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Blood Nutrient Concentrations and Tumor Growth *in Vivo* in Rats: Relationships during the Onset of an Acute Fast[1]

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ABSTRACT

The rate of tumor growth *in vivo* in adult rats (250- to 350-g total body weight) is stimulated during an acute fast. No tumor growth stimulation is observed in fasted immature rats (less than about 200-g total body weight). The different tumor growth responses in rats of these two age groups appear to depend on the increased availability to the tumor of nutrients from host fat stores in adult rats. Immature rats, which lack significant fat stores, show neither hyperlipemia nor ketosis during fasting. These experiments were performed to determine the relationship between blood fat store-derived nutrient concentrations and the onset of stimulated tumor growth in fasted adult rats. Animals were matched for tumor size and growth during a period of *ad libitum* feeding preceding the fast. Tumor growth was documented by increased size and incorporation of [*methyl*-^{3}H]thymidine into tumor DNA. Mobilization of host fat stores leading to increased blood concentrations of free fatty acids, glycerol, ketone bodies, and triglycerides started about 7 h after food was removed and reached its maximum after about 15 h. Increased rates of tumor growth and incorporation of thymidine into tumor DNA correlated closely with the higher circulating nutrient concentrations. Both the nutrient concentrations and tumor growth were decreased by refeeding. These findings suggest that the availability of nutrients derived from host fat stores may be rate limiting for tumor growth *in vivo*.

INTRODUCTION

In a previous paper we described the effect of an acute fast on tumor growth in adult and immature rats (1). Removal of food stimulated the rates of tumor enlargement and incorporation of [^{3}H]thymidine into tumor DNA in adult rats but had no effect on tumor growth in immature rats. New, faster tumor growth persisted in the adult rats as long as food was withheld but ceased at refeeding. The most important difference between these tumor-bearing rats appeared to be the lipolytic and ketotic responses that were observed in the adult rats; immature rats did not show these responses, apparently because they lack significant fat stores. We suggested that a host fat store-derived nutrient or other factor is rate limiting for tumor growth *in vivo* and that the concentration of this putative nutrient is increased in the blood of fasted adult tumor-bearing rats.

In this study we have examined relationships between nutrients mobilized from host fat stores and tumor growth. The kinetic values of increase in arterial blood free fatty acid, glycerol, ketone body, and triglyceride concentrations were compared with those in tumor DNA synthesis and growth in adult tumor-bearing rats during the onset of the fast. The kinetic values of decrease in these nutrient concentrations were also compared with that in the rate of DNA synthesis after refeeding. The results are further support for an important role of fat store-derived nutrients in tumor growth *in vivo*.

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MATERIALS AND METHODS

Animals, Tumors, and Tumor Transplantation. Adult and immature Harlan Sprague-Dawley rats were obtained from colonies established here. The rats were fed a standard laboratory chow (Charles River rat, mouse, and hamster formula; Agway, Inc., Syracuse, NY), had water *ad libitum*, and were maintained at a constant temperature of 23°C in a room with lights from 6 a.m. to 6 p.m. At the time of tumor implantation the adult rats were 3 to 5 mo old and weighed about 250 to 350 g, and the immature rats were 30 to 45 days old and weighed less than 175 g. All experiments were performed with the Jensen sarcoma, a fast-growing tumor that gains about 1.5 g/day in fed animals (1). This tumor was originally obtained from Dr. Artemio A. Overjera, DCT Tumor Repository, Frederick Cancer Research Facility, National Cancer Institute, Frederick, MD, and has been carried in our laboratory for about 3 yr.

Tumors were grown s.c. as tissue-isolated implants in the left inguinal fossa (2). A 3-mm cube of tumor was attached with a small suture to the end of a vascular stalk composed of the truncated superficial inferior epigastric artery and vein. The implant and the end of the stalk were enclosed in a parafilm envelope and placed beneath the skin, and the incision was closed. As the implant grows the vascular supply and venous drainage are through the epigastric vessels. Tissue-isolated tumors do not generally show large volumes of central necrosis as frequently as do ordinary s.c. implants, which must obtain their blood supply from the periphery.

Assays and Reagents. Blood samples were obtained by cardiac puncture from tumor-bearing rats lightly anesthesized with ether. Samples were drawn into syringes moistened with a saturated solution of disodium EDTA. Every attempt was made to draw blood from the left side of the heart. About 1 of 15 to 20 heart punctures did not yield oxygenated blood and was repeated. Acetoacetic and 3-hydroxybutyric acids and glucose were measured fluorometrically and spectrophotometrically, respectively, in perchloric acid extracts of the whole blood, as previously described (3). Glycerol and triglycerides were measured in plasma before and after enzymatic hydrolysis, respectively, by the method of Wahlefeld (4). Plasma free fatty acids were measured as described by Bergmann *et al.* (5) using chemicals obtained from Sigma and Eastman Chemical Co.

Arterial and tumor venous blood samples were collected *in situ* from heparinized, nembutal-anesthetized rats (2, 3). Plasma arteriovenous differences across the tumor for free fatty acids, glycerol, and triglycerides were calculated from the plasma concentrations and the blood plasma flow rates.

Measurement of Tumor Weight *in Situ*. Tumor mass in the living animal was estimated from measurements made through the skin (1, 6). The accuracy of this technique has been verified (1, 6). Host carcass weight was calculated by subtracting the estimated tumor weight from total animal plus tumor weight.

Experimental Design for Measurement of the Kinetics of Incorporation of [*methyl*-^{3}H]Thymidine. Tumors were implanted in 15 to 20 male rats of the same age and body weight. The matched animals were either littermates or were from different litters born on the same day, and the tumor cubes used as implants were taken from the same tumor. All inoculated rats were housed in a single large cage. After tumor growth was established, 8 to 12 rats were removed and placed in individual cages. Tumor weights *in situ* were estimated daily; the rates of tumor growth were very uniform in these matched animals (1). When the estimated tumor weights were 7 to 8 g, the animals were divided at random into two or three groups of four animals each. Food was removed from each animal in a group at 3 a.m. after a 9-h period of darkness and feeding.

The kinetics of tumor DNA synthesis was studied from the onset of the fast to 72 h postfast. The mean of the data from a group of four rats was a single time point. Animals in the zero time group were fed *ad libitum*, anesthetized with ether, and given injections of [*methyl*-^{3}H]-thymidine (1-μCi/g total body weight as a single injection in the external jugular). The neck incision was closed with a wound clip, and the animals were killed 1 h later (zero time). Animal groups for subsequent time points were treated similarly with injection of [^{3}H]-thymidine 1 h before sacrifice and tumor collection at the designated time after start of the fast. In some experiments animal groups were refed after 48 h of starvation. These rats were sacrificed, and the labeled tumors were collected at 2, 4, and 6 h after refeeding. Important time points were repeated; consequently, each time point represents the mean ± SD of from 4 to 12 matched animals.

The collected tumors were weighed, and a portion was fixed in formalin for histological examination and autoradiography. Another weighed portion was homogenized in cold saline with a Brinkmann polytron homogenizer for determination of DNA content and measurement of thymidine incorporation. The methods used were described previously (1).

Experimental Design for Measurement of Arterial Blood Nutrient Concentrations. Groups of matched, tumor-bearing rats were prepared as described above. A single arterial blood sample was collected by cardiac puncture from each of the four animals in a group. Collections were made from different groups at zero time and at subsequent time points after food was removed. The same time points were used as in the thymidine incorporation experiments.

Statistical Analysis. Significance of means was tested by Student's *t* test (7). Tumor and host carcass weights and analytical data are expressed as the mean ± SD.

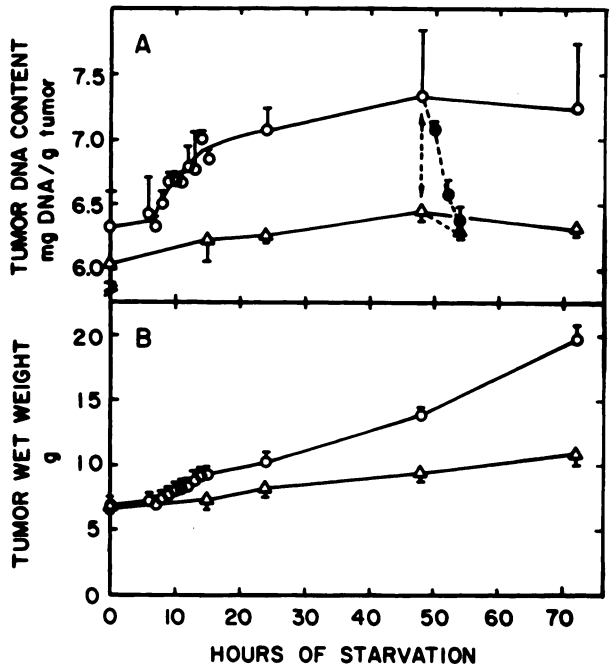

Fig. 2. DNA contents and wet weights of Jensen sarcomas removed from host rats before and at different times after removal of food. These are the same adult (○) and immature (△) animal groups shown in Fig. 1. *Points*, mean for 4 to 12 rats; *bars*, SD. Tumor protein contents (mg of protein/g of tumor wet weight) were 170 ± 18 at t = 0, 186 ± 25 at 12 h, 174 ± 28 at 24 h, 181 ± 17 at 48 h, and 178 ± 11 after 72 h.

RESULTS

Rates of Tumor DNA Synthesis during the Onset of an Acute Fast. The rates of DNA synthesis were measured in the Jensen sarcoma *in vivo* before and during onset of an acute fast and after refeeding. [^{3}H]Thymidine (1-μCi/g total body weight) was administered i.v. as a pulse 1 h before sacrifice of the host. Progress curves, which describe the kinetics of thymidine incorporation during these periods, are shown in Fig. 1. Data are presented for rates of DNA synthesis in Jensen sarcomas growing in adult and immature rats. Removal of food had no effect on the rate of tumor DNA synthesis in adult rats for the first 6 to 7 h. However, thymidine incorporation began to increase 8 h after onset of the fast and reached a value of about 1200 dpm/μg of DNA by 14 h. This rate was sustained for 72 h, as long as food was withheld in these experiments. During the first day of the fast, [^{3}H]thymidine incorporation per μg of tumor DNA and per g of tumor wet weight was increased 2.5 times. The shift from the rate seen in the fed animals (zero time) to the new rate occurred entirely within a period of from 7 to 15 h after the start of the fast. Fig. 2 shows that the tumor DNA content (mg of DNA/g of tumor wet weight) and rate of growth of tumor mass were also increasing during this period. Tumor protein content (mg of protein/g of tumor wet weight; see legend to Fig. 2), however, did not change. The water contents (100 minus dry weight/wet weight) of Jensen sarcomas growing in fed and fasted rats were 81.1 (n = 2) and 82.8 ± 2.6 (n = 15), respectively. Increases in the wet weights of Jensen sarcomas growing in adult rats were about 4 g/day during the fast (Fig. 2*B*) and about 1.5 g/day in fed, adult or immature rats (1) and fasted, immature rats (Fig. 2*B*).

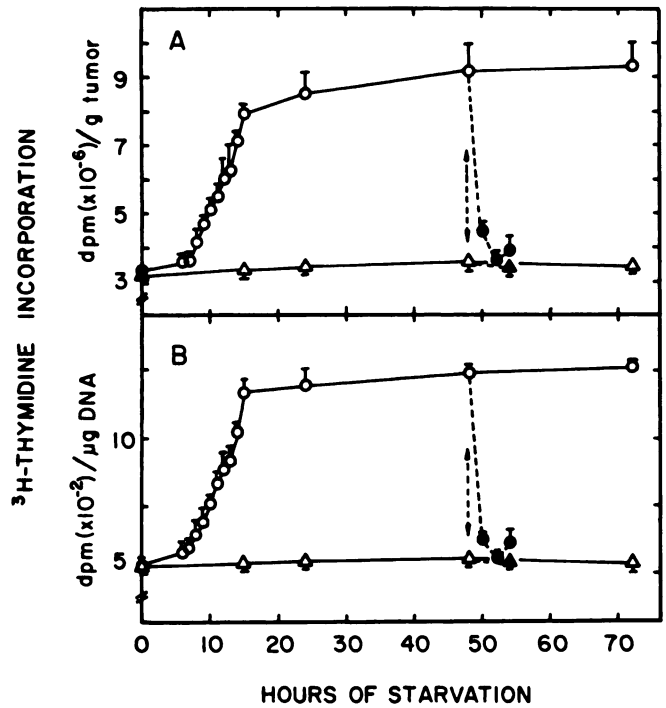

Fig. 1. Incorporation of [^{3}H]thymidine into the DNA of Jensen sarcomas *in vivo* measured before and at different times after removal of food from the host rats. Tumors were growing in adult (○) and immature (△) Sprague-Dawley rats matched for tumor and host body weights. Food was removed at zero time, but water was available *ad libitum*. The rats were pulse labeled with an i.v. injection of [^{3}H]thymidine (1 μCi/g body weight) 1 h before tumor harvest. Analytical and counting procedures were performed as described in "Materials and Methods." After 48 h of starvation some animal groups were refed for 2, 4, and 6 h (●, adult fasted-refed host rats; ▲, immature fasted-refed host rats). *Points*, mean for 4 to 12 tumors; *bars*, SD.

Jensen sarcomas growing in starved immature rats showed none of the changes in thymidine incorporation that were observed in adult animals. A smaller but significant ($P < 0.05$) increase in DNA content after 1, 2, and 3 days of starvation was seen in tumors growing in immature male rats (Fig. 2*A*).

Refeeding reversed the effects of an acute fast on the rate of tumor DNA synthesis and DNA content. An 80% inhibition of the [^{3}H]thymidine incorporation rate due to starvation occurred

Table 1 *[^{3}H]Thymidine incorporated per nucleus in tumors from 2 day fasted and 2 day fasted-2 h refed rats*

Jensen sarcomas, labeled with [^{3}H]thymidine *in vivo* during the experiment shown in Fig. 1, were prepared for autoradiographic analysis as described in "Materials and Methods." Tumors were collected from one group of host rats after 2 days of starvation and from a second group after 2 days of starvation and 2 h of refeeding. Two tumors were collected at each of these time points. The sections were exposed to the emulsion for 7 days. At least 500 labeled nuclei were counted under oil immersion in each section. Labeled nuclei contained 5 or more autoradiographic grains and were grouped depending on the number of grains/nucleus.

	No. of grains/nucleus (% of total cells counted)						Specific radioactivity of tumor DNA (dpm/μg)
	5–9	10–14	15–19	20–24	25–29	>30	
2 Day fasted rats	19.6	21.9	17.0	13.0	9.3	19.2	1210.0
	23.2	25.9	20.3	14.9	7.7	7.9	1225.8
2 Day fasted-2 hour refed rats	25.4	25.6	22.2	10.8	6.6	9.4	641.9
	34.8	27.5	14.1	9.6	5.8	8.5	636.8

2 h after eating was resumed (Fig. 1). Tumor DNA contents declined less abruptly but still returned to the levels observed in fed animals in about 6 h. The abrupt decrease in the rate of thymidine incorporation that occurred 2 h after refeeding suggests that less radioactive thymidine was incorporated per labeled tumor nucleus in the refed rats. Table 1 shows that there may be a trend toward fewer grains/nucleus in the fasted-refed rats. A greater number of observations, however, would be required to determine if the trend is significant. A decrease in the amount of [^{3}H]thymidine incorporated/nucleus in the fasted-refed rats could occur if the S phase of the cell cycle were longer in refed rats and/or if the thymidine specific activity available to the tumor were lower. Resolution of these possibilities is beyond the intended scope of this study and will be examined in subsequent experiments. We have already presented evidence which suggests that the thymidine specific activity available to the growing tumor is the same in fed and 2 day-fasted adult rats (1).

Glucose and Fat Store-derived Blood Nutrient Concentrations in Adult Rats following an Acute Fast. Glucose, ketone body, free fatty acid, glycerol, and triglyceride concentrations in arterial whole blood and plasma are shown in Fig. 3. The samples were collected from adult Jensen sarcoma-bearing rats before and during the onset of an acute fast. Blood levels of these nutrients were unchanged during the first 6 h of the fast, but the concentrations of the fat store-derived nutrients began to increase gradually after 8 h and reached maxima after 15 to 24 h. These values were sustained until the end of the period of starvation (72 h). Arterial blood glucose levels gradually declined throughout this period. Refeeding increased blood glucose concentrations and decreased the fat store-derived nutrient levels to those found in the fed rats (at the start of the fast). Similar results have been observed by others (8) in normal male Sprague-Dawley rats during fasting and refeeding. Blood nutrient concentrations were not examined in the immature tumor-bearing rats because we demonstrated earlier (1) that these younger rats show increases in neither blood free fatty acids nor ketone bodies during an acute fast. Blood glucose levels decrease, however, during starvation in immature rats (1).

Free Fatty Acid, Glycerol, and Triglyceride Utilization by the Jensen Sarcoma *in Vivo*. Measurements of the arteriovenous difference made across tissue-isolated Jensen sarcomas growing in fasted, adult host rats indicated that the tumors remove these nutrients from the host arterial blood (Table 2). On the average, 42% of the free fatty acids, 45% of the glycerol, and 59% of the triglycerides were removed from the arterial blood during one pass through the tumor. Previously (3), we showed that 30% of the 3-hydroxybutyric and 52% of the acetoacetic acids were removed from the arterial blood during a single pass through the tumor. Since the concentrations of these nutrients are increased from 2 to 20 times (depending on the nutrient; see

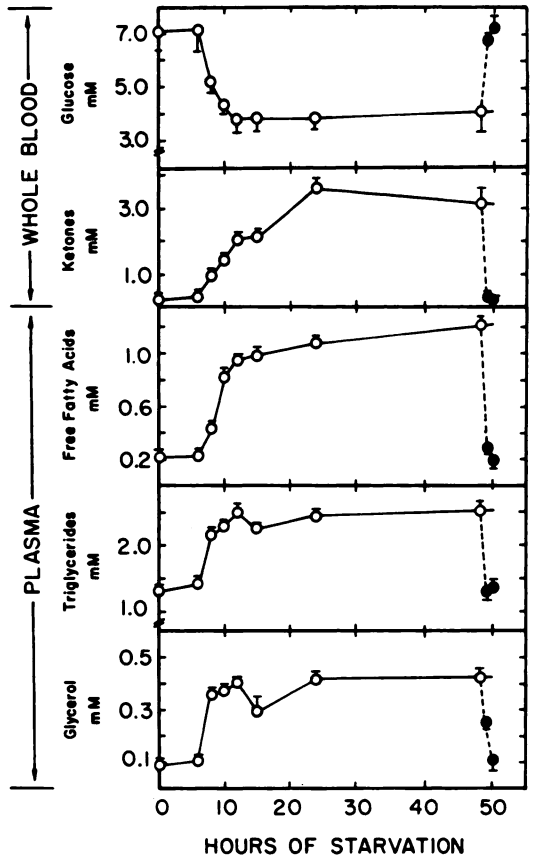

Fig. 3. Arterial whole blood glucose and ketone body, and plasma free fatty acid, triglyceride, and glycerol concentrations in Jensen sarcoma-bearing rats before and at different times after removal of food (zero time). Blood samples were obtained by cardiac puncture. Some rat groups (●) were refed for 1 and 2 h after 48 h of starvation. *Points*, mean for 4 to 12 rats; *bars*, SD.

Fig. 3) in the arterial blood of fasted, adult host rats, the tumor has access to this rich carbon source for growth and energy production.

DISCUSSION

Tumor growth in adult rats is stimulated by underfeeding or acute starvation (1). Results accumulated to date suggest that the rate of tumor growth in fed animals is limited by the availability of a substance that is enriched in the blood of the underfed or fasted adult rat. Because tumor growth is stimulated in fasted adult and not in immature rats and because hyperlipemia and ketosis occur only in the adult rats, we suggest that the rate-limiting substance is derived from the fat stores of the adult host rat. Other data (1) that support this proposal are the following. (*a*) The rate of tumor growth appears to be directly

Table 2 *Arterial and tumor vein plasma free fatty acid, glycerol, and triglyceride concentrations measured across tissue-isolated Jensen sarcomas*

Arterial and venous blood samples were collected across the tumors as described in "Materials and Methods." Mean tumor weights and blood flow rates were 15.5 ± 0.4 g and 0.11 ± 0.01 ml/min, respectively. The host rats were fasted for 2 days.

	Free fatty acids			Glycerol			Triglycerides		
Experiment	Arterial (mM)	Venous (mM)	Utilization rate[a]	Arterial (mM)	Venous (mM)	Utilization rate	Arterial (mM)	Venous (mM)	Utilization rate
1	1.24	0.75	2.2	0.52	0.39	0.6	1.04	0.5	2.4
2	1.15	0.50	3.0	0.66	0.36	1.4	1.89	0.6	5.9
3	1.26	0.70	2.4	0.48	0.29	0.7	2.39	0.56	7.8
4	1.24	0.78	2.1	0.77	0.54	1.1	2.07	0.75	6.0
5	1.32	0.83	2.2	0.66	0.26	1.8	2.14	1.18	4.3
6	1.27	0.75	2.2	0.66	0.32	1.3	2.10	1.05	4.4

[a] Utilization rates are nmol/min/g of tumor. Sixty % of the whole blood samples was plasma.

related to the blood levels of nutrients derived from host fat stores. (*b*) Refeeding slowed the tumor growth rate to the rate observed in fed rats. (*c*) No stimulation of tumor growth occurred in chronically underfed adult rats. (These animals have diminished fat stores and showed neither hyperlipemia nor ketosis following an acute fast.) (*d*) Tumors *in vivo* utilize ketone bodies, free fatty acids, glycerol, and triglycerides.

In this study we measured the rates of tumor growth and DNA synthesis and the concentrations of glucose and the fat store-derived blood nutrients at frequent intervals over the first day of an acute fast, when the stimulation of tumor growth is initiated, and during refeeding, when the growth stimulus is subsiding. If tumor growth in the fed rat is limited by the availability of fat store-derived nutrients, the increase in tumor DNA synthesis should coincide with the increases in the blood concentrations of these nutrients. The results show that there is an excellent match in the timing of these events. A new rate of [^{3}H]thymidine incorporation into tumor DNA began 6 to 7 h after the onset of the fast and at the same time that the concentrations of the arterial blood free fatty acids, glycerol, triglycerides, and ketone bodies were beginning to increase. The increasing rates of DNA synthesis and circulating arterial nutrient concentrations simultaneously reached a plateau 15 to 24 h after the start of the fast. Finally, after refeeding the rate of tumor DNA synthesis and the concentrations of the fat store-derived blood nutrients quickly returned to the values observed in fed rats. Thus, the kinetics of the increases and decreases in the fat store-derived blood nutrients concentrations and the kinetics of the increases and decreases in tumor DNA synthesis correlate and suggest a cause and effect relationship.

The apparent very tight coupling between the circulating concentrations of fat store-derived nutrients and the rates of tumor DNA synthesis and growth is remarkable. This implies that the cellular machinery in the tumor required for utilization of the rate-limiting substance and initiation of growth is already in place and active. Whether the supply of this substance in the fed adult rat is from the host or from the tumor is an interesting question for future study. A similar association between increases in these nutrients and rates of tumor DNA synthesis and growth is observed during the onset of streptozotocin-induced diabetes mellitus in adult rats.[3] In this instance, however, the increase in the rate of DNA synthesis begins as early as 2 to 4 h after drug administration. Mobilization of fat stores also begins more quickly after drug-induced diabetes (9) than after starvation (Ref. 8; see Fig. 2). Induction of diabetes in immature tumor-bearing rats increased neither the rate of tumor growth nor the concentrations of the fat store-derived nutrients. Again, immature tumor-bearing rats appear to lack the resources required for the stimulation of tumor growth.

Identification of the rate-limiting substance that is responsible for the stimulation of tumor growth in adult rats is potentially important and of interest. Although our results, including the kinetic studies described here, favor a critical role for one or more of the nutrients released from host fat stores, the mechanism is not yet established. A different hormonal milieu provoked by fasting might cause all of the changes seen. Satisfactory descriptions will have to explain not only the positive effect of an acute fast on tumor growth in the adult rat but also the lack of a response in the immature rat. There are several reports in the literature that describe no effect by starvation on tumor growth in immature rats (1, 10). Also, the effects of starvation on tumor growth in other species (10, 11) may be completely different from those observed in adult rats. A mechanism that adequately explains these diverse findings will aid in understanding relationships in nutrition, diet, and cancer.

[3] L. A. Sauer and R. T. Dauchy. Stimulation of tumor growth in adult rats *in vivo* during acute streptozotocin-induced diabetes, submitted for publication.

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REFERENCES

1. Sauer, L. A., Nagel, W. O., Dauchy, R. T., Miceli, L. A., and Austin, J. E. Stimulation of tumor growth in adult rats *in vivo* during an acute fast. Cancer Res., *46:* 3469–3475, 1986.
2. Sauer, L. A., Stayman, J. W., III, and Dauchy, R. T. Amino acid, glucose, and lactic acid utilization *in vivo* in rat tumors. Cancer Res., *42:* 4090–4097, 1982.
3. Sauer, L. A., and Dauchy, R. T. Ketone body, glucose, lactic acid, and amino acid utilization by tumors *in vivo* in fasted rats. Cancer Res., *43:* 3497–3503, 1983.
4. Wahlefeld, A. W. Triglycerides. Determination after enzymatic hydrolysis. *In:* H. U. Bergmeyer (ed.), Methods of Enzymatic Analysis, Vol. 4, pp. 1831–1835. New York: Academic Press, Inc., 1974.
5. Bergmann, S. R., Carlson, E., Dannen, E., and Sobel, B. E. An improved assay with 4-(2-thiazolylazo)resorcinol for nonesterified fatty acids in biological fluids. Clin. Chim. Acta, *104:* 53–63, 1980.
6. Morrison, D. S. *In vivo* estimation of size of experimental tumors. J. Natl. Cancer Inst., *71:* 407–408, 1983.
7. Snedecor, G. W., and Cochran, W. G. Statistical Methods. Ames, IA: The Iowa State University Press, 1976.
8. McGarry, J. D., Meier, J. M., and Foster, D. W. The effect of starvation and refeeding on carbohydrate and lipid metabolism *in vivo* and in perfused rat liver. J. Biol. Chem., *248:* 270–278, 1973.
9. Meier, J. M., McGarry, J. D., Faloona, G. R., Unger, R. H., and Foster, D. W. Studies of the development of diabetic ketosis in the rat. J. Lipid Res., *13:* 228–233, 1972.
10. White, F. R. The relationship between underfeeding and tumor formation, transplantation, and growth in rats and mice. Cancer Res., *21:* 281–290, 1961.
11. Stragand, J. J., Braunschweiger, P. G., Pollice, A. A., and Schiffer, L. M. Cell kinetic alterations in murine mammary tumors following fasting and refeeding. Eur. J. Cancer, *15:* 281–286, 1978.