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Alteration in Pyruvate Metabolism in the Liver of Tumor-bearing Rats[1]

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ABSTRACT

Weight loss associated with tumor burden has been postulated to be due to an energy imbalance resulting from increased hepatic gluconeogenesis secondary to Cori cycle activity. The mechanisms which control pyruvate metabolism are inherent to the control of gluconeogenesis in the liver. Therefore, the metabolism of pyruvate was evaluated in a transplanted tumor model in rodents which has previously shown an increased rate of hepatic gluconeogenesis. Female Lewis-Wistar rats received a s.c. injection of a suspension of mammary tumor cells in the left flank. Tumor-bearing rats were allowed *ad libitum* food consumption, and non-tumor-bearing controls were pair-fed to the consumption of their tumor-bearing cohorts. At Days 12, 13, and 14 following inoculation, tumor-bearing and non-tumor-bearing controls were used for *in vivo* body composition analysis or subjected to isolated liver perfusion. Animals were not fasted prior to sacrifice. Pyruvate use by the livers of tumor-bearing and pair-fed non-tumor-bearing rats was evaluated in the presence of 8 mM glucose and 5 mM lactate. Pyruvate clearance was increased by 270%, and pyruvate intake was increased by 212% compared to pair-fed non-tumor-bearing rats. Oxidation of pyruvate to CO_2 was increased 130%, and pyruvate conversion to lactate was increased by 197% above that seen in pair-fed non-tumor-bearing rats. Gluconeogenesis from pyruvate was increased by 184% in tumor-bearing rats.

The increased gluconeogenesis in tumor-bearing rats above that of control animals at a 5 mM lactate concentration suggests that some factor, other than substrate supply, may stimulate gluconeogenesis in tumor-bearing rats. Although the use of pyruvate was greater in tumor-bearing rats, the disposal of pyruvate carbon into CO_2, lactate, and glucose was proportionally the same in both groups. Therefore, these data suggest that the increased metabolism of pyruvate in tumor-bearing rats is controlled by a mechanism affecting cellular pyruvate transport.

INTRODUCTION

In patients with growing tumors, the syndrome of cancer cachexia is generally defined by the symptoms of anorexia, weight loss, emaciation, and progressive alteration of vital functions (6, 29, 30, 32). Weight loss is the most common phenomenon associated with cancer cachexia. In some cases, there are clear physical reasons for weight loss such as a gastrointestinal obstruction, organ destruction, or malabsorption. In other cases, weight loss has been shown to be due primarily to anorexia (5, 6, 30, 31). Frequently, the weight loss associated with tumor burden cannot be explained. In these cases, the weight loss has been postulated to be due to an energy imbalance resulting from increased gluconeogenesis secondary to increased Cori cycle activity (11, 15, 28). In a fasting tumor-bearing rat model, which closely resembles human cancer cachexia, we have previously shown increased rates of gluconeogenesis (25). The rate of gluconeogenesis is accelerated by starvation (7). Therefore, alterations in the rate of gluconeogenesis in tumor-bearing rats *versus* pair-fed non-tumor-bearing rats may be magnified in the fed state. Since the final common precursor for gluconeogenesis is pyruvate, the mechanism that regulate pyruvate metabolism are inherent to the control of gluconeogenesis in the liver (4). As a consequence, the metabolism of pyruvate was evaluated in fed tumor-bearing and pair-fed non-tumor-bearing rats.

MATERIALS AND METHODS

Animals/Tumor Model. Female Lewis-Wistar rats (Microbiological Associates, Bethesda, MD) weighing 160 to 200 g, received a s.c. injection of a suspension of mammary tumor cells (approximately 1.5×10^6 cells) into the left flank. This tumor line is a transplantable adenocarcinoma initially arising in this strain after treatment with the alkylating agents, dimethyl-β-aziridinopropionamide (21). Untreated, this tumor grows rapidly, reaching massive proportions and causing host death 4 to 6 weeks following implantation (1). The s.c. location permits noninvasive determinations of tumor volume by 3-dimensional caliper measurement. Host carcass weight was determined by subtracting tumor weight, as determined by tumor volume, from total body weight. Rats of the same strain and weight range served as pair-fed controls. Tumor-bearing rats were allowed *ad libitum* food consumption of commercial RMH 3000 rat chow (Agway, Inc., Syracuse, NY) with free access to water. Controls were pair-fed to the consumption of their tumor-bearing cohorts. Treatment (injection of 0.5 ml of Hank's buffer into the left flank), feeding, and sacrifice of the pair-fed controls were staggered by 24 hr in order to match the conditions of the tumor-bearing animals. Weight, food consumption, and tumor volume were determined daily. At Days 12, 13, and 14, rats from each group were used for *in vivo* body composition analysis or subjected to isolated liver perfusion as described below. Tumor weights at these times were 8.6 ± 0.5 (S.E.), 9.9 ± 0.6, and 11.7 ± 1.2 g, respectively. Additional rats were used to study tumor lactate content.

Body Composition. Fed tumor-bearing and pair-fed non-tumor-bearing rats were anesthetized by i.p. injection of 5 mg/100 g body weight of sodium pentobarbital (Sodium Nembutal; Abbott Laboratories, North Chicago, IL). Following the acquisition of a 2-ml blood sample via cardiac puncture, a portion of the liver was rapidly frozen by the freeze clamp technique of Wollenberger *et al.* (34). The tumor was excised, and the carcasses of tumor-bearing and pair-fed non-tumor-bearing rats were analyzed for water, fat, and nitrogen. The carcasses were homogenized in 9 volumes of water using a Model PT 45 Polytron homogenizer (Brinkman Instruments, Westbury, NY). An aliquot of the homogenate was removed and dried in a vacuum oven for the gravimetric determination of water. Fat was extracted from the dried homogenate by the method of Folch *et al.* (9) and total carcass fat determined gravimetrically. Another aliquot of homogenate was used for nitrogen determination by a semimicro Kjeldahl method (14).

In a separate group of animals, following adequate anesthesia with sodium pentobarbital, the entire tumor was excised and immediately frozen in liquid nitrogen. Total tumor weight was determined.

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Perfusion Studies. Hepatic gluconeogenesis from pyruvate was determined using an isolated perfused liver preparation (8). Animals were not fasted overnight. Perfusate was oxygenated using a membrane oxygenator as described by Hamilton *et al.* (13). The perfusate medium has been described previously (20) and contained 4% bovine serum albumin (Pentex; Fraction V), 8 mM glucose, 5 mM lactate, 0.1 mM pyruvate, and [1-^{14}C]pyruvate (0.015 μC/ml). The final concentration of amino acids used in the perfusate was constructed to mimic the concentration of amino acids in arterial blood from fed rats (2). Washed, aged, human erythrocytes were added to give a final hematocrit of 30%. Livers were perfused at a flow rate of 4 ml/min via a single-pass system for 40 min following a 20-min stabilization period. During the 40 min, arterial samples were drawn at 20-min intervals and venous samples collected in 5-min intervals. At the end of perfusion, a portion of the liver was rapidly frozen by the freeze clamp technique of Wollenberger *et al.* (34) and stored at −20°.

Analytical Procedures. The arterial and venous perfusate samples were analyzed for pCO_2, pO_2, and pH using a blood gas analyzer (IL Model 213; Instrumentation Laboratory, Lexington, MA). Perfusate glucose concentration was determined using an automated assay (10). Serum and perfusate lactate and pyruvate concentrations were determined fluorometrically (18).

Separation of radiolabeled glucose, lactate, amino acids, and pyruvate in perfusate was accomplished by the use of anion-cation exchange columns as described by Kreisberg *et al.* (17). Columns were eluted into vials, and 20 ml of aqueous scintillation cocktail (Aquasol 2; New England Nuclear, Boston, MA) were added to the vials. Duplicate samples were counted in a liquid scintillation counter.

The $^{14}CO_2$ production from [1-^{14}C]pyruvate was measured by injecting arterial and venous perfusate samples (1 ml), obtained simultaneously, into counting vials with center wells containing 0.2 ml of Hyamine hydroxide (New England Nuclear) and filter paper wicks. After injection of 1 ml of 0.7 M perchloric acid into the main compartment, the vials were incubated in a shaking water bath at 37° for 1 hr. The center wells are then counted in 10 ml of Econofluor (New England Nuclear).

Labeled glycogen was measured according to the method of Chan and Exton (3), and nonradiolabeled glycogen was determined according to the procedure of Passonneau and Lauderdale (23). Frozen tissue was homogenized in frozen 0.7 M perchloric acid using a Polytron homogenizer. The tumor lactate was determined by fluorometric enzyme analysis (18).

Statistical Analysis. Mean and S.E. are calculated for each parameter. The means were then analyzed by unpaired Student's *t* test (27).

RESULTS

Carcass weight in tumor-bearing and pair-fed non-tumor-bearing animals stabilized at 10 days following inoculation with tumor. Rats allowed *ad libitum* food consumption continued to gain weight (Chart 1). Total weight gain (carcass plus tumor) of tumor-bearing rats was similar to that of *ad libitum* fed controls.

Food consumption of the tumor-bearing and pair-fed non-tumor-bearing rats was identical. Food consumption was reduced beginning at Day 10 following tumor inoculation (Chart 2).

Changes in the body composition of tumor-bearing and pair-fed non-tumor-bearing rats are presented in Table 1. Water, fat, and nitrogen content were not different. In the fed state, there was an increase in serum lactate in tumor-bearing rats above that seen in pair-fed non-tumor-bearing rats [2.64 (±0.41) mM/liter *versus* 1.56 (±0.19) mM/liter; $n = 4$; $p < 0.05$]. Analysis of lactate concentration in tumors of different sizes showed an increase in concentration of lactate with increased tumor size (Table 2). Hepatic glycogen content of tumor-bearing and pair-fed non-tumor-bearing rats was not different. Pyruvate use by the livers of tumor-bearing and pair-fed non-tumor-bearing rats

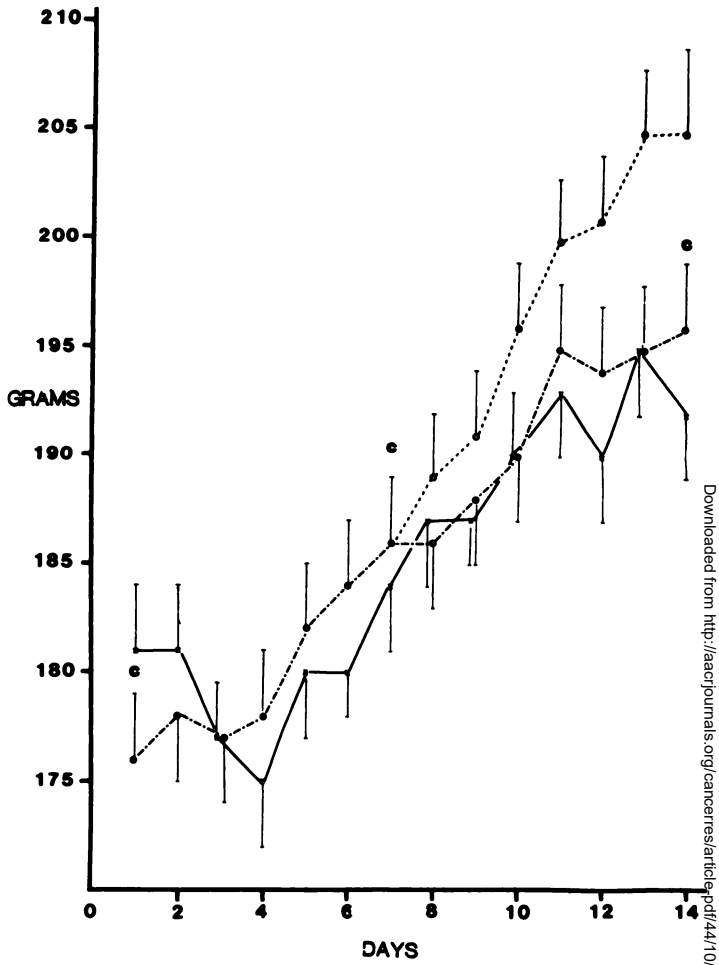

Chart 1. Carcass weight gain in tumor-bearing, pair-fed non-tumor-bearing, and *ad libitum*-fed non-tumor-bearing rats. Values are the mean of 12 rats for each day in the tumor-bearing (carcass plus tumor, – – – –; carcass minus tumor, –·–) and pair-fed non-tumor-bearing groups (——) and 4 rats for each day in the *ad libitum*-fed non-tumor-bearing groups (c). Total weight gain (carcass and tumor) for tumor-bearing rats is also shown. *Bars*, S.E.

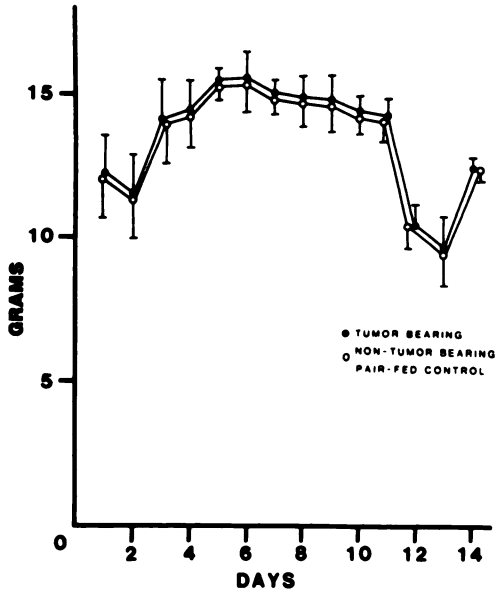

Chart 2. Daily food consumption of tumor-bearing (●) and pair-fed animals (○). Values are the mean for 12 rats in each group for each day. Pair feeding of non-tumor-bearing rats was staggered by 24 hr. *Bars*, S.E.

Table 1

Body composition of tumor-bearing and pair-fed non-tumor-bearing rats

	% of H_2O	% of nitrogen	% of fat
Tumor-bearing (n = 4)	67.8 ± 1.4[a]	3.0 ± 0.1	11.5 ± 0.2
Pair-fed non-tumor-bearing (n = 4)	67.3 ± 1.7	3.1 ± 0.1	9.9 ± 0.8

[a] Mean ± S.E.

Table 2

Lactate content of tumors

Tumor lactate concentration was determined in various size tumors; $p < 0.05$ in all comparisons.

Tumor wt (g)	No. of tumors	Lactate (μmol/g)
0.25– 1.5	11	4.95 ± 0.28[a]
5.7 –10.5	7	9.01 ± 0.40
11.8 –21.4	9	13.6 ± 0.92

[a] Mean ± S.E.

in the presence of 8 mM glucose and 5 mM lactate is shown in Table 3. Pyruvate clearance was increased by 270%, and pyruvate uptake was increased by 212% compared to pair-fed non-tumor-bearing rats. Oxidation of pyruvate to CO_2 was increased 130%, and pyruvate conversion to lactate was increased by 197% above that seen in pair-fed non-tumor-bearing rats. Gluconeogenesis from pyruvate was increased by 184% in tumor-bearing rats.

Incorporation of radioactivity from pyruvate into glycogen or amino acids was not detectable. Although the use of pyruvate was greater in tumor-bearing rats, the disposal of pyruvate carbon into CO_2, lactate, and glucose was proportionally the same in both groups as shown in Table 4.

DISCUSSION

Weight loss associated with tumor burden can be accounted for by several factors including anorexia and interference with the assimilation of nutrients. In this study, carcass weight stabilized at approximately the same level in both tumor-bearing and pair-fed non-tumor-bearing rats at 10 days following tumor inoculation (Chart 1). Concurrently, a reduction in food consumption occurred in tumor-bearing rats which continued throughout the course of the study (Chart 2). Therefore, it appears that the decreased food consumption was the major cause of carcass weight stabilization in rats with this size tumor. Morrison (22) has reported weight loss associated with a decrease in food intake in several transplantable tumors in rats. His conclusions were that a reduction in food intake occurred because of a deterioration of extrahypothalamic feeding controls (*i.e.*, impaired response to: caloric density of food; reduced environmental temperature; amino acid deficiency; and taste perception) and a loss of capacity for motor activity.

Total weight gain (carcass plus tumor) continues at a rate comparable to that of *ad libitum*-fed non-tumor-bearing rats (Chart 1). The increase in total body weight in tumor-bearing rats occurs in spite of a reduced food intake. This finding could be a result of accumulation of water in the tumor, increased efficiency in energy use by the tumor, or decreased activity of tumor-bearing rats. The water content of tumors at 11 days following inoculation is 80.1% (±3.9) and does not change with increased tumor size.[3] Therefore, the increased total body weight in tumor-bearing rats in spite of reduced food intake cannot be explained by an increase in tumor water content. A role for increased efficiency in energy use by the tumor has been proposed by Sauer and Dauchy (24) who have shown tumor growth during starvation to be supported by increased availability and use of ketone bodies, increased efficiency in the use of glucose and glutamine, and the use of lactate as an energy source. Finally, as noted above, Morrison (22) has proposed a loss of capacity for motor activity in tumor-bearing rats.

As was predicted, the difference in the rate of gluconeogenesis between the tumor-bearing and non-tumor-bearing rats is augmented in the fed state. The unusual finding of the accelerated rate of gluconeogenesis in tumor-bearing rats in the fed state has been reported previously (25, 26). Lowry *et al.* (19) have shown that a higher rate of glucose infusion is necessary to suppress gluconeogenesis in fasted tumor-bearing rats than in fasted non-tumor-bearing rats. Several investigators have postulated the increased gluconeogenesis to be a result of increased substrate supply (*e.g.*, lactate) (11, 15, 16, 33). As shown in this study and in our previous work (25), the difference in serum lactate between tumor-bearing and non-tumor-bearing rats is maintained regardless of food intake. This finding has been reported by other investigators (26). Serum lactate has been shown to progressively rise with increasing tumor burden (26). The elevated serum lactate in the present study may be related to the increase in tissue lactate content with increasing tumor size (Table 2). The glycolytic activity of the tumor is probably the major source of this lactate since the arterial-venous difference across the tumor has shown that 50% of the glucose carbon taken up by the tumor appears as lactate in the venous blood (12, 26). The perfusate lactate concentration used in the present study has been shown to maximally stimulate gluconeogenesis in the liver of normal rats (8). However, increased gluconeogenesis in tumor-bearing rats above that of control animals at this lactate concentration suggests that some factor, other than substrate supply, may stimulate gluconeogenesis in tumor-bearing rats.

One such factor may be found in the increased pyruvate clearance and uptake demonstrated in the tumor-bearing rats in this study (Table 3). Pyruvate metabolism occurs in cytoplasmic and mitochondrial compartments (4). Entry of pyruvate into either compartment is dependent upon 2 unrelated specific carriers. The carrier which exists in the plasma membrane is pH-dependent, and is specific for many short-chain carboxylic acids including ketone bodies. However, the K_ms of this carrier for pyruvate and lactate during net uptake are 2 and 10 mM, respectively (4). Since the perfusate pyruvate concentration was well below the K_m for the carrier, and was not different among the groups, it is unlikely that this carrier was rate-limiting for pyruvate transport in this study (assuming an absence of a tumor-specific modification of this carrier).

The mitochondrial carrier makes pyruvate available as a substrate for both pyruvate dehydrogenase and pyruvate carboxylase. Supply of pyruvate to pyruvate carboxylase is possibly the limiting factor during gluconeogenesis (4). The mitochondrial carrier is specific for short-chain carboxylic acids with keto-groups or halides in the 2- or 3-position. Therefore, the mitochondrial carrier is important for transport of ketone bodies as well as pyruvate. The K_m for pyruvate ranges from 0.2 to 0.6 mM, depending upon conditions used to determine this value. Maximal activity of the carrier is pH-dependent, and transport increases as the pH rises. The rate of transport is slow compared to other mitochondrial transport systems. In addition, glucagon

[3] J. D. Shearer, G. P. Buzby, J. L. Mullen, E. Miller, and M. D. Caldwell, unpublished observations.

Table 3

Pyruvate use by the isolated perfused liver of tumor-bearing and pair-fed non-tumor-bearing rats

Rats were perfused in the fed state as described using a single-pass system.

Treatment	No. of rats	Pyruvate clearance (ml/min/100 g body wt)	Pyruvate uptake (nmol/min/100 g body wt)	$^{14}CO_2$	[^{14}C]Pyruvate to [^{14}C]lactate (nmol/min/100 g body wt)	[^{14}C]Glucose
Tumor-bearing	6	0.581 ± 0.093[a,b]	132.7 ± 6.3[b]	31.5 ± 1.1[b]	99.5 ± 3.2[b]	6.7 ± 0.34[b]
Pair-fed Non-tumor-bearing	6	0.157 ± 0.121	42.6 ± 7.7	13.7 ± 0.5	30.5 ± 3.2	2.5 ± 0.15

[a] Mean ± S.E. of 9 samples/animal taken at 5-min intervals for 40 min. Pyruvate clearance was calculated as follows: (arterial-venous difference × flow rate)/(arterial concentration × body wt/100).

[b] $p < 0.05$; statistical analysis was performed using an unpaired *t* test of means.

Table 4

Proportional use of pyruvate in the isolated perfused liver of tumor-bearing and pair-fed non-tumor-bearing rats

Shown is the percentage of conversion of [1-^{14}C]pyruvate to $^{14}CO_2$, [^{14}C]lactate, and [^{14}C]glucose in isolated perfused liver of tumor-bearing and pair-fed non-tumor-bearing rats. Values are the mean percentage of 9 samples/animal taken at 5-min intervals for 40 min. Statistical analysis was performed using an unpaired *t* test of means.

Treatment	No. of rats	% of conversion of pyruvate to CO_2	% of conversion of pyruvate to Lactate	% of conversion of pyruvate to Glucose
Tumor-bearing	6	24.2 ± 1.5[a]	76.4 ± 4.4	5.2 ± 0.4
Pair-fed Non-tumor-bearing	6	25.8 ± 4.4	75.9 ± 16.7	4.5 ± 0.8

[a] Mean ± S.E.

is thought to stimulate gluconeogenesis by increasing mitochondrial pyruvate transport (4). Therefore, it has been suggested that pyruvate transport may be a limiting factor under certain conditions and subject to control (4).

Since accelerated gluconeogenesis in tumor-bearing animals appears to be mediated by some effector other than substrate supply, it is possible that a mechanism for stimulating mitochondrial pyruvate transport may be controlling the rate of gluconeogenesis in the liver of tumor-bearing rats.

In conclusion, the major cause of carcass weight stabilization in this tumor model is a decrease in food consumption. There is no apparent selective mobilization of carcass fat, protein, or hepatic glycogen in tumor-bearing rats. The increased oxidation of pyruvate to CO_2 and the conversion of pyruvate to lactate and glucose in tumor-bearing rats is apparently related to increased clearance and uptake of pyruvate. Therefore, it appears that the increased metabolism of pyruvate in the tumor-bearing rat is controlled by a mechanism affecting cellular pyruvate transport. Direct evidence for the site of this altered hepatic pyruvate transport awaits further study.

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