Pergamon

Advan. Enzyme Regul., Vol. 35, pp. 101–115, 1995

0065-2571/95/$29.00

0065-2571(94)00016-6

TUMOR METABOLISM: THE LESSONS OF MAGNETIC RESONANCE SPECTROSCOPY

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INTRODUCTION

Since oncogene expression may alter the metabolism of tumors, it is of fundamental importance to determine how tumor metabolism differs from that of normal tissue. Such an understanding may offer hope of therapy prior to the time we are able to manipulate gene expression in the living cell. *In vivo* tumors are often partially hypoxic due to compromised vasculature with consequent poor response to chemotherapy and/or radiation therapy. What effect does this chronic hypoxia have on tumor metabolism and how does this affect strategies for new therapeutic approaches?

About 60 years ago Warburg (1) demonstrated that tumor cells had a great capacity for aerobic glycolysis, which leads to increased lactate production; for many years after Warburg's classic work, it was generally assumed that tumors had an acidic intracellular pH (pH_i). In the intervening years, microelectrode measurements of tumor pH appeared to validate the assumption that the pH of tumor cells would be low, but these measurements are now acknowledged to largely reflect pH_ϵ, the pH of the interstitial (or extracellular) fluid. Some normal tissues (e.g., exercising muscle) also form large amounts of lactic acid, which is rapidly removed, but because tumors have poorly organized vasculature, large extracellular spaces and heterogeneous blood perfusion, the lactic acid formed cannot always be removed quickly. pH_ϵ values of tumors range from 5.5–7.3 (2–4).

With the advent of Magnetic Resonance Spectroscopy (MRS), a non-invasive *in vivo* measure of tissue pH became available and the first pH measurements of animal (5) and human (6) tumors were made and demonstrated to be neutral to alkaline. (In this context neutrality is taken

to be pH 7.0 throughout this paper.) The pH_{MRS} measurement is based on the pH-dependent chemical shift difference between the $^{31}P_i$ signal and an endogenous reference signal (7). At physiological pH, the P_i signal reflects the relative concentrations of the two phosphate species ($H_2PO_4^-$ and HPO_4^-) present.

Unless two P_i peaks can be resolved in the MRS spectrum, the MRS measurement of tissue pH is a composite value of pH_i and pH_ϵ. In normal tissues, it is generally considered that the pH measured in this way is intracellular, since the amount of P_i present in the extracellular fluid can be neglected. This assumption might not hold for tumors, however, since their extracellular volume can be much larger than in normal tissues. However, calculation of the proportion of P_i signal coming from the intracellular volume is possible if total tissue water content and the fractional vol. of extracellular water are known. It has been demonstrated in animal tumors that if the extracellular vol. does not exceed 55%, the pH_{MRS} is largely representative of pH_i (8).

Many NMR studies in both human and animal tumors have confirmed the initial findings (5, 6) that pH_i is on average on the alkaline side of neutrality — pH 7.1–7.2 (2, 9) — which is *similar* to that in most normal tissues. This means that tumor pH_i under steady state conditions is regulated to physiological levels (4).

The major difference with regard to pH between normal tissue and tumors lies in pH_ϵ. In normal tissue, pH_ϵ at approximately 7.4 is *alkaline* in comparison to pH_i, whereas in tumors pH_ϵ is *acid* in comparison to pH_i — on average 0.5 pH units more acid (measured by microelectrodes (2, 10, 11)) and this has now been confirmed with the aid of an MRS visible extracellular marker (12) where pH_i and pH_ϵ can be measured simultaneously and non-invasively by MRS (see Results and Ref. 13).

Over the years there were many proposals to develop drugs that would localize in what were thought to be 'acidic' tumor cells. Now that it is clear that pH_i is more alkaline than pH_ϵ, it is evident that drugs that were intended to partition preferentially across the cell membrane will actually partition into the acidic extracellular fluid. For some purposes, however, this may not matter. Conjugates that release free drug at acid pH (14) would benefit from being localized in the extracellular fluid.

Various strategies for altering pH_i and pH_ϵ have been attempted in the quest for new anticancer strategies for solid tumors. For instance, hyperglycemia can be induced to increase lactic acid production and thus lower pH_i (11, 15, 16) or the Na^+/H^+ exchanger can be inhibited by amiloride and its analogues (17, 18). Compounds such as hydralazine can be administered to reduce blood flow to tumors (19) and make the tumors more anoxic and therefore (presumably) more acidic. By these strategies, it is hoped that pH can be exploited to activate cytotoxic agents selectively

in tumors (10,20) or that the distribution of drugs that are weak acids or bases (11) will be affected in such a way that they will be taken up more effectively by the tumor than by the normal surrounding tissue.

A basic understanding of the tissue biochemistry underlying these strategies is essential if these approaches are to be successful and MRS has already made a significant contribution in overturning a dogma of 50 years standing about tumor pH. Unlike microelectrode measurements, which are invasive and can only sample a few microenvironments within a tumor, MRS is non-invasive and produces vol. average measurements. This may not seem ideal, since tumors are known to be heterogeneous, but there are some advantages in this volume averaging, particularly when comparisons are to be made with more classical techniques for analysis of ions and metabolites in the *whole* (freeze-clamped) tumor. These values are then directly comparable to the MRS and any heterogeneity is averaged out. Other consequences of the finding that $pH_i > pH_\epsilon$ in tumors have been investigated with a mixture of 'state of the art' magnetic resonance techniques and classical biochemical techniques, including Warburg manometry and the findings are reported and discussed below.

METHODS

Tumors. The Morris hepatoma 9618a was grown subcutaneously in Buffalo rats. Tumor volumes were <2% of body weight.

MR measurements. MR measurements were made on a SISCO 200–330 at 4.7T using image-guided localized spectroscopy by ISIS (Image Selected *In vivo* Spectroscopy) technique (21), a 2–3 cm diameter surface coil, adiabatic pulses, a recycle time of 3 secs and a gradient strength of 7.5 Gauss/cm. pH_i was measured from the difference in chemical shift between the P_i resonance and that of α-NTP at −7.57 ppm. For pH_ϵ measurements rats were injected with 3-aminopropyl phosphonate (3-APP) (1.5 g/kg) i.p. 30 min before spectra were collected and pH_ϵ was measured from the chemical shift difference between 3-APP and α-NTP (12). Spectra were obtained with an interleaved ISIS localization acquired in 320 scans (total) with the transmitter frequency set on α-NTP in one spectrum (upper) and on 3-APP in the other (see Fig. 1). This was necessary because the chemical shift difference between 3-APP and α-NTP is > 30ppm and thus there would have been a significant chemical shift artefact (22). The consequence of this would be that the volumes from which the α-NTP and 3-APP signals were obtained would have been displaced by 2 mm (in three dimensions). In an 8 mm cube, this would have meant that they overlapped by only 43%. Using the interleaved ISIS acquisition (23) ensured that the two vol. were identically positioned.

Tumor and liver extracts. Tumor and liver extracts were made after the MRS examination from rapidly dissected freeze-clamped tissues (liver first, then tumors). Total tissue water was measured by assessing the distribution of ^3H_2O and extracellular space was measured using ^{14}C inulin, which distributes only in the extracellular spaces (24).

Metabolite and ion measurements. Aliquots of the neutralized extracts were used in the following assays:-P_i, lactate, pyruvate, ATP, ADP, AMP, DHAP, 3PG, essentially by the methods described in Bergmeyer (25), citrate and isocitrate (26), Na^+ and K^+ by atomic absorption spectroscopy, HCO_3^- by classical Warburg manometry, total Mg^{2+}, Ca^{2+} and Cl^-, as described in (27).

Calculation of ion gradients. Because of the absence of information on the composition of the extracellular vol. surrounding the hepatomas, where samples from venous drainage confined to tumor tissue alone cannot be obtained, we have used rat plasma values as estimates of extracellular values: 155 mM for Na^+, 5.9 mM for K^+ and 1.51 mM for lactate.

RESULTS

pH_i, pH_ϵ and Lactate of Tumors and Liver

Representative spectra collected from the hepatomas are shown in Figure 1. pH_i was calculated from the chemical shift difference between P_i and α-NTP in the upper spectrum and pH_ϵ was calculated from the chemical shift difference between the α-NTP in the upper spectrum and the 3-APP peak in the lower spectrum (23). The mean pH_i of hepatoma 9618A was 7.12 ± 0.02 (n = 8), and pH_ϵ was 6.8 ± 0.07 (n = 3). Similar findings have also occurred for nitrosomethylurea-induced mammary tumors. Liver, on the other hand, which we have considered as a control tissue for the hepatoma, had a pH_i similar to that of tumor (7.26 ± 0.02 (n = 8)), but a significantly more alkaline pH_ϵ of 7.4 (3). In summary, the pH gradient in tumors ($pH_i > pH_\epsilon$) is the *reverse* of that observed in liver ($pH_i < pH_\epsilon$).

Compared to liver, many tumors have high steady state lactate levels (8, 28), up to four times higher in a Walker sarcoma (8) and three times higher in the hepatoma (3.7 ± 0.55 μmol/g wet wt compared to 1.24 ± 0.27 in liver n = 8). Because H^+ and $lactate^-$ move together on the monocarboxylate carrier, the distribution of H^+ and $lactate^-$ across the plasma membrane tends to assume the relationship $[H^+]_i[lactate^-]_i/[H^+]_\epsilon[lactate^-]_\epsilon = 1$ (29, 30). In normal liver $[H^+]_i/[H^+]_\epsilon > 1$, therefore $[lactate^-]_i/[lactate^-]_\epsilon$ must be <1. In hepatomas, however,

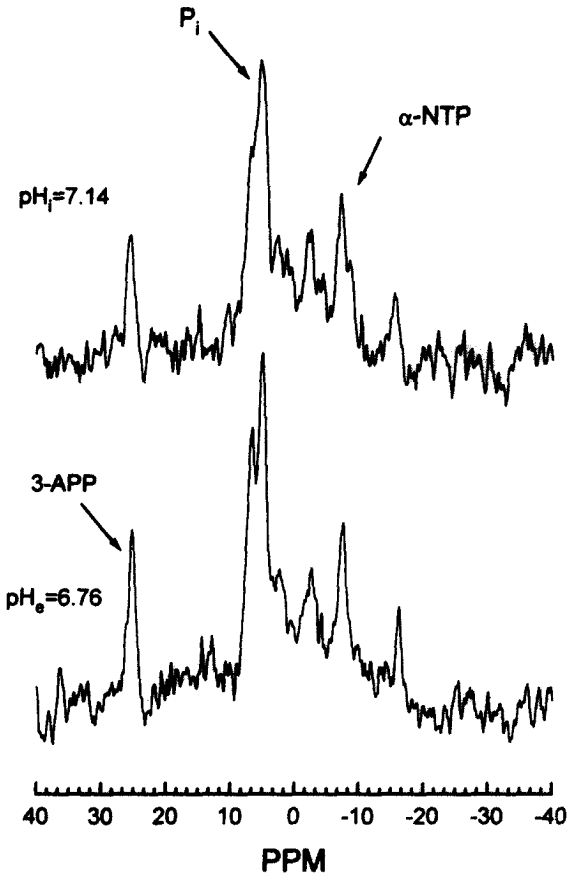

FIG 1. pH_i and pH_ϵ of Morris hepatoma 9618a measured by ^{31}P MRS. ^{31}P MR spectra were obtained with an interleaved ISIS localization acquired with the transmitter frequency set on α-NTP in the upper spectrum and on 3-APP in the lower spectrum, 30 mins after the rats were injected with 3-aminopropyl phosphonate (3-APP). pH_i was measured from the chemical shift difference between α-NTP and P_i in the upper spectrum. pH_ϵ was measured from the chemical shift difference between 3-APP in the lower spectrum and α-NTP in the upper spectrum to correct for the chemical shift artefact (for further details see text).

the situation is reversed and $[H^+]_i/[H^+]_\epsilon$ is <1, so that $[lactate^-]_i/[lactate^-]_\epsilon$ becomes >1. This means that high pH_i in comparison to pH_ϵ (as found in tumors), would be expected to be accompanied by high intracellular lactate.

Tissue Ion Measurements

A better understanding of the underlying differences in pH between tumors and normal tissues may stem from knowledge of the ionic

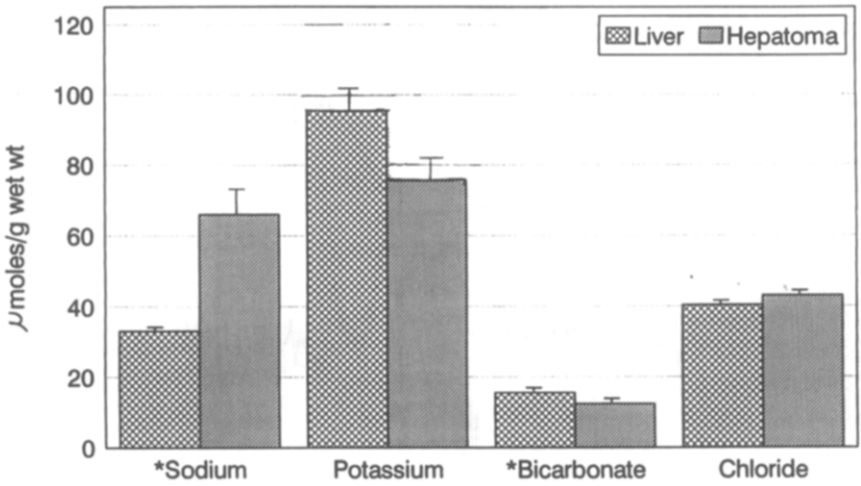

FIG 2. Tissue ions in liver and hepatoma. The results are expressed as means ± S.E.M (n = 5–7) μmol/g wet wt tissue. * indicates $p < 0.05$ when compared to liver.

composition of the intra- and extracellular compartments of solid tumors. One expected consequence of the reversed pH gradient observed in tumors is a redistribution of other charged species across the membrane. Significantly higher amounts of Na^+ were found in all the tumor types studied so far (13) when compared to liver (for hepatoma and liver content see Fig. 2). A decrease in K^+ was also observed as was a decrease in the HCO_3^{2-} content; however, Cl^- did not change significantly (see also Fig. 2).

Total P_i, measured in acid extracts, was 2-fold higher in the hepatoma (Fig. 3), as was free P_i measured from the MR spectra (for details see Ref. 8). Free P_i (MRS visible) has been demonstrated in many tissues including tumors to be lower than total (i.e., acid extractable) P_i (31). The origin of the MR invisible pool is still unclear, but it is likely to represent either bound P_i or P_i, which has been sequestered in the mitochondria where it is invisible to MR spectroscopy. Total Mg^{2+} was lower in the tumor than in the liver, but not significantly. However, free $[Mg^{2+}]$ calculated from the citrate/isocitrate ratio and the measured pH_i, (assuming equilibrium in the aconitase reaction (26, 32)) was <0.1 μmol/g wet wt in tumors and 0.55 μmol g wet wt in normal liver (Fig. 3). Tumors, of course, may have much decreased Krebs cycle activity due to lack of oxygen and/or fewer mitochondria and, therefore, these equilibrium assumptions may not hold. However, free $[Mg^{2+}]$ was also assessed by measuring the chemical shift difference between the α- and β- ATP peaks in the ^{31}P MR spectrum

(33, 34, 13). The absolute values from the ^{31}P MR spectra indicated a slightly higher $[Mg^{2+}]$ value than the calculated value. This is because the functional dependence of $[Mg^{2+}]$ on the α–β chemical shift difference makes it very sensitive to the choice of the value of α–β for the fully complexed $MgATP^{2-}$ form. However, the relative differences between the liver and tumors indicated that free$[Mg^{2+}]$ of the tumors was 4–5-fold lower than the liver values, similar to the difference calculated from the citrate/isocitrate ratios.

Total Ca^{2+} was 8-fold higher in the hepatoma than in the liver (Fig. 3). In spite of large inter-tumor variability, this difference was significant. Free cytoplasmic Ca^{2+} is of the order of 2×10^{-7}M (27) and therefore is unlikely to be the cause of the massive increase in Ca^{2+} content observed in these tumors, which is associated with an increase in tissue P_i (see Fig. 3) content and likely to be due to the formation of insoluble calcium phosphate.

The Phosphorylation and Redox State of Hepatoma and Liver

Concomitant with the high lactate content found in tumors is a low pyruvate content leading to NAD^+/NADH ratios that were >10-fold lower in the hepatoma than in the liver (see Table 1). This, in turn, is associated with a low $[ATP]/[ADP] \times [P_i]$ ratio. Many ^{31}P MRS studies of growing tumors have demonstrated decreasing ATP/P_i ratios with increasing size

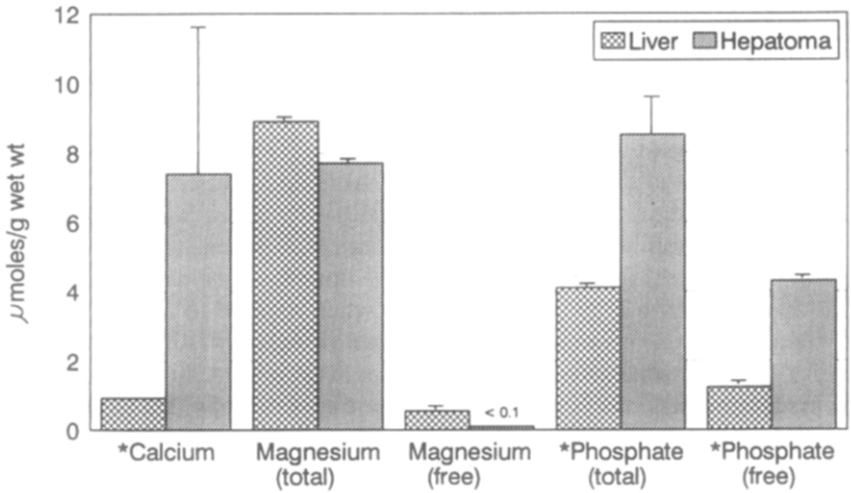

FIG 3. Tissue Ca^{2+}, Mg^{2+} and P_i in liver and hepatoma. The results are expressed as means ± S.E.M ($n = 5$) μmol/g wet wt tissue. * indicates $p < 0.05$ when compared to liver. Free Mg^{2+} was calculated from the citrate/isocitrate ratio according to Ref. (26). Free P_i was measured from the MR spectrum (8).

TABLE 1. ATP CONTENT, REDOX AND PHOSPHORYLATION RATIOS

Metabolite or metabolite ratio	Liver	Hepatoma
[lactate]/[pyruvate]	6.8	88
$[NAD^+]/[NADH]$	1074	83
ATP (μmol/g wet wt)	2.9	0.82
$[ATP]/[ADP]\cdot[P_i]\ M^{-1}$	14420	1060

(for review see Ref. 35) and this has been confirmed in acid extracts made from tumors, including hepatomas (36). In comparison to normal tissue (liver), the $[ATP]/[ADP] \times [P_i]$ ratio of the hepatoma was 14 times lower. This was caused by a 4-fold higher free $[P_i]$ (Fig. 3) and a 3-fold lower ATP content (Table 1).

Calculation of Ion Gradients

Apart from pH_i and pH_ϵ values, from which it is possible to calculate the gradient of the $[H^+]$ between the intra- and extracellular compartments (assuming similar H^+ activity in both compartments), all the ion content measurements have been expressed as tissue content (i.e., μmol/g wet wt), which is adequate to indicate the *direction* of the differences between hepatoma and liver. However, in order to appreciate the *magnitude* of the differences in the ion gradients (i.e., $[ion]_i/[ion]_\epsilon$), it is necessary to allow for relative vol. of intra- and extracellular water (Fig. 4). There is slightly more water per g wet wt of tissue in the hepatoma than in the liver, but the major difference between the two tissues lies, as would be expected, in the extracellular vol., which was 39% in the tumors compared to only 23% in the liver. In addition to knowledge of the distribution of water, it is necessary to know the extracellular ion concentration. In liver, the assumption that plasma [ion] (corrected for plasma water) is an estimate of $[ion]_\epsilon$ appears reasonable. However, this approximation may have limitations when applied to tumors because of their heterogenous nature, compromised vascularity (for discussion see Ref. 13) and possible presence of substances in the extracellular matrix, such as proteoglycans, which could bind many cations. Notwithstanding these limitations, when corrections are made for extracellular contributions using the values of $[ion]_\epsilon$ mentioned in the method, and allowing for intracellular water, calculations showed that Na^+ gradient was approximately six times lower in the hepatoma than in the liver (Table 2). On the assumption that the distribution of Cl^- between the intra- and extracellular compartments represents the membrane potential (27) and that this is −28 mV in both

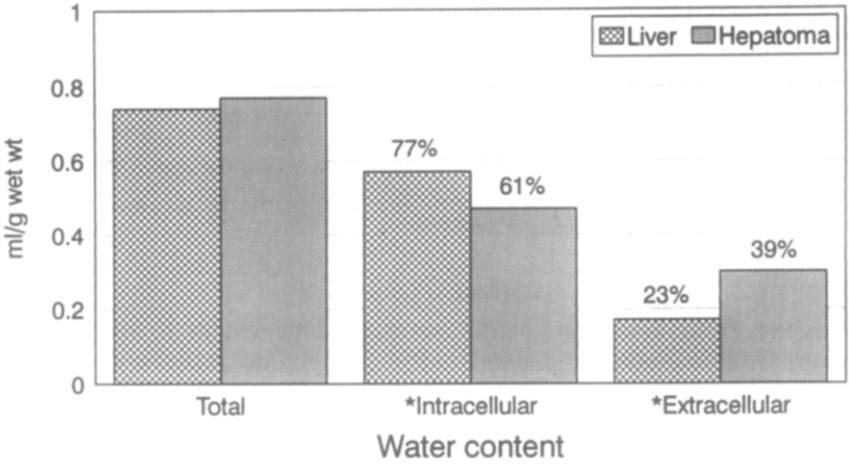

FIG 4. Total, intra- and extracellular water content of liver and hepatoma. The results (n = 7) are expressed as ml/g wet wt for total water with intra and extracellular water expressed as a percentage of the total. * indicates $p < 0.05$ when compared to liver.

liver and hepatoma, the ΔG of the gradients (e/i) of some of the ions has been calculated (Table 2) along with the ΔG of ATP hydrolysis in both hepatoma and liver. The energy of the gradients of any ion between intra- and extracellular phases is given by a concentration work term and an electrical term and can be represented as:

$$\Delta G[ion]_{e/i} = RTln \frac{[ion]_e}{[ion]_e} - zFE_N$$

where R, T and F are the gas constant, the temperature in degrees Kelvin and the Faraday constant, respectively, z is the charge on the ion in question and E_N is the membrane potential in volts. The increase in the

TABLE 2. NA^+ GRADIENT AND ΔG OF THE GRADIENTS OF NA^+, H^+, MG^{2+} AND ATP HYDROLYSIS

Ratio or ΔG(kJ/mol)	Liver	Hepatoma
$[Na^+]e/i$	24	3.7
Na^+ $\Delta G[e/i]$	11	7.3
H^+ $\Delta G[e/i]$	2.2	4.6
Mg^{2+} $\Delta G[e/i]$	5.3	11.9
$\Delta G[ATP]/[ADP]\cdot[P_i]$	−57.3	−52.7

energy of the H^+ gradient concomitant with the decrease in the energy of the Na^+ gradient are compatible with an increase in the Na^+/H^+ exchange activity in the tumor. The chemical and electrical energies of the ion gradients are equal in magnitude and opposite in sign to the chemical energy of ATP hydrolysis and show that approximately one-third of the energy of ATP hydrolysis is used to maintain the gradients of Na^+, H^+ and Mg^{2+} in liver and approximately one-half in the hepatoma.

DISCUSSION

In the 1930s Warburg noted "the remarkable extent to which living tumor cells are able to convert carbohydrate into lactic acid." Thirty years later, in the 1960s, George Weber demonstrated that the lactate production of tumors correlated with the degree of differentiation and growth rate (37). In the 1990s, MRS studies with ^{13}C glucose and ^{13}C alanine *in vivo* and *in vitro* have demonstrated that glucose (and not alanine) was taken up in a de-differentiated subclone of a hepatoma and metabolized to lactate, whereas alanine was the preferred substrate in the well-differentiated subclone (38). All these findings support the view that many fast growing tumors produce copious amounts of lactate—why do they favor lactate production over oxidation?

In Warburg's experiments in tumor slices, he demonstrated that even in the presence of O_2, lactate was produced. It is well known that the amount of ATP yielded from the formation of lactate from glucose is approximately 18 times less than from complete oxidation of glucose. In most normal tissues (liver, kidney) the complete oxidation of glucose is ensured by the Pasteur effect, i.e., the inhibition of glycolysis in the presence of oxygen. In many *in vivo* tumors, tissue O_2 pressures are lower than those in normal tissue (39, 40), although there is still thought to be enough O_2 available for functioning of the respiratory chain. With the poor vasculature observed in many solid tumors it is likely that the O_2 is not always available in the sites where it is needed. In tissue-isolated tumors described by Kallinowski *et al.* (41), between 50 and 75% (depending on tumor type) of the O_2 available was not used by the tumor. For this reason it is difficult to extrapolate from the many experiments on isolated cells or mitochondria bathed in oxygen-saturated medium to the *in vivo* situation.

Hexokinase (EC 2.7.1.1), the enzyme that brings glucose into the glycolytic pathway, is not only present at much higher levels in many rapidly growing tumors (42), but a large fraction is frequently bound to the mitochondria. This allows direct and more efficient interaction between mitochondrial oxidative phosphorylation and glycolysis and may well give the tumor cell the ability to produce more pyruvate than can be oxidized by the available mitochondria, since many tumor types have less

(approximately 50%) mitochondria than their normal tissue counterparts (42). This reduction in mitochondrial number also makes them poor competitors for available ADP and P_i with glycolysis. Impairment of the transfer of reducing equivalents generated by glycolysis in tumors is another confounding factor (43), which leaves the [NAD]/[NADH] ratio poised to convert excess pyruvate into lactate.

The reason for the high glycolytic rate of tumor cells has puzzled biochemists for a long time and is still largely unresolved (for review see Ref. 44), although the extent of it appears to be associated with the differentiation status of the tumor type and consequent degree of hypoxia *in vivo*. To survive, the tumor cell needs to maintain its pH_i relatively neutral—how does it do this in the face of this excessive production of anionic lactate produced from electroneutral glucose? To maintain electroneutrality, the export of lactate$^-$ would require that a cation (H^+) would accompany this movement and would account for the reversal of the proton gradient.

There are many different modes by which ions exchange, co-transport or diffuse across the selectively permeable plasma membrane in normal cells (45). Ionic gradients between a neutral extracellular and electronegative intracellular compartment are characteristic of the normal state of living cells. Changing the ionic composition of the extracellular fluid or damage of tissue, by almost any means, results in a stereotypic increase in $[Na^+]_i$ (46, 47), a loss of $[K^+]_i$ and swelling of cells (48). The data indicate that in tumors similar abnormalities of ion distribution are found and that the change in gradient of one ion (H^+) results in alterations to the gradients of many ions.

The energy to maintain the ion gradients is derived from cellular metabolic processes and one way to describe these interactions is to consider them as a Gibbs–Donnan equilibrium system. This method has successfully described the ionic gradients of a closed system: the perfused rat heart (27). In an *in vivo* tumor, which is essentially an open system, it is difficult to measure the relevant (i.e., intra-tumor) extracellular concentrations of many of the ions due to the difficulties in sampling tumor extracellular fluid and thus the system cannot be analyzed totally. Nevertheless, the equilibrium approach allows us to examine the results and draw certain conclusions about the metabolism of tumors. The reversal of the proton gradient would also be compatible with elevated levels of Na^+, Ca^{2+} and P_i observed in the hepatoma, and the lower tumor Mg^{2+}, K^+ and $MgATP^{2-}$ compared to liver. The lower $MgATP^{2-}$ would help to maintain osmotic equilibrium in the face of the loss of the Na^+ gradient. The lower ΔG of the Na^+ gradient in the tumors provides less energy for the Na^+/Ca^{2+} exchange, which would cause an accumulation of Ca^{2+}. This uptake of calcium cations would

be accompanied by phosphate anions in approximately similar amounts, with resultant formation of calcium phosphate precipitates in the tumors. Tumor calcification is indeed a hallmark of many tumors, forming the basis of diagnostic tests for cancer, most notably mammography. Dystrophic calcification is also observed in many other chronically injured tissues, such as scars from myocardial infarction (49). If the primary cause of tumor calcification is the ion balance changes due to the chronic hypoxia that exists in tumors, other chronically hypoxic tissues would be expected to show similar effects.

Chronic hypoxia up-regulates the expression of a number of stress proteins. Lactate dehydrogenase (EC 1.1.1.27) is expressed at high levels in many human tumors and under well-oxygenated conditions is synthesized as a soluble form whereas hypoxia induces synthesis of a form that binds to membranes (50). The major hypoxic stress protein p34 has been demonstrated to be an LDH isozyme (51). There is also evidence that hypoxia induces other enzymes of the glycolytic pathway, which would favor glycolysis (52). Taken together, these findings suggest that induction of these stress proteins enables the tumor cells to withstand prolonged hypoxia, perhaps at the expense of more efficient energy transformation from glucose, but at least by maintaining pH_i relatively neutral.

The initial observation by this relatively novel and non-invasive MRS technique indicated that tumors were not 'acid', as had been inherent in Warburg's work for 50 years or so. This finding has subsequently led to studies that offer new insights into major properties of tumors that are important both diagnostically and therapeutically.

SUMMARY

For many years after Warburg's classic work, it was generally assumed that tumors produced large amounts of lactic acid and consequently had an acidic intracellular pH_i. However, with the advent of Magnetic Resonance Spectroscopy (MRS), a non-invasive *in vivo* measure of tissue pH became available and demonstrated that in both human and animal tumors, pH_i was higher (>7.0) than pH_ϵ (<6.8), in contrast to normal tissues (e.g., liver) in which pH_i (~7.2) is lower than pH_ϵ (~7.4). This result has been confirmed in animal tumors using an MRS-visible extracellular marker, 3-aminopropyl phosphonate. The pH gradient across the tumor cell membrane is part of an interrelated system of ionic gradients and measurements made by both ^{31}P MRS and by conventional analysis in Morris hepatoma 9618a and in livers demonstrated that the following ions also changed: compared with liver the Na^+ content was 2-fold higher, K^+ was 20% lower, total Ca^{2+} was 8-fold higher (7.4 μmol/g wet wt) and total Pi 2-fold higher (8.5 μmol/g wet wt), suggesting the presence of

insoluble calcium phosphate, HCO_3^- was lower, total Mg^{2+} was similar in both tissues, but free $[Mg^{2+}]$ (calculated by two different methods) was ~5-fold lower in the hepatoma, as was $[ATP]/[ADP][P_i]$. Because of an inadequate blood supply, tumors are often hypoxic with impaired Krebs cycle activity, low $[ATP]/[ADP][P_i]$ and rely mainly on glycolysis for energy. The rapid production and subsequent export of anionic lactate$^-$ from the tumor cell would be accompanied by H^+. This would account for reversal of the proton gradient and activation of the Na^+/H^+ exchange. The elevated $[Na^+]_i$ would decrease the Na^+/Ca^{2+} exchange, which would in turn tend to cause the accumulation of Ca^{2+} (and P_i). Such calcification is a very common feature of tumor pathology. The data indicate the change in gradient of one ion (H^+) involves alterations in the linked equilibria of many ions and also of energy metabolites and offers new insights into properties of tumors important both diagnostically and therapeutically.

ACKNOWLEDGEMENTS

The authors acknowledge the Cancer Research Campaign, UK (grant SP 1971/0402) for supporting this research. They would also like to thank Franklyn Howe, Loreta Rodrigues, Cheryl McCoy, Lindsay Bashford and David Winterbourne for help in various ways.

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