significantly influenced (Table III).

In MCF-7 cells the total inhibition of cell proliferation by AMP was correlated with a drastic reduction of glucose consumption (p < 0.001, Table III), whereas lactate production was not significantly affected. Pyruvate and glutamine consumption as well as glutamate production were enhanced in AMP-arrested MCF-7 cells (p < 0.05). In contrast to MCF-7 cells there was a strong reduction of pyruvate consumption in AMP-arrested MDA-MB-453 cells compared with proliferating cells (p < 0.001, Table III), whereas glucose consumption and lactate production were not affected by AMP treatment in MDA-MB-453 cells. Glutamine consumption (p < 0.05) and glutamate production (p < 0.001) were enhanced in AMP-arrested MDA-MB-453 cells.

Effect of Glucose, Galactose, and AMP on Intracellular Metabolite Levels in MCF-7 and MDA-MB-453 Cells—Measurements of the intracellular metabolite concentrations revealed a strong correlation between lactate, NAD concentrations, and cell density. For the lactate concentration in MCF-7 cells, the slope of the regression line was -30.3 with an intercept of 53.7 nmol/mg protein (n = 17, r = -0.876). For the NAD concentration a slope of -0.7 was calculated with an intercept of 3.2 nmol/mg protein (n = 41, r = -0.754). This dependence on cell density has been taken into account for the following comparison of the metabolite concentrations between the different cell groups. For intracellular pyruvate concentration no such correlation could be demonstrated. All measurements were done at the same cell density of about 5 million cells/dish.

Proliferating MDA-MB-453 cells cultured in medium with 5 mM galactose and 0.5 mM glucose had a higher lactate and pyruvate content than proliferating MCF-7 cells cultured in medium with 5 mM glucose (Fig. 3A). The absolute NAD levels did not significantly differ between the two cell lines (Fig. 3B). However, there was a great difference in the ratio of free NADH:NAD between the two cell lines. In proliferating MCF-7 cells the NADH:NAD ratio was 1:160, whereas in proliferating MDA-MB-453 cells the ratio was 1:920. MCF-7 cells had a 5-fold higher NADH content than MDA-MB-453 cells (Fig. 3B). In MCF-7 cells inhibition of cell proliferation by glucose starvation (0.5 mM glucose) or total inhibition of cell proliferation by AMP led to a drastic reduction in the intracellular lactate and NADH concentrations, whereas the pyruvate concentration increased (Fig. 3, A and B). Furthermore, in AMP-arrested MCF-7 cells the NAD content was reduced (Fig. 3B), and the NADH:NAD ratio was 1:1000. Glucose starvation had no significant effect on the NAD level and the ratio between NADH and NAD was 1:2400. In MDA-MB-453 cells lactate and pyruvate levels were not significantly different between AMP-arrested cells and controls. NAD as well as NADH levels decreased under AMP treatment (Fig. 3B). The NADH:NAD ratio was 1:1400 in AMP-arrested MDA-MB-453 cells compared with 1:920 in proliferating cells.

Comparison of Glycolytic Enzymes between MCF-7 and MDA-MB-453 Cells—A correlation between specific glycolytic enzyme activities and cell density was found in both cell lines. In Fig. 4 this relationship is shown for glyceroldehyde-3-phosphate dehydrogenase in both cell lines. Glyceroldehyde-3-phosphate dehydrogenase activity increased with cell density. Significant correlations between the specific enzyme activity and...
cell density were also found for the mitochondrial hexokinase (slope $= 2.2$ in both cell lines), the cytosolic hexokinase (slope $= 2.3$ in both cell lines), 6-phosphofructo-1-kinase (slope in MCF-7 cells $= 90.0$; in MDA-MB-453 cells $= 52.8$), glucose-6-phosphate dehydrogenase (slope in MCF-7 cells $= 0.3$; in MDA-MB-453 cells $= -0.4$), 6-phosphogluconate dehydrogenase (slope $= 21.4$ in both cell lines), enolase (slope $= 105.6$ in both cell lines), and pyruvate kinase (slope in MCF-7 cells $= -0.1$; in MDA-MB-453 cells $= 0.35$).

The differences in the glycolytic flux rates between proliferating MCF-7 and proliferating MDA-MB-453 cells correlate with a striking difference in the specific glycolytic enzyme activities (Table IV). MCF-7 cells, which had the higher glycolytic capacity, had much higher specific 6-phosphofructo-1-kinase, glyceraldehyde-3-phosphate dehydrogenase, and pyruvate kinase activities than MDA-MB-453 cells. On the other hand, MDA-MB-453 cells contain cytosolic glycerol-3-phosphate dehydrogenase, which was not detectable in MCF-7 cells. Furthermore, MDA-MB-453 cells had a higher specific cytosolic hexokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and enolase activity than MCF-7 cells (Table IV).

In tumor cells, the pyruvate kinase type M2 isoenzyme exists in two different forms, an active tetrameric form and an inactive dimeric form (36, 49). In MCF-7 cells the ratio between the inactive dimeric and the active tetrameric form of pyruvate kinase was 6:1. AMP had no effect on this relationship in MCF-7 cells. MDA-MB-453 cells had the same ratio between the two forms of pyruvate kinase when cultured in a medium with 5 mM glucose. Culture of MDA-MB-453 cells in a medium with 5 mM galactose and 0.5 mM glucose led to a shift to the inactive dimeric form. Under these conditions the ratio between the dimeric and tetrameric form of pyruvate kinase was 10:1 in MDA-MB-453 cells.

Comparison of the Glutaminolytic Enzyme Activities between MCF-7 and MDA-MB-453 Cells—The measurement of the glutaminolytic enzyme activities revealed a strong dependence of glutamate dehydrogenase activity as well as malate dehydrogenase activity (measured in NADH to NAD direction) on cell density in both cell lines. The slope of glutamate dehydrogenase is 66 in MCF-7 cells and 28 in MDA-MB-453 cells. The slope of malate dehydrogenase is 0.5 in MCF-7 cells and 0.3 in MDA-MB-453 cells.
In a first statistical step the dependence of specific enzyme activities on cell density was tested. The comparison of MCF-7 and MDA-MB-453 cells (cell type) as well as the effect of AMP on the specific enzyme activities (AMP) was performed by a two-factor (co)variance analysis under consideration of dependence from cell density. Interaction is the result of a statistical test which tests whether there is a difference in the effect of AMP between MCF-7 and MDA-MB-453 cells. For the statistical analysis of MDH Ma. the values had to be transformed by logarithm because the distribution was skewed to the right.

Concerning the glutaminolytic enzymes, the greatest difference between the two cell lines was found in the case of malate dehydrogenase. MCF-7 cells showed a 7-fold higher malate dehydrogenase activity when measured in NADH → NAD direction (MDH Ox.) than MDA-MB-453 cells (Table V). Measured in NAD → NADH direction (MDH Ma.) MDA-MB-453 cells had a slightly higher activity.

### Table IV

| Enzymes | Specific activities (x ± S.E.) |
|---------|-----------------------------|
|         | MCF-7 cells | AMP-arrested | MDA-MB-453 cells | AMP-arrested | Significance |
|         | x̄ ± S.E. | x̄ ± S.E. | x̄ ± S.E. | x̄ ± S.E. | p | p | p |
| HK mitochondria | 7.8 ± 0.2 | 6.4 ± 0.3 | 7.5 ± 0.3 | 6.1 ± 0.5 | NS | 0.001 | NS |
| HK cytosol | 37.6 ± 1.1 | 38.0 ± 1.6 | 50.5 ± 1.3 | 44.8 ± 2.6 | 0.001 | NS | NS |
| G6PDH | 700.0 ± 27.7 | 1900.0 ± 34.6 | 3770.0 ± 53.7 | 4630.0 ± 180.2 | 0.001 | 0.001 | 0.001 |
| PGDH | 89.2 ± 2.8 | 98.0 ± 4.1 | 120.9 ± 3.2 | 140.4 ± 6.6 | 0.001 | 0.001 | 0.001 |
| PFK | 120.8 ± 3.6 | 156.2 ± 3.4 | 69.6 ± 3.4 | 67.8 ± 9.9 | 0.001 | 0.001 | 0.001 |
| G3PDH | ND | ND | 15.8 ± 1.9 | 36.6 ± 4.5 | 0.001 | 0.001 | 0.001 |
| GAPDH | 4330.0 ± 120.0 | 6730.0 ± 180.0 | 2030.0 ± 150.0 | 2190.0 ± 290.0 | 0.001 | 0.001 | 0.001 |
| EN | 904.5 ± 15.5 | 924.8 ± 22.4 | 1037.3 ± 17.6 | 1431.3 ± 36.5 | 0.001 | 0.001 | 0.001 |
| PK | 2390.0 ± 70.7 | 3040.0 ± 67.4 | 750.0 ± 31.3 | 980.0 ± 25.3 | 0.001 | 0.001 | 0.001 |
| LDH | 4270.0 ± 61.0 | 4455.0 ± 73.1 | 4240.0 ± 178.9 | 6970.0 ± 224.5 | 0.001 | 0.001 | 0.001 |

### Table V

| Enzymes | Specific activities (x ± S.E.) |
|---------|-----------------------------|
|         | MCF-7 cells | AMP-arrested | MDA-MB-453 cells | AMP-arrested | Cell type | AMP | Interaction |
|         | x̄ ± S.E. | x̄ ± S.E. | x̄ ± S.E. | x̄ ± S.E. | p | p | p |
| GLDH | 54.7 ± 2.4 | 80.5 ± 2.7 | 82.5 ± 4.4 | 111.5 ± 13.8 | 0.001 | 0.001 | NS |
| GOT mitochondrial | 80.5 ± 2.0 | 101.8 ± 2.9 | 36.2 ± 1.8 | 66.7 ± 2.7 | 0.001 | 0.001 | NS |
| GOT cytosolic | 158.5 ± 4.6 | 1076.7 ± 5.3 | 155.8 ± 5.5 | 177.2 ± 12.0 | 0.001 | 0.001 | 0.05 |
| MDH Ox. | 2960.0 ± 67.3 | 3830.80.0 ± 165.5 | 400.0 ± 10.4 | 280.0 ± 9.5 | 0.001 | 0.001 | 0.001 |
| MDH Ma. x̄, DF=1 | 35.5 ± 1.1 | 46.8 ± 1.1 | 53.7 ± 1.1 | 61.7 ± 1.1 | 0.001 | 0.001 | 0.01 |
| ME mitochondria | 1.0 ± 0.3 | 0.9 ± 0.3 | 1.2 ± 0.1 | 0.7 ± 0.1 | NS | 0.001 | 0.05 |
| ME cytosol | 37.4 ± 0.8 | 37.0 ± 1.1 | 19.2 ± 0.8 | 11.8 ± 0.8 | 0.001 | 0.001 | 0.01 |

Concerning the glutaminolytic enzymes, the greatest difference between the two cell lines was found in the case of malate dehydrogenase. MCF-7 cells showed a 7-fold higher malate dehydrogenase activity when measured in NADH → NAD direction (MDH Ox.) than MDA-MB-453 cells (Table V). Measured in NAD → NADH direction (MDH Ma.) MDA-MB-453 cells had a slightly higher activity.

The ratio between malate dehydrogenase activity measured in the NADH → NAD direction and the NAD → NADH direction was 83:1 in MCF-7 cells, whereas MDA-MB-453 cells had a ratio of 7:1. This difference is a result of the different malate dehydrogenase isoenzyme features and kinetic properties of the isoenzymes in the two cell lines. Both cell lines basically have two malate dehydrogenase isoenzymes that can be distinguished by their different isolectric points. The cytosolic isoenzyme has an isolectric point of 5.0, the mitochondrial isoenzyme of 9.5 (Fig. 5, A and B). In MCF-7 cells a third form of malate dehydrogenase can be detected. This form has an isolectric point of 7.8 and represents a precursor of the mitochondrial isoenzyme located in the cytosol (Fig. 5A). This form of malate dehydrogenase could not be found in MDA-MB-453 cells.

In contrast to MCF-7 cells, MDA-MB-453 cells showed a broad mitochondrial peak, which ranged from pl 8.5 up to pl 10.5 (Fig. 5, A and B). Furthermore in MCF-7 cells the pl 7.8 form of malate dehydrogenase was associated with p36, whereas the pl 5.0 and the pl 9.5 forms were not (61). In MDA-MB-453 cells p36 was found in considerably higher amounts than in MCF-7 cells, but these were spread over all malate dehydrogenase fractions (data not shown).

The cytosolic and mitochondrial malate dehydrogenase isoenzymes had different capacities when measured in the NADH → NAD direction or in the NAD → NADH direction in MCF-7 and MDA-MB-453 cells (Table VI). In MCF-7 cells the cytosolic isoenzyme (pl 5.0) preferred the NADH → NADH conversion, whereas the mitochondrial forms (pl 7.8 and pl 9.5) had the greatest capacity when measured in the NAD → NADH direction (Table VI). Measured in the NADH → NAD direction only 26% of the total malate dehydrogenase activity was measured at pl 5.0, and 72% of the total malate dehydrogenase activity was found in the fractions corresponding to the mitochondrial forms. Measured in the NADH → NAD direction this ratio was just the other way around. In MDA-MB-453 cells the mitochondrial isoenzyme showed almost no capacity for the NADH → NAD direction (8.5%). Ninety percent of the activity was found in the cytosolic fractions. Measured in the NADH → NAD direction, the ratio between the cytosolic and the mitochondrial isoenzyme was 10:1 (Fig. 5, A and B).
Effect of AMP on Tumor Cell Metabolism

Fig. 5. Isoelectric focusing of proliferating MCF-7 and proliferating MDA-MB-453 cells. A, malate dehydrogenase measured in NADH → NAD direction (= oxaloacetate → malate direction); B, malate dehydrogenase measured in NAD → NADH direction (= malate → oxaloacetate direction). In both cell lines the same number of cells per isoelectric focusing (= 3.5×10⁶ cells) were applied for the calculation. ○ - ○, MCF-7 cells; ■ - ■, MDA-MB-453 cells.

Glutamate dehydrogenase and cytosolic glutamate oxaloacetate transaminase activities were slightly higher in MDA-MB-453 cells than in MCF-7, whereas MCF-7 cells had higher mitochondrial glutamate oxaloacetate transaminase and cytosolic malic enzyme activities (Table V).

Effect of AMP on Glycolytic and Glutaminolytic Enzymes in MCF-7 and MDA-MB-453 Cells—Addition of AMP led to an enhancement of most of the specific glycolytic enzyme activities in both cell lines (Table IV). Glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and pyruvate kinase activities were enhanced after AMP treatment in both cell lines. Differences between the two cell lines were seen in the case of 6-phosphofructo-1-kinase, glyceraldehyde-3-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, enolase, and lactate dehydrogenase (interaction p ≤ 0.001). In AMP-arrested MCF-7 cells 6-phosphofructo-1-kinase and glyceraldehyde-3-phosphate dehydrogenase activity were enhanced when compared with untreated proliferating cells. In contrast, in MDA-MB-453 cells AMP treatment had no effect on these enzyme activities. Glyceral-3-phosphate dehydrogenase activity increased in AMP-arrested MDA-MB-453 cells, whereas MCF-7 cells contained no cytosolic glyceral-3-phosphate dehydrogenase. Enolase and lactate dehydrogenase activities were enhanced in AMP-treated MDA-MB-453 cells but were not involved in AMP-treated MCF-7 cells. The only glycolytic enzyme that was reduced under AMP treatment in both cell lines was mitochondrial hexokinase. Cytosolic hexokinase was not affected by AMP treatment in either of the cell lines.

In glutaminolysis, inhibition of cell proliferation by AMP was coupled with an increase of glutamate dehydrogenase and mitochondrial glutamate oxaloacetate transaminase activities in both cell lines (Table V). The mitochondrial and cytosolic isoenzymes of malic enzyme were reduced in AMP-treated MDA-MB-453 cells, whereas in MCF-7 cells AMP treatment had no effect on the two isoenzymes. The cytosolic glutamate oxaloacetate transaminase activity was not affected by AMP treatment in either cell line.

Comparison of the Phosphotyrosine Content in MCF-7 and MDA-MB-453 Cells—Staining with anti-phosphotyrosine antibodies after SDS-polyacrylamide gel electrophoresis of cytosolic supernatants from MCF-7 and MDA-MB-453 cells cultured under different conditions. For each sample the same protein amount (30 μg) was applied on the gel. gluc, glucose; gal, galactose.

Discussion

Comparison of Metabolism between MCF-7 and MDA-MB-453 Cells—Glycolysis—MCF-7 cells have a much higher glycolytic flux rate than MDA-MB-453 cells (Tables I–III, Fig. 2, A and B). The different glycolytic flux rates in MCF-7 and MDA-MB-453 cells can be correlated with different enzyme expres-

TABLE VI

Distribution of the cytosolic and mitochondrial malate dehydrogenase isoenzymes in MCF-7 and MDA-MB-453 cells, calculated as “area under the curve” of the different peaks after isoelectric focusing Compare Fig. 5, A and B. n = 4.

| Malate dehydrogenase | Area under the curve [%]: x ± S.E. |
|----------------------|-----------------------------------|
| MCF-7 cells          | MDA-MB-453 cells                  |
| NADH → NAD direction |                                   |
| Cytosolic form       | 26.1 ± 1.2                        |
| Mitochondrial forms  | 71.9 ± 2.6                        |
| NAD → NADH direction |                                   |
| Cytosolic form       | 70.2 ± 7.5                        |
| Mitochondrial forms  | 31.2 ± 3.9                        |

DISCUSSION

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FIG. 6. Comparison of the phosphotyrosine content in MCF-7 and MDA-MB-453 cells depending on the culture conditions. Immunostaining with anti-phosphotyrosine antibodies after SDS-polyacrylamide gel electrophoresis of cytosolic supernatants from MCF-7 and MDA-MB-453 cells cultured under different conditions. For each sample the same protein amount (30 μg) was applied on the gel. gluc, glucose; gal, galactose.

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| Cytosolic form       | 70.2 ± 7.5                        |
| Mitochondrial forms  | 31.2 ± 3.9                        |
by the malate-aspartate shuttle. The cytosolic glycerol-3-phosphate dehydrogenase, the enzyme of the glycerol 3-phosphate shuttle, is not
measurable. The increase in AMP totally inhibits glycolysis (broken line). The inhibition of glycolysis by AMP is caused by the decrease of the NAD and NADH levels. The reduced NAD and NADH levels inhibit the conversion of pyruvate to lactate by lactate dehydrogenase. As a consequence, NAD cannot be regenerated and glyceraldehyde-3-phosphate dehydrogenase is inhibited, thereby strongly inhibiting glycolysis. The malate-aspartate shuttle and glycolysis are stimulated in AMP-arrested MCF-7 cells. B, effect of AMP on the metabolism of MDA-MB-453 cells. Proliferating MDA-MB-453 cells consume pyruvate as main substrate for lactate production and respiration. Glucose and glutamine are used in equal amounts. Therefore, MDA-MB-453 cells have a much lower glycolytic capacity and flux rate than MCF-7 cells. In contrast to MCF-7 cells MDA-MB-453 cells contain cytosolic glycerol-3-phosphate dehydrogenase activity, whereas the cytosolic malate dehydrogenase activity is low. Therefore, in MDA-MB-453 cells the glycerol 3-phosphate shuttle is mainly responsible for the transport of the cytosolic hydrogen in the mitochondria. In AMP-arrested MDA-MB-453 cells the glycerol 3-phosphate shuttle is working although NAD and NADH levels are reduced. As a consequence, NAD can be regenerated for the glyceraldehyde-3-phosphate dehydrogenase reaction and glycolysis is not inhibited.

**Glutaminolysis and Malate-Aspartate Shuttle**—Active proliferating MCF-7 and MDA-MB-453 cells consumed glutamine in equal amounts (Table III). Accordingly, there is no great difference in the glutaminolytic enzyme equipment between the two cell lines. An exception is malate dehydrogenase, measured in the NADH → NAD direction (Table V). Cytosolic and mitochondrial malate dehydrogenase isoenzymes are part of the malate-aspartate shuttle (Fig. 7A). MCF-7 cells had a 7-fold higher total malate dehydrogenase activity than MDA-MB-453 cells when measured in the NADH → NAD direction (=oxaloacetate → malate direction) due to an increase in the mitochondrial malate dehydrogenase activity (Tables V and VI, Fig. 5, A and B) (61). In MCF-7 cells the amount of the mitochondrial malate dehydrogenase is so high that the precursor of the mitochondrial isoenzyme is retained in the cytosol presumably by the interaction with a protein referred to as p36 (annexin II).

In MCF-7 cells the p36-associated form of malate dehydrogenase, which has an isoelectric point of 7.8, might be responsible for the increase of cytosolic malate and for the flow of glycolytic hydrogen derived from the glyceraldehyde-3-phosphate dehydrogenase reaction from the cytosol into the mitochondria (Fig. 7A) (61). The availability of cytosolic malate determines whether glutamine is converted to lactate or excreted as glutamate (2, 21, 23, 61, 71). In MCF-7 cells, which contain the pH 7.8 form of malate dehydrogenase, the glycolytic flux rate increases the flow of glutamine to lactate via glutaminolysis (Table I) (2, 61). Therefore, under glucose starvation (0.5 mM glucose) glutamine consumption was reduced, whereas glutamate production increased (Table III). MDA-MB-453 cells had a low mitochondrial malate dehydrogenase activity and no p36-associated precursor of the mitochondrial malate dehydrogenase in the cytosol. In complete contrast to MCF-7 cells, in MDA-MB-453 cells the flow of glutamine to lactate was reduced, and glutamate production was enhanced at high glucose concentrations (Table III). Such a difference in the interaction between cell proliferation and glutamine and glucose consumption has also been reported for other cell lines, and it has been
demonstrated that this interaction can be overcome by inhibitors of the malate-aspartate shuttle (19–23, 28–30, 71–74). Therefore, under glucose starvation, energy production from glutaminolysis is drastically reduced in MCF-7 cells but increases in MDA-MB-453 cells (Table III). In MDA-MB-453 cells but not in MCF-7 cells the increased glutamine consumption can compensate for the reduced glycolytic energy production and represents a commitment for cell proliferation under glucose starvation.

Influence of Metabolism on Cell Proliferation, Carbohydrate Consumption for Glycolysis and Synthetic Processes—The different metabolic behavior of the two cell lines correlates with different nutrient requirements. MCF-7 cells are unable to grow in a galactose medium, whereas MDA-MB-453 cells need galactose for optimal growth (Fig. 1, A and B). The inability to grow in media, which contain galactose, fructose, or other carbohydrates instead of glucose, is commonly linked with the inability to grow under glucose starvation (75–78). Those cells are characterized by high NADH levels (75, 77–80). The high NADH levels are caused by a disruption of the glycerol 3-phosphate shuttle and a reduction of gluconolysis as found in MCF-7 cells (77).

High NADH levels inhibit the enzyme UDP-galactose epimerase, which regulates the flow of glucose 6-phosphate to galactose or the inverse reaction (75). MDA-MB-453 cells have an active glycerol 3-phosphate shuttle and have much lower NADH levels than MCF-7 cells (Table IV and Fig. 3B). In MDA-MB-453 cells the UDP-galactose epimerase favors the flow of galactose to glucose 6-phosphate and into glycolysis (75). Galactose is preferentially used for synthetic processes and is not converted to pyruvate and lactate for energy production (2, 19, 20, 31, 81). Another factor that regulates the ability to grow under glucose starvation is pyruvate kinase. For this purpose, proliferating cells express a particular pyruvate kinase isoenzyme, termed type M2. Under glucose starvation this enzyme stays primarily in the inactive dimeric form to guarantee that sufficient glycolytic carbons are channeled into synthetic processes (34–36). In the case of an over-supply of glycolytic carbons, high fructose 1,6-bisphosphate levels induce the association of the enzyme to the active tetrameric form and lead to an increased flow of the glycolytic carbons to pyruvate (34–36). In accordance, MDA-MB-453 cells, which are able to grow under glucose starvation, had a much lower pyruvate kinase activity and a lower amount of the active tetrameric form than MCF-7 cells. Indeed, in MDA-MB-453 cells only 35% of glucose was converted to lactate, whereas in MCF-7 cells 85% of glucose consumed was channeled to lactate independent of the glucose concentration in the medium (Tables I and II, Fig. 2, A and B). HT-29 cells, which like MCF-7 cells need high glucose concentrations to grow, are also characterized by a high pyruvate kinase activity and an 85% conversion of glucose to lactate. Accordingly, specific selected HT-29 cells, which like MDA-MB-453 cells are able to grow under glucose starvation, have a much lower pyruvate kinase activity than the wild type, and only 65% of the glucose consumed is converted to lactate (76, 82).

Effect of AMP on the Metabolism of MCF-7 and MDA-MB-453 Cells—The addition of AMP into the culture medium totally inhibits proliferation of both cell lines (Fig. 1, A and B). However, the effect of AMP on the metabolism of the two cell lines is completely different. In MDA-MB-453 cells AMP treatment had no effect on glucose consumption or lactate production but led to an increase in the amount of glucose converted to pyruvate and lactate from 35 to 65%, whereas the amount of glucose used for synthetic processes decreased (Tables II and III). The availability of glycolytic pyruvate led to a reduction in the consumption of extracellular pyruvate in AMP-arrested MDA-MB-453 cells. In contrast, in MCF-7 cells AMP treatment led to a drastic reduction of glucose consumption and glucose to pyruvate conversion (Tables I and III). On the other hand, lactate production was not influenced in AMP-arrested MCF-7 cells. Therefore, AMP-arrested MCF-7 cells need more extracellular pyruvate and glutamine for energy production than proliferating MCF-7 cells. The mass of the lactate produced in AMP-arrested MCF-7 cells is derived from the increased degradation of the amino acid glutamine (Table III).

Measurements of glycolytic enzyme activities in AMP-arrested MCF-7 cells revealed that, in contrast to the inhibition of the glycolytic flux rate, most glycolytic enzymes were upregulated under AMP treatment (Table IV). An increase of some glycolytic enzyme activities was also found in AMP-arrested MDA-MB-453 cells, in which the glycolytic flux rate was not affected by AMP (Table IV). Therefore, the alteration in the glycolytic flux rate by AMP is not induced by an increase of the glycolytic enzyme activities. Furthermore, the glycolytic enzyme complex and the ratio between the dimeric and tetrameric forms of pyruvate kinase were not affected by AMP. Therefore, the main difference in the response of these two cell lines to AMP must be caused by the different shuttle systems (Fig. 7, A and B). MDA-MB-453 cells contain the cytosolic glycerol 3-phosphate shuttle, whereas MCF-7 cells do not. In proliferating MCF-7 cells the mass of hydrogen produced in the cytosolic glyceraldehyde-3-phosphate dehydrogenase reaction must be excreted as lactate. The reduction of NAD and free NADH levels under AMP treatment reduce lactate dehydrogenase activity. Thus the generation of NAD in the cytosol is limited, and the glyceraldehyde-3-phosphate dehydrogenase reaction is inhibited. As a consequence, total glycolysis is inhibited in AMP-arrested MCF-7 cells (Fig. 7A) (49). In contrast to MCF-7 cells, MDA-MB-453 cells contain cytosolic glycerol-3-phosphate dehydrogenase, which was further activated under AMP treatment (Table IV). In MDA-MB-453 cells the hydrogen produced in the glyceraldehyde-3-phosphate dehydrogenase reaction is transported into the mitochondria by the glycerol 3-phosphate shuttle and is not excreted as lactate (Fig. 7B). The slope of the regression line between lactate production and pyruvate consumption dropped to zero under AMP treatment (Table II). Therefore, the drop in NAD levels reduces the flow of extracellular pyruvate to lactate by the lactate dehydrogenase reaction but does not affect the glyceraldehyde-3-phosphate dehydrogenase reaction and glucose consumption in MDA-MB-453 cells.

Metabolism and Phosphotyrosine—In MCF-7 and MDA-MB-453 cells, inhibition of cell proliferation either by AMP treatment or by glucose starvation (MCF-7 cells) or galactose starvation (MDA-MB-453 cells) correlates with a decrease in the phosphotyrosine content in several cytosolic proteins (Fig. 6). One of these proteins has been characterized as glyceraldehyde-3-phosphate dehydrogenase (61). The decrease in phosphotyrosine is either induced by an inhibition of tyrosine kinases or an activation of tyrosine phosphatases. The complex interaction between protein kinases and phosphatases makes it difficult to define exactly which protein kinase or phosphatase is altered. However, there are previous reports that tyrosine kinases are modified by phosphometabolites such as fructose 1,6-bisphosphate and P-ribose-P. Both metabolites inhibit the pp60v-src kinase activity and the epidermal growth factor receptor kinase (83, 84). Since levels of both metabolites decrease under glucose starvation, phosphotyrosine should increase but not decrease under those conditions. Glycolytic phosphometabolites and their synthetic products, e.g. glucose 6-phosphate, phosphoserine, and ribose-5-P, can be used as.....
substrates by tyrosine phosphatases instead of 
phosphotyrosine (85). A decrease in those metabolites 
may make the phosphate in tyrosine more accessible to phospha-
tases. Another candidate for the inhibition of tyrosine phospho-
rilation is Ap4A, which is a potent inhibitor of pp60c-src kinase (84). However, the reduction of the glycolytic carbon flow to 
synthetic processes and the decrease in the NADH levels cor-
relate best with the decrease in phosphotyrosine and the inhi-
bition of cell proliferation (2, 47, 86). Therefore, it is likely that 
a synthetic product of the glycolytic carbons such as NAD 
and/or NADH or AMP itself either regulates tyrosine kinases 
or phosphotyrosine (84). However, the reduction of the glycolytic carbon flow to 
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**General Conclusions**—The MCF-7 and MDA-MB-453 cell 
system represents a valuable model for identification of meta-
bolites that regulate the protein kinase cascade and cell prolif-
eration. In addition, the data presented in this paper provide 
useful suggestions regarding the therapeutic consequence 
linked to the individual metabolic characteristics of those 
cells.

MCF-7 cells have a high glycolytic capacity that allows sur-
vival under hypoxic conditions (26). Under glucose starvation 
cell proliferation is reduced (Fig. 1A). Cell proliferation is a 
process that consumes a great deal of energy with a 2–4-fold 
increase over nonproliferating cells (87–89). Therefore, the in-
hibition of cell proliferation under glucose starvation saves 
energy, and all of the ATP produced by glycolysis is used for 
the survival of the cells. MCF-7 cells have an optimal metabolism 
to survive in solid tumors with variable glucose supply (2). This 
advantage of MCF-7 cells is linked to the disadvantage that 
glycolytic energy production is highly sensitive to reduction in 
NAD levels (49).

In MDA-MB-453 cells glycolytic energy production is not 
impaired by lowering of the NAD levels. Several tumor therapeu-
dic drugs and peroxides produced by natural killer cells 
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Effect of AMP on Tumor Cell Metabolism

80. Ibsen, K. H., and Schiller, K. W. (1971) Arch. Biochem. Biophys. 143, 187–203
81. Wenzel, A., and Schneider, F. (1989) Biol. Chem. Hoppe-Seyler 370, 205–209
82. Denis, C., Cortinovis, C., Terrain, B., Viallard, V.; Paris, H., and Murat, J. C. (1984) Int. J. Biochem. 16, 87–91
83. Vicario, P. P., and Bennun, A. (1989) Biochem. Soc. Trans. 17, 1110–1111
84. Glossmann, H., Presek, P., and Eigenbrodt, E. (1981) Mol. Cell. Endocrinol. 23, 49–63
85. Zhang, Z.-Y. (1995) J. Biol. Chem. 270, 16052–16055
86. Navas, P., Sun, I. L., Morre, D. J., and Crane, F. L. (1986) Biochem. Biophys. Res. Commun. 135, 110–115
87. Skog, S., Tribukait, B., and Sundius, G. (1982) Exp. Cell Res. 141, 23–29
88. Talha, S., and Harel, L. (1983) Exp. Cell Res. 149, 471–481
89. Guppy, M., Greiner, E., and Brand, K. (1993) Eur. J. Biochem. 212, 95–99
90. Gaal, J. C., Smith, K. R., and Pearson, C. K. (1987) Trends Biochem. Sci. 12, 129–130
91. Richter, C., Schweizer, M., Cossarizza, A., and Franceschi, C. (1996) FEBS Lett. 378, 107–110
92. Hyslop, P. A., Hinshaw, D. B., Halsey, W. A., Jr., Schraufstatter, I. U., Sauerheber, R. D., Spragg, R. G., Jackson, J. H., and Cochrane, C. G. (1988) J. Biol. Chem. 263, 1665–1675