Stimulation of Tumor Growth in Adult Rats in Vivo during an Acute Fast

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ABSTRACT

These experiments investigate an increase in tumor growth that occurs in adult rats in vivo during an acute fast. The effects of feeding, fasting, and underfeeding on the growth of Morris hepatomas 5123C and 7288CTC in Buffalo rats and of Walker carcinoma 256 and Jensen sarcoma in Sprague-Dawley rats were studied. Animals were matched for tumor size and growth during a period of ad libitum feeding preceding the fasting or underfeeding. Tumor growth was documented by increased size and incorporation of [methyl-3H]thymidine into tumor DNA. Fasting increased the rate of growth of the tumors 3 to 4 times over that measured in fed rats. This effect began during the first day of fasting and ended abruptly on refeeding. After refeeding tumor growth slowed to the rate in fed rats. Tumors from fed or fasted rats were not different in cellularity or dry weight/g wet weight.

A positive growth response in the tumor required lipolysis and ketosis in the host. No stimulation was observed during an acute fast in either immature rats or in mature rats whose weights had been reduced by underfeeding. These animals have small fat stores and show no increase in arterial blood free fatty acid or ketone body concentrations during an acute fast. Finally, underfeeding of adult rats raised the blood concentrations of these nutrients to values that were intermediate between those in fasted and fed rats. Tumor growth rates in these rats were intermediate between those in fasted and fed rats. The results support the proposal that an increase in availability of free fatty acids and/or ketone bodies is the stimulus that increases the rate of tumor growth during an acute fast.

INTRODUCTION

During studies on tumor nutrient utilization in vivo in fed and fasted adult rats (1), we observed an increase in the growth rate of transplantable tumors when the host rat was fasted. This was an unexpected finding; earlier studies had shown that the tumor growth rate was about the same during fasting (2-7) or underfeeding (8, 9) when compared to growth in fed animals. Our observation appeared to be the first time that an increase in tumor growth was noted during an acute fast.

The ketone body and free fatty acid concentrations are increased in the arterial blood of adult host rats during an acute fast, and the rate of tumor utilization of these nutrients is increased (1). We suggested that the ketone bodies and free fatty acids were important carbon sources for synthesis of tumor protoplasm and that the increased availability of these nutrients promoted the increase in the rate of tumor growth. An important corollary of this hypothesis is that tumor growth in vivo in the fed state is limited by the availability of these nutrients. In this study we examined this phenomenon further. We have documented the increased rate of tumor growth in adult rats during an acute fast and examined the relationship between the arterial blood concentrations of ketone bodies and free fatty acids and tumor growth. Different dietary manipulations were performed to influence the concentrations of ketone bodies and free fatty acids in host arterial blood. The results support a direct association between the rate of tumor growth in vivo and the abundance of these nutrients in host arterial blood.

MATERIALS AND METHODS

Animals, Tumors, and Tumor Transplantation. Adult and immature male Buffalo and 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 on at 6 a.m. and off at 6 p.m. Unless otherwise indicated the animals were 3 to 5 months old and weighed about 250 to 350 g at the time of tumor implantation.

Morris hepatomas 5123C and 7288CTC, the Walker carcinoma 256, and the Jensen sarcoma were used in these experiments. We have carried these tumors in our laboratory for from 2 to 9 years.

Tumors were grown s.c. either as tissue-isolated or non-tissue-isolated implants. Tissue-isolated tumors were grown in the left inguinal fossa on a vascular stalk composed of the truncated superficial inferior epigastric artery and vein as described previously (10). A carefully measured 3-mm cube of tumor was attached to the end of the stalk with a small suture, and the implant and vascular stalk were enclosed in a parafilm envelope. The skin incision was closed with a few sutures. For the non-tissue-isolated implants a 3-mm cube of tumor tissue was placed under the shaved skin through a small incision in the midline between the scapulae. The wound was closed by a suture. Tissue-isolated tumors were used in most experiments because these tumors do not show large volumes of central necrosis as frequently as do non-tissue-isolated tumors.

Assays and Reagents. Blood samples were obtained by cardiac puncture from tumor-bearing animals lightly anesthetized with ether. Aqueoetric and 3-OH butyric acids and glucose were measured fluorometrically and spectrophotometrically, respectively, in perchloric acid extracts of whole blood using enzymatic methods (1, 10). Enzymes, nucleotides, and other chemicals used in these assays were purchased from Sigma Chemical Co. Plasma free fatty acids were measured as described by Bergmann et al. (11). Chemicals used in this assay were obtained from Sigma and Eastman Chemical Co.

[methyl-3H]thymidine (6.7Ci/mmol) was obtained from Research Products International, M. Prospect, IL. Liquiscint was purchased from National Diagnostics, Somerville, NJ. Kodak N2B2-Nuclear Track Emulsion, D-19 Developer, and Rapid Fixer are products of Eastman Kodak Company, Rochester, NY and were prepared and used as directed.

Method for Measurement of Tumor Weight in Situ. Tumor mass in the living animal was estimated from measurements made through the skin essentially as described by Morrison (12). The rat was lightly anesthetized with ether, and the tumor dimensions were measured with calipers (Manostat brand, dial-type with metric scale and vernier, purchased from Fisher Scientific). Measurements were made through the tumor long axis and then through two other axes at right angles to the long axis and to each other. Two mm were subtracted from each measurement to compensate for the skin thickness. The three dimensions were multiplied. The product, which has units of cm² but is not a true volume, is directly proportional to tumor wet weight (12). Tumor weight in situ was estimated by substituting the product value in an equation for a standard linear regression line derived from a plot of the product of the three dimensions and weights measured on excised tumors (Fig. 1). Carcass weight was calculated by subtracting the estimated tumor weight from total animal plus tumor weight. Similar procedures have been used by others (3, 5-7) for estimation of host carcass and tumor weights in vivo.
STIMULATION OF TUMOR GROWTH IN VIVO

 Experimental Design for Tumor Growth Studies. Tumors were implanted in 10 to 25 male animals of the same age and body weight. Animals were either littermates or from different litters born on the same day. The tumor cubes used as implants were taken from the same tumor. All inoculated animals were housed together in a large cage. After tumor growth was established, six to sixteen rats with the same sized tumors were selected from the large cage and distributed randomly among individual cages. Animals entered the control or experimental groups (3 to 5 rats/group) at this time. Tumor weights in situ in each animal were estimated daily to measure tumor growth rates. All animals had free access to food, and the amount of food consumed by each animal was measured daily. Tumor-bearing rats matched as described above show very uniform rates of tumor growth in ad libitum fed animals, and it was not necessary to change the experimental design to correct for atypical tumor growth in individual rats. After 3 to 5 days one group was allowed continued access to food; all of the food was removed from a second group. Animals in the food restricted groups, if part of the experiment, received 15, 50, or 60% of the known daily food intake. All animals had free access to water. The fast or underfeeding began at 6 to 8 a.m. after a normal overnight period of feeding and lasted for 3 to 7 days. The length of the fast depended on the tumor growth rate, the time required for the tumor weight differences or other data to be statistically significant, and the health of the tumor-bearing animals. Starvation causes a severe weight loss and endangers the health of animals bearing fast growing tumors, especially immature rats without significant fat stores. Estimated tumor and host carcass weights in the fasted or underfed animals were compared to estimated tumor weights in the ad libitum fed rats. In a few experiments the fasted animals were re-fed for a period of 3 to 4 days. At the end of the experiment the animals were sacrificed, and the excised tumors and host carcasses were weighed. In some experiments arterial blood samples were collected by cardiac puncture from the fed, underfed, and fasted animals during the experimental period.

 Experimental Design for Measurement of Incorporation of [methyl-3H]thymidine. Two animals, pair-matched for tumor and host carcass weights, were used in these experiments. Food was removed from one animal and the other was fed ad libitum. After 2 days both animals were lightly anesthetized with ether, and [methyl-3H]thymidine (1 μCi/g total body plus tumor weight) was injected i.v. (in the external jugular) as a single injection. The neck incision was closed with wound clips, and the animals were returned to their cages. One h later the animals were sacrificed by ether anesthesia. The skin over the tumors was incised, and the tumors were removed and weighed. A portion of each tumor was placed in phosphate-buffered formalin for autoradiographic analysis, another portion was removed and weighed for estimation of dry weight, and the remainder was weighed and a 20% homogenate prepared in cold saline using a glass-Teflon Potter-Elvehjem homogenizer.

 Duplicate one-half ml portions of the homogenate were precipitated with 4 volumes of cold 0.4 N perchloric acid, and the mixture was placed in an ice bath for 20 min. The precipitate was recovered by centrifugation, and the DNA in the pellet was hydrolyzed and assayed as described by Leyva and Kelley (13). Portions of the hydrolysates were counted in a Beckman LS100 Liquid Scintillation Counter. Quenching was corrected by the channels ratio method and by internal standardization. Radioactivity in tumor DNA is reported as dpm/g tumor wet weight and as dpm/μg tumor DNA.

 Autoradiography. The formalin fixed tumor pieces were embedded in paraffin; 2-μm sections were cut and fixed to glass microscope slides. The paraffin was extracted, and the slides were dipped in autoradiographic emulsion, dried, and then stored at 4°C in the dark for 3 to 20 days. The period of exposure depended on the tumor growth rate; faster growing tumors incorporated more radioactivity (14) and were exposed for shorter periods. Autoradiograms were developed according to the instructions for the emulsion. Developed slides were counterstained with Harris’ hematoxylin for 3 min with Phloxin-eosin for 1 min. Silver grains over the nuclei of labeled cells were counted under oil immersion.

 Statistical Analysis. Significance of means was tested by Student’s t-test for paired samples (15). Tumor and host carcass weights and analytical data are expressed as means ± SE. Sample sizes required for demonstration of significant differences in tumor weights and incorporates of radioactive thymidine in matched ad libitum fed and fasted rats were estimated as described by Snedecor and Cochran (15).

 RESULTS

 Effects of Feeding and Fasting on Tumor Growth in Adult Rats. Figs. 2 and 3 show mean estimated tumor and host carcass weights during feeding and during periods of feeding and fasting for tumors growing in adult rats. The effect of feeding, fasting, and refeeding on tumor and host weights in Walker carcino-256-bearing Sprague-Dawley rats is also shown in Fig. 2. Tu- mor growth in the fed rats continued at a fairly constant rate: 1.7 g/day for the fast growing Jensen sarcoma, about 1 g/day...
for Walker 256 carcinoma and hepatoma 7288CTC, and about 0.5 g/day for the slower growing hepatoma 5123C. In each experiment the rates of growth during fasting, as estimated from the slopes of the lines connecting tumor weights, were increased about 3 to 4 times over growth rates measured in the same animals before the fast and in the fed rat groups. The increase in growth rate began during the first day of the fast, continued throughout the duration of the fast, and decreased immediately on refeeding. Increments in tumor growth during the period of the fast were, on the average, about 4 g/day for Jensen sarcoma, 2.5 to 3 g/day for Walker carcinoma 256 and hepatoma 7288CTC, and about 1 g/day for hepatoma 5123C. The mean tumor weights measured at the end of the experiments are listed in the legends of Figs. 2 and 3. These weights were close to the estimated mean weights and confirm the basic accuracy of the method for estimating tumor weights in vivo. Host carcass weights decreased 50 to 85 g during a 4 to 7 day fast.

Incorporation of Thymidine into Tumors in Fed and Fasted Adult Rats. The rates of incorporation of \[^3\text{H}\]thymidine into tumors, kidney, and lymph nodes from adult animals fed or fasted for 2 days are shown in Table 1. Six to 15 matched pairs of animals were studied. The rate of incorporation of radioactive thymidine into tumor mass increased directly with the tumor growth rate, in agreement with the findings of Lea et al. (14). Fasting increased the amount of thymidine incorporated into tumor mass in all four tumors. The rates of incorporation were increased 250 to 350% when the increment in incorporation due to fasting was expressed as a percentage of the incorporation in tumors growing in fed animals. That is, the stimulation to increase incorporation of thymidine due to fasting was about the same in all four tumors. Thymidine incorporation into kidney was not influenced by fasting, and incorporation into lymph nodes, a more actively proliferating normal tissue, was increased slightly but significantly. As expected, the rates of incorporation of \[^3\text{H}\]thymidine into tumor DNA also increased during the acute fast (Table 1). The tumor dry weight/wet weight ratios and qualitative estimates of cellularity, determined by examination of microscopic sections, were not different in tumors harvested from fed or fasted rats. In a few experiments the radioactive thymidine was injected into the fed and fasted rats the evening before the morning of tumor collection to determine if a diurnal variation in mitotic activity observed in some hepatomas (16) might influence these results. Equal rates of thymidine incorporation into tumor DNA were seen following the evening and morning injections, a finding that decreases the possibility of diurnal differences. The data from the evening and morning injections were pooled.

The increase in labeling observed in tumors during fasting could be caused by an increase in the specific activity of radioactive thymidine in tumors of fasted rats. To account for this possibility we categorized the labeled cells by counting the numbers of grains/nucleus by autoradiography in tissue sections of tumors from the fed or fasted rats. The numbers of cells collected in each of the grains/nucleus categories were the same for the tumors. If the specific activity of the thymidine pool was higher in tumors growing in fasted rats, more radioactive thymidine would have been accumulated by each cell, and increased numbers of cells would have been counted in the higher grains/nucleus categories. Since the number of cells in each category was not different, the specific activity of radioactive thymidine available to the dividing tumor cells must have been the same in the fed and fasted rats.

Comparison of an Acute Fast on Tumor Growth and Host Lipolysis and Ketosis in Immature and Adult Rats. Fig. 4 shows the effect of an acute fast on growth of hepatoma 7288CTC and Jensen sarcoma in immature and adult Buffalo and Sprague-Dawley rats, respectively. The young and adult rats were implanted on the same day with tumor from a single donor and were matched for tumor size and for tumor growth rates during the initial period of ad libitum feeding. The rate of tumor growth in the immature rats did not change during the period of the acute fast, a finding that is in agreement with the results of previous investigators (2-9). Tumor growth in the pair-matched adult rats showed the typical marked increase in growth rate. Table 2 shows that the metabolic responses of the young and adult host animals shown in Fig. 4 were also different; immature rats showed neither a lipolytic nor a ketotic response to the fast. In adult rats, the concentrations of free fatty acids and ketone bodies in the arterial blood were increased on the first day of the fast and remained high throughout the 3-day fast.

Two other types of experiments were performed to test the relationship of host arterial blood free fatty acid and ketone body concentrations to tumor growth. The first experiments were designed to increase the arterial blood concentrations of the ketone bodies and free fatty acids to submaximal levels. Groups of matched adult tumor-bearing rats were either fed, fasted, or underfed. The quantity of food given the underfed rats was determined by trial and error; the final regimens used were 15, 50, or 60% of the normal ad libitum food intake for that animal. Fig. 5 shows that the tumor growth rates observed in the underfed animals were intermediate between the slower rates observed in fed rats and the faster rates observed in the starved rats. Table 3 shows that these regimens increased the arterial free fatty acids and ketone body concentrations in the underfed rats to values intermediate between the arterial blood concentrations in fed and fasted hosts. Thus, the rates of tumor growth correlated positively with host arterial blood free fatty acid and ketone body concentrations.

The second experiments (not shown) were designed to examine tumor growth in adult rats after weight reduction. Nine
ad libitum. The 9 rats fed the restricted diet lost weight rapidly. The results were as follows: (a) the percentage of the tumor weight of about 225 g. The fed rats gained about 10 g during the first few days and then more gradually to a plateau intake for 10 days. Three other 300-g rats continued to be fed ad libitum. The 9 rats fed the restricted diet lost weight rapidly during the first few days and then more gradually to a plateau weight of about 225 g. The fed rats gained about 10 g during these 10 days. Jensen sarcomas were implanted in the 12 rats, and the feeding and 50% underfeeding diets were continued. The results were as follows: (a) the percentage of the tumor implants that grew decreased from 100% for the fed rats to 55% for the underfed rats; (b) Palpable tumors did not appear in the diet restricted rats until 7 days after implantation compared to 2–3 days for tumors implanted in fed rats; and (c) once established the tumors grew at about the same rate in the caloric restricted rats as in fed rats. [These results were described several years ago by Sweet et al. (17), Rous (8), and Tannenbaum (18) and are now generally accepted as characteristics of tumor implantation and growth in chronically underfed animals (see Ref. 19 for a review)]. One-half of the underfed rats with growing tumors (mean body weight was about 200 g) was fasted for 2 days, and one-half was continued on the 50% underfed diet. Arterial blood samples were collected from these rats during the two days. The fasted animals lost only 10 g carcass weight and showed no lipolytic or ketotic response, indicating an absence of significant fat stores. There was no stimulation of tumor growth in the fasted rats.

### DISCUSSION

In this study we examined the effects of acute fasting and underfeeding on tumor growth in rats. The research was prompted by an observation made earlier (1) that the rate of tumor growth in adult rats is increased during an acute fast. The experiments elucidated the following characteristics of the effect: a new rate of tumor growth is turned on by fasting or underfeeding and turned off by refeeding; the increased rate of tumor growth is observed only in animals that contain significant fat stores; an increase in the rate of tumor growth during an acute fast is dependent on a lipolytic and ketogenic response in the host; and the extent to which the tumor growth rate increases is directly dependent on the arterial concentrations of the free fatty acids and/or the ketone bodies. The results suggest that the increased rate of tumor growth during an acute fast is dependent on the increased availability to the tumor of the ketone bodies and free fatty acids (1). The growth rate of a tumor is a function of the growth fraction and the cell cycle time. Either or both of these parameters may be altered by fasting.

These findings are of special interest because previous investigators (2–9) had shown only that tumors maintain about the same growth rate in the starved or underfed rat as in the fed rat. An acceleration of tumor growth was not observed. Consequently, the results reported here could be interpreted to be

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**Table 1 Incorporation of methyl-$^3$HJthymidine in vivo in Morris hepatomas 5123C and 7288CTC, Walker carcinoma 256, and Jensen sarcoma growing in fed and fasted rats**

Tumor implantation, matching of host rats for tumor size and growth, and labeling in vivo were as described under "Materials and Methods." The numbers in parentheses are the number of tumors examined. Rats were fasted for 2 days.

<table>
<thead>
<tr>
<th>Tumor type or tissue</th>
<th>Tumor generation time</th>
<th>Tumor wt (increment due to fasting as a % of fed value)</th>
<th>Thymidine incorporation</th>
<th>dpm ($\times 10^3$/g wet wt)</th>
<th>dpm/µg DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fed</td>
<td>Fasted</td>
<td>Increment due to fasting as a % of fed value</td>
<td>Fed</td>
</tr>
<tr>
<td>Hepatoma 5123C (7)</td>
<td>6–8 weeks</td>
<td>69</td>
<td>345 ± 100$^a$</td>
<td>1214 ± 170$^d$</td>
<td>251</td>
</tr>
<tr>
<td>Hepatoma 7288CTC (6)</td>
<td>2 weeks</td>
<td>110</td>
<td>701 ± 151</td>
<td>2741 ± 811</td>
<td>291</td>
</tr>
<tr>
<td>Walker carcinoma (15)</td>
<td>10 days</td>
<td>130</td>
<td>790 ± 228</td>
<td>3557 ± 990$^d$</td>
<td>350</td>
</tr>
<tr>
<td>Jensen sarcoma (9)</td>
<td>5–7 days</td>
<td>350</td>
<td>1623 ± 488</td>
<td>5710 ± 1588$^d$</td>
<td>252</td>
</tr>
<tr>
<td>Kidney (13)</td>
<td></td>
<td>1172 ± 181</td>
<td>1813 ± 170$^d$</td>
<td>55</td>
<td>142 ± 15</td>
</tr>
</tbody>
</table>

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* Time between transplants.
* Fasted – fed.
* Mean ± SE.
* Means in fed and fasted animals are different ($P < 0.05$).
host animal during stimulation of tumor growth and the fact

The key findings are the need for lipolysis and ketosis in the fasting. However, our data demonstrate that no conflict exists.

The 50% and 85% underfed and fasted groups was 23, 29, and 39 g, respectively. The average weight loss in g in the 85% underfed group, and 27.4 ± 2.1 g in the fasted group. The measured 4.1 ± 0.2 g in the fed group, 7.7 ± 0.7 g in the 50% underfed group, 16.0 ± 1.5 g in the 85% underfed group, and 27.4 ± 2.1 g in the fasted group. The measured weight was about 2 g, a group (n = 3) was continued fed (A), two groups were fed rations equal to either 50% (•) or 15% (O) of the normal daily food intake, and the fourth group was fasted (A). On day 9, the measured mean tumor weight was about 8 g, a group (n = 3) was fed a ration equal to 40% of the normal

animals. Average carcass weights decreased 82 g in the fasted rats and 33 g in the animals, 20.9 ± 0.3 g in the 60% underfed animals, and 32.4 ± 2.3 g in the fasted

data were listed in the text and in the legend of Fig. 4.

Table 2 Arterial whole blood glucose, ketone body, and plasma free fatty acid concentrations in immature and adult fed and fasted tumor-bearing rats

<table>
<thead>
<tr>
<th>Glucose (mm)</th>
<th>Acetoacetate (mm)</th>
<th>3-OH butyrate (mm)</th>
<th>Free fatty acids (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fed</strong></td>
<td><strong>Fasted</strong></td>
<td><strong>Fed</strong></td>
<td><strong>Fasted</strong></td>
</tr>
<tr>
<td>Adult rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>6.20</td>
<td>5.47</td>
<td>0.04</td>
</tr>
<tr>
<td>Day 8</td>
<td>6.64</td>
<td>5.01</td>
<td>0.15</td>
</tr>
<tr>
<td>Day 9</td>
<td>6.41</td>
<td>4.23</td>
<td>0.07</td>
</tr>
<tr>
<td>Day 10 (n = 3)</td>
<td>6.25 ± 0.3*</td>
<td>5.24 ± 0.8</td>
<td>0.09 ± 0.004</td>
</tr>
<tr>
<td>Immature rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>6.70</td>
<td>5.43</td>
<td>0.07</td>
</tr>
<tr>
<td>Day 8</td>
<td>6.18</td>
<td>5.25</td>
<td>0.15</td>
</tr>
<tr>
<td>Day 9</td>
<td>6.56</td>
<td>4.40</td>
<td>0.09</td>
</tr>
<tr>
<td>Day 10 (n = 3)</td>
<td>5.96 ± 0.2</td>
<td>4.71 ± 0.4</td>
<td>0.10 ± 0.001</td>
</tr>
<tr>
<td>Jensen sarcoma-bearing Sprague-Dawley rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 5</td>
<td>6.45</td>
<td>5.15</td>
<td>0.02</td>
</tr>
<tr>
<td>Day 6</td>
<td>6.50</td>
<td>4.42</td>
<td>0.02</td>
</tr>
<tr>
<td>Day 7</td>
<td>6.43</td>
<td>4.54</td>
<td>0.03</td>
</tr>
<tr>
<td>Day 8 (n = 3)</td>
<td>6.35 ± 0.1</td>
<td>3.82 ± 0.3</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>Immature rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 5</td>
<td>4.96</td>
<td>3.47</td>
<td>0.04</td>
</tr>
<tr>
<td>Day 6</td>
<td>6.49</td>
<td>4.36</td>
<td>0.02</td>
</tr>
<tr>
<td>Day 7</td>
<td>7.55</td>
<td>4.33</td>
<td>0.04</td>
</tr>
<tr>
<td>Day 8 (n = 3)</td>
<td>6.96 ± 0.2</td>
<td>4.43 ± 0.2</td>
<td>0.07 ± 0.003</td>
</tr>
</tbody>
</table>

* Mean ± SE.

Fig. 5. Effects of acute underfeeding and fasting on tumor growth in male adult Sprague-Dawley rats bearing the Jensen sarcoma and Walker carcinoma 256. Tumor weights were estimated daily in animals that were matched for tumor size and growth as described in “Materials and Methods.” Final tumor weights were measured on the excised tumors at the end of the experiment. In the experiment with the Jensen sarcoma, nine animals matched for tumor size and growth were fed ad libitum for 4 days. On day 5, when the estimated mean tumor weight was about 8 g, a group (n = 3) was fed a ration equal to 40% of the normal daily food intake (A), another group was fasted (A), and the third group was fed ad libitum (A). On day 11, mean tumor weight was 15.4 ± 0.3 g in the fed animals, 20.9 ± 0.3 g in the 60% underfed animals, and 32.4 ± 2.3 g in the fasted animals. Average carcass weights decreased 82 g in the fasted rats and 33 g in the 60% underfed rats. The measured mean tumor weights in the fed animals and in the fasted and 60% underfed animal groups were different (P < 0.05). In the experiment with Walker 256 carcinoma, 12 animals matched for tumor size and growth were fed ad libitum for 4 days. On day 5, when the estimated mean tumor weight was about 2 g, a group (n = 3) was continued fed (A), two groups were fed rations equal to either 50% (O) or 15% (C) of the normal daily food intake, and the fourth group was fasted (A). On day 9, the measured mean tumor weight was 4.1 ± 0.2 g in the fed group, 7.7 ± 0.7 g in the 50% underfed group, 16.0 ± 1.5 g in the 85% underfed group, and 27.4 ± 2.1 g in the fasted group. The measured mean tumor weights in the fed and fasted, the fed and 85% underfed, and the fed and 50% underfed groups were different (P < 0.05). The average weight loss in the 50% and 85% underfed and fasted groups was 23, 29, and 39 g, respectively.

Bass, SE.

in conflict with these long held views on tumor growth during fasting. However, our data demonstrate that no conflict exists. The key findings are the need for lipolysis and ketosis in the host animal during stimulation of tumor growth and the fact that these metabolic responses to fasting do not occur to a significant extent in immature tumor-bearing rats. In the earlier studies (2–9) the animals used were immature (less than about 200 g body weight), and tumor growth was measured as an increase in tumor weight. No differences were noted between growth in the fasted and ad libitum fed control rats. The extent of lipolysis and ketosis in the host tumor-bearing animals was not described in these studies but, as judged from our results, it seems reasonable to assume that host fat stores in these young rats were not sufficient to generate and sustain the needed arterial blood levels of free fatty acids and ketone bodies. We suggest, therefore, that selection and use of immature animals is the reason previous investigators did not observe the effect of fasting reported here in adult rats. The remarkable difference between the rates of tumor growth in immature and adult rats during an acute fast (Fig. 4) indicates that host animal age is an important consideration in design of experiments in diet, nutrition, and cancer.

It is interesting to note that Reilly et al. (5) and Goodgame et al. (6) did find that short-term starvation increased the rate of incorporation of radioactive thymidine into tumor DNA and that Lowry et al. (20) reported that tumor-bearing animals fed a protein deficient diet also showed increased rates of tumor DNA synthesis. Reilly et al. (5) concluded that starvation might cause an increase in tumor growth. However, no definitive explanation was available for how an increase in incorporation of radioactive thymidine into tumor DNA could occur in the absence of tumor growth. The 200-g rats used by these investigators (5–7, 20) should have small fat stores and might show a transient and low rise in arterial blood ketone body and free fatty acid concentrations. Perhaps a brief, subthreshold stimulation to growth such as might occur at lower arterial blood nutrient levels is insufficient to provide for real growth but sufficient to promote DNA synthesis and a division that remains arrested in S phase (21). As an alternate consideration,
there may be differences in tumors such that slower growing, carcinogen-induced sarcomas do not show an effect due to fasting.

In important experiments, Halperin et al. (22) showed that fasting did not decrease the rate of lipogenesis in Morris hepatoma 7777. The rate of lipogenesis in host liver, however, was decreased approximately 80%. Pyruvate dehydrogenase in hepatoma 7777 was in the active form in either fed or fasted host rats. Under these conditions, an increase in the availability of substrates for tumor lipogenesis, such as occurs during fasting, would be expected to stimulate tumor growth. [The growth rate of hepatoma 7777, as with the other Morris hepatomas studied here, is stimulated by fasting.] Finally, it should be noted that enzymes for activation of the ketone bodies are found in tumor cells. Though absent in normal liver, succinyl CoA-acetoacetyl CoA transferase, a mitochondrial enzyme, is progression linked in the Morris hepatomas (23). Other rapidly growing tumors that have lower activities of the transferase contain the cytoplasmic ATP-dependent acetoacyl-CoA synthetase. It is not yet known what role the free fatty acids and ketone bodies play in the growth of rat tumors in vivo. Presumably, they serve as carbon sources for lipogenesis and cholesterol synthesis and oxidation.

The factors that regulate tumor growth in vivo in fed animals are not well-defined, and the regulation of tumor growth in starved animals is even less well understood. Although our data point to an effect of free fatty acids and/or ketone bodies in the stimulation of tumor growth during fasting, the exact mechanism has not been established. Other more complex explanations need to be considered. For example, tumor growth may increase in adult rats if their immune functions are suppressed during an acute fast. Immature rats, which might have a less fully developed immune system, may have a smaller immuno-suppressive response and decrease in immunosurveillance. Also, hormonal fluctuations during the feeding-fasting transition (24) may be influencing tumor growth. Presumably, these fluctuations do not occur or are different in immature tumor-bearing rats. Experiments are under way to examine these alternate mechanisms.

The finding that the supply of free fatty acids and ketone bodies may be rate limiting for tumor growth in rats will need to be confirmed in human tumors. New chemotherapeutic agents designed to interrupt the metabolism of these important nutrients could aid in the control of tumor growth.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Charles A. Ellsworth, Department of Pathology, for help in photographing the sections of tumors from fed and fasted rats. Thanks are also due Dr. William E. Bowers for several helpful discussions during the course of this research.

REFERENCES

4. Buzby, G. P., Mullen, J. L., Stein, T. P., Miller, E. E., Hobbs, C. L., and

### Table: Effects of fasting on tumor growth in vivo

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Glucose (mmol/L)</th>
<th>Free fatty acids (mmol/L)</th>
<th>Acetoacetate (mmol/L)</th>
<th>3-OH butyrate (mmol/L)</th>
<th>2565-4256</th>
<th>4A-4256</th>
<th>D-4256</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morris 7777</td>
<td>Fed</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>Fasted</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.01</td>
</tr>
</tbody>
</table>

*ND: not determined.*

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Stimulation of Tumor Growth in Adult Rats *in Vivo* during an Acute Fast


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