Tumor-selective Modification of Cellular Microenvironment in Vivo: Effect of Glucose Infusion on the pH in Normal and Malignant Rat Tissues

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ABSTRACT

The pH distributions in transplanted neural (TV1A, BT1A) and hepatic (HV1A3) tumors and in brain and kidney of BDIX rats were analyzed as a function of serum glucose concentration (SGC), tumor size, and tissue architecture. Tissue damage during pH measurements in vivo could be minimized by the use of pH microelectrodes with tip diameters of ≤10 μm. In normoglycemic rats, the pH in TV1A tumors was only slightly lower than in brain or kidney. However, at 6 hr after the induction of hyperglycemia by continuous i.v. infusion of glucose, the average pH in TV1A tumors had fallen to 6.7 at an SGC of 27 mm and to 6.1 at an SGC of 50 mm. A similar glucose-mediated pH reduction was observed in BT1A and HV1A3 tumors. No significant increase in tissue acidity occurred in brain and kidney. The pH in tumors had reached its minimum at 2 hr after the onset of high-dose glucose infusion (SGC, 50 mm) and could be maintained at this level in hyperglycemic rats for at least 48 hr. In hyperglycemic hosts, an increased retention of acidic metabolites in the tumor tissue with decreasing vascular density was reflected by a tumor size (age)-dependent pH reduction and a higher degree of intratumal pH variation. In partially necrotic tumors, pH values as low as 5.2 were recorded. Oral administration of NaHCO3 to tumor-bearing rats had no effect on the average pH in TV1A tumors.

INTRODUCTION

The development of tumor-selective modalities of cancer treatment requires the exploitation of cellular properties that specifically distinguish cancer cells from normal cells. This property is the capacity of most malignant but not of normal cells for aerobic glycolysis (1, 35). However, due to the low GC3 in the interstitial fluid of solid neoplasms (15), tumor tissue generally does not use this potential at a maximum rate under normal conditions in vivo (15, 31). In normoglycemic rats, an average GC of ~30 μM was measured in the interstitial fluid of transplanted tumors whereas the corresponding values in normal tissues and aortic serum were ~300-fold higher (15). The pH in tumors had reached its minimum at 2 hr after the induction of hyperglycemia by continuous i.v. infusion of glucose, the average pH in TV1A tumors had fallen to 6.7 at an SGC of 27 mm and to 6.1 at an SGC of 50 mm. A similar glucose-mediated pH reduction was observed in BT1A and HV1A3 tumors. No significant increase in tissue acidity occurred in brain and kidney. The pH in tumors had reached its minimum at 2 hr after the onset of high-dose glucose infusion (SGC, 50 mm) and could be maintained at this level in hyperglycemic rats for at least 48 hr. In hyperglycemic hosts, an increased retention of acidic metabolites in the tumor tissue with decreasing vascular density was reflected by a tumor size (age)-dependent pH reduction and a higher degree of intratumoral pH variation. In partially necrotic tumors, pH values as low as 5.2 were recorded. Oral administration of NaHCO3 to tumor-bearing rats had no effect on the average pH in TV1A tumors.

MATERIALS AND METHODS

Animals. Adult male rats of the inbred BDIX strain (8) weighing 250 to 300 g were used in all experiments. The rats were housed 2/cage and maintained on a standard laboratory diet (Alma, Kempten, Germany) with tap water ad libitum.

Transplanted Tumors. The TV1A tumors originate from a malignant neurinoma of the trigeminal nerve induced by a transplacental pulse of N-ethyl-N-nitrosourea to a fetal (18th day of gestation) BDIX rat (19, 20). The glioblastoma-like BT1A tumors were obtained after s.c. implantation into BDIX rats of malignant BT1C cells (20). The BT1C cell line originates from fetal BDIX rat brain cells malignantly transformed in cell culture after transplacental exposure to N-ethyl-N-nitrosourea in vivo on the 18th day of gestation (19). The HV1A3 tumors are a subline of a transplantable tumor which originally arose in a BDIX rat in the form of multiple “spontaneous” intraabdominal tumor nodules around an i.p. diffusion chamber (containing human leukemic cells). The primary tumor was histologically classified as an adenocarcinoma (possibly originating from the liver). Later passages and the subline used here show histological features of an anaplastic carcinoma containing a high component of fibrous tissue. Unless otherwise stated, all tumors used were free of necrotic areas as confirmed by histology. Two to 3 weeks (TV1A, BT1A) and 4 to 5 weeks (HV1A3) prior to the experi-

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2 To whom requests for reprints should be addressed.
3 The abbreviations used are: GC, glucose concentration; pHex, extracellular pH; SGC, serum glucose concentration.

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ments, tumors of transplantation passages 21 to 36 (TV1 A), 2 to 5 (BT1 A), and 14 to 18 (HV1 A3) were transplanted s.c. to both flanks of BDIX rats.

**Hyperglycemia by Continuous i.v. Infusion of Glucose.** For long-term i.v. infusion of sterile 40% glucose solution into unanesthetized and unrestrained rats, a technique described by Steiger et al. (28) was modified (Chart 1). Catheters were made of polyethylene tubing (length, 15 cm; outer diameter, 1 mm; Braun, Melsungen, Germany). The intravascular portion of the catheters (29 mm) was drawn out to reduce the outer diameter to ~0.5 mm. To secure the catheter in its i.v. position, the tubing was enlarged at 26 mm from its tip to an outer diameter of ~2 mm (length, ~3 mm) in an electrical heating coil. Under light ether anesthesia, the catheter was inserted through a phlebotomy into the right jugular vein and advanced 30 mm into the right atrium (28). A bent cannula was inserted through the skin in the midscapular area and advanced s.c. to the cervical skin incision. The free end of the catheter was then passed through the cannula and connected to the infusion apparatus after removing the cannula, and the infusion apparatus was fixed to the dorsum of the rat by a piece of polyvinyl tubing slipped over the catheter and fastened with skin suture clamps. Glucose solution was continuously infused with the aid of syringe pumps (Model 975; Harvard Apparatus Co., Inc., Millis, Mass.). Routinely, the rats were connected to the infusion apparatus 1 day prior to the experiments. To maintain the patency of the catheters, 0.9% NaCl solution was infused at a rate of 0.6 ml/hr until glucose infusion began. The amount of glucose infused depended on the desired SGC and the total infusion time (Charts 2, 3, and 13). To raise the normal SGC of adult BDIX rats [6 ± 1 (S.D.) mm] to an SGC of 27 ± 7 or 50 ± 8 mm, 0.7 ml of a 20% glucose solution was initially infused i.v. within 1 min and a 40% glucose solution was then continuously infused at a rate of 3.4 ml/hr or 4.7 ml/hr. To maintain an SGC of ~50 mm for 40 to 50 hr, the infusion rate (40% glucose solution) was adjusted individually for each rat according to SGC determinations at intervals of 3 hr to 5 hr (Chart 3; Table 1). SGCs were determined enzymatically in 10-µl samples of deproteinized tail vein blood, using a commercially available kit (glucose test combination, hexokinase method; Boehringer Mannheim, Mannheim, Germany).

**pH Measurements.** Hinke-type glass microelectrodes were used for the pH measurements. The construction and sensing properties of these pH microelectrodes as well as the equipment and technique used for pH analyses in tissues have been described in detail in the preceding paper (18). Consisting of a tip of pH-sensitive glass (diameter, ≤10 µm; length, ~20 µm) fused into a lead glass capillary, these microelectrodes had a sensitivity (slope) of 58 to 60 mV/pH unit at 37°C, a response time of ≤3 sec (95% of the signal difference in test buffers of pH 4.00 and 9.18 at 22°C), and a drift of ≤0.01 pH unit/hr. Reference electrodes (type 373; Ingold, Frankfurt am Main, Germany) were connected to the tissue surfaces (test buffers) via KCI interfaces (2 m) and open glass capillaries filled with an aqueous solution of 0.9% NaCl and 1% agar. An electrometer (Model 616; Keithley Instruments, Inc., Cleveland, Ohio) served as the amplifier. All calibrations and tissue measurements were performed in an electrically shielded cage. The microelectrodes were calibrated at 37°C in test buffers of pH 4.03 to 9.09 (Ingold) and recalibrated at ≤4-hr intervals. Under microscopic control, the microelectrodes were inserted into the tissues with the aid of a microinjection apparatus.
Tumor-selective pH Reduction by Glucose Infusion

Effect of continuous i.v. glucose infusion on the SGC of BDIX rats: 48-hr infusion. Sterile 40% glucose solution was infused at a rate of 4.7 ml/hr from 0 to 3 hr. Thereafter, the infusion rate was adjusted for each rat individually according to its actual SGC and to the data listed in Table 1. Points, means for 10 rats; bars, S.D.

Table 1
Effect of various rates of i.v. infusion of a 40% glucose solution on the SGC of hyperglycemic BDIX rats

<table>
<thead>
<tr>
<th>Infusion rate (ml/hr)</th>
<th>SGC (mM) after continuous i.v. glucose infusion at the indicated rate for 3 hr</th>
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<tr>
<td>1.2</td>
<td>28 to 33 mM</td>
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<td>1.7</td>
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<td>3.4</td>
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<td>4.7</td>
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* SGC at t = 0 hr.

of automated modified micromanipulators (Leitz, Wetzlar, Germany) at a continuous speed of $500 \mu$m/min, to a depth of 5 mm (tumors weighing 1.0 to 2.5 g, 10 mm (tumors weighing 4 to 6 g, 6.5 mm (kidney), and 4 mm (brain, right cerebral hemisphere). Electrode signals were continuously recorded and pH frequency distributions were calculated from single-point measurements (100-µm steps). Unless otherwise stated, all tumors and normal tissues were used for pH measurements only once.

For statistical evaluation, the pH frequency distributions were compared by variance analysis at a 0.5% level of significance.

RESULTS

Effect of i.v. Glucose Infusion on the pH in TV1A Tumors. At the normal SGC of BDIX rats (6 ± 1 mM; Chart 2), the mean pH in TV1A tumors weighing 1.0 to 2.5 g was 7.0 (range, 6.8 to 7.1; Chart 4A). At 6 hr after the induction of hyperglycemia (SGC, 27 ± 7 mM) by i.v. infusion of glucose, the pH frequency distribution was broadened (pH 6.5 to 7.1) and shifted to a slightly lower mean value (pH 6.9; Chart 4B; $p < 0.005$). Elevation of the SGC to 50 ± 8 mM within 6 hr resulted in a further reduction of the mean pH in TV1A tumors to 6.5 (range, 6.0 to 7.0; Chart 4C; $p < 0.005$). A decrease of the intratumoral pH as a function of host blood glucose level could also be demonstrated within individual TV1A tumors, when the SGC was elevated stepwise between consecutive insertions of the pH microelectrode into different tumor areas. As shown in Chart 5, a gradual rise of the SGC from 27 to 50 mM was paralleled by a decrease of the mean pH in an individual TV1A tumor from 6.9 to 6.2 ($p < 0.005$).

Effect of Tumor Size on the pH in TV1A Tumors of Hyperglycemic Hosts. As shown in the preceding paper (18), the pH frequency distributions in TV1A tumors weighing 1.0 to 2.5 g (mean pH, 7.0) or 4 to 6 g (mean pH, 6.9) do not differ markedly under normal conditions in vivo. However, in hyperglycemic hosts, the average reduction of pH values was more pronounced in TV1A tumors weighing 4 to 6 g than in tumors weighing 1.0 to 2.5 g. At 6 hr after the induction of hyperglycemia (27 mM), pH values between 6.1 and 7.2 (mean, 6.7) were measured in TV1A tumors weighing 4 to 6 g (Chart 4B; $p < 0.005$). The glucose-mediated pH shift was further enhanced to a mean pH value of 6.1 (range, 5.5 to 6.7) when the SGC was elevated to 50 mM (Chart 4C; $p < 0.005$). These data indicate that, under identical conditions in terms of SGC, the concentration of H⁺ ions in TV1A tumors weighing 4 to 6 g is by a factor of 1.6 (SGC, 27 mM) and 2.5 (SGC, 50 mM) higher than in tumors weighing 1.0 to 2.5 g. An SGC-dependent pH reduction could also be demonstrated in individual TV1A tu-
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Chart 6. Effect of i.v. glucose infusion on the pH in TV1A tumors: frequency distributions of single-point pH values in tumors weighing 4 to 6 g. A: untreated controls; SGC, 6 mM; 10 tumors; number of pH measurements, 1000. B: SGC, 27 mM; 6-hr glucose infusion; 8 tumors; number of pH measurements, 800. C: SGC, 50 mM; 6-hr glucose infusion; 12 tumors; number of pH measurements, 1200.

Chart 7. Effect of i.v. glucose infusion on the pH in TV1A tumors: frequency distributions of single-point pH values in an individual TV1A tumor at 2 different SGCs. Tumor weight, 4.3 g. A: untreated controls; see legend of Chart 6; SGC, 6 mM; tumor weight, 4 to 6 g; 10 tumors; number of pH measurements, 1000. B: SGC, 27 mM; 6-hr glucose infusion; number of pH measurements, 100. C: SGC, 50 mM; 6-hr glucose infusion; number of pH measurements, 100.

Chart 8. Effect of i.v. glucose infusion on the pH in BDIX rat brain: frequency distributions of single-point pH values in the right cerebral hemisphere. A: SGC, 6 mM; 5 brains; number of pH measurements, 200. B: SGC, 50 mM; 6-hr glucose infusion; 6 brains; number of pH measurements, 240.

Effect of i.v. Glucose Infusion on the pH in Normal Organs of BDIX Rats. Since TV1A and BT1A tumors are of neuroectodermal origin, the brain of BDIX rats was chosen as a normal control tissue. In addition, the pH in BDIX rat kidney was analyzed (18). In contrast to the tumors, only a minor decrease in pH was observed in brain and kidney after i.v. glucose infusion. The pH frequency distributions measured in the brains of normoglycemic BDIX rats cover a range of pH 6.6 to 7.3 (mean, pH 7.0; Chart 8A). As shown in Chart 8B, this pH histogram remained almost unaltered by i.v. glucose infusion. At 6 hr after the induction of hyperglycemia (SGC, 50 mM), the pH values in the brain were still in the range of pH 6.6 to 7.2 (mean, pH 6.9). Similar results were obtained for the kidneys of hyperglycemic BDIX rats. At an SGC of 50 mM, the average pH measured in renal tissue (7.1; range, 6.7 to 7.3) was only reduced by 0.1 unit to 7.0 (range, 6.7 to 7.2; Chart 9). The small shift to the left of the histograms of brain and kidney observed at this SGC corresponded to a pH reduction in blood (7.23; normal value, 7.38) and urine (5.9; normal value, 7.1). The data thus demonstrate that the capacity of malignant cells for aerobic glycolysis can be exploited to decrease the pH selectively in tumor tissues.

Effect of Long-Term (48-Hr) i.v. Glucose Infusion on the pH in TV1A Tumors. In cell culture systems, the degree of the cytotoxic effects of low pH (either per se or in combination with second treatment modalities) has been shown to be dependent on the exposure time of the target cells (9, 13, 14). We, therefore, investigated whether by continuous glucose infusion a reduced pH could be maintained in TV1A tumors for >6 hr. Unanesthetized tumor-bearing BDIX rats were infused with glucose solution for ~48 hr. Within 3 and 10 hr, the average SGC was increased to ~30 and 40 mM, respectively, and the final value of 50 mM was gradually reached during the following 38 hr (Chart 3). pH measurements began at 0.5 hr before the end of the infusion period. The pH frequency distribution measured in TV1A tumors weighing 4 to 6 g ranged from pH 5.6 to 6.9 (mean, 6.2; Chart 10B). This distribution is very similar to that obtained after 6 hr of i.v. glucose infusion (Chart 6C), indicating that a glucose-mediated pH reduction in tumors can be maintained for at least 48 hr. In comparison to TV1A tumors weighing 4 to 6 g, the pH histogram of TV1A tumors weighing 8 to 13 g was shifted to slightly lower values (pH 5.3 to 6.7; mean, 6.1; Chart 10C). This tumor size dependence of pH reduction is in agreement with the results obtained after 6 hr of glucose infusion in tumors weighing 4 to 6 g versus tumors weighing 1.0 to 2.5 g. The lowest pH values were recorded in TV1A tumors containing necrotic areas where the mean pH was 5.6 (range, 5.2 to 6.0) after 48 hr of glucose infusion (Chart 10D).

pH in BT1A and HV1A3 Tumors of Hyperglycemic Hosts. As shown in Chart 11, the pH frequency distributions measured...
Tumor-selective pH Reduction by Glucose Infusion

Chart 9. Effect of i.v. glucose infusion on the pH in BDIX rat kidney: frequency distributions of single-point pH values. A: SGC, 6 mM; 5 kidneys; number of pH measurements, 325. B: SGC, 50 mM; 6-hr glucose infusion; 8 kidneys; number of pH measurements, 520.

Chart 10. Effect of i.v. glucose infusion on the pH in TV1A tumors of hyperglycemic BDIX rats: frequency distributions of single-point pH values after 48 hr of glucose infusion. A: SGC, 6 mM; tumor weight, 4 to 6 g; 10 tumors; number of pH measurements, 1000. B: SGC, 50 mM; tumor weight, 4 to 6 g; 13 tumors; number of pH measurements, 1300. C: SGC, 50 mM; tumor weight, 8 to 13 g; 8 tumors; number of pH measurements, 800. D: SGC, 50 mM; tumors containing macroscopically detectable necrotic areas; tumor weight, 4 to 6 g; 4 selected tumors; number of pH measurements, 400.

in BT1A and HV1A3 tumors at an SGC of 50 mM are in good agreement with the corresponding pH histogram for TV1A tumors. The average pH in BT1A tumors and in HV1A3 tumors (nonneural origin) was 6.4 (range, 6.1 to 6.8) and 6.1 (range, 5.8 to 6.4), respectively. These data indicate that glucose-mediated pH reduction is not restricted to a particular tumor type and may (e.g., in the HV1A3 tumors) be even more pronounced than in the TV1A tumors.

Local pH Variations in TV1A Tumors in Hyperglycemic Hosts. Local variations of pH were observed in all tumors analyzed in this study. As outlined in the preceding paper (18), these intratumoral pH fluctuations did not, at a physiological SGC, correlate with any macroscopically recognizable property of the tumor tissue. However, in hyperglycemic hosts, the average local pH in TV1A tumors varied according to the position (insertion depth) of the pH microelectrode within a given tumor, the magnitude of these pH differences being dependent on tumor size. To analyze the local pH profiles in TV1A tumors, the microelectrodes were automatically advanced from the tumor periphery to the tumor center, and the continuously recorded electrode signals were directly plotted in relation to the electrode position. Mean pH values were calculated from single-point measurements (500-μm steps). The pH frequency distribution for individual TV1A tumors (1.0 to 2.5 g) shows that the pH variations observed at physiological SGC (18) were augmented after i.v. infusion of glucose (Chart 4). However, when the average local pH for a group of such tumors is plotted as a function of the insertion depth of the microelectrode, no significant pH difference is seen between different tumor areas (SGC, 50 mM; not shown). In contrast, in tumors weighing 4 to 6 g, a gradual decrease of the average pH was observed at an SGC of 50 mM when the pH microelectrode was moved from the tumor periphery (~pH 6.35) to the tumor center (~pH 5.9) (Chart 12). After 48 hr of glucose infusion (SGC, 50 mM), the average pH in peripheral tumor areas was not significantly different from the value measured after 6 hr. However, in central tumor areas, the concentration of acidic metabolites was apparently somewhat diminished under these conditions, thus reducing the pH difference between tumor periphery and center from 0.45 to 0.25 pH unit (Chart 12).

Kinetics of pH Reduction in TV1A Tumors of Hyperglycemic Hosts. To investigate the time course of pH reduction in tumors of hyperglycemic hosts, the SGC of tumor-bearing BDIX rats was raised from 6 to ~50 mM within 30 min. The initial infusion rate (9.4 ml/hr) was then reduced for each rat individually according to SGC determinations at intervals of 15 min, in order to maintain a constant SGC of 50 mM. pH microelectrodes were inserted 5 mm into tumors weighing 4 to 6 g, 10 min prior to the onset of glucose infusion. The presence of a microelectrode in a constant position within the capillary network of a tumor did not give rise to pH variations, indicating that the local H+ ion concentration was not significantly altered by the use of a microelectrode with a tip diameter of ≤10 μm. As shown in Chart 13, rapid elevation of the SGC in the tumor-bearing hosts resulted in an almost concomitant decrease of pH in the tumor tissue. Within 30 min after the onset of glucose

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Chart 12. Local pH variations in TV1A tumors of normo- and hyperglycemic hosts: tumor weight, 4 to 6 g. Mean values of single-point pH values at distance intervals of 500 μm. SGC, 6 mM; 8 tumors. SGC, 50 mM; 6-hr glucose infusion; 10 tumors. SGC, 50 mM; 48-hr glucose infusion; 13 tumors. pH values measured near tumor surface are not included. Standard deviations range between 1.2 and 7.6% of the mean values given in the graph.

Insertion Depth (mm)

5.8 6.0 6.2 6.4 6.6 6.8 7.0

Chart 13. Kinetics of glucose-mediated pH reduction in TV1A tumors: tumor weight, 4 to 6 g; 10 tumors. At a normal SGC, the pH microelectrode was advanced 5 mm into the TV1A tumors and fixed in this position (t = 0 min). Glucose infusion was initiated at t = 10 min. Points, means; shaded area, S.D. infusion, the average pH was reduced from ~6.85 to 6.6. The pH continued to decline after the SGC had reached a plateau (SGC, 50 mM) and reached a value of 6.15 (range, 5.75 to 6.5) after a total infusion time of 120 min. This value (measured at the same insertion depth in each of the tumors) is in good agreement with the pH histogram obtained by multiple measurements in TV1A tumors after 6 hr of glucose infusion (mean, pH 6.1; range, 5.5 to 6.7; Chart 6C) and significantly different from the starting value (p < 0.005).

Effect of p.o. Administration of NaHCO₃ on the pH in TV1A Tumors. Gullino et al. (16) have reported that the pH in the interstitial fluid of s.c.-transplanted rat tumors (collected in intratumoral diffusion chambers) could be reduced by either p.o. or i.v. administration of NaHCO₃ to the tumor-bearing animals. We tried to reproduce this effect, since it might provide a possibility to further augment the glucose-mediated reduction of pH. NaHCO₃ was added at a concentration of 0.1% to the drinking water of TV1A tumor-bearing BDIX rats, beginning 3 days prior to the pH measurements (16). As compared to untreated controls, the pH histograms of TV1A tumors growing in NaHCO₃-supplemented hosts were somewhat broadened with pH values ranging from 6.8 to 7.2 (Chart 14B). However, no reduction of the average pH value (7.0) was observed.

DISCUSSION

Since the early work of Voegtl et al. (33) in 1935, a number of studies have been performed on the effect of parenteral glucose administration on tissue pH (see Refs. 2, 8, 12, 28, 35, 45-47 in Ref. 18, and Ref. 6). The limitations of the techniques applied in these studies have been discussed in Ref. 18. Using pH microelectrodes with tip diameters considerably smaller than those previously applied, the present data confirm qualitatively the results of the earlier studies. For example, Von Ardenne et al. (34) published pH values in the range of 5.9 to 6.68 in metastases of the DS carcinosarcoma in hyperglycemic rats, and Calderwood and Dickson (6), using pH electrodes, reported a decline of the mean pH in solid rat Yoshida sarcomas from 7.19 to 6.63 within 4 hr after i.p. injection of 6 g of glucose per kg body weight. In the present study, we have investigated in detail the dependence of intratumoral pH on different levels of hyperglycemia, the tumor size (age), and the administration of NaHCO₃, and we have analyzed

Chart 14. Effect of p.o. administration of NaHCO₃ on the pH of TV1A tumors. NaHCO₃ (0.1%) was added to the drinking water of tumor-bearing BDIX rats for 3 days. At the end of this period, single-point pH measurements were performed in tumors weighing 1.0 to 2.5 g. A: untreated controls; 10 tumors; number of pH measurements, 500. B: TV1A tumors after administration of NaHCO₃; 8 tumors; number of pH measurements, 400.
the local intratumoral pH distributions. In contrast to normal tissues, a pronounced and SGC-dependent pH reduction was induced in various transplanted rat tumors. In TV1A tumors, a maximum pH reduction occurred within 2 hr after the onset of i.v. glucose infusion, which could be maintained for at least 48 hr.

The present data show that a metabolic property common to most cancer cells, the capacity for aerobic glycolysis (1, 35), can be exploited to modify the cellular microenvironment selectively in malignant tissues. Contrary to normal cells, malignant cells produce lactic acid at a rate proportional to extra-cellular GC (11, 35). In normoglycemic rats, however, the GC in the interstitial fluid of transplantable tumors is by a factor of ~300 lower than in the interstitial fluid of normal tissues or aortic serum (15), thus preventing cancer cells to use their high glycolytic potential at a maximum rate in vivo (15, 31). On the other hand, the GC in tumor interstitial fluid and glucose consumption in transplanted tumors can be increased markedly by parenteral administration of glucose (15). At an elevated extracellular GC, lactic acid accumulates in the interstitial space of tumors until a new steady state is reached between increased production and concentration-dependent clearance of lactic acid from the tissue. Since the pH in tumors is a function of lactate concentration (26), a stepwise increase in SGC from 6 to 27 mM and to 50 mM was reflected by a corresponding pH reduction from 6.9 to 6.5 and 6.1, respectively, in the present TV1A tumors (4 to 6 g). In contrast, the pH histograms of BDIX rat brain and kidney remained essentially unaltered even at an SGC as high as 50 mM, indicating that the pH can be modified selectively in malignant tissues by appropriate modifications of SGC. It remains an important question whether in primary and metastatic tumors invading normal tissues a reduced pH would still be observed in the normal tissue adjacent to the malignant cells. This question could not be studied in the present s.c.-transplanted tumors which were generally separated from the surrounding tissue by a fibrous capsule. Moreover, the fragile microelectrodes were not suited for penetration of these capsules. However, it will be of interest to investigate this problem in, e.g., primary rat kidney tumors induced by N,N-dimethylnitrosamine (23).

As indicated by the broad distributions of pH values in the TV1A, BT1A, and HV1A3 tumors of hyperglycemic rats, the pH in malignant tissues is not only a function of the SGC but is modulated by additional factors. Of particular importance are the rate of tumor blood flow in the tumor tissue and the rate of diffusive and convective transport of glucose and acidic metabolites into and out of the interstitial space. Compared with the vasculature of normal tissues, the vascular system of tumors is characterized by morphological and functional defects (30). During tumor growth, a reduction of intravascular volume and vascular surface area is accompanied by an increase in intercapillary diffusion ranges and an exponential decline of tumor blood flow (30, 32). This, in turn, leads to a reduced clearance of acidic metabolites as the tumors increase in size, especially if the cellular production rate of lactic acid is increased. In the present study, this was mirrored by a size (age)-dependent reduction of the mean intratumoral pH in hyperglycemic hosts. At an SGC of 50 mM (27 mM), the average pH of TV1A tumors weighing 1.0 to 2.5 g was 6.5 (6.9) while it was reduced to 6.1 (6.5) in tumors weighing 4 to 6 g.

The tumor size-dependent variations of substrate and metabolite transport in malignant tissues are superimposed by marked local inhomogeneities. Fluctuations of tumor blood flow in the order of 1:700 have been observed in single tumors of the same size (21). As tumors grow larger, local variations of tumor vasculature generally become more apparent and give rise to the formation of necrotic areas (see, e.g., Ref. 12). We did not perform morphometric analyses of the tumors investigated in this study. However, the development of central necrotic areas in TV1A tumors of ≥10 g indicates that in these tumors the vascular density is higher in peripheral areas (12). Local pH variations were observed in all tumors analyzed in the present study. In normoglycemic rats, local pH differences did, however, not exceed 0.3 unit, and no general relation between the position (insertion depth) of the pH microelectrode and the local pH was detected. Obviously, the relatively small amounts of acidic metabolites produced in tumors of normoglycemic hosts can, on average, be cleared from all tumor areas to a similar degree. In small well-vascularized TV1A tumors (1.0 to 2.5 g), the average pH-ion concentration as well as local pH variations within individual tumors was increased after i.v. infusion of glucose, but the mean local pH values were again approximately constant throughout the tumors. In contrast, the reduction of vascular density in the centers of larger TV1A tumors (4 to 6 g) apparently resulted in a retarded clearance of acidic metabolites at elevated SGCs as compared to the better perfused tumor periphery. This is indicated by a higher average reduction of pH in the tumor centers (—1.0 unit) than in peripheral areas (—0.55 unit). In TV1A tumors containing necrotic areas, pH values as low as 5.2 were measured.

Glucose-mediated decrease of pH in malignant tissues is not confined to tumors of a certain origin or histological type, as indicated by the essentially similar pH histograms for TV1A, BT1A, and HV1A3 tumors in hyperglycemic BDIX rats. While malignant cells converting glucose to lactic acid at a rate similar to the glycolytic rates of most normal cells in differentiated tissues have been described (25), the majority of cancer cells of both animal and human origin appear to be characterized by a glycolytic capacity exceeding that of their normal counterparts (1, 35). It may, therefore, be assumed that by i.v. infusion of glucose the pH could be selectively reduced also in human neoplasms. The present experiments seem to indicate that systemically administered glucose is nontoxic in rats even at high concentrations and over prolonged periods. In humans, SGC levels of 25 mM have been maintained for 24 hr by i.v. infusion of glucose without serious side effects (22). Higher levels may be tolerated since the neurological symptoms of hyperglycemic nonketotic coma are not due to an elevated SGC per se but rather to a substantial loss of water which can be replaced under clinical conditions. In this respect, it is of interest that in the present TV1A rat tumors maximum pH reduction was reached as early as 2 hr after the onset of glucose infusion.

A controlled tumor-selective decrease of pH may be of practical interest in various respects. In tissue culture, the proliferation and survival of malignant cells is sensitive to changes of pH4.7, 9. At an SGC of 50 mM, ~90% of the pH values measured in TV1A tumors weighing 4 to 6 g were ≤6.3. At this pH4, the proliferation of various animal and human tumor cell lines in vitro is severely depressed or completely inhibited (4, 9). Similarly, the clonogenicity of cultured human malignant
cells is reduced to ~1% of the optimal values at this pH (9). In a recent study, we have shown that the fraction of clonogenic cells isolated from s.c.-transplanted TV1A tumors was reduced by a factor of ~18 after 4 hr of i.v. glucose infusion resulting in an SGC of 50 mm (17). The latter study further indicated that by temporarily raising the extracellular GC, rat tumor cells become sensitized to an independent treatment modality in vivo. Thus, the fraction of clonogenic cells in TV1A tumors exposed to hyperthermic treatment (42°C; 1 hr) was reduced by a factor of 2.5 × 10^3 in hyperglycemic animals (SGC, 50 mm) whereas the corresponding reduction factor for TV1A tumors in normoglycemic BDIX rats was ~10 (17). Poorly vascularized tumor regions not readily accessible for oxygen and cytotoxic drugs exhibit particularly low pH values during hyperglycemia. Glucose-mediated cytotoxicity may thus be most pronounced in these “sanctuaries” of clonogenic cells capable of repopulating a tumor after therapy. As indicated by cell culture data (24), a reduced pH may also partially prevent the development of thermotolerance. With or without concomitant hyperthermia, a tumor-selective pH reduction may be combined with other treatment modalities, preferentially with drugs activated at subphysiological pH (3, 5, 29) or encapsulated in liposomes sensitive to acidic pH (36). Since the cytotoxic effects will depend on the exposure time of the malignant target cells to the respective therapeutic agents, it is of interest that in TV1A tumors a selective decrease of pH could be maintained for at least 48 hr.

While the pH in malignant tissues can obviously be reduced by an elevated SGC to a considerable extent, it could be advantageous to further decrease the pH by additional means. In the present study, p.o. administration of NaHCO₃ (16) did not change the average pH in TV1A tumors. However, a further pH reduction may be possible either selectively by administration of glucose in combination with insulin or nonselectively, e.g., by i.v. infusion of NH₄Cl (2). Studies to investigate the effect of insulin on the pH in transplanted tumors of hyperglycemic rats (Max Planck-Institut für Systemphysiologie, Dortmund, Germany) for generously

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