Glucose Uptake, Lactate Release, Ketone Body Turnover, Metabolic Micromilieu, and pH Distributions in Human Breast Cancer Xenografts in Nude Rats


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ABSTRACT

Glucose uptake, lactate release, ketone body utilization, spatial distribution of glucose, lactate, and ATP concentrations as well as tissue pH distributions were systematically investigated in s.c. and/or “tissue-isolated” human breast cancer xenografts in T-cell-deficient nu/nu rats. Large variations in all parameters were detected within and between tumors indicating a very nonuniform substrate turnover. Glucose was taken up by all xenografts. Glucose consumption rates increased with increasing glucose availabilities, implying that the glucose uptake is mainly determined by the efficiency of nutritive tumor blood flow. The average uptake was 0.37 ± 0.05 μmol/g/min in medullary and 0.26 ± 0.05 μmol/g/min in squamous cell carcinomas of the breast. At wet weights below 5 g, medullary breast cancers consumed more glucose than squamous cell carcinomas (P < 0.05). Most tumors (97%) released lactate in an amount linearly related to glucose consumption. The lactate production of medullary (0.33 ± 0.05 μmol/g/min) and squamous cell (0.35 ± 0.05 μmol/g/min) breast cancers was similar. In general, the xenografts utilized ketone bodies. β-Hydroxybutyrate was consumed by 82% and acetoacetate by 73% of the tumors, the uptake rates being linearly related to the respective availabilities. The mean uptake of β-hydroxybutyrate was 3.48 ± 0.27 μmol/g/min and that of acetoacetate 2.56 ± 0.27 μmol/g/min. No significant differences were seen between medullary and squamous cell breast cancers. The β-hydroxybutyrate/acetoacetate ratio in the tumorous venous blood rose with decreasing tumor blood flow indicating the development of hypoxia at advanced growth stages. Glucose, lactate, and ATP levels were all very heterogeneously distributed in medullary and squamous cell tumors as compared with normal tissue. No relationship was evident between the spatial distribution of concentrations of these three substrates. The xenografts were acidic compared with pH values in normal subcutis. The mean tissue pH in medullary breast cancers was 6.81 ± 0.25 (SD). Compared with these values, the tissue pH distribution in squamous cell breast cancers was shifted to significantly higher values. The mean pH of the latter tumors was 7.04 ± 0.19 (P < 0.001). From the experimental data presented there is clear indication that the metabolism of the xenografts investigated was mainly determined by the efficiency of nutritive blood flow, i.e., by substrate availability, and not by the metabolic demand of the cancer cells.

INTRODUCTION

Based on in vitro experiments, Warburg (1) postulated a high aerobic glycolysis to be the outstanding biochemical characteristic of neoplastic tissue. The investigation of this hypothesis under in vivo conditions was permitted by the development of “tissue-isolated” tumor preparations using kidney, ovary, testis, or an inguinal fat pedicle as implantation sites (2–8). In these studies, blood flow, oxygen consumption, glucose uptake, and lactate production of malignant rat tumors were measured. Recently, the inguinal implantation site was used to study the turnover of further substrates like ketone bodies and amino acids (9–12). These in vivo studies revealed that a particular metabolic micromilieu develops in many malignant rodent tumors due to severe restrictions of convective and diffusive transport. This metabolic micromilieu is characterized by hypoxia and even anoxia, a general deprivation of nutrients and an insufficient removal of metabolic waste products, predominantly lactic acid, causing tissue acidosis (13). For human tumors in vivo, very few direct determinations of substrate turnover are available (14, 15). Since the available data were obtained from tumors with widely varying histologies, no correlation between metabolic micromilieu and treatment response is possible. Furthermore, systematic investigation of patients’ tumors, i.e., measurements on tumors from one cell line at different growth stages, cannot be performed due to the need of immediate therapeutic intervention. Therefore, the xenotransplantation of human tumors as “tissue-isolated” preparations into nude rats was performed in order to gain further insight into the nutrient uptake as a potential modulator of tumor growth and cancer-related cachexia. As a first approach, breast cancers were investigated due to their frequency and multimodal treatment. Regional distributions of important substrates and metabolites (glucose, lactate, and ATP) were determined using s.c. xenotransplants of the same tumor lines. Furthermore, tissue pH distributions were measured in these tumors. Knowledge of tumor pH is important since tissue acidosis can influence the proliferation of malignant and normal cells (16), the substrate utilization (17), the metastatic ability (18), and the efficacy of radiotherapy, chemotherapy and hyperthermia (19, 20). The study reported here provides for the first time detailed data on in vivo metabolism and regional metabolic micromilieu of human tumor tissue. It is concluded that this approach may help in evaluating therapeutically relevant parameters to be determined in human tumors in patients.

MATERIALS AND METHODS

Animals and Tumors. Homozygous, athymic nude rats (WAG/Fra- nnu/rnu) were obtained from a colony established at the Department of Animal Experimentation, University Clinics, Frankfurt (FRG). Breeding and maintenance of these rats have been described previously (21, 22). In brief, the rats were maintained under defined environmental conditions (temperature, 25 ± 1°C; relative humidity >70%; 12-h light-dark cycles). The rats were supplied with a highly digestible, energy- and protein-rich diet (Altromin 1414, Altromin, Lage/Lippe, FRG) and drinking water (pH 2.5) ad libitum. At the day of investigation, food was removed at 8:00 a.m. The experiments began 2–4 h later. Tumor tissue was obtained from pre- and postmenopausal women during surgery for breast cancer at the Department of Gynaecology and Obstetrics, University Clinics of Frankfurt (FRG). After histological confirmation of the diagnosis, the tumor tissue was directly transplanted to nude (nu/nu) mice and serially passaged thereafter. From this “tumor bank,” xenografts in the fourth to 93rd generation were grafted into nude rats. Six medullary and two squamous cell carcinoma lines were investigated. Glucose uptake, lactate release, and ketone body utilization were determined using “tissue-isolated” preparations
as previously described (21, 23). Measurements of blood flow, oxygen consumption, and tissue oxygenation as well as histological studies were performed as reported by Vaupel et al. (21). Spatial distributions of glucose, lactate, and ATP concentrations as well as tissue pH distributions were measured in s.c. flank tumors (21). The tumors were used at average wet weights of 2–3 g (4 to 6 weeks after transplantation). At that time, the rats were up to 110 days old (mean body weight ± SD, 280 ± 44 g).

Experimental Protocol for the Investigation of "Tissue-isolated" Tumors. During pentobarbital-Na anesthesia (Nembutal, Ceva, Paris; 35–40 mg/kg i.p.) catheters were placed into the external jugular vein, the common carotid artery, and the tumor vein (former inferior superficial epigastric vein) using an operating microscope when necessary (Olympus MTX; Olympus Optical Co. Ltd., Tokyo, Japan). The mean arterial blood pressure was continuously monitored via the arterial cannula (Statham pressure transducer, type P23 ID, Gould blood pressure monitor, type SP 1400, Gould, Oxnard, California). Arterial and tumor-venous blood samples were taken repeatedly under steady state conditions. Any blood loss due to sampling was adequately replenished by fresh donor blood via the catheter in the jugular vein. Fresh donor blood was derived from nude rats of the same litter, which were maintained under identical conditions and implanted with tissue of the same cell lines. Arterial blood was taken from the carotid artery of heparinized animals (350 USP units/kg) about 1 h before use, stored on ice (T = 4°C) and warmed to body temperature for infusion.

Total blood flow through the tumors was directly determined by timed collection of blood draining from the tumor vein. Thereby, outflow of blood from the tumor-venous cannula per unit time was collected in vials with known weight (3810; Eppendorf, Hamburg, FRG). Volume flow was calculated taking into account the weight of the collected blood, a density of 1.06 g/ml and the wet weight of the tumor excised after the completion of the experiment. During the collection period, resistance to flow within the catheter did not exceed 5 mmHg·min/ml.

Throughout the experiments, the animals were placed on a thermostated heating pad in order to keep the rectal temperature at 37 ± 1°C. At the end of the experiments, the tumors were excised, weighed, and examined by standard histological techniques.

Determination of Glucose, Lactate, and Ketone Body Turnover. The arterial and tumor-venous blood samples were deproteinated immediately with ice-chilled perchloric acid (glucose, 0.33 m; lactate and ketone bodies, 1 m). The substrate concentrations were determined enzymatically. For measurements of blood glucose levels, a hexokinase/G6P-DH-method was used (gluco-quant; Boehringer Mannheim, Mannheim, FRG). The lactate concentrations were determined by an enzymatic test according to Hohorst (24). The ketone bodies were measured with enzymatic micromethods (25). From the actual TBF3 and the substrate concentrations, the following parameters (per unit weight) were calculated (26):

\[
\text{Availability (or supply)} = \text{artrial concentration} \times \text{TFB} \tag{A}
\]

Consumption (or uptake) or release rate = arterial-tumor-venous concentration difference × TBF \tag{B}

Utilization = arterial-tumor-venous concentration difference \tag{C}

The abbreviation used is: TBF, tumor blood flow.

Concomitantly, an enzyme cocktail was prepared, containing all enzymes and cofactors necessary to link the substrates of interest to the luciferase luminescence reaction via NAD(P)/NAD(P)H redox systems. This solution was rapidly frozen and stored at −25°C as well. A sandwich, formed from frozen sections of the tissue and of the enzyme mixture, was placed upon a photographic film under a cover glass. Upon thawing, the enzymes diffusing into the tissue sections initiated luminescence. In complete darkness, the photon emission is used for film exposure. After conventional processing, the films were evaluated by microdensitometry and specific image analysis. Thus, regional distributions of glucose, lactate, and ATP have been obtained on a relative scale in serial sections, i.e., at adjacent locations, from human breast cancer xenografts.

Measurements of Tissue pH Distributions in s.c. Xenografts. Tissue pH measurements were performed with steel-sheathed, miniaturized needle glass pH electrodes (type MI 408 B; Microelectrodes Inc., Londonderry, NH). The performance characteristics of these electrodes both in buffer solutions and tissues as well as the setup used have been described previously (31). In the experiments reported here, the pH electrode was inserted into the tumor tissue to an initial depth of 0.25–0.50 mm after careful removal of the overlying skin and subcutis. The reference electrode (macro calomel electrode; type 303, Ingold, Frankfurt/M., FRG) was routinely placed into a s.c. pocket approx. 4 cm away from the insertion site of the pH electrode in order to minimize any possible influence of different biopotentials of the tissues. The insertion sites were moistened with 0.9% NaCl-solution (T = 34°C). After obtaining stable readings (equilibration time, 10–15 min), the pH electrodes were advanced in steps of 0.5 mm. 60–80 values were measured in each tumor (two to four electrode tracks). For control values, pH measurements were performed in s.c. tissue at the lower abdominal wall of nude rats. Here, the pH electrodes were inserted through a skin incision and progressively pushed forward at step intervals of 1–2 mm. Three to five values were taken per animal.

Arterial blood samples were repeatedly collected from these animals for determination of respiratory gas and acid-base status (O2 and CO2 partial pressures and pH values; blood gas analyzer, type MT 33, Eschweiler, Kiel, FRG). The arterial hematocrit was measured by capillary tube centrifugation. The oxyhemoglobin saturation of the arterial blood was obtained nomographically according to Bork et al. (32). Here again, any blood loss due to sampling was adequately compensated with fresh donor blood.

Statistical Evaluation. For the data obtained, descriptive statistical parameters were calculated. Differences within and between groups of tumors were statistically evaluated using the Kruskal-Wallis H test and the Mann-Whitney U test. Means ± SE are given if not indicated otherwise. Linear regression lines were calculated using a commercially available least square fit program (Statworks; Cricket Software, Philadelphia). Hyperbolic curves were fitted using a nonlinear maximum likelihood least-squares estimation technique (software written by Dr. P. Okunieff, Department of Radiation Medicine, Massachusetts General Hospital, Harvard Medical School, Boston).

The pH data obtained were grouped in relative frequency histograms in order to get an insight into the distribution of pH values within the tissues. For statistical tests, pH data measured in a single animal or tumor were represented by their median pH value.

RESULTS

Glucose Uptake, Lactate Release, and Ketone Body Utilization of "Tissue-isolated" Human Breast Cancer Xenografts. A total of 67 tissue-isolated preparations of human breast cancer xenografts in nude rats were investigated. 43 tumors out of 67 xenografts were medullary mammary carcinomas and 24 tumors were squamous cell carcinomas of the breast. Relevant parameters related to the tumors investigated are compiled in Table 1. In both tumor types, glucose consumption rates were linearly related to glucose availabilities (Fig. 1). At relatively constant arterial glucose concentrations the glucose supply was mainly determined by nutritive blood flow. Blood flow through
Table 1 Glucose, lactate and ketone body concentrations in the arterial and tumor-venous blood, arterio-tumor-venous concentration differences, arterial availability and consumption or release rates measured in "tissue-isolated" human breast cancers xenografted into nude rats

Total TBF and oxygen consumption per unit tumor mass (V_{O2}) of these tumors are given for comparison (21). Values are means ± SE.

<table>
<thead>
<tr>
<th>Breast cancer xenografts</th>
<th>Medullary</th>
<th>Squamous cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of tumors</td>
<td>43</td>
<td>24</td>
</tr>
<tr>
<td>Animal age (days)</td>
<td>97 ± 1</td>
<td>96 ± 2</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>287 ± 7</td>
<td>264 ± 8</td>
</tr>
<tr>
<td>Tumor growth period (days)</td>
<td>34 ± 1</td>
<td>29 ± 1</td>
</tr>
<tr>
<td>Tumor wet weight (g)</td>
<td>2.35 ± 0.28</td>
<td>2.23 ± 0.27</td>
</tr>
<tr>
<td>TBF (ml/g/min)</td>
<td>0.17 ± 0.02</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>V_{O2} (µl O2/g/min)</td>
<td>10.4 ± 1.2</td>
<td>7.7 ± 1.3</td>
</tr>
<tr>
<td>Arterial [gl] (mM)</td>
<td>7.86 ± 0.36</td>
<td>8.28 ± 0.45</td>
</tr>
<tr>
<td>Tumor-venous [gl] (mM)</td>
<td>5.24 ± 0.22</td>
<td>5.25 ± 0.25</td>
</tr>
<tr>
<td>avD, (mM)</td>
<td>2.63 ± 0.22</td>
<td>3.02 ± 0.34</td>
</tr>
<tr>
<td>Glucose supply (µmol/g/min)</td>
<td>1.25 ± 0.14</td>
<td>0.85 ± 0.14</td>
</tr>
<tr>
<td>Glucose consumption (µmol/g/min)</td>
<td>0.37 ± 0.04</td>
<td>0.26 ± 0.03</td>
</tr>
<tr>
<td>Arterial [la] (mM)</td>
<td>4.98 ± 0.29</td>
<td>5.43 ± 0.52</td>
</tr>
<tr>
<td>Tumor-venous [la] (mM)</td>
<td>7.41 ± 0.37</td>
<td>9.12 ± 0.66</td>
</tr>
<tr>
<td>vaD, (mM)</td>
<td>2.43 ± 0.24</td>
<td>3.69 ± 0.42</td>
</tr>
<tr>
<td>Lactate supply (µmol/g/min)</td>
<td>0.79 ± 0.09</td>
<td>0.43 ± 0.06</td>
</tr>
<tr>
<td>Lactate release (µmol/g/min)</td>
<td>0.31 ± 0.04</td>
<td>0.30 ± 0.04</td>
</tr>
<tr>
<td>Arterial [acac] (µM)</td>
<td>118 ± 6</td>
<td>129 ± 9</td>
</tr>
<tr>
<td>Tumor-venous [acac] (µM)</td>
<td>100 ± 4</td>
<td>108 ± 8</td>
</tr>
<tr>
<td>avD,low (µM)</td>
<td>19 ± 2</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>acac consumption (nmol/g/min)</td>
<td>3 ± 1</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Arterial [β-hb] (µM)</td>
<td>61 ± 4</td>
<td>85 ± 15</td>
</tr>
<tr>
<td>Tumor-venous [β-hb] (µM)</td>
<td>42 ± 5</td>
<td>41 ± 6</td>
</tr>
<tr>
<td>avD,low (µM)</td>
<td>19 ± 4</td>
<td>44 ± 15</td>
</tr>
<tr>
<td>β-hb supply (nmol/g/min)</td>
<td>10 ± 1</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>β-hb uptake (nmol/g/min)</td>
<td>4 ± 1</td>
<td>5 ± 3</td>
</tr>
</tbody>
</table>

* Abbreviations: gl, glucose; [], concentration; avD, arterio-tumor-venous concentration difference; la, lactate; vaD, tumor-venous-arterial concentration difference; acac, acetoacetate; β-hb, β-hydroxybutyrate.

Both types of breast cancers investigated were compromised already in small tumors as evidenced by a flow-limited oxygen consumption and large tissue areas with hypoxia and anoxia (21). Therefore, the glucose uptake of both medullary and squamous cell breast cancers investigated was primarily limited by the delivery of this substrate and not by the metabolic demand of the cancer cell lines (Fig. 2). Since the tissue oxygenation, the oxygen consumption and the glucose uptake were governed by nutritive blood flow (21), linear relationships between the oxygen consumption, and the glucose uptake were found (Fig. 3). The medullary carcinomas had higher blood flow and glucose supply rates than squamous cell carcinomas and had correspondingly higher rates of glucose uptake at comparable tumor sizes (Fig. 4, 0.41 versus 0.26 µmol/g/min...
The glucose consumption per unit tumor mass exhibited considerable intratumor variability in both medullary and squamous cell carcinomas which is comparable to previously measured intratumor blood flow variability (21). The glucose uptake of the medullary breast cancers decreased from 1.02 to 0.03 μmol/g/min with tumor sizes increasing from 0.4 to 7.1 g. The respective consumption rates of squamous cell cancers diminished from 0.66 to 0.02 μmol/g/min as tumor sizes enlarged from 0.2 to 5.5 g. During tumor passage of the blood (and neglecting the impact of possible shunt flow), about one third of the glucose available was extracted from both medullary and squamous cell breast cancers, the glucose utilization being comparable in both tumor types (medullary, 0.32 ± 0.02; squamous cell, 0.35 ± 0.03). The glucose extraction was found to be independent of tumor wet weight, tumor blood flow or oxygen consumption.

All but two tumors released lactate, the release rate of medullary tumors being similar to that of squamous cell carcinomas. For glucose consumption rates less than 0.75 μmol/g/min the lactate release was linearly related to the glucose uptake (Fig. 5). Two xenografts with consumption rates above this value showed a net lactate uptake. No significant relationship between the lactate turnover and the tumor wet weight or the arterial lactate concentration was detected. The glycolytic rate can be determined as the ratio of the lactate release and the glucose uptake assuming (a) steady state conditions and (b) glucose to be the only substrate source of lactate. With these assumptions, it was found that in the smaller tumors (approximately 1 g) 50% of the glucose taken up was released as lactate. This ratio increased with tumor growth. In large breast cancer xenografts (tumor wet weights, 5–8 g), an average of two moles of lactate was produced for every mole of glucose taken up, yielding a mean glycolytic rate of 100%. However, 10 of the 67 xenografts released more lactate than could be accounted for by glycolysis alone, indicating lactate production from substrates other than glucose (e.g., amino acids).

Ketone Bodies. δ-Hydroxybutyrate and acetoacetate were predominantly consumed by both medullary and squamous cell breast cancer xenografts, the uptake being linearly related to the availability of the respective substrate (Fig. 6). From these results it has to be concluded that, similar to oxygen and glucose, the uptake of ketone bodies is mainly determined by nutritive tumor blood flow. The uptake of δ-hydroxybutyrate combined with that of acetoacetate was linearly related to the oxygen consumption of the breast cancer xenografts (2P < 0.001). The ratio of δ-hydroxybutyrate and acetoacetate in the tumor-venous blood is assumed to be an indicator of the intramitochondrial redox state (10). This ratio was low in small tumors (tumor size below 1 g) with high flow values and shifted towards higher values as tumor blood flow was reduced at advanced growth stages. This indicated an enlargement of hypoxic areas as the breast cancer xenografts increased in size. Similar results have been obtained with O2-sensitive microelectrodes using s.c. transplants of the same human breast cancer lines (21).

Spatial Distribution of Glucose, Lactate, and ATP Concentrations in s.c. Breast Cancer Xenografts. Glucose, lactate, and ATP levels were heterogeneously distributed in medullary and squamous cell tumors as compared with normal tissue (Fig. 7), and extended areas with very low substrate concentrations (almost at the background level) were obvious in the tumor tissue. The distributions of single substrates in successive sections of the same tumor were very similar. However, no correlations between the distribution profiles of glucose, lactate or ATP measured in consecutive tissue slices were detected indicating complex interrelationships between the substrate supply, the removal of metabolic waste products and the energy status of malignant tumors.

Tissue pH Measurements in s.c. Breast Cancer Xenografts.
Fig. 7. Spatial distribution of glucose (A, D), lactate (B, E) and ATP (C, F) concentrations registered in consecutive sections of a human medullary breast cancer xenograft (A–C; wet weight: 2.4 g) and of rat skeletal muscle (D–F). The values are obtained relative to the maximal concentration in the experimental series. The high lactate concentrations in the skeletal muscle are attributed to twitching activities prior to the biopsy procedure. According to in vitro calibration studies (29), the highest ATP concentrations correspond to approximately 2.0 μM.
pH distributions were investigated in 36 medullary and 24 squamous cell breast cancer xenografts. Relevant systemic parameters of the host animals as well as statistical data on the measured tissue pH values are listed in Table 2. Compared to s.c. tissue at the abdominal wall of nude rats, the pH distributions in both types of human mammary carcinomas were shifted towards more acidic values (Fig. 8, 2P < 0.001). In the squamous cell breast cancers higher pH values were found than in the medullary tumors (2P < 0.001). The appearance of necrotic areas was noted in the squamous cell tumors at earlier growth stages than in the medullary cancers (21). In both tumor types, marked intertumor pH variations were observed. A trend was found towards an alkalinization of the median tumor pH with increasing tumor size which was statistically not significant. The pH values measured within single tumors exhibited very heterogeneous distributions with large pH gradients in some tumors and very flat pH profiles in others.

**DISCUSSION**

The "tissue-isolated" implantation of human tumor xenografts permitted for the first time the detailed and systematic investigation of substrate turnover by human tumor tissues under well-defined systemic conditions. So far, no striking differences on substrate turnover of xenografted human breast cancers compared to isotransplanted rodent tumors are obvious. Data on blood flow and oxygen consumption of primary human breast cancers as compared with breast cancer xenografts indicate that functional characteristics regarding blood flow and oxygen supply are retained during xenotransplantation (21). The usefulness of "tissue-isolated" tumor models for the investigation of tumor metabolism has been demonstrated previously using rodent tumor transplants (2-12, 33, 34). Besides the investigations described, the model may be used for the detailed analysis of other substrates, for the investigation of intratumor pharmacokinetics of anticancer agents and for the determination of production rates of different tumor-specific substances (e.g., tumor angiogenesis factor, α-fetoprotein or carcinoembryonic antigen). For such measurements, the concentrations of the substrates of interest have to be obtained with such accuracy as to permit the calculation of arterial-tumor-venous concentration differences. The nude rat was chosen as tumor host since its size permits experiments under well-defined physiological conditions. However, the usefulness of these animals is limited by a possible host-versus-graft reaction which starts about 4 months after birth. Since meaningful data can only be obtained before the onset of this reaction, relatively fast growing human tumors had to be chosen.

In the present study, the glucose consumption of xenografted medullary and squamous cell breast cancers was high, being comparable to brain tissue (0.25-0.70 μmol/g/min, 35, 36). This might be explained by changes of enzyme patterns occurring in transformed cells during carcinogenesis. Immortalization and transformation lead, among other changes, to an increased glycolytic and glutaminolytic capacity. In this way, malignant cells are able to meet the increased energy requirements caused by the enhanced nucleic acid and phospholipid synthesis (37). These metabolic changes are brought about by the induction of isoenzymes different from those expressed in normal cells, a process which may be stimulated by oxygen deprivation (38). As a result, the glycolytic capacity both in the presence and absence of oxygen is elevated under in vitro conditions. Data on human tumor tissue in vivo are scanty. Measurements of arterial-tumor-venous concentration differences of sarcoma-bearing limbs (14) and head and neck cancers in patients (15) indicated an increased glucose utilization by human tumors as compared with tissues at the site of tumor growth. Since tumor blood flow values were not determined in these studies no conclusions can be drawn regarding the actual glucose consumption of the tumors investigated. Using noninvasive PET techniques (44) glucose uptake of various lung tumors was found to be 8 times higher than tumor-free lung tissue. The mean glucose consumption rates of xenografted human breast cancers were well within the range of values.

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**Table 2. Mean arterial blood pressure (MABP), respiratory gas parameters within the arterial blood, and relevant parameters of the statistical evaluation of measured tissue pH values**

<table>
<thead>
<tr>
<th>Values are means ± SE.</th>
<th>s.c. human breast cancer xenografts</th>
<th>Medullary carcinoma</th>
<th>Squamous cell carcinoma</th>
<th>Control (subcutis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of experiments</td>
<td>36</td>
<td>24</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>No. of pH readings</td>
<td>2633</td>
<td>1580</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>223 ± 5</td>
<td>198 ± 10</td>
<td>221 ± 8</td>
<td></td>
</tr>
<tr>
<td>Tumor growth period (days)</td>
<td>34 ± 1</td>
<td>31 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor weight (g)</td>
<td>4.7 ± 0.5</td>
<td>2.8 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>124 ± 1</td>
<td>124 ± 3</td>
<td>125 ± 2</td>
<td></td>
</tr>
<tr>
<td>Arterial pO2 (mmHg)</td>
<td>84 ± 2</td>
<td>82 ± 2</td>
<td>80 ± 1</td>
<td></td>
</tr>
<tr>
<td>Arterial pCO2 (mmHg)</td>
<td>39 ± 1</td>
<td>37 ± 1</td>
<td>39 ± 2</td>
<td></td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.38 ± 0.01</td>
<td>7.39 ± 0.01</td>
<td>7.39 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Arterial So2 (Sat. %)</td>
<td>95 ± 1</td>
<td>96 ± 1</td>
<td>96 ± 1</td>
<td></td>
</tr>
<tr>
<td>Arterial hematocrit (v/v)</td>
<td>0.42 ± 0.01</td>
<td>0.46 ± 0.02</td>
<td>0.44 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Mean tissue pH</td>
<td>6.81 ± 0.04</td>
<td>7.04 ± 0.04</td>
<td>7.26 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Median tissue pH</td>
<td>6.82</td>
<td>7.03</td>
<td>7.28</td>
<td></td>
</tr>
<tr>
<td>Modal class</td>
<td>6.8-6.9</td>
<td>7.0-7.1</td>
<td>7.3-7.4</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>5.78-7.59</td>
<td>6.51-7.62</td>
<td>6.97-7.95</td>
<td></td>
</tr>
</tbody>
</table>

*So2, O2 saturation of hemoglobin.

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**Fig. 8. Cumulative frequency distribution (histograms) of pH values measured in the subcutis of nude rats (A) and in medullary (B) and squamous cell breast cancers (C). Broken line, median pH value. N = number of animals or tumors, respective. n = number of measurements.
reported earlier for various rodent tumors (range, 60-888 nmol/g/min; 4, 7, 9-12, 45). Similar to oxygen consumption (21), glucose uptake rose with increasing availability, indicating nutritive tumor blood flow to be the rate-limiting factor in these tumors. Similar results have been reported earlier for isografted rodent tumors (4, 7, 9-12). The direct relationship of oxygen consumption and glucose uptake which was found for human breast cancer xenografts further suggests that both respiration and glycolysis were governed by nutritive blood flow as previously indicated in studies on rodent tumors (5). Since tumor blood flow decreased significantly at advanced tumor growth stages, a reduced glucose uptake is expected at larger tumor sizes. An increase of the glycolytic rate from 50 to 100% indicates an enlargement of hypoxic tumor areas with maintained glucose supply (46). This finding is supported by theoretical analyses of supply radii around tumor microvessels taking into account actual in vivo data (46).

Theoretically, in tumors with adequate nutritive flow maximum glucose uptake should occur which corresponds to the glycolytic capacity of the tumor cell line investigated. Thus, "saturation" of the glucose uptake should be encountered at very high availabilities and therefore, a hyperbolic relationship of glucose consumption and supply has to be expected. Such a relationship is substantiated by fitting the data to the following formula

\[ V_{g\text{-actual}} = \frac{V_{g\text{-maximal}} \times \text{glucose supply}}{K_m + \text{glucose supply}} \]  

where \( V_{g\text{-actual}} \) = actual glucose consumption, \( V_{g\text{-maximal}} \) = maximal glucose uptake, and \( K_m \) = glucose availability at half-maximal glucose uptake.

In medullary breast cancer xenografts, \( V_{g\text{-maximal}} \) is expected to be 1.57 \( \mu \text{mol/g/min} \) (Fig. 9, top; average \( K_m \), 3.46 \( \mu \text{mol/g/min} \) and 0.76 \( \mu \text{mol/g/min} \) in squamous cell carcinomas (Fig. 9, bottom; mean \( K_m \), 1.20 \( \mu \text{mol/g/min} \)). Such an analysis may permit the evaluation of inherent differences of the glucose demand of different tumor cell lines in situ.

Lactate is assumed to be mainly a glycolytic product since a direct relationship between glucose consumption and lactate production was found. However, a lactate release exceeding the amount that could be produced even at maximal glycolytic rates from the amount of glucose utilized indicates other sources for glycolysis, e.g., amino acids. In vitro studies (35) and some in vivo results (9, 10) suggested that glutamine might be the most likely precursor for the excess lactate production. However, this was not the case in the present study since there was a net glutamine release from these breast cancer cell lines (46). This finding has to be expected considering that (a) oxygen is necessary for the oxidative breakdown of glutamine to pyruvate ("glutaminolysis"; 47, 48) and (b) large areas with hypoxia and even anoxia are present in subcutaneous breast cancer xenografts from the cell lines used here (21). Ketone bodies were predominantly taken up by the xenografted tumors. Ketone body uptake by breast cancers is not surprising since this pathway can also be activated in the mammary gland during lactation for milk synthesis (49). However, under conditions of low fumarate levels, acetoacetate formation has been reported for mammary gland homogenates (50). In some breast cancers, this mechanism could aggravate the tissue acidosis observed.

Ketone body utilization was demonstrated previously in head and neck cancers (15), and for a variety of rodent tumors (9, 51). In the present study, the uptake rates of both \( \beta \)-hydroxybutyrate and acetoacetate were related to the respective supply values, suggesting that the capacity of human breast carcinomas to consume ketone bodies is substantial. However, the overall contribution of ketone bodies to the energy status of breast cancer xenografts is probably minimal due to the small amount absorbed as compared with glucose. Nevertheless, ketone body turnover may be monitored as an indicator of the tumor oxygenation status. This is indicated by changes of the \( \beta \)-hydroxybutyrate/acetoacetate-ratio with increasing tumor wet weight leading to reduced nutritive blood flow and oxygen availability per unit weight at advanced growth stages.

The spatial distributions of glucose, lactate, and ATP concentrations were very heterogeneous due to the nonuniform distribution of nutritive blood flow (13). The lactate production was governed by glucose uptake, which in turn depended on glucose availability and oxygen consumption. From these substrates and possible other sources, ATP is formed to meet the energy requirements of rapidly growing cancer cells. Thus, the ATP concentration depends on the substrate supply as well as on its rate of metabolic breakdown. Noninvasive measurements using nuclear magnetic resonance and positron emission tomography indicated that the energy status of malignant tumors is mainly governed by tumor size (52, 53). However, for the assessment of tumor response to treatment, the evaluation of changes of the energy status in micronegions of malignant tumors might be superior to integrated values over the whole tumor mass (54).

The pH values obtained in human mammary carcinoma...
Metabolism of human breast cancer xenografts in vivo

The weight-related decrease of tumor blood flow in human mammary carcinomas with increasing tumor wet weight (21) is not accompanied by a significant drop in the interstitial pH, although an impaired intratubular drainage function in these tumors is evident with increasing tumor hypoxia. Obviously, the severe restrictions of active and passive transport with progressive tumor growth led to a decrease of glucose availability and, subsequently, to a decrease of glucose turnover. As the lactate production usually correlated well with the glucose consumption one may conclude that the drop of tumor blood flow led to a restriction of the substrate available for glycolysis. Therefore, increasing tumor wet weight must not necessarily be accompanied by a continual pH drop.

From the results obtained it is concluded that in the breast cancer xenografts investigated nutritive tumor blood flow but not the metabolic demand of the cancer cells determine the glucose uptake, the lactate release, and the ketone body turnover. The spatial distribution of glucose, lactate, and ATP within tumor microregions is very inhomogeneous due to a nonuniform distribution of tumor perfusion within the tumor mass. Tissue acidosis is a result of pronounced microcirculatory and metabolic alterations during tumor growth.

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References


Glucose Uptake, Lactate Release, Ketone Body Turnover, Metabolic Micromilieu, and pH Distributions in Human Breast Cancer Xenografts in Nude Rats


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