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Regulation of Energy Metabolism in Morris Hepatoma 7777 and 7800[1]

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ABSTRACT

The pathway of fat oxidation in two experimental hepatomas was studied in order to demonstrate that a specific deficit in the energy metabolism of a tumor might contribute to the cachexia of the host. Forty-eight male Buffalo rats were divided into four groups of 12 each. One group was implanted s.c. with Morris hepatoma 7777 and one group was implanted with Morris hepatoma 7800, whereas the other two groups served as controls. All groups were fed standard rat chow diet *ad libitum* until the tumors reached 2 cm in diameter. The animals were then fasted for 24 hr prior to sacrifice and excision of tumor and liver for assays. During the period of tumor growth, the animals bearing the 7777 hepatoma lost weight, but the weight of the 7800 hepatoma-bearing rats did not differ significantly from that of the control animals. The livers of both groups of animals showed evidence of fatty acid oxidation *in vivo* and *in vitro*, and, as expected, during fasting, pyruvate dehydrogenase was inactivated and the rate of fatty acid synthesis was low. A qualitatively similar picture was seen with the better-differentiated 7800 hepatoma. In contrast, the 7777 hepatoma exhibited low levels of fatty acyl coenzyme CoA, no appreciable activity of carnitine palmitoyl transferase and fortified homogenates of the tumor were unable to oxidize palmitate. In keeping with these observations, pyruvate dehydrogenase remained in the active form, and fatty acid synthesis continued unabated in the fasted state in these tumors. Ketone bodies could not be oxidized by fortified homogenates of the liver or by either tumor, probably due to the lack of 3-ketoacid thiotransferase, which was undetectable in these tissues. We hypothesize that flow-through pyruvate dehydrogenase during fasting in Morris hepatoma 7777, occurring as a result of the defect in fat oxidation, contributes to the weight loss of these animals.

INTRODUCTION

The principal pathway for ATP synthesis in tissues with mitochondria requires substrate oxidation in the citric acid cycle coupled with flux of reducing power down the electron transport system. Acetyl-CoA, the substrate for the citric acid cycle, can be derived from 2 major groups of precursors, fat-derived substrates or pyruvate precursors. In order to oxidize the latter, the fuel must be metabolized via PDH.[3] PDH is a multienzyme complex which has 2 major types of control, feedback inhibition of PDH by its products and conversion of PDH to an inactive form via phosphorylation. As a general rule, the activity of PDH is decreased when fat is being oxidized (for review, see Ref. 9, 12, 18, and 20).

Morris hepatoma 7777 is a poorly differentiated and rapidly growing transplantable tumor. It grows rapidly but locally, resulting in the death of the host, usually within 4 to 5 weeks after implantation (for review, see Ref. 16). Bloch-Frankenthal *et al.* (4) have shown that homogenates of this tumor could not oxidize fatty acids readily, and we have shown that PDH activity of this tumor was not decreased during fasting (11).

Since PDH is the critical enzyme for the regulation of carbohydrate oxidation (9, 12, 18, 20), the purpose of this study was to evaluate PDH control in 2 different Morris hepatomas with different degrees of differentiation in order to define the factors that might regulate PDH in these tumors. The results to be reported indicate that there is an inverse correlation between the capacity for fat oxidation and PDH activity in these tumors. The implications of these findings with respect to weight loss in the host are discussed.

MATERIALS AND METHODS

Rats. Forty-eight male Buffalo rats (Simonson Laboratories, Gilroy, Calif.) weighing 243 ± 3 (S.E.) g received 2 s.c. Morris hepatoma 7777 or 7800 implants. The tumors are nonmetastasizing transplantable hepatomas initially arising in this strain of rats after treatment with the alkylating agent, 2-*N*-fluorenylphthalamic acid. Untreated, these tumors grow rapidly, reaching a size of about 10% of body weight within 4 to 5 weeks (for review, see Ref. 16).

All animals were allowed free access to Purina laboratory chow and water and were weighed 3 times/week. When the tumor measured approximately 2 cm in diameter, food was withheld for 24 hr, and the tumor-bearing and control rats were anesthetized with diethyl ether prior to excision of the tumors and the liver. The tumor was dissected free of surrounding normal tissue and necrotic areas, and one portion was frozen in liquid nitrogen for enzyme and metabolite assays. A second portion was used for the substrate oxidation studies.

Fat Oxidation. The liver or tumor was homogenized in ice-cold 250 mM mannitol (500 mg tissue per ml), filtered through a nylon mesh, and used as quickly as possible. A portion of the homogenate corresponding to 100 mg of tissue was incubated for 20 min at 37° in a volume of 2 ml containing 50 mM potassium phosphate buffer (pH 7.4), 4 mM ATP, 95 mM potassium chloride, 3 mM magnesium sulfate, 1 mM NAD, 0.1 mM sodium palmitate, 0.5 mg of cytochrome *c* per ml, 20 mg of albumin per ml, and 0.1 μCi of sodium [*U*-^{14}C]palmitate per ml. The assay was terminated by the addition of 0.5 ml of 10% perchloric acid, and the $^{14}CO_2$ produced was trapped in center wells on filter paper impregnated with Hyamine hydroxide. The water-soluble products of fat oxidation were obtained by ex-

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tracting the radioactive lipid with 4 washes of petroleum and diethyl ether (95:5) (2 ml/ml of incubation medium). Controls were done with perchloric acid added prior to the incubation.

Acetoacetate Oxidation. A portion of the tissue homogenate corresponding to 50 to 100 mg of tissue was incubated for 30 min at 37° in 2 ml of 50 mM potassium phosphate buffer (pH 7.4), containing in addition 3 mM magnesium sulfate, 4 mM ATP, 1 mM NAD, 5 mM acetoacetate, 0.5 mg of cytochrome *c*, and 0.1 μCi of [3-^{14}C]acetoacetate per ml of incubation medium. The [3-^{14}C]acetoacetate ethyl ester was hydrolyzed immediately before use by incubation at pH 10 to 12 for 30 min at 45–60°. The assay was terminated with 0.5 ml of 10% perchloric acid, and the $^{14}CO_2$ produced was trapped in center wells on filter paper impregnated with Hyamine hydroxide. Assays were carried out on liver, kidney, and hepatomas in each rat. Controls were done with perchloric acid added prior to the incubation. Blanks were incubated in parallel but did not have tissue added.

Enzyme Assays. β-Hydroxybutyrate dehydrogenase was assayed as described previously (2), and the 3-ketoacid thiotransferase was measured by the method of Williamson *et al.* (26). Carnitine palmitoyltransferase was measured in the homogenate as described by Halperin and Pande (10), using the forward isotope exchange assay of Bremer *et al.* (6). The kinetics for the mitochondrial citrate transporter was determined as described by Cheema-Dhadli and Holperin (8). The contents of acid-soluble and acid-insoluble CoASH were determined by the method of Allred and Guy (1).

PDH Activity. Samples of liver and tumor from anesthetized animals were obtained for assay of PDH by *in situ* clamping with liquid nitrogen-cooled Wallenberger clamps. The samples were weighed and homogenized (125 mg/ml) in a 10 mM phosphate buffer (pH 7.4), containing 1 mM EDTA, 1 mM dithiothreitol, and 1% bovine serum albumin. The PDH activity was subsequently assayed in triplicate by incubation with [1-^{14}C]pyruvate, as described by Taylor *et al.* (24). The assays were linear for 3 min and directly proportional to the tissue weights from 75 to 300 mg/ml using 0.2 ml of tissue homogenate per 0.5-ml assay. All assays were done at a pyruvate concentration of 0.2 mM because higher concentrations of pyruvate resulted in an increase in PDH activity in the absence of magnesium and calcium. As described previously (11), complete activation was achieved by incubation with 3 mM Ca^{2+} and 10 mM Mg^{2+}. Blanks were run with addition of tissue-free media.

Chemicals. Cytochrome *c*, CoA, L-palmitoylcarnitine, DL-carnitine, glutathione, acetoacetyl-CoA, dithiothreitol, fatty acid-free bovine serum albumin, and pyruvate were obtained from Sigma Chemical Company, St. Louis, Mo. Thiamine pyrophosphate was obtained from Calbiochem, San Diego, Calif. NAD, sodium succinate, and ATP were obtained from Boehringer/Mannheim, New York, N. Y. Hyamine hydroxide, [1-^{14}C]pyruvate, L-[^{14}C]carnitine, and sodium [*U*-^{14}C]palmitate were obtained from New England Nuclear, Boston, Mass. The remainder of the chemicals were of the highest purity.

RESULTS

Change in Body Weight (Table 1). Control rats gained 2 to 3 g/day on *ad libitum* diet, and the weight gain was almost linear over the time of observation. In rats implanted with the weight over the 3-week period, whereas the rats bearing the Morris hepatoma 7800 gained weight at a rate comparable to the controls. It should be remembered that the total tumor weight at the time of sacrifice was approximately 8 g in these animals.

Table 1

Effect of tumor implantation on body weight

Rats were fed *ad libitum*. The control rats were matched for age and initial weight and were treated in an identical fashion except that no tumor was implanted.

Time after implantation (wk)	Body wt of rats (g)			
	Control	Hepatoma 7777	Control	Hepatoma 7800
1	267 ± 9[a]	263 ± 6	256 ± 9	257 ± 6
2	285 ± 10	257 ± 6[b]	270 ± 9	273 ± 6
3	303 ± 7	237 ± 6[b]	300 ± 9	288 ± 6
4			318 ± 9	309 ± 7
5			332 ± 9	310 ± 10

[a] Mean ± S.E. of 12 rats.
[b] $p < 0.01$ *versus* the control rats.

Fatty Acid Oxidation (Table 2). Rats were fasted for 24 hr prior to sacrifice. The rates of oxidation of palmitate to water-soluble products and $^{14}CO_2$ by fortified homogenates of liver and tumor are shown in Table 2. The rate of palmitate oxidation was comparable in the livers of both groups of tumor-bearing animals; therefore the results were combined. In homogenates of Morris hepatoma 7800, palmitate oxidation to these products occurred at a rate which was approximately one-third that in the liver, whereas there was almost no oxidation observed in homogenates of Morris hepatoma 7777. Consistent with these observations, there were proportionate reductions in acid-insoluble CoA and K_T (the citrate concentration required for half-maximal rates of citrate transport) for the mitochondrial citrate transporter in these hepatomas. Carnitine palmitoyltransferase activity was present in the liver and in Morris hepatoma 7800 but not in Morris hepatoma 7777. When acetate incorporation into fatty acids was determined in tissues from fed and fasted rats, the rate of fatty acid synthesis decreased on fasting by about 90% in the liver but by only 25% in Morris hepatoma 7800. There was no significant decrease in this rate in Morris hepatoma 7777 (2).

Ketone Body Metabolism. Acetoacetate oxidation by fortified kidney homogenates was linear over the 40-min period of incubation and was similar in rats bearing hepatoma 7777 and 7800 (results not shown). In contrast, there was no detectable $^{14}CO_2$ production in homogenates of the liver or of either hepatoma. Consistent with these observations was the absence of any detectable 3-ketoacid thiotransferase activity in these tissues, whereas it was observed in homogenates of cardiac muscle and of kidney (results not shown). There was considerable β-hydroxybutyrate dehydrogenase activity in liver (5.1 ± 0.7 units/g, wet wt; $n = 12$), whereas approximately one-fourth as much was found in Morris hepatoma 7800 and less than one-tenth as much was found in Morris hepatoma 7777.

PDH Activity (Table 3). The activity of PDH was measured in separate portions of liver and tumor from fasted rats bearing the above-mentioned Morris hepatomas. The activity of PDH was measured before and after incubation with 3 mM Ca^{2+} and 10 mM Mg^{2+}, conditions which result in activation of the enzyme *in vitro* (11). The percentage of enzyme present in the active form was low in the liver in the fasted animals; it was

Table 2

Metabolism of fatty acids in liver and hepatoma

For details, see "Materials and Methods." All data were derived from rats fasted for 24 hr except where indicated.

	Oxidation of palmitate[a]	Acetate to neutral lipid (ng atoms carbon/120 min/g wet wt)		Acid-insoluble CoA (pmol/mg protein)		Carnitine palmitoyltransferase[b]	K_T mitochondrial citrate transporter (μM)
		Fed	Fasted	Fed	Fasted		
Liver	365 ± 26[c]	400 ± 67	68 ± 11[d]	720 ±80	1050 ± 50[d]	N	497 ± 34
Hepatoma							
7777	4 ± 2	582 ± 47	545 ± 75	180 ± 20	190 ± 20	0	<75
7800	113 ± 15	280 ± 20	210 ± 20[d]	286 ± 30	320 ± 25[d]	1.5 N	264 ± 31

[a] Units for [*U*-^{14}C]palmitate oxidation in fortified homogenates are μmol converted to water-soluble products plus CO_2 over the 20-min incubation period.

[b] The isotopic exchange assay for carnitine palmitoyltransferase is recorded in dpm, an arbitrary number. We have called this value N for liver and have expressed the value in the tumors relative to that observed in liver.

[c] Mean ± S.E. of 12 rats.

[d] $p < 0.01$ for fed *versus* fasted.

Table 3

Activity of PDH in liver and hepatomas in fasted rats

PDH was measured as described in "Materials and Methods." There were 7 animals for the hepatoma 7777 series and 11 animals for the 7800 series.

	Activity					
	Liver			Hepatoma		
Rats with hepatoma implanted	Basal PDH (milliunits/g wet wt	Activated PDH (milliunits/g wet wt)	% in activated form	Basal PDH (milliunits/g wet wt)	Activated PDH (milliunits/g wet wt)	% in activated form
7777	13 ± 2[a]	141 ± 19	9 ± 1	28 ± 4[b]	54 ± 8	56 ± 7[b]
7800	11 ± 1	123 ± 11	9 ± 1	11 ± 2	90 ± 13	12 ± 2

[a] Mean ± S.E.

[b] $p < 0.01$ for the basal or percentage in the active form liver *versus* hepatoma.

also very low in the Morris hepatoma 7800. In contrast, the PDH in Morris hepatoma 7777 showed a much higher percentage of PDH in the active form.

DISCUSSION

Metabolism in the Fasted State. In the fasted state, normal individuals must consume fuels stored in the body for immediate energy needs. Of the available endogenous fuels, only adipose tissue triglyceride provides an energy store of any quantitative significance. Glycogen stores can satisfy the energy needs for less than 1 day. All body proteins appear to have specific functions (contractile or structural elements, enzymes, etc.); hence, fatty acid oxidation is essential for survival during prolonged hypocaloric states (for review, see Ref. 21). There are 3 primary sites of regulation of the pathway of fatty acid oxidation: (*a*) adipose tissue lipolysis (hormone-sensitive lipase); (*b*) the fatty acyl-CoA crossroad in the cytoplasm; and (*c*) the carnitine-mediated acyl group entry into mitochondria (Chart 1). As a result of oxidation of fat-derived substrates, the critical enzymes for carbohydrate and protein oxidation will be inhibited, the most important of these being PDH. In addition, fat oxidation leads to the inhibition of fatty acid synthesis (for review, see Ref. 3). Therefore, in order to evaluate the pathway of fatty acid oxidation, one can examine free fatty acid delivery, the tissue long-chain fatty acyl-CoA level, the carnitine-mediated pathway of fatty acyl group transfer into mitochondria, PDH activity, and the capacity for fatty acid synthesis.

Fat Oxidation in Liver and Hepatomas. In the livers of fasted rats bearing Morris hepatomas, the long-chain fatty acyl-CoA content was high and increased further on fasting (Ref. 11; Table 2). This suggests that there was an adequate supply of circulating free fatty acid substrates in these animals and that there was no defect in fat oxidation residing in adipose tissue. Fatty acid oxidation leads to inactivation of PDH (9, 12, 18, 20) and inhibition of fatty acid synthesis (3, 5, 13). Thus, the observations that PDH was in the inactive form and fatty acid synthesis was markedly suppressed in the livers from these fasted rats (Table 2) suggest that fat oxidation was active in this organ. Consistent with these findings were the appreciable levels of Z protein (15), carnitine palmitoyltransferase activity, and the oxidation of palmitate by fortified homogenates of liver (Table 2).

Bloch-Frankenthal *et al.* (4) had shown previously that fortified homogenates of liver could oxidize fatty acids, whereas those from Morris hepatoma 7777 were much less active. We have confirmed these observations and extended them to the Morris hepatoma 7800 series (Table 2). When fat oxidation was evaluated in the 2 hepatomas, it appeared that there was virtually no fatty acid oxidation in hepatoma 7777 in that fortified homogenates of this tumor did not oxidize palmitate nor was there a rise in the long-chain fatty acyl-CoA content during fasting (11); in fact, the long-chain fatty acyl-CoA content was very low in this tumor. Furthermore, both Z protein (15) and carnitine palmitoyltransferase activity (Table 2) were markedly reduced in the neoplastic tissue. In addition, there was no indirect evidence for active fatty acid oxidation which would have been suggested by a fall on PDH activity or an inhibition of fatty acid synthesis (3, 13, 15). In contrast, hepatoma 7800 had intermediate values between liver and hepatoma 7777 for the parameters measured. Palmitate was oxidized by fortified homogenates, the long-chain fatty acyl-CoA levels were about one-third of that in the liver, and the latter increased significantly upon fasting. Carnitine palmitoyltrans-

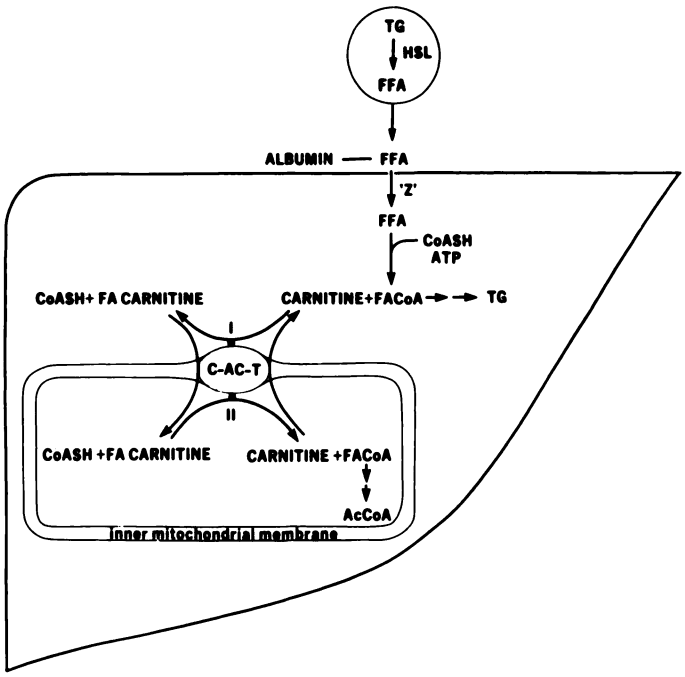

Chart 1. Pathway for fat oxidation. *Circle* at *top* represents adipose tissue, in which stored triglycerides (*TG*) are broken down by hormone-sensitive lipase (*HSL*), releasing free fatty acids (*FFA*). Free fatty acids are then bound to circulating albumin. The large structure represents the liver. Uptake of free fatty acids by the hepatocyte involves binding to Z protein (*Z*). In the cytosol, there is thioesterification of free fatty acids to form fatty acyl-CoA (*FACoA*), which stands at a metabolic crossroads in that it may serve as a substrate for triglyceride synthesis or it may be shuttled into the mitochondria for oxidation to acetyl-CoA (*AcCoA*). The carnitine-mediated transfer of the fatty acyl group across the inner mitochondrial membrane is depicted sequentially involving formation of fatty acyl carnitine (*FA carnitine*), transport of this species, and regeneration of FACoA, involving the carnitine acyl transferases I and II and the carnitine-acyl carnitine translocase (*C-AC-T*).

ferase activity was about 50% higher in hepatoma 7800 than in the liver (Table 2), whereas the Z protein was not much higher than in hepatoma 7777 (15). Indirect evidence for fatty acid oxidation was also evident in fasting rats bearing Morris hepatoma 7800 because PDH was inactivated to the same degree as in the liver. From the above data, it appears that hepatoma 7777 is unable to oxidize fat, whereas hepatoma 7800 is able to achieve appreciable rates of fat oxidation despite the fact that both Z protein and acid-insoluble CoA levels were both much lower than in the liver. In summary, during fasting, it appears that fat oxidation is an important pathway in the liver and in hepatoma 7800, whereas it does not play such a role in hepatoma 7777. In the following sections, we shall discuss the role of alternate fuels and the metabolic consequences of low rates of fat oxidation in a tissue during the fasted state.

Oxidation of Ketone Bodies. The first alternate fuel to consider is the other major fat-derived substrate, the ketone bodies. All tissues had the capacity to interconvert β-hydroxybutyrate and acetoacetate, but they are unable to oxidize acetoacetate, probably because 3-ketoacid thiotransferase was not detectable. Hence, the liver and these tumors would be unable to derive a significant number of calories from circulating ketoacids during the fasted state.

Oxidation of Proteins or Carbohydrates. In order to derive the majority of energy from the oxidation of proteins or carbohydrates, these nutrients must first be converted to pyruvate and then oxidized via PDH to acetyl-CoA, the substrate for the citric acid cycle. Only the ketogenic amino acids and a small component of certain glucogenic amino acids can be metabolized to produce ATP without requiring flux through PDH (27). It follows that the nonglycolytic ATP synthesis in Morris hepatoma 7777 during the fed and fasted state requires carbon flux through PDH. In contrast, in the liver during fasting very little carbon would flow through PDH, thus preserving the body protein content. There is yet a further drain of the body proteins and carbohydrates because of the specific metabolism of Morris hepatoma 7777. Since this tumor has an active fatty acid synthesis pathway both during the fed and fasted state (11), this could result in the conversion of protein to fat during fasting. Since this is an irreversible pathway, there will be the net loss of lean body mass during fasting as a result of carbon flux through this pathway.

Change in Body Weight in Hepatoma-bearing Rats. It is well known that animals bearing tumors usually reduce their dietary intake of calories (for review, see Refs. 14, 17, and 19); however, one cannot attribute the weight loss solely to this factor. Although the basal energy expenditure may be somewhat increased in animals with tumors, this factor also contributes to but cannot entirely explain the profound loss of weight (22, 23, 25). Characteristically, animals implanted with Morris hepatoma 7777 (Table 1) lost a considerable amount of weight but still had visible fat stores; hence, an important component of the marked weight loss observed must have come from a loss of lean body mass. One should appreciate that muscle, for example, had only 0.8 kcal/g, wet weight, whereas a similar weight of adipose tissue has more than 10 times as many calories (for review, see Ref. 21). Hence, if a fasted rat used 8 kcal in a time period and derived all of these calories from its lean body mass, the weight loss would have been 10 g, whereas only 1 g would have been lost if fat stores could have supplied all of this energy. Thus, the obligatory oxidation of nonfat precursors by Morris hepatoma 7777 may also contribute to the excessive weight loss of the host for the reasons outlined above. In contrast, the oxidation of fatty acids by Morris hepatoma 7800 and the consequent inactivation of PDH in these tissues would be expected to minimize the loss of lean body mass of these animals (Table 1). Clearly, the problem of cachexia in cancer is very complex, and many factors are involved in any given circumstance.

In summary, the rapidly growing poorly differentiated Morris hepatoma 7777 is not able to oxidize fat; hence, it must derive all of its calories from protein or carbohydrate sources and thus requires a considerable flux through PDH. During fasting, this would result in the loss of lean body mass and much larger weight loss per number of calories oxidized. In contrast, the slower-growing and better-differentiated hepatoma 7800 is able to convert its PDH to the inactive form during fasting, presumably due to the fact that this tumor can oxidize fatty acids at an appreciable rate. Hence, animals with this tumor show a much smaller and much later weight loss than do those bearing hepatoma 7777. Details of energy metabolism in other tumors must await further investigations but might provide a rationale for future therapy. For example, a pulse attack on PDH during fasting might place hepatoma 7777 at a disadvantage with respect to ATP synthesis. Other tissues can be spared by presenting alternate fat-derived substrates, minimizing the requirement for flux through PDH (prolonged fasting or

fat only diets). In a recent study (7), the consumption of a high-fat diet did lead to a relative decrease in tumor mass, possibly for the reasons outlined above.

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