Parenteral Level of Glucose Intake on Glucose Homeostasis, Tumor Growth, Gluconeogenesis, and Body Composition in Normal and Tumor-bearing Rats

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ABSTRACT

To determine the effects of different levels of glucose intake on glucose homeostasis, gluconeogenesis, body composition, and tumor growth, we gave 8 days of total parenteral feeding of a defined liquid formula diet to groups of Buffalo rats, with and without a transplanted Morris 7777 hepatoma. The level of glucose intake was held at levels which ranged from 0 to 9.5 g/100 body weight per day while the levels of all other nutrients were held constant. Measurements were made on tumor growth rate, terminal blood plasma glucose and whole blood lactate levels, gluconeogenesis, body and organ weight, muscle nitrogen content, liver glycogen, and urine analysis. Tumor-bearing rats (TB) at low glucose intake but not non-tumor-bearing rats (NTB) were found to be dependent on gluconeogenesis for maintenance of blood glucose homeostasis (normoglycemia). Body weight was dependent on glucose intake level in both TB and NTB rats but with glucose intake rates of 5.7 g/100 g/day being the point between weight loss or gain. However, under these feeding conditions, tumor growth rate was not dependent on the glucose intake rate. The weight of epididymal fat pad and the size of fat cells were positively correlated with glucose intake rate in both TB and NTB rats, but the fat pad weight in TB rats showed a greater dependence on the rate of glucose intake than did in the NTB rats. Glucose intake of 3.8 g/100 g/day or less leads to significant loss of muscle mass and loss of muscle nitrogen (protein) in TB but not in NTB rats. Some liver glycogen was detected in all groups of rats except those TB rats with zero glucose intake. TB rats with high glucose intake (5.7 to 9.5 g/100 g/day) had higher blood lactate and lower urine pH than did NTB rats. Thus, TB rats at low glucose intake (3.8 g/100 g/day or less), as opposed to NTB rats, demonstrated a significant dependence on gluconeogenesis for glucose homeostasis, mobilized more of their liver glycogen, and catabolized more of their muscle proteins to supply the increased energy needs of the growing tumor and to maintain normoglycemia.

INTRODUCTION

The parenteral delivery by pump of a defined liquid formula diet allows us to make direct correlations between the glucose intake rate, which allows precise control over calorie intake, and the measurement of a number of experimental parameters in TB and in NTB rats. In this study, the level of glucose was held constant at levels which ranged from 0 to 9.5 g/100 g/day, while keeping the delivery of the remainder of nutrients constant. This experimental approach allowed us to control nutritional variables and to get a better understanding of glucose metabolism, gluconeogenesis, and the depletion of adipose and muscle as an energy source in TB and NTB rats.

It was our hope that the integration of findings from such a study would give us important new information on the role of glucose or calorie intake rate on the metabolism of TB and NTB rats. The establishment of significant differences between the TB and the NTB rats should provide us with clues as to how the tumor influences host metabolism, and should also provide basic information that can eventually be used to design therapeutic interventions to prevent the wasting of body mass due to the presence of the tumor (cancer cachexia), or to suppress tumor growth.

The role of nutrition on tumor-host metabolism has been the subject of several recent symposia and a review article. The interested reader is referred to these sources as an excellent background summary of this field (3, 12, 18, 19).

MATERIALS AND METHODS

The dispersing of animals into groups was done by assigning numbers to all rats, then dispersing the animals into groups by selection of random numbers from a random-number table. Male Buffalo rats, 7 weeks old, were initially divided into 2 groups. One group of rats were inoculated in the right flank with an 0.25-ml suspension of 10⁷ viable Morris 7777 hepatoma cells, as reported previously (9). The Morris 7777 hepatoma cells were obtained from Henryka Brania of the McArdle Laboratory for Cancer Research in Madison, Wis. The second group of rats were given the same volume of lactated Ringer’s solution. After 3 weeks, the rats inoculated with tumor and those not inoculated with tumor cells were randomly divided into groups to receive different levels of glucose. The animals were housed individually in metabolic cages with water available, and were exposed to a 14-hr light and 10-hr dark cycle from the time they were 7 weeks of age.

Animals were fed a liquid diet parenterally (i.v.). The components of the elemental diet are listed in Table 1. Any other required trace elements should be available as diet or environmental contaminants. The glucose levels in the i.v. solution ranged from 0 to 9.5 g/100 g/day. The i.v. solution was administered at a flow rate of 0.4 ml/hr/100 g the first day, 0.6 ml/hr/100 g the second day, and 0.79 ml/hr/100 g from Day 3 through the end of the experiment at Day 10.

The cannulation procedure has been described previously (10). Briefly, parenterally fed rats have a Silastic catheter tube inserted into the superior vena cava or the right atrium via the external jugular vein. The other end of the catheter exits the body between the scapulae. The rats were placed in a padded harness and attached to swivel apparatus (Instec Labs, Philadelphia, Pa.) via a tightly wound steel shielding cable, housing the plastic infusion tubing. The rats were infused at a constant continuous rate with a Holter Model 903 peristaltic pump (Critikon, Inc., Tampa, Fla.).
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RESULTS

Rate of Parenteral Glucose Intake on Tumor Growth Rate.

Three weeks after s.c. implantation of tumor cells on the right flank of each rat, a palpable and measurable tumor was present in each rat. At this time the TB rats weighed an average of 255 ± 9 (S.E.) g and the NTB rats weighed an average of 255 ± 5 g. Thus the tumor was found not to have caused a significant effect on the total body weight at the beginning of the feeding treatment period (F = 0.0003; p, not significant).

Daily measurements of tumor size during the 8-day course of the experiment were made and were subjected to a least-squares linear and to a least-squares logarithmic regression analysis. The linear regression model showed a higher correlation coefficient value for 9 of the 10 tumors, as compared to the correlation coefficient value for the logarithmic model. The linear regression data were therefore selected for presentation, as shown in Table 2.

Tumor growth under the different rates of glucose intake gave tumor growth rate slopes which range from 122 to 183 sq mm increase in cross-sectional area per day. The goodness of fit of the growth slopes to a linear model is indicated by the high correlation coefficient values (range, 0.90 to 0.99). The table also lists the intercept values of each tumor growth slope, which gives a good measure of the initial cross-sectional area of the tumor (range, 586 to 1405 sq mm). Past studies from this laboratory showed a high correlation between cross-sectional area of the tumor and tumor weight (9). It is therefore possible for us to get an accurate estimate of tumor weight based on its cross-sectional area. Using this procedure, we estimated the mean initial tumor weight to be 18.25 ± 1.90 g, which amounted to an average of 7.26% of the body weight. At the termination of the experiment the mean tumor weight was 46.30 ± 1.94 g, which amounted to an average of 18.68% of the initial body weight. The data showed that each tumor more than doubled in

Table 1

<table>
<thead>
<tr>
<th>Electrolytes</th>
<th>mg/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium</td>
<td>4.06</td>
</tr>
<tr>
<td>Potassium</td>
<td>60.36</td>
</tr>
<tr>
<td>Calcium</td>
<td>3.4</td>
</tr>
<tr>
<td>Phosphate</td>
<td>3.411</td>
</tr>
<tr>
<td>Sodium</td>
<td>44.64</td>
</tr>
<tr>
<td>Chloride</td>
<td>1.79</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Multivitamin mix (M.V.I.) (5-ml vial, use 0.50 ml)</th>
<th>mg/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid (vitamin C)</td>
<td>50</td>
</tr>
<tr>
<td>Vitamin A (retinyl palmitate)</td>
<td>1000</td>
</tr>
<tr>
<td>Vitamin D (ergocalciferol)</td>
<td>100</td>
</tr>
<tr>
<td>Thiamine hydrochloride (vitamin B1)</td>
<td>5</td>
</tr>
<tr>
<td>Riboflavin (as 5-phosphate) (vitamin B2)</td>
<td>1</td>
</tr>
<tr>
<td>Pyridoxine hydrochloride (vitamin B6)</td>
<td>1.5</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>10</td>
</tr>
<tr>
<td>Desiprenadrol</td>
<td>2.5</td>
</tr>
<tr>
<td>Vitamin E (DL-α-tocopheryl acetate)</td>
<td>0.5</td>
</tr>
<tr>
<td>Folic acid</td>
<td>2</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Beginning at the start of the third week after tumor inoculation, which was the same time the rats were started on the different i.v. diets, body weight, tumor size, water intake, and urine output were measured daily. Tumor size was determined by measuring the long and short axes with vernier calipers; the cross-sectional area of the tumor was calculated by using the formula for an ellipse. The rate of tumor growth was also measured for each rat, as reported by Cameron et al. (8). Samples of urine were analyzed for pH, glucose, and ketone, using BILL-Labstix (Ames Multistix, Elkhart, Ind.).

On Day 10 the rats were anesthetized with an i.p. injection of sodium pentobarbital (37 mg/kg in 0.9% NaCl solution). Blood samples (0.7 ml) were then obtained from the left external jugular. For blood plasma glucose determination, 0.2 ml of blood was placed in a test tube with an anticoagulant, sodium heparinate, and centrifuged for 5 min. The plasma was drawn off, frozen, and later assayed (26). For determination of lactate, 0.5 ml of whole blood was put directly into 1.0 ml of cold 6% HClO4. The acidified samples were centrifuged for 10 min, and the protein-free supernatants were neutralized with 0.3 ml 20% KOH. The samples were placed on ice for 10 min, after which they were centrifuged to remove the KClO4 precipitate. Samples of the supernatant were assayed enzymatically for lactate (16). Immediately after the initial blood sample was taken the rats were given s.c. injections of sodium 3-mercaptopicolinate, a gift from Dr. N. DiTullio of Smith Kline & French Laboratories, Philadelphia, Pa., at 15 mg/100 g (75 mg/ml in 0.9% NaCl solution). A further blood sample was collected from the anesthetized rats 1 hr after administration of the sodium 3-mercaptopicolinate, and was subsequently analyzed for glucose and lactate, as described above. To validate the 3-mercaptopicolinate method of assessment of gluconeogenesis, 2 separate groups of normally fed and 24-hr starved rats were given injections of this drug. The 24-hr starved rats, but not the normally fed rats, showed significant increases in whole blood lactate (data not presented). This gives a clear indication that the 24-hr starved rats, but not the normally fed rats, are dependent on gluconeogenesis. Thus, this method detects and measures gluconeogenesis in our laboratory, as previously reported by others (24).

After the last blood sample was taken, the rats were killed by ether overdose. The following organs were dissected and weighed at the time the rats were killed: liver, heart, epididymal fat pad, and gastrocnemius muscle. Sections of the liver and the epididymal fat pad were fixed in 10% neutral buffered formalin for later histological analysis. The gastrocnemius muscle was frozen on dry ice. To obtain the dry weight the muscles were weighed, then heated in a vacuum oven at 100° at 3 dynes/sq cm until a stable weight was obtained, usually taking between 2 and 3 days. The nitrogen concentration of the dried muscle was determined using microdistillation apparatus (Labconco Corp., Kansas City, Mo.) by the micro-Kjeldahl method (2).
size during the parenteral feeding treatment period. This tumor normally causes death of untreated rats between 8 and 10 weeks after its inoculation. To determine if the glucose intake rate influenced the tumor growth rate, a least-squares linear regression analysis was performed and is presented at the bottom of Table 2. The results of this analysis showed that the glucose intake rate did not determine or directly correlate with the tumor growth rate under these experimental conditions.

Thus tumor growth rate was not found to be dependent on the glucose intake rate when the intake of all other nutrients in the liquid formula diet were held constant.

Rate of Parenteral Glucose Intake on Change in Body Weight and on Terminal Plasma Glucose and Whole Blood Lactate Levels in TB and NTB Rats. Table 3 summarizes the results and the analysis of results of the rats given 8 days of parenteral feeding at different glucose infusion rates on the percentage of change in body weight, terminal blood glucose level, and terminal blood lactate level for both the TB and NTB rats.

The least-squares linear regression analysis revealed that glucose intake rate was positively and significantly correlated to body weight change as a result of the 8-day course of the parenteral feeding treatments. The intercept values of the regression analysis showed that TB and NTB rats, when placed on zero glucose intake rate, lost between 19 and 20% of their initial weight. Statistical analysis for differences in slope of body weight changes did not show significant differences between the TB and NTB rats in their weight response to increasing level of glucose intake. The regression analysis showed that for each g increase in glucose intake per 100 g per day there resulted a 3.47 to 3.59% increase in body weight. It should be noted that terminal body weight in the TB rats includes the weight of a substantial tumor mass (average of 46.3 ± 1.94 g). Clearly, the terminal body weight of TB rats less the tumor mass would be less than the terminal body weight of NTB rats.

Thus, overall body weight increases in both TB and NTB was dependent on the glucose intake rate when the intake of all other nutrients was held constant. Under these experimental conditions a glucose intake rate of 5.7 g glucose per 100 g/day was the transition point between weight loss and weight gain.

Terminal plasma glucose level in either the TB or the NTB rats, as shown in Table 3, when correlated to the glucose intake rate by means of least-squares linear regression analysis were not found to be significantly correlated. This indicated that glucose homeostasis in the plasma was maintained in both TB and NTB rats, regardless of the rate of glucose infusion. Urinalysis during the last 4 days of parenteral feeding showed no indication of glucose, even in the rats on the highest levels of glucose infusion.

The terminal blood lactate level in NTB rats, as shown in Table 3, was not significantly correlated with the glucose intake rate, as determined by least-squares linear regression analysis, but terminal blood lactate level in TB rats was significantly correlated with the glucose intake rate. A statistical comparison between the regression analysis slopes of the TB and NTB rats showed a significantly higher slope value in the TB rats (p < 0.005). It was also noted that blood lactate level at zero glucose intake was lower in TB rats (intercept values, 0.448 mm) than in the NTB rats (intercept value, 1.113 mm), as seen by comparison of the regression analysis of slope intercept values (Table 3). Thus, terminal lactate level was found to be dependent on the glucose intake rate in TB but not in NTB rats.

Urine pH might be anticipated to decrease as blood levels of lactate are increased above normal. Regular urine pH measure-
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Table 4
Assessment of gluconeogenesis as measured by the change in blood plasma glucose and blood lactate levels 1 hr after injection of 3-mercaptopicolinate (expressed as a percentage of initial blood level prior to 3-mercaptopicolinate injection)

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumor condition</th>
<th>Glucose intake rate (g/hr/100 g/day)</th>
<th>n</th>
<th>Glucose</th>
<th>Lactate</th>
<th>Lactate/glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No tumor</td>
<td>0-3.8</td>
<td>5</td>
<td>83.8 ± 7.0a</td>
<td>82.6 ± 14.8</td>
<td>1.02 ± 0.21</td>
</tr>
<tr>
<td>2</td>
<td>No tumor</td>
<td>5.7-9.0</td>
<td>4</td>
<td>108.3 ± 5.6</td>
<td>88.5 ± 22.5</td>
<td>0.85 ± 0.24</td>
</tr>
<tr>
<td>3</td>
<td>Tumor</td>
<td>0-3.8</td>
<td>4</td>
<td>81.5 ± 3.66</td>
<td>214.3 ± 46.5b</td>
<td>2.59 ± 0.49b</td>
</tr>
<tr>
<td>4</td>
<td>Tumor</td>
<td>5.7-9.0</td>
<td>5</td>
<td>92.2 ± 6.5</td>
<td>137.8 ± 9.6</td>
<td>1.56 ± 0.21</td>
</tr>
</tbody>
</table>

Results of analysis of variance

<table>
<thead>
<tr>
<th>F</th>
<th>p</th>
<th>2.826</th>
<th>4.321</th>
<th>5.153</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>&lt;0.10</td>
<td>&lt;0.05</td>
<td>&lt;0.025</td>
<td></td>
</tr>
</tbody>
</table>

a Mean ± S.E.
b Significantly different from Groups 1, 2, and 4, p < 0.05.
c Significantly different from Groups 1, 2, and 4, p < 0.05.

Rate of Parenteral Glucose Intake on Gluconeogenesis in TB and NTB Rats. To help determine the mechanism for maintenance of normoglycemia in the TB and NTB rats, as shown in Table 3, we subsequently measured their gluconeogenesis activity. The assessment of gluconeogenesis was done by inhibiting gluconeogenesis with 3-mercaptopicolinate, and then taking blood samples for blood glucose and lactate levels 1 hr later. Table 4 summarizes the data and the analysis of the data from these measurements. A statistical analysis of variance of the percentage of change in plasma glucose and blood lactate levels from immediately before to 1 hr after the 3-mercaptopicolinate injection was performed. The analysis of TB and NTB rats on low glucose intake (0 to 3.8 g/100 g/day) and high glucose intake (5.7 to 9.5 g/100 g/day) showed that the TB rats on high glucose had a significantly (p < 0.001) lower pH (7.07 ± 0.15) than the other 3 groups of rats (range, 7.86 ± 0.14 to 7.91 ± 0.16). The other 3 groups were not significantly different.

Rate of Parenteral Glucose Intake on Gluconeogenesis in TB and NTB Rats. To help determine the mechanism for maintenance of normoglycemia in the TB and NTB rats, as shown in Table 3, we subsequently measured their gluconeogenesis activity. The assessment of gluconeogenesis was done by inhibiting gluconeogenesis with 3-mercaptopicolinate, and then taking blood samples for blood glucose and lactate levels 1 hr later. Table 4 summarizes the data and the analysis of the data from these measurements. A statistical analysis of variance of the percentage of change in plasma glucose and blood lactate levels from immediately before to 1 hr after the 3-mercaptopicolinate injection was performed. The analysis of TB and NTB rats on low glucose intake (0 to 3.8 g/100 g/day) and those high glucose intake (5.7 to 9.5 g/100 g/day) revealed that the percentage of change in blood lactate in the TB rats was significantly higher than the change in blood lactate in the NTB rats with either high or low glucose intake. Analysis of the ratio of the change in lactate to the glucose in each rat showed that the TB rats on low glucose intake have a significantly higher ratio than all of the other groups of rats. These results gave the indication that TB rats on the low glucose intake rates were dependent on gluconeogenesis for glucose homeostasis, whereas the NTB rats on low glucose intake rates were not dependent on gluconeogenesis for maintenance of glucose homeostasis.

Rate of Parenteral Glucose Intake on Organ Weights and Body Composition in TB and NTB Rats. Table 5 summarizes the data and the analysis of data of 8 days of parenteral feeding at different glucose infusion rates on organ weights, expressed as a percentage of initial body weight for both the TB and NTB rats. Table 5 lists the results of a least-squares linear regression analysis between the glucose intake rate and the terminal organ weights for TB and NTB rats. To determine if the terminal organ weights of TB rats showed a different response to glucose intake rate than did the NTB rats, a statistical analysis for differences in the regression slopes was performed. Statistically different organ weight responses to glucose intake were determined to exist between the TB and the NTB rats in the epididymal fat pad, gastrocnemius muscle, and liver (see the bottom of Table 5). The regression slopes of the heart weights were not shown to be significantly different between the TB and NTB rats.

Specifically, the weights of the fat pad, the gastrocnemius, and the liver in the TB rats were shown to be significantly more dependent on the glucose intake rate than was the case in the NTB rats.

Further assessments were made on the fat pad, the gastro-
cnemius, and the liver to better understand the nature of differences in the organ weights in TB and NTB rats. Histological cross-sections through the middle of the epididymal fat pad were examined in all rats. The number of fat cells in unit areas near the central vessels was scored with a microscope and with the aid of an ocular grid on coded histological slides. A statistical analysis of variance of the data showed a significant difference to exist, and a Student-Newman-Keuls multiple comparison test showed that a decrease in number of fat cells (adipocytes) per unit area occurred as the glucose intake rate was increased from zero to 5.7 g/100 g/day. No significant differences in numbers of fat cells occurred at higher glucose intake rates. Histological examination showed that the number of fat cells per unit area was related to the observed size of the fat cell. These results indicated that the individual fat cells of both TB and NTB rats decreased their size by loss of stored fat (lipolysis), and that this loss of fat is dependent on glucose intake at rates below 5.7 g/100 g/day.

Urinalysis showed a significant development of ketonuria in those TB rats with low glucose intake rates (0 to 3.8/100 g/day) during the last 2 to 3 days of the experiment, compared to TB rats on higher glucose intake or to any of the NTB rats (p < 0.001).

To further assess differences in the gastrocnemius muscle mass under different levels of glucose intake, the muscle from each rat was dried to a constant weight and the water content was calculated by subtraction of the dry weight from the wet weight. The results, expressed as percentage of water, are given in Table 6. Analysis of variance showed no significant differences in the water content between TB or NTB rats, and no significant differences between the low and the high glucose intake rate groups (Table 6). Analysis of the nitrogen content of these same muscles was performed as a means of determining protein metabolism. Table 6 lists the results of this study and a statistical analysis of the data. The analysis showed that the muscles from the group of TB rats on a low glucose intake (0 to 3.8 g/100 g/day) had significantly less nitrogen than the muscles from any of the other groups of rats. The results showed that under these experimental conditions, the maintenance of muscle nitrogen (protein) is dependent on adequate glucose intake rate (>3.8 g/100 g/day) in TB rats as compared to the NTB rats.

Histological sections were made on the left lobe of the liver of all rats in the study. One slice of histological sections was digested with α-amylase and another set of sections was treated the same, except that the α-amylase was omitted from the procedure. The slide sets were both stained with a PAS-staining reaction and were coded for analysis. No liver treated with α-amylase was found to have a positive PAS-staining reaction. PAS-positive staining was found in the livers of 3 of 10 TB rats and in the livers of 9 of 13 NTB rats. A PAS-positive staining reaction was found in 1 of 5 of the TB rats but in 7 of 9 NTB rats with glucose intake of >3.8 g/100 g/day. A PAS-positive staining reaction was found in none of the TB rats on zero glucose intake, but a positive PAS reaction was found in 4 of 5 NTB rats on zero glucose (this difference was significant at p < 0.05).

Histological evidence of liver cell vacuolization was seen in several TB and NTB rats with glucose intake rates >8.92 g/100 g/day, but was not observed in the livers of rats with lower glucose intake rates. Pieces of those livers that showed vacuolization were processed for histological staining with the lipid stain, oil red O. The vacuoles gave a positive oil red O staining reaction, and the vacuolization of the liver cells was attributed to fat.

**DISCUSSION**

The finding that tumor growth rate was not dependent on the glucose intake rate when all of the other nutrients were held constant was unexpected, based on our past studies on the rat Morris 7777 hepatoma system. These past studies repeatedly showed that total parenteral feeding of the TB rats stimulated tumor growth in comparison to TB rats given cereal-based stock chow ad libitum (7–9). Experimental results similar to those reported here have been reported by Buzby et al. (6). They, too, found that tumor growth rate was not dependent on the glucose intake rate when the intake of all other nutrients (amino acids and minerals) was held constant. Reasons that the tumor growth rate was not dependent on glucose intake rate can be proposed. As our present experimental findings (Table 3) show that the host maintained normoglycemia regardless of the glucose intake rate, it seems likely that growth of the tumor was not limited by the availability of blood glucose. Perhaps the ad libitum feeding of TB rats with a large rapidly growing Morris 7777 hepatoma with stock chow, as opposed to the total parenteral alimentation with high levels of amino acids, did not provide adequate amino acid intake to meet the needs of the growing tumor.

Further support for this idea is provided in a study by Buzby et al. (6), which showed that parenteral feeding of amino acids without glucose, as compared to a cereal-based stock chow ad libitum, stimulated tumor growth. It seems likely that when the size of the tumor and its nutrient demands reach a critical point, the plasma glucose level or perhaps the level of amino acids would become limiting to tumor growth. Singh et al. (24) have shown in rats that when the weight ratio between a transplantable sarcoma and the body weight reached a critical ratio of 0.31 to 0.50, the blood plasma glucose level became significantly lower. It seems likely that tumor growth rate might be limited by hypoglycemia under such extreme tumor conditions.

Let us briefly review and integrate the tumor-associated changes in host tissues and the metabolic abnormalities seen in response to the level of glucose intake. Drawings to help integrate and explain many of the observed findings of the present study are put forth in Chart 1. This chart depicts the effects of different levels of glucose intake rate on TB and NTB rats when the intake of all other nutrients was held constant. Remember
that TB and NTB rats were shown to maintain normoglycemia regardless of the glucose intake rate. Chart 1 indicates that the weight of the epididymal fat stores decrease by lipolysis in both TB and NTB rats as the glucose (energy) intake rate drops below 5.7 g/100 g/day. Apparently this lipolysis is occurring in response to the low calorie intake in the diet. That no ketonuria was found in NTB rats with little or no glucose intake, but was observed during the last 2 to 3 days in TB rats with no glucose intake, suggests that fatty acid oxidation was complete in NTB rats but may not have been complete in the TB rats. Perhaps the demand for glycerol for gluconeogenesis in the TB cachectic host may limit its availability for lipogenesis and be associated with incomplete oxidation of fatty acids, resulting in ketonuria. In fact it has been previously suggested that suppression of free fatty acid oxidation reflects utilization of glycrol for the acceleration of gluconeogenesis (21). That gluconeogenesis, as a source of glucose production, is an energy-inefficient process has been repeatedly suggested in TB individuals (1, 4, 5, 13, 14, 17, 20, 22-25, 27).

No evidence of significant gluconeogenesis was found in NTB rats with little or no glucose intake, and yet normoglycemia was maintained. In this case the NTB rats appear to mobilize and efficiently use lipid stores as a major energy source, and the NTB rats are apparently able to maintain normoglycemia without measurable gluconeogenesis, probably by lowering their energy requirements. In other words, glucose utilization may be very slow in this particular group of NTB rats. The sustained parenteral intake of amino acids, even with no glucose intake, supported maintenance of muscle protein in these NTB rats, unlike what one expects from NTB rats during sustained total starvation (4, 25).

As illustrated in Chart 1, muscle mass and muscle protein content (as measured by nitrogen content) were maintained in all TB and NTB rats, except those TB rats on 3.8 g/100 g/day or less of glucose. An implication of this finding is that the TB rats on low glucose intake have catabolized muscle protein to mobilize amino acids, such as alanine. Such amino acids are utilized for the accelerated gluconeogenesis observed in these same TB rats (Table 4), but are also utilized for tumor growth.

What causes the gluconeogenesis seen only in TB rats on low glucose intake? In addition to the possible control of gluconeogenesis: (a) by substrate levels; (b) by blood plasma glucose levels; (c) by the extent to which phosphate groups are attached (phosphorylation) to enzymes that synthesize glucose (which is usually controlled by rapidly acting hormones like glucagon); and (d) by effectors of several of the enzymes that synthesize glucose. Cohen et al. (11) have reported evidence that the slow-acting thyroxine hormone stimulates the rate of glucose production via gluconeogenesis. This stimulation involves the mitochondrial enzyme that converts α-glycerophosphate to dihydroxyacetone (glycerophosphate dehydrogenase), which is the next step in the gluconeogenic pathway. Studies on the role of thyroxine...
in the control of gluconeogenesis in TB and NTB individuals may therefore prove fruitful. Waterhouse (27) has recently reported that the metabolism of branched-chain amino acids, such as leucine, is not under normal control in malnourished cancer patients, and that this imbalance may be linked to the frequently observed high gluconeogenesis in cancerous individuals.

That uncontrolled gluconeogenesis is an important factor in cachetic cancer conditions is suggested by the fact that the blocking of gluconeogenesis in TB rats by an inhibitor of the enzyme phosphoenolpyruvate carboxykinase, combined with total parenteral hyperalimentation, proved successful in preventing cancer cachexia under experimental conditions (7, 15). How the host meets the ever-increasing nutrient needs of the growing tumor is at least partially explained by our findings; however, the mechanism causing these changes remain to be characterized. It is reasonable to expect that elucidation of the control of gluconeogenesis in TB individuals will add greatly to our understanding and to our treatment of cancer cachexia.

REFERENCES


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