Ketone Body, Glucose, Lactic Acid, and Amino Acid Utilization by Tumors in Vivo in Fasted Rats

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ABSTRACT

Arteriovenous differences for acetocetate, ß-hydroxybutyrate, glucose, lactic acid, and glutamine and other amino acids were measured across Morris hepatomas 5123C, 7777, and 7288CTCF and Walker sarcocarcinoma 256 in vivo in rats fasted for 2 days. The acetocetate and ß-hydroxybutyrate concentrations in arterial whole blood of fasted tumor-bearing rats were 0.52 ± 0.06 and 1.82 ± 0.19 mm (S.E., n = 38), respectively. Both ketone bodies were utilized by the tumors, and the rates of utilization were directly related to the rates of supply. The mean utilization rates for acetocetate and ß-hydroxybutyrate were 13.9 ± 2.9 (range, 0 to 64; n = 30) and 24.7 ± 4.4 (range, 0 to 145; n = 38) nmol/min/g tumor wet weight, respectively. Eight of the tumors produced acetocetate, presumably from utilization of substrates found that both acetoacetate and ß-hydroxybutyrate were utilized by rat tumors in vivo and that the rates of utilization were directly proportional to the rates of supply. The mean rates of glucose and glutamine utilization for all tumors in fasted rats were 101 ± 11 (range, 3 to 313) and 8.2 ± 1.1 (range, 0 to 25.1) nmol/min/g tumor wet weight, respectively. Thirty-six percent of the glucose and 25% of the glutamine supplied to the tumors was utilized. Comparison (by linear regression and analysis of covariance) of the rates of supply and utilization of glucose and glutamine in tumors growing in fasted versus fed rats indicated that these substrates are utilized more efficiently by tumors growing in fasted animals. Lactic acid was either utilized or produced, depending on the arterial whole-blood concentration. Production or utilization occurred, respectively, when the arterial lactate concentration was less or greater than 1 to 3 mm. The arterial whole-blood amino acids (except glutamine) were utilized at rates that were directly proportional to the rates of supply. The mean concentrations of glucose and glutamine in the arterial whole blood of fasted tumor-bearing rats (n = 38) were 6.55 ± 0.3 and 0.76 ± 0.02 mm, respectively; both of these substrates were utilized at rates that were directly proportional to the rates of supply. The mean rates of glucose and glutamine utilization for all tumors in fasted rats were 101 ± 11 (range, 3 to 313) and 8.2 ± 1.1 (range, 0 to 25.1) nmol/min/g tumor wet weight, respectively. Thirty-six percent of the glucose and 25% of the glutamine supplied to the tumors was utilized. Comparison (by linear regression and analysis of covariance) of the rates of supply and utilization of glucose and glutamine in tumors growing in fasted versus fed rats indicated that these substrates are utilized more efficiently by tumors growing in fasted animals. Lactic acid was either produced or utilized, depending on the arterial whole-blood concentration. Production or utilization occurred, respectively, when the arterial lactate concentration was less or greater than 1 to 3 mm. The arterial whole-blood amino acids (except glutamine) were utilized at rates that ranged from 1 to 4 nmol/min/g tumor wet weight. The results indicate that energy production for tumor growth in fasted rats is supported, in part, by an increased availability of ketone bodies, by an increased efficiency of utilization of glucose and glutamine, and, under certain circumstances, by utilization of lactic acid.

INTRODUCTION

Tumors have the remarkable ability to sustain a rapid rate of growth during starvation or semistarvation of the host (2, 16). The tumor growth occurs as the host tissues, especially fat and skeletal muscle, decrease in weight (4, 5), and it is obvious that the nutrient needs of the tumor are adequately supplied and that they are extracted from the diminishing resources of the host. The host-derived metabolic fuels utilized by the tumor in the fasted animal are not known. Arterial blood glucose, the putative major fuel for fast-growing tumors, is decreased in concentration during fasting, and, as we (26) and others (9) have shown, the rate at which tumors utilize glucose in vivo is directly dependent on the rate of supply. Consequently, arterial glucose is unlikely to be the only fuel utilized by a tumor during fasting. Fenselau et al. (6) made the important observation that succinyl-CoA:acetoacyl-CoA transferase, the initial enzyme for utilization of acetoacetate by peripheral tissues, is increased in activity with increased hepatoma growth rate. ß-Hydroxybutyrate dehydrogenase is present but relative to liver is reduced in activity in Morris hepatomas (20). These in vitro results suggested that ketone bodies may be important fuels for tumor growth in vivo in the fasted or semifasted animal. In this study, we measured the arteriovenous differences for the ketone bodies, lactic acid, glucose, and amino acids across 4 rat tumors in fasted rats. We found that both acetocetate and ß-hydroxybutyrate were utilized by rat tumors in vivo and that the rates of utilization were directly proportional to the rates of supply. Lactate was utilized at arterial lactate levels greater than 1 to 3 mm. Evidence is also presented which suggests that arterial glucose and glutamine are utilized more efficiently by tumors growing in fasted rats relative to tumors growing in fed rats. These properties of the tumor enhance access to available nutrients and may help explain the rapid tumor growth during fasting.

MATERIALS AND METHODS

Animals, Tumors, and Reagents. Adult male and female Buffalo and Harian Sprague-Dawley rats were obtained from colonies established here. The rats were fed a standard laboratory chow (Charles River Rat, Mouse, Hamster Formula: Agway, Inc., Syracuse, N. Y.) and water ad libitum and were subjected to alternate 12-hr periods of dark and light. Tumor implantation, growth of "tissue-isolated" tumors, preparation of the animal for tumor harvest, and collection of arterial and venous blood samples were as described previously (26). The Morris hepatomas were originally obtained from the late Dr. Harold P. Morris, Morris Hepatoma Program, Howard University Cancer Center, Washington, D. C. The Walker sarcoma 256 was obtained from the E. G. and G. Mason Research Institute, Worcester, Mass. These tumors have been carried in this laboratory for about 4 years. The mean tumor blood flow rate was 107 ± 4 µl/min (n = 38). Food was removed from fasted animals at 8 a.m. after the overnight feeding period, and the tumors were harvested 48 hr later.

Blood Sample Preparation and Assay. Perchloric and trichloroacetic acid extracts of arterial and venous whole blood were prepared as described previously (26). Acetocetate (18) and ß-hydroxybutyrate (33) were assayed fluorometrically by enzymatic methods using an Eppendorf photometer with fluorometric attachment. Acetoacetate was measured immediately after neutralization of the perchloric acid extract. Glucose, lactic acid, and glutamine and other amino acids were measured across Morris hepatomas 5123C, 7777, and 7288CTCF and Walker sarcocarcinoma 256 in vivo in rats fasted for 2 days. The acetocetate and ß-hydroxybutyrate concentrations in arterial whole blood of fasted tumor-bearing rats were 0.52 ± 0.06 and 1.82 ± 0.19 mm (S.E., n = 38), respectively. Both ketone bodies were utilized by the tumors, and the rates of utilization were directly related to the rates of supply. The mean utilization rates for acetocetate and ß-hydroxybutyrate were 13.9 ± 2.9 (range, 0 to 64; n = 30) and 24.7 ± 4.4 (range, 0 to 145; n = 38) nmol/min/g tumor wet weight, respectively. Eight of the tumors produced acetocetate, presumably from utilization of the substrates found that both acetoacetate and ß-hydroxybutyrate were utilized by rat tumors in vivo and that the rates of utilization were directly proportional to the rates of supply. Lactate was utilized at arterial lactate levels greater than 1 to 3 mm. Evidence is also presented which suggests that arterial glucose and glutamine are utilized more efficiently by tumors growing in fasted rats relative to tumors growing in fed rats. These properties of the tumor enhance access to available nutrients and may help explain the rapid tumor growth during fasting.
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Expression and Evaluation of Results. For interpretation of the arteriovenous difference measurements, we assume that the tumor is in a steady state with regard to nutrient supply and metabolism and that this steady state continues during the period of sample collection, usually about 5 min. Utilization or production of a nutrient by the tumor occurs when the arteriovenous difference is positive or negative, respectively. Utilization (or use) of a nutrient includes inter alia oxidation to CO$_2$ and/or conversion via biosynthesis to form protoplasm and secretions. Only the overall process of utilization was measured in these experiments. Utilization and production rates are given in nmol substrate utilized or produced per min per g tumor, wet weight and were calculated from the arteriovenous differences and the tumor blood flow rate (µl/min/g). Supply rates are expressed as nmol/min/g tumor, wet weight and were calculated from the arterial whole-blood concentration and the tumor blood flow rate. Errors in assay of amino acids and other substrates and in measurement of tumor blood flow rates and the effects of these errors on calculations of utilization and production rates are the same as those described earlier (25). Data are presented as mean ± S.E. Groups of results were compared by linear regression and analysis of covariance (26). The same caveats are in effect here with regard to interpretation of data by linear regression as were in effect in the previous report (26).

RESULTS AND DISCUSSION

Ketone Body Utilization. Ketone bodies were utilized by 37 of the 38 tumors examined. The rates of utilization by Morris hepatomas 5123C, 7777, and 7288CTCF and Walker sarcocarcinoma 256 were directly dependent on the rate of supply (Chart 1, A and B). The mean rate of acetoacetate and β-hydroxybutyrate utilization for all tumors was 13.9 ± 2.9 (n = 30) and 24.7 ± 4.4 (n = 38) nmol/min/g tumor, wet weight, respectively. Eight of the tumors produced acetoacetate at slow rates (not shown), presumably from β-hydroxybutyrate utilized (see below). The fastest rate of acetoacetate utilization (64 nmol/min/g tumor, wet weight) was observed in a hepatoma 7288CTCF, and the fastest rate of β-hydroxybutyrate utilization (145 nmol/min/g tumor, wet weight) occurred in a hepatoma 5123C (not shown in Chart 1B). The rate of β-hydroxybutyrate supply to the latter tumor was 403 nmol/min/g tumor, wet weight, a value that agrees reasonably well with the predicted supply rate (482 nmol/min/g tumor, wet weight) based on the linear regression analysis. It is interesting that, in the Morris hepatomas, the activity of succinyl-CoA:acetoacetyl-CoA transferase is increased (6) and that the activity of β-hydroxybutyrate dehydrogenase is decreased (20) during tumor progression. Of the hepatomas studied here, the CoA transferase activity has been reported to be most active in hepatoma 7288CTCF (6). Total enzyme activity of the homogenate was 5.1 µmol/min/g tumor, wet weight (measured in the direction of acetoacetate formation). Assuming an equivalent reaction rate in the direction of acetoacetyl-CoA formation, the fastest acetoacetate utilization rate observed in a hepatoma 7288CTCF was less than 5% of the enzyme activity measured in vitro. The mean β-hydroxybutyrate dehydrogenase activity of hepatoma 5123C has been reported to be 2.5 µmol/min/g tumor, wet weight (20). The maximum rate of β-hydroxybutyrate utilization by hepatoma 5123C in this study was 145 nmol/min/g tumor, wet weight, a rate that is less than 10% of the maximum in vitro enzyme activity. It is worth noting that the in vitro enzyme activity determinations were made at saturating levels of substrates and other cofactors and were performed with isolated hepatoma mitochondria solubilized by either detergent treatment (6) or sonication (20). The in vitro enzyme activity measurements indicate that the specific enzyme content of the hepatoma mitochondria is more than adequate to explain the observed ketone body utilization rates and that the potential exists for even greater utilization rates than those measured here. The apparent straight-line relationship between ketone body utilization and supply illustrated in Chart 1 indicates that the rate of transport of the substrates into the tumor cells and the availability of required cofactors in the mitochondria are also adequate. The enzyme phenotype for mitochondrial energy production that develops during tumor progression in hepatomas results from both the synthesis of new enzymes, e.g., CoA transferase, and the persistence of constitutive enzymes, e.g., β-hydroxybutyrate dehydrogenase. Though adequate for the needs of the tumor, the final enzyme activities in the tumor mitochondria may be low relative to the activities found in mitochondria of the parent organ or in mitochondria of other specialized organs as with, for example, the β-hydroxybutyrate dehydrogenase in liver (20, 32), kidney, and brain (32), and CoA transferase in kidney (32) and heart muscle (6, 32). The NAD(P)- dependent malic enzyme (25) and glutaminase (17), 2 other mitochondrial enzymes newly synthesized by Morris hepatomas during tumor progression, are additional examples. Both of these enzymes are much higher in activity in mitochondria from small intestinal mucosa (22, 24), a tissue in which glutamine is known to be a major respiratory fuel (34).

Arterial whole-blood concentrations of acetoacetate and β-hydroxybutyrate in several 2-day-fasted control Buffalo rats and tumor-bearing Buffalo and Sprague-Dawley rats are listed in Table 1. Total arterial whole-blood ketone body concentrations for control and tumor-bearing animals were 3.75 ± 0.85 mm (range, 2.0 to 8.7; n = 7) and 2.33 ± 0.22 mm (range, 0.4 to 7.1; n = 38), respectively, suggesting that the presence of the tumor decreased the ketogenic response of the host to starvation. The fat stores of tumor-bearing rats are known to decrease during tumor growth (4, 5), and the lower arterial whole-blood ketone body concentrations in tumor-bearing animals probably result, in part, from a decreased availability of substrate for ketogenesis.

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**Chart 1.** Regression of the rate of acetoacetate and β-hydroxybutyrate utilization on the rate of supply in rat tumors in fasted rats in vivo. Each point represents the determination for a single tumor. A least-squares fit to the data points. A, acetoacetate [y = 0.53x − 0.92, n = 30, r = 0.961 (p < 0.05)]; B, β-hydroxybutyrate [y = 0.33x − 0.4, n = 38, r = 0.869 (p < 0.05)].
The acetoacetate/β-hydroxybutyrate ratios in the arterial blood of the 38 animals examined ranged from 0 to 1.42. In some animals, significant differences were noted between the acetoacetate/β-hydroxybutyrate ratio in the carotid arterial blood and the ratio in the tumor venous blood. Assuming that these ratios are measures of the oxidation-reduction state in the animal carcass and in the tumor, respectively, we may conclude that the tumor is able to maintain an oxidation-reduction state different from that of the rest of the animal. An interesting relationship was noticed between the arterial and venous acetoacetate/β-hydroxybutyrate ratio differences across the tumor and whether a tumor was a lactate producer or a lactate utilizer. For the purposes of this discussion, we divided the tumors into 3 groups. Group 1 tumors were termed strong lactate producers and showed lactate production rates and other data for 6 representative tumors (Experiments 69, 93, 97, 104, 112, and 113) from this group. Both ketone bodies were utilized by the hepatomas and by the Walker sarcoma. In this group, an average of 56% of the arterial acetoacetate and of 30% of the β-hydroxybutyrate was removed during one pass through the tumor. Group 3 tumors were more oxidized (p < 0.05) than the animal carcass, the arterial whole-blood lactate concentration was high, and the tumors utilized arterial lactate acid. Data for 6 representative experiments from this tumor group are shown in Table 1C. Arterial whole-blood acetoacetate concentrations were lower in these animals, and, as expected, 8 of these tumors released lactate into the venous blood. Utilization of β-hydroxybutyrate remained high, however, and 30% of the β-hydroxybutyrate supplied to the tumors was utilized. Group 2 tumors were intermediate between the tumors in Groups 1 and 3, and the oxidation-reduction states of the tumor and the host were not different (p > 0.05). Three representative experiments from this tumor group are shown in Table 1B. Arterial whole-blood lactate concentrations were lower in these animals, and, as expected, 8 of these tumors released lactate (see Experiments 74, 81, 105, and 107) into the venous blood. Utilization of β-hydroxybutyrate remained high, however, and 30% of the β-hydroxybutyrate supplied to the tumors was utilized. Group 2 tumors were intermediate between the tumors in Groups 1 and 3, and the oxidation-reduction states of the tumor and the host were not different (p > 0.05). Three representative experiments from this tumor group are shown in Table 1B. Tumors in Groups 1, 2, and 3 did not differ significantly in oxidation-reduction state, suggesting that the tumor attempts to maintain a relatively fixed oxidation-reduction state despite wide fluctuations in the oxidation-reduction state of the host. This could provide a stable environment ensuring continued reductive biosynthesis and growth. We assume that the acetoacetate and β-hydroxybutyrate reached equilibrium during passage through the tumor and that the ratio is an accurate measure of the tumor oxidation-reduction state. The high enzyme activities (see above) of the tumors relative to the ketone body supply rates to the tumors suggest that this is a reasonable assumption. It is not clear why more than one-half of the control (5 of 7)
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and about half of the tumor-bearing fasted rats had acetoacetate: ß-hydroxybutyrate ratios of less than 0.25. Considering that certain animals may be more susceptible to respiratory depression and tissue hypoxia during pentobarbital anesthesia, we routinely supplied all anesthetized animals with oxygen through a loose-fitting nose cone (26). In other experiments, we found arterial whole-blood lactate concentrations of 2 mw and lower and low acetoacetate: ß-hydroxybutyrate ratios in anesthetized rats breathing 100% oxygen in a closed system with respiration assisted via a rodent respirator (data not shown). It does appear, therefore, that tissue hypoxia is not responsible for the elevated lactate and ß-hydroxybutyrate levels. The acetoacetate: ß-hydroxybutyrate ratio is decreased in rats by starvation. The animal carcass, particularly skeletal muscle, becomes more reduced as a response to fasting (8) and may explain the low acetoacetate: ß-hydroxybutyrate ratios and high lactic acid concentrations observed. A more reduced oxidation-reduction state in muscle during fasting might help conserve muscle protein (8). In addition to the oxidation-reduction state data discussed above, other metabolic data indicate that the Group 3 tumor is more oxidized than the rest of the body. For example, lactate, ß-hydroxybutyrate, glucose, and glutamine were all utilized (and presumably oxidized) at substantial rates in these tumors (Table 1C). It seems very unlikely, therefore, that the tumors listed in Table 1C were anoxic. Gullino et al. (10) have shown that a decrease in oxygen availability decreases the rate of glucose utilization by tumors in vivo.

Lactic Acid Production and Utilization. In a previous study (26) performed with fed tumor-bearing rats, we found that tumors in vivo could either produce or utilize lactic acid. Which of these processes occurred depended on the arterial lactic acid concentration. Production of lactate was most often observed at lactic acid concentrations below 2 mw, and lactate utilization generally occurred at lactate levels above 2 mw. In the range of 1 to 3 mw arterial lactate, tumors were often found that neither removed nor added (despite the fact that glucose utilization had occurred) significant amounts of lactate to the blood. Chart 2 shows that an identical relationship was observed in the tumors growing in the 38 fasted rats studied here. The data for both the fasted and fed animals (see Ref. 26) were combined in Chart 2 to illustrate the identical nature of the results obtained and to emphasize the frequency with which in vivo lactate utilization occurred. A total of 71 tumors were sampled; 24 were found to utilize arterial whole-blood lactate. Since lactic acid is a metabolic end product, it can reenter metabolism only through oxidation via lactic dehydrogenase; therefore, the capacity for NADH plus pyruvate oxidation in these tumors must be high. For example, assuming that 1 and 2 mol of pyruvic acid were formed, respectively, from each mol of arterial lactate and glucose utilized, the hepatoma 7288CTCF listed in Table 1C (Experiment 98) may have oxidized more than 650 nmol of pyruvate per min per g of tumor. These oxidations occurred simultaneously with the utilization of 10 and 20 nmol/min/g of tumor of glutamine and ß-hydroxybutyrate, respectively. Unlike normal liver, the pyruvate dehydrogenase activity of fast-growing Morris hepatomas is not decreased by fasting (11).

Several of the tumors that produced lactate at low rates (Group 2 tumors) were high-glucose utilizers (see Table 1B, Experiments 70, 78, and 92). The great potential for lactate production was not expressed, presumably because the oxidation-reduction states of tumor and host were similar and because arterial lactate-derived and glucose-derived NADH and pyruvate generated intracellularly were being utilized at the same time, and only a small amount of lactate was released into the tumor vein. Windmueller and Spaeth (34) have shown in rat small intestinal mucosa that arterial lactate may be utilized at the same time that glucose-derived lactate is released into the venous blood. When the arterial lactate concentration was greater than 1.2 to 1.6 mw, net lactate utilization occurred simultaneously with glucose utilization (34). In tumor, as in small intestinal mucosa, it would appear that the arterial lactic acid concentration plays an important role in determining if intracellular lactate (as NADH and pyruvate) is oxidized or is formed and released.

More than 50 years ago, Warburg (29) described the production of large amounts of lactic acid from glucose by tumor slices incubated aerobically. Since then, numerous other in vitro experiments confirmed this finding, and a high rate of aerobic glycolysis became an acknowledged biochemical hallmark of tumor tissues (15). More recently, however, high rates of aerobic glycolysis were described in normal tissues (3, 13, 19, 21, 23), and then, following the development of the Morris hepatomas, it was discovered that slow-growing tumors were poor lactate producers (1). Although these findings did alter the view that aerobic glycolysis was associated only with malignant cells, the idea that a high rate of lactate production is a property of a fast-growing tumor cell has persisted and is supported by in vitro experiments (31). We believe, however, that this idea can no longer be considered as an accurate representation of glucose and lactate metabolism by tumors in vivo. In the total of 71 tumors examined in vivo (Chart 2), about 50% either utilized arterial lactate or released only a small amount of lactic acid into the venous blood. These tumors included Morris hepatoma 7288CTCF, a fast-growing hepatoma, and Walker sarcocarcinoma 256, an undifferentiated tumor. High arterial lactate concentrations inhibited release of lactate even though glucose had
been utilized (Table 1C). On the other hand, low arterial lactate concentrations promoted lactate release (Table 1B). Since in vitro experiments of glucose oxidation and lactate production are most often performed in the absence of added lactate, high rates of lactate formation are expected (Chart 2). There are no discrepancies between results obtained in vitro and the in vivo results shown in Chart 2 if it is recognized that, in the presence of high arterial lactate, tumor tissues may remove lactate from the arterial blood. The uptake, metabolism, and oxidation of lactate utilized by tumors in vitro have been described by Spencer and Lehninger (28), Katz et al. (14), and Weinhouse (30), respectively.

Glucose Utilization. The mean arterial whole-blood glucose concentrations in fasted control and tumor-bearing rats were 4.83 ± 0.36 mm (n = 8) (Table 1) and 6.55 ± 0.3 mm (n = 38), respectively. The same relative differences were observed in control and tumor-bearing fed rats (26), suggesting that the presence of the tumor increases the arterial whole-blood glucose concentration of the fasted host. All 38 of the tumors growing in the fasted rats utilized glucose; the rate of glucose utilization for each tumor plotted against the rate of glucose supply to the tumor via the arterial blood is shown in Chart 3. Chart 3 also shows regressions of the rates of glucose utilization on the rates of glucose supply for tumors growing in the fasted rats (---) and for tumors growing in fed animals (---), data taken from Ref. 26. The same apparent direct dependence of the glucose utilization rate on the rate of supply observed in vivo in tumors growing in fed rats (9, 26) was observed for tumors growing in fasted rats. The results for the 2 animal groups were not identical, however, as shown by the shift to the left of the regression line for tumors grown in the fasted rats. Comparison of the 2 regression lines by analysis of covariance (27) indicated that the difference between the regression lines (and, hence, between the sample populations) is significant (p < 0.05). At any rate of glucose supply, tumors growing in fasted rats increased their glucose utilization rates by about 50 nmol/min/g tumor over those of tumors growing in fed rats. This apparent increase in efficiency of glucose utilization by tumors in fasted animals may compensate for the decreased arterial glucose concentration observed in fasted (6.55 mm, n = 38) versus fed (7.4 ± 0.4 mm, n = 26; Ref. 26) tumor-bearing rats.

Amino Acid Utilization. Glutamine, the most abundant amino acid in rat arterial blood, was the most rapidly utilized. The rate of glutamine utilization by tumors growing in fasted rats appeared to be directly dependent on the rate of glutamine supply to the tumor. An identical relationship was noted previously for tumors growing in fed rats (26). Chart 4 shows the rates of glutamine utilization by 38 tumors growing in fasted rats plotted against the rates of glutamine supply to the tumors. The regression line for these data points is shown by the solid line. The dashed line is the regression line for the data obtained for tumors growing in fed animals (data not shown; see Ref. 26). As was noted for the relationship between glucose utilization and supply by tumors growing in fed and fasted animals (Chart 3), the regression line for glutamine utilization on glutamine supply for fasted rats was shifted to the left of that for fed rats. These results suggest that tumors growing in fasted rats are able to increase the efficiency of the processes by which arterial glutamine and glucose are utilized. Since the rate-limiting steps for glucose and glutamine utilizations in vivo are not known, no decisions can be made about the mechanisms of these apparent activations of substrate uptake.

Arterial whole-blood amino acid levels in fasted control and tumor-bearing Buffalo rats are listed in Table 2. The concentrations of threonine, serine, glycine, and alanine were increased, and the concentration of lysine was decreased in the blood of the hepatoma 7288CTCF-bearing animals. Serine was the only amino acid the concentration of which was increased in the blood of hepatoma 5123C-bearing rats. Amino acid utilization and production by tumors growing in fasted rats (not shown) were similar to those observed for tumors growing in fed rats (26). Each of the amino acids measured was utilized by hepatoma 5123C (n = 9); the utilization rates ranged from 0 to 3 nmol/min/g tumor, except for the utilization rates of lysine and glutamine, which were 5.5 and 8.2, respectively. Similar rates of amino acid utilization were noted in the faster-growing tumors, hepatoma 7288CTCF (n = 9) and Walker sarcoma 256 (n = 14).
Table 2
Amino acid levels in arterial whole blood of normal and tumor-bearing fasted
Buffalo rats

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Normal 2-day-fasted rats</th>
<th>5123C hepatoma-bearing rats</th>
<th>7288CTC hepatoma-bearing rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(μmol/liter)</td>
<td>(μmol/liter)</td>
<td>(μmol/liter)</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>49 ± 5</td>
<td>37 ± 3</td>
<td>45 ± 6</td>
</tr>
<tr>
<td>Threonine</td>
<td>79 ± 5</td>
<td>110 ± 13</td>
<td>133 ± 18^c</td>
</tr>
<tr>
<td>Serine</td>
<td>152 ± 10</td>
<td>198 ± 7^d</td>
<td>221 ± 16^a</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>239 ± 16</td>
<td>234 ± 13</td>
<td>283 ± 31</td>
</tr>
<tr>
<td>Glutamine</td>
<td>724 ± 50</td>
<td>775 ± 37</td>
<td>807 ± 64</td>
</tr>
<tr>
<td>Glycine</td>
<td>241 ± 15</td>
<td>255 ± 29</td>
<td>351 ± 25^f</td>
</tr>
<tr>
<td>Alanine</td>
<td>148 ± 10</td>
<td>200 ± 20</td>
<td>224 ± 27^g</td>
</tr>
<tr>
<td>Lysine</td>
<td>68 ± 7</td>
<td>64 ± 7</td>
<td>76 ± 7</td>
</tr>
<tr>
<td>Leucine</td>
<td>99 ± 12</td>
<td>95 ± 10</td>
<td>119 ± 15</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>55 ± 6</td>
<td>60 ± 4</td>
<td>58 ± 8</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>49 ± 3</td>
<td>50 ± 4</td>
<td>62 ± 7</td>
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<tr>
<td>Lysine</td>
<td>518 ± 28</td>
<td>513 ± 22</td>
<td>442 ± 13^h</td>
</tr>
<tr>
<td>Ammonia</td>
<td>154 ± 31</td>
<td>210 ± 25</td>
<td>239 ± 27</td>
</tr>
</tbody>
</table>

^a n = 8. ^b n = 9. ^c Significant difference at p < 0.05 level as compared to normal 2-day-fasted rats.

Again, glutamine and lysine were the most rapidly utilized. Low production rates (less than 3 nmol/min/g tumor) for glycine and alanine were noted in 5 of the 9 hepatomas 7288CTC and in 5 of the 14 Walker sarcomas 256. Production of these amino acids by a few of these tumors also occurred in fed rats (26) as well. The source of these amino acids is not known.

In this paper, we have shown that tumors growing in fasted rats are able to utilize the ketone bodies supplied via the arterial blood. To our knowledge, this is the first demonstration of the utilization of these substrates by tumors in vivo. Fields et al. (7) were unable to demonstrate ketone body oxidation in hepatoma 7777 in vitro, a tumor that we found utilized ketone bodies in vivo (Chart 1). Although we assumed that the utilized acetoacetate and β-hydroxybutyrate were oxidized via established mitochondrial reactions (6, 20), we have no evidence as yet that the oxidations occurred. The same statement may be made concerning the in vivo lactic acid, glucose, and glutamine utilizations. Experiments in which radioactive ketone bodies (and other substrates) are infused into the tumor arterial blood followed by isolation, identification, and measurement of CO2 and other products in the tumor venous blood are required to determine the extent of substrate oxidation in vivo. Despite our present lack of information on the extent of the oxidation of these substrates, it seems likely that the utilization of the ketone bodies and lactic acid and amino acids and the apparent enhanced utilization of glucose and glutamine described herein are responsible for the rapid rate of tumor growth observed in the fasted host (2, 16). In a subsequent report,3 we will show that the growth rate of s.c. tumor implants and of tumors implanted on the epigastric vascular pedicle (26) is increased by fasting. Refeeding decreases the rate of tumor growth to the prefasted growth rate. Holley (12) has proposed that the growth rate of tumors is directly related to the intracellular concentrations of the nutrients needed for energy production and for the synthesis of cell constituents. An accelerated tumor growth rate during acute fasting, therefore, could result from both increased substrate availability (ketone bodies and lactate) and from an increased efficiency of utilization of substrates (glucose and glutamine). An increased efficiency of glucose and glutamine utilization during fasting is important, because it suggests that the signals generated in the host during fasting which cause hypometabolic changes in some host tissues will promote a hypermetabolic state in the tumor.

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