Reversal of Tumor-Induced Biochemical Abnormalities by Insulin Treatment in Rats [1,2,3,4]

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ABSTRACT—In F344 rats bearing transplantable 3-methylcholanthrene (CAS: 56-49-5)-induced sarcomas, plasma concentrations of immunoreactive insulin were decreased following the development of mild or severe anorexia. Plasma levels of immunoreactive glucagon and lactate were elevated in severely anorectic tumor-bearing (TB) rats, while plasma glucose concentrations remained normal. Both groups of TB rats exhibited decreased plasma levels of serine, glutamine, citrulline, and tryptophan and increased concentrations of alanine. Plasma levels of proline and phenylalanine were also elevated in the severely anorectic TB rats. In a second experiment, 7 daily treatments with insulin corrected the anorexia for 6 days and increased body weights of TB rats. Plasma concentrations of lactate and immunoreactive glucagon were decreased, and the abnormal plasma concentrations of glutamine, proline, analine, and phenylalanine were altered toward normal following the insulin treatments. Therefore, these data are consistent with insulin treatments benefiting the TB host by 1) increasing feeding, 2) increasing body weight, 3) reducing tumor glycolysis and metabolism, 4) reducing gluconeogenesis, and 5) reducing host catabolism, while not stimulating tumor growth. Thus insulin therapy may have potential benefits in cancer treatment by shifting glucose metabolism toward the host and away from the tumor.—JNCI 1986; 77:497-503.

Anorexia and cachexia are common features of neoplastic disease that complicate therapeutic intervention. Although nutritional support of an anorectic patient can increase the supply of nitrogen and total calories to normal levels, there is some controversy as to whether the host benefits from this nutritional support (*1, 2*). Insulin resistance has been reported as one of the many biochemical abnormalities in cachectic hosts (*3*). TB rats also exhibit abnormalities in this hormone, with plasma concentrations of immunoreactive insulin being decreased (*4, 5*). Systemic administration of large doses of insulin has been observed to stimulate feeding in anorectic TB rats (*6*) and to increase host weight without affecting tumor growth (*7*). However, we have little information concerning possible biochemical anticachectic effects of insulin treatment of anorectic TB rats. To provide such data, in the present experiments we compared plasma concentrations of amino acids, glucose, lactate, glucagon, and insulin in insulin-treated normal and TB rats. The addition of another control group in which individual body weights were reduced by restrictive feeding to match the individual carcass weights (body wt minus tumor wt) of the TB rats allowed better separation of the biochemical changes due to undernutrition alone from those resulting from effects of the tumor.

MATERIALS AND METHODS

Experiment I.—The purpose of this experiment was to compare changes in plasma amino acids, glucose, insulin, and glucagon in TB rats during mild and severe anorexia with FF and MCW-PF control rats.

The subjects in this experiment were 32 adult (225-250 g), male, F344 rats (Charles River Laboratories, Wilmington, MA). These rats were divided into 4 weight-matched groups of 8 subjects each. Transplantable MCA (CAS: 56-49-5)-induced sarcomas were induced in 16 of these rats as previously reported (*8*) by the sc injection of 2×10^6 (0.2 ml) viable tumor cells. The other 16 rats received control injections of normal saline and were assigned to the FF or PF groups. TB and FF rats had ad libitum access to standard rat chow pellets, while each rat of the PF group was fed the amount of food consumed by its weight-matched TB pair during the preceding 24 hours. All rats had unlimited access to water. These rats were housed in individual plastic cages in a temperature- and humidity-controlled environment under a 12-hour light-dark cycle. Food intake, water intake, and body weight were monitored daily. All measurements of food were corrected for spillage, which was minimal. One group of 8 TB rats (TB-16) was sacrificed by decapitation 2 days after the development of significant anorexia (food intake, 1 g/100 g body wt less than FF controls), which occurred 14 days after the induction of tumors. Each of the remaining 8 TB rats (TB-28) was sacrificed during severe anorexia when food intake fell between 3 and 4 g/100 g body weight. One FF rat and 1 PF control rat were sacrificed along with each severely anorectic TB rat. For the reduction of the body weights of the PF rats to match the individual carcass weights of the TB rats, the food allowed these

ABBREVIATIONS USED: FF = freely feeding; MCA = 3-methylcholanthrene; MCW = matched carcass weight; PF = pair-fed; TB = tumor-bearing.

[1] Received August 29, 1985; revised March 31, 1986; accepted April 14, 1986.

[2] Supported by Public Health Service grant CA-36325 from the Division of Extramural Activities, National Cancer Institute.

[3] Preliminary reports of these results were presented to the American College of Surgeons in 1983 and 1984 and published in the *Surgical Forum* volumes 34:441-443 and 35:66-69.

[4] The National Research Council's guide for the care and use of laboratory animals was followed in all experiments.

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animals was gradually reduced on a daily basis. These carcass weights were determined as previously reported (*9*) by estimating tumor weights (*TW*) from planar measurements of tumor length (*L*) and width (*W*) and applying the empirically derived formula *L* (mm) × *W* (mm) × 0.0199 = *TW* (g).

After the rats were decapitated, blood was collected from the neck wound and centrifuged (1,500×g, 4°C) for 15 minutes. For the determinations of free amino acid concentrations, 0.5 ml of plasma was deproteinized by 1.5 ml of 5% sulfosalicylic acid (pH 1.7), containing thienylalanine as an internal standard. After whirling, the samples were centrifuged (30,000×*g*, 4°C) and filtered (0.45 μm). Amino acid levels were quantified on this filtrate (50 μl) with an automated Beckman 121-MB amino acid analyzer employing a three-buffer, single-column, lithium citrate system. Plasma concentrations of glucose were determined by the glucose oxidase method (*10*). Concentrations of insulin in the plasma (200 μl) were assayed by radioimmunoassay (*11*) employing [^{125}I]insulin (New England Nuclear Corp., Boston, MA) as a tracer and antibody GP25 (*12*). Plasma glucagon levels were also determined by radioimmunoassay, with glucagon being radioiodinated (^{125}I) with the use of chloramine-T (*13*). Labeled glucagon was purified by chromatography on an SP-C25 column (*14*), and immunoreactivity was measured in ethanolic extracts of plasma (200 μl) with antibodies specific to the C-terminal and the N-terminal to central region of the peptide (*15*).

Experiment II.—This second study was conducted to assess the effects of daily insulin administration on food intake, body weight, and biochemical parameters in TB and control rats.

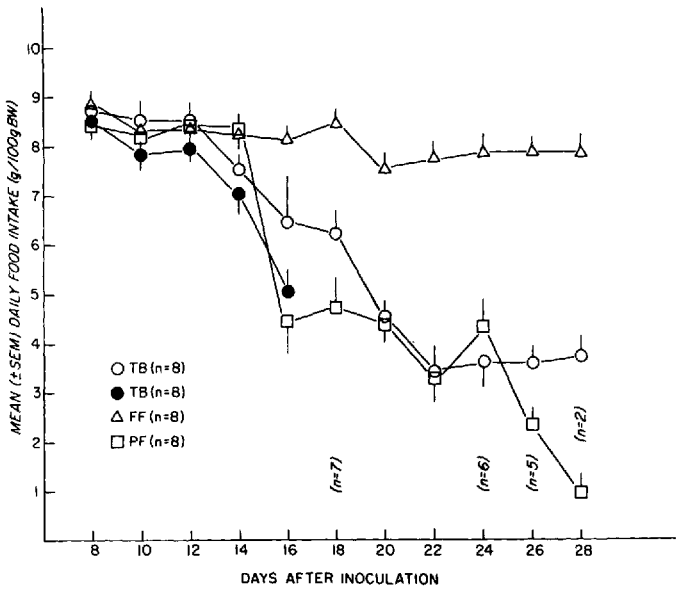
Sarcomas were induced by MCA in 36 male F344 rats (225-250 g). An additional 26 rats were subjected to control manipulations. These animals were maintained under environmental conditions identical to those of the first experiment. Equal numbers of individual rats with MCA-induced tumors were treated either with NPH insulin (17-20 U/kg/day, sc; E. R. Squibb & Sons Inc., Princeton, NJ) or with saline for 7 days, beginning 2 days after the onset of anorexia (food intake decreased by 1 g/100 g body wt). Similar treatments were administered to 2 groups of non-TB control animals at the same time. After 7 days of treatment, the animals were decapitated. The plasma of these rats was subjected to the same biochemical analyses as in the first study. In addition, plasma concentrations of lactate were determined in this study by the lactate oxidase method.

Statistical analyses.—Statistical evaluations were accomplished with the use of analysis-of-variance techniques, with individual means compared by *t*-tests.

RESULTS

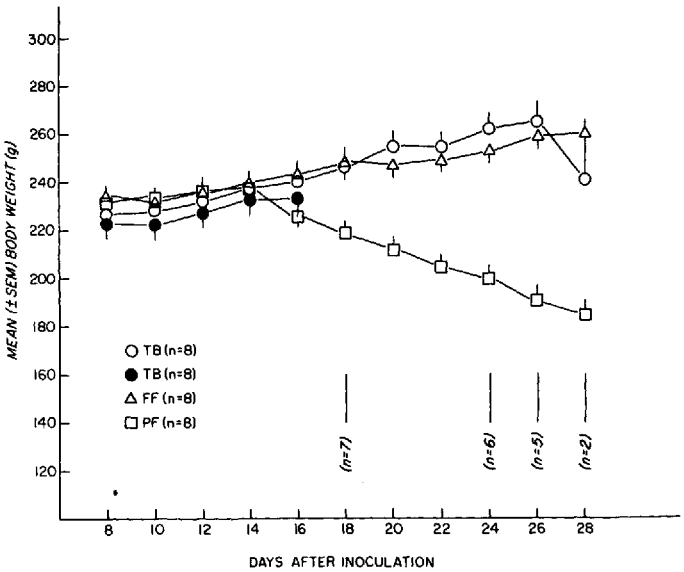
Experiment I

As may be observed in text-figure 1, 1 group of TB rats exhibited significantly decreased food intake 14 days after tumor induction and was sacrificed on day 16. The



TEXT-FIGURE 1.—Mean daily food intake by TB, FF, and MCW-PF (PF) control rats. Eight TB rats (*solid circles*) were sacrificed during mild anorexia, while the remaining TB rats (*open circles*) were sacrificed after the development of severe anorexia. BW = body wt.

remaining TB rats exhibited statistically significant ($P<.01$) anorexia as a group on day 17. Individual TB rats met the severe anorexia criterion on days 18, 24, 26, and 28 and were sacrificed accordingly, along with the appropriate control rats. There was no difference in body weights between the TB and FF groups (text-fig. 2). However, the body weight loss by the MCW-PF group was a good indication of the decrease in host weight of the TB-28 group. At sacrifice, these rats had a mean tumor weight of 71 ± 6 g, which constituted 28%



TEXT-FIGURE 2.—Mean daily body weights of mildly anorectic (*solid circles*) and severely anorectic (*open circles*) TB rats as well as FF and MCW-PF (PF) control rats.

TABLE 1.—*Mean (± SEM) plasma concentrations of glucose, immunoreactive insulin, and pancreatic (C) and total (N) glucagon in FF and MCW control rats and in MCA-induced sarcoma-bearing rats sacrificed during mild (TB-16) or severe (TB-28) anorexia*

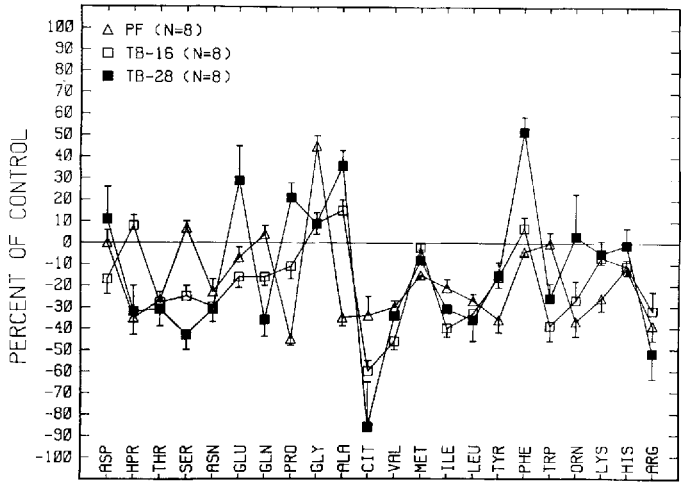
Group	No.	Glucose, mg/100 ml	Insulin, μU/ml	C-glucagon, pg/ml	N-glucagon, pg/ml
FF	8	129±3	3.6±0.6	73±8	171±16
MCW-PF	6	102±6[a]	1.3±0.1[a]	51±11	107±5[a]
TB-16	8	122±3	2.2±0.2[b]	86±14	176±12
TB-28	7	118±9	1.3±0.1[a]	169±15[a]	233±26[b]

[a] $P<.01$ vs. FF.
[b] $P<.05$ vs. FF.

of their total body weight. This tumor burden yielded a mean carcass weight of 183 g and closely matched the body weight of the PF group (184 g). The rats that were sacrificed during mild anorexia had a mean tumor weight of 22±2 g, constituting 9% of their total body weight (233±6 g).

As indicated in table 1, plasma levels of glucose were significantly ($P<.01$) decreased in MCW-PF rats but were not altered in either TB group. Plasma concentrations of immunoreactive insulin were significantly decreased in PF, TB-16, and TB-28 rats (table 2). Levels of N-terminal immunoreactive glucagon (G_n) were significantly reduced in the PF rats, while both C- and N-terminal immunoreactive glucagon concentrations were significantly increased in the severely anorectic TB-28 rats (table 1).

Significant tumor-specific changes in plasma amino acid concentrations may be observed in text-figure 3. Alterations in amino acid levels in TB rats that are not observed to the same degree in the MCW control rats appear to be tumor-specific in that they do not result from undernutrition alone. Thus both groups of TB rats exhibited significantly ($P<.01$) elevated levels of alanine and decreased plasma concentrations of serine, glutamine, citrulline, and tryptophan. In addition, the severely anorectic TB rats had significantly elevated levels of proline and phenylalanine. Plasma amino acid alterations that appear to be due to undernutrition included decreases in hydroxyproline, threonine, asparagine, proline, alanine, citrulline, valine, leucine, isoleucine, tyrosine, ornithine, lysine, and arginine. Only glycine concentrations were elevated in the MCW control animals.

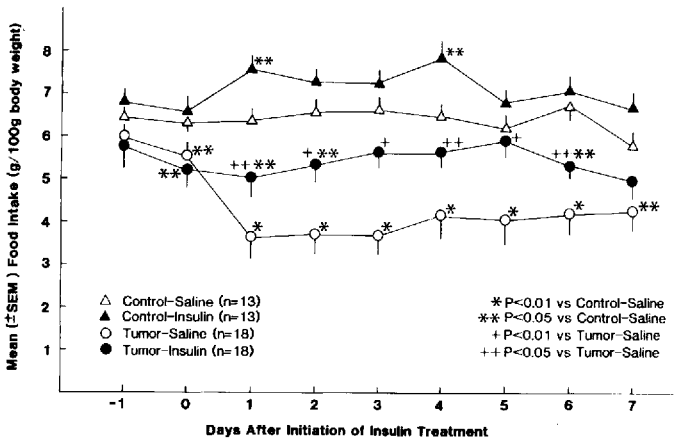


TEXT-FIGURE 3.—Mean (± SEM) plasma concentrations of amino acids in mildly anorectic (TB-16), severely anorectic (TB-28), and MCW-PF (PF) rats expressed as percentages of FF control values. ASP = aspartic acid; HPR = hydroxyproline; THR = threonine; SER = serine; ASN = asparagine; GLU = glutamic acid; GLN = glutamine; PRO = proline; GLY = glycine; ALA = alanine; CIT = citrulline; VAL = valine; MET = methionine; ILE = isoleucine; LEU = leucine; TYR = tyrosine; PHE = phenylalanine; TRP = tryptophan; ORN = ornithine; LYS = lysine; HIS = histidine; ARG = arginine.

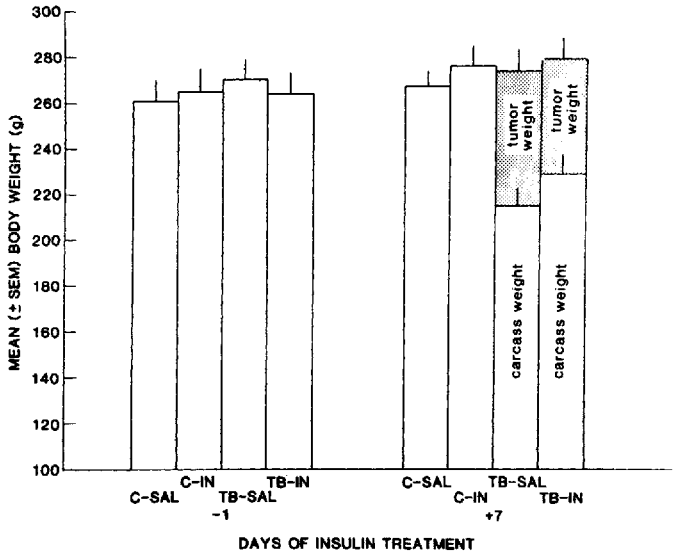
Experiment II

The purpose of this experiment was to investigate which of these tumor-induced biochemical abnormalities could be reversed toward normal by the daily administration of insulin. Text-figure 4 illustrates that the daily injection of insulin significantly increased food intake in anorectic TB rats for 6 days. After the third dose of insulin, there was no significant difference in food intake between TB and saline-treated control rats. However, by the 6th treatment day, the insulin-treated TB rats were again consuming significantly less food than were the control rats.

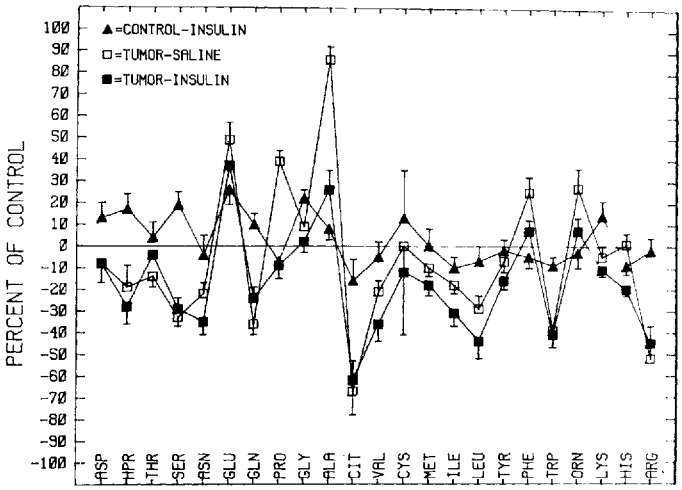
As indicated in text-figure 5, the insulin-treated TB rats gained significant ($P<.01$; paired t-test) body weight during the insulin treatments, while the body weights of



TEXT-FIGURE 4.—Mean daily food intake by TB and control rats following the daily administration of saline or insulin.



TEXT-FIGURE 5.—Mean body weights of TB and control (C) rats prior to and following 7 days of treatment with saline (SAL) or insulin (IN). Body weights of TB rats on day 7 are separated into carcass and tumor weights.



TEXT-FIGURE 6.—Mean (± SEM) plasma concentrations of amino acids in TB and control rats treated with insulin or saline 24 hr previously. Data are expressed as percentages of saline-treated FF control values. *See* legend to text-fig. 3 for explanation of abbreviations. CYS = cysteine.

the saline-treated TB group were not significantly increased during this period. The insulin-treated TB rats also exhibited a slight increase in carcass weight (229±8 g vs. 215±9 g) and a moderate decrease in tumor weight (50±4 g vs. 59±5 g) as compared with the carcass weight and tumor weight in the saline-treated TB rats. However, neither of these differences was statistically significant.

Plasma levels of glucose were significantly increased in the insulin-treated control rats and somewhat decreased in the TB rats 24 hours after the last insulin injection (table 2). TB rats also exhibited significantly ($P<.01$) increased plasma concentrations of lactate (table 2), which were significantly ($P<.01$) reduced by the insulin treatments. Twenty-four hours after the last insulin injection, plasma levels of immunoreactive insulin were still elevated in insulin-treated TB and control rats (table 2). As in the first experiment, plasma concentrations of both C-terminal and N-terminal immunoreactive glucagon were significantly ($P<.01$) elevated in anorectic TB rats. However, treatment with insulin significantly ($P<.05$) reduced both measures of glucagon toward normal (table 2). Plasma amino acid alterations in anorectic TB rats that were determined by the first experiment to be tumor specific were observed again in the present study (text-fig. 6). Thus concentrations of serine, glutamine, citrulline, and tryptophan were decreased, while levels of proline, alanine, and phenylalanine were elevated in anorectic TB rats. Treatment with insulin significantly ($P<.01$) altered glutamine, proline, alanine, and phenylalanine toward normal (text-fig. 6).

DISCUSSION

Alterations in plasma concentrations of peptides, amino acids, and glycolytic metabolites in anorectic TB animals result from the complex interaction of several factors. The presence of a malignant tumor places demands on the host to supply nutrients to the neoplastic tissue. Although the tumor appears to prefer glucose as a fuel (*16*), a portion of which it metabolizes to lactate (*17*), the host does not meet this demand by increasing food intake. Rather, the host supplies nutrients to the tumor by less efficient means through catabolism of fat (*18*) and lean body tissue (*19*) to form glucose from glycerol and free amino acids released by muscle and by recycling the lactate manufactured by the tumor (*20*).

TABLE 2.—*Mean (± SEM) plasma concentrations of glucose, lactate, immunoreactive insulin, and pancreatic (C) and total (N) glucagon in TB and FF control rats treated with insulin (I) or saline (S) for 7 days*

Group	No.	Glucose, mg/100 ml	Lactate, mg/100 ml	Insulin, μU/ml	C-glucagon, pg/ml	N-glucagon, pg/ml
FF-S	13	122±3	25±3	49±6	64±6	78±12
FF-I	13	154±10[a]	30±4	65±14	71±7	79±10
TB-S	18	116±6	69±5[a]	51±7	138±15[a]	141±11[a]
TB-I	18	95±14	41±3[b,c]	70±10	92±12[d]	109±5[d]

[a] $P<.01$ vs. FF-S.
[b] $P<.01$ vs. TB-S.
[c] $P<.05$ vs. FF-S.
[d] $P<.05$ vs. TB-S.

The net result of these catabolic alterations is the cachexia of cancer. Therefore, plasma concentrations of these various compounds result from host metabolism, the effects of anorexia, catabolism of fat and lean tissue, products taken up and released by the tumor, and hormonal alterations associated with these manifold changes.

Many biochemical alterations observed in the plasma of anorectic TB rats in the present experiments are consistent with increased catabolism of muscle, redistribution of nitrogen, undernutrition, and elevated gluconeogenesis. Plasma amino acid alterations characteristic of undernutrition include reduced concentrations of the branched chain amino acids (valine, leucine, and isoleucine) in the TB and MCF-PF groups as well as decreased alanine and increased glycine in the PF group (*21*). Increased concentrations of alanine in the TB rats may reflect increased release of this amino acid from catabolic muscle (*21*). However, in experiments that measured arteriovenous differences of in vivo tumors, Sauer et al. (*22*) reported that Walker 256 carcinosarcomas released alanine into the venous circulation. Therefore, alanine synthesized by the tumor may contribute to the elevated plasma concentrations of this amino acid in anorectic TB rats. These authors also report that each of the tumor strains investigated removed large amounts of glutamine from the arterial blood. This removal of blood glutamine accounts for the reduced plasma concentrations in TB rats in the present study and is consistent with previous reports of glutamine (*23*) and nitrogen (*24*) uptake by tumor tissue.

Immunoreactivity to both pancreatic (G_c) and total (G_n) glucagon was significantly increased in the severely anorectic TB rats. This elevation in glucagon levels is reflective of increased gluconeogenesis, which should increase blood glucose levels in response to the demand of the tumor. Increased gluconeogenesis has been reported in TB rats even prior to the onset of overt cachexia (*25*). The major amino acid precursors for the synthesis of glucose are alanine, threonine, serine, glycine, proline, glutamine, and asparagine (*26*). Each of these amino acids with the exception of serine, glycine, and glutamine was decreased in the MCW-PF rats. In TB rats, levels of all of these amino acids were decreased with the exception of the levels of proline, glycine, and alanine. This pattern of gluconeogenic amino acids is similar to that previously reported for rats bearing Walker 256 carcinosarcomas (*27*). Reasons why all the gluconeogenic precursors are not reduced in these rats may be partial adaptation to undernutrition by the PF rats in the case of glycine (*28*) and the increased catabolism of muscle in the TB rats as well as the release of amino acids by the tumor itself. Thus TB rats may be making glucose from alanine, but the increased release of alanine from muscle and tumor may more than compensate for any reductions due to gluconeogenesis.

Amino acids associated with the urea cycle (ornithine, citrulline, and arginine) were also reduced in TB and MCW-PF rats. Plasma levels of ornithine were significantly reduced in early anorexia and returned to normal when the rats were severely anorectic, while reductions in citrulline and arginine became greater as the anorexia increased. Again, similar decreases in citrulline and arginine concentrations have been reported in plasma of rats bearing Walker 256 tumors (*27*). However, no alterations in these amino acids were reported for rats that were PF as controls. This difference emphasizes the need for a more stringent control for undernutrition, as we have employed in the MCW group in the present study. Therefore, these differences in urea cycle amino acids may result from undernutrition with concomitant reduction in excess nitrogen to be excreted as urea. Sarcoma-bearing mice have also been reported to excrete 50% less urea than control mice (*29*). This reduction in urea may reflect the tumor's affinity for nitrogen as indicated by the tumor's increased uptake of glutamine and other amino acids (*22*).

Anorectic TB rats also exhibited decreased plasma concentrations of tryptophan. Since the majority of plasma tryptophan is normally bound to albumin (*30*), this decrease in tryptophan levels is probably due to a reduction in plasma albumin levels (*31*) and displacement of tryptophan from albumin by increased concentrations of free fatty acids (*32*). We (*31*) and others (*27*) have observed decreased concentrations in total plasma tryptophan and increased levels of free (unbound) tryptophan in both Walker 256 and MCA-induced tumor strains.

The many biochemical abnormalities associated with cancer cachexia contribute to and may cause the anorexia of cancer. A recent report (*33*) employing a parabiotic model, in which TB and control rats shared a small portion of circulating blood, demonstrated reduced food intake in the non-tumor-bearing half of the pair as anorexia developed in the TB partner. Thus the factors that contribute to cancer anorexia appear to be humorally mediated.

The daily administration of NPH insulin to anorectic TB rats effectively stimulated feeding for 6 days. This feeding effect of insulin on anorectic TB rats has been reported previously for several different tumor strains (*6, 7, 34*). At present, we are uncertain why the feeding effect in rats with MCA-induced tumors can be maintained for only 6 days. Our attempts to maintain insulin-induced feeding in TB rats for longer periods have invariably resulted in the death of the animals. Additional unpublished results from our laboratory indicate that insulin still elicits hypoglycemia even when it no longer stimulates feeding in TB rats. Therefore, adaptive changes in the central nervous system to the glucoprivation may occur after several days of insulin treatment.

Following the insulin treatments both TB and control rats gained significant body weight, while the difference in body weight gain across the 7 days of the experiment for the saline-treated TB group was statistically nonsignificant. Although the tumor weights were slightly lower and the carcass weights were slightly higher in the insulin-treated TB group, neither of these differences was statistically significant. Therefore, it

appears that insulin treatment stimulated feeding and led to significant increases in body weight in TB rats without stimulating tumor growth. Similar effects of insulin on food intake, body weight gain, and tumor growth have been reported recently by other laboratories employing a variety of transplantable tumors (*7, 34*).

Insulin also had several significant effects on the biochemical perturbations observed in severely anorectic TB rats in the first experiment. Plasma levels of lactate were significantly reduced in the insulin-treated TB rats. Since lactate is a major product of glycolysis in the tumor (*17*), we suggest that insulin reduces aerobic glycolysis in tumor tissue by depriving the tumor of glucose. Thus in the presence of large doses of insulin the available glucose is driven into muscle and relatively less is available for tumor use. This effect of insulin may be a major reason why weight can be gained by the host while not stimulating tumor growth rate. Considering this effect of insulin, its addition to parenteral nutrition sources may correct the increase in tumor growth observed with conventional hyperalimentation techniques (*35*).

Insulin treatment may also have reduced muscle catabolism in TB rats, since the concentrations of several of the abnormally elevated amino acids (proline, alanine, and phenylalanine) were reduced by the insulin treatments. Insulin has been reported to reduce plasma amino acid concentrations by increasing their uptake into muscle and by reducing amino acid efflux from muscle (*21*). Although alanine is thought not to be greatly affected by insulin (*21*), it is apparent that the elevated concentration of alanine observed in TB rats was significantly reduced by the insulin treatments. Insulin also reduced the elevations in proline and phenylalanine and increased the low plasma levels of glutamine in TB rats. These effects could be direct effects of insulin on reducing muscle catabolism or may be secondary to the reduction in metabolism in tumor tissue. Therefore, glutamine uptake and alanine release by tumor tissue may be reduced in the presence of insulin.

Although we did not assess gluconeogenesis, the decreased levels of glucagon in the insulin-treated TB rats suggest that insulin also has reduced gluconeogenesis. Insulin has been reported to reduce the conversion of alanine to glucose in the perfused liver (*36*). Unger and Orci (*37*) have also suggested that insulin reduces plasma glucagon levels by direct inhibition of release at the level of the pancreas. Working with perfused livers, Cherrington et al. (*38*) reported that the effects of increased concentrations of insulin and glucagon are additive in reducing plasma levels of alanine. The drop in alanine is due to the reduction in release of alanine from peripheral tissue, which is due to insulin, and the increase in alanine extraction by liver, which is stimulated by glucagon. They further reported that the ability of increased glucagon to stimulate gluconeogenesis in the liver was blocked by the addition of insulin, but the added insulin did not further reduce the gluconeogenetic rate to below control levels.

Therefore, these experiments suggest that exogenous insulin has several potential benefits for the TB host. The anorexia of cancer is blocked for a period of time, weight may be gained by the host without stimulating tumor growth, aerobic glycolysis and metabolism by the tumor may be reduced, catabolism of lean body tissue may be lessened, and rate of gluconeogenesis may be lowered. Future research should be directed toward the determination of whether lowered doses of insulin will have similar beneficial effects for the TB host.

More importantly, these studies demonstrate that alterations in metabolism in animals with tumors are not inexorable and that metabolic changes to favor the host rather than the tumor may be delivered by alterations in substrate.

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