Sequential Hepatic Triglycerides in Tumor-bearing Mice

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Summary

The hepatic triglycerides were serially determined following transplantation of glioma (Zimmerman ependymoma) in C57 black mice. During the first 2 days after tumor homogenate inoculation there was a sharp and progressive fall in hepatic triglycerides. Thereafter there was a transient increase in hepatic lipids followed by a return to the low levels previously observed. These biochemical changes could not be directly correlated with the size of the transplant.

The effects of starvation on hepatic triglyceride levels in animals with tumors 10, 5, and 0 days, respectively, after transplantation of tumor were determined. The sequential hepatic triglyceride patterns resembled the controls; however, they were progressively diminished in time and amplitude. Variations in the quantitative triglyceride patterns on successive analysis can be related to the combined sequential effects of the presence of this tumor in conjunction with the effects of starvation.

Introduction

Recently we have described the lipid patterns in experimentally transmissible ependymal glial tumors in mice (8). The triglycerides were the major lipids in the gliomata (approximately 10 mg/gm of tissue). Later it was observed that, although the neoplasm remained localized in the subcutis, it did influence host lipid metabolism (7). In addition to loss of subcutaneous fat, there was a reduction in the triglycerides in liver and cardiac muscle. Further studies indicated that in tumor-bearing animals, there was a quantitative decrease in the rate of triglyceride synthesis in the liver (7). However, the triglyceride levels (mg/gm) in the liver had been measured only in tumor-bearing mice in which the tumor was approximately 20 days old. It then appeared necessary to sequentially determine the hepatic triglyceride levels in relation to the growth of the transplantable tumor. In addition, mice with tumors of varying ages were starved in order to evaluate this effect on hepatic triglyceride levels.

Materials and Methods

A colony of C57 black mice with transplantable glioma (Zimmerman ependymoma) has been established. For transplantation, a viable tumor was removed aseptically and transferred to a glass homogenizer to which an equal amount of saline was added. This loosely fitting unit a tumor cell suspension was prepared that readily passed through a 22-gauge needle. A 0.1-ml amount of this cell suspension was injected s.c. in the shoulder region. Previous experience indicated that with this technic, 100% transplantability of the tumor occurred.

Determination of hepatic triglycerides in groups of tumor-bearing mice sacrificed on 0, 2, 4, 5, 7, 10, and 20 days, respectively, were performed. At each of these time intervals, 3 animals were used and the individual livers were analyzed separately.

Two other groups of 10 tumor-bearing and control animals were established and all were sacrificed at 10 days after the tumor group had been inoculated. At sacrifice, the animals were decapitated and blood was collected and pooled from each group for analysis of serum triglycerides and nonesterified fatty acids.

The following series of experiments concerned with the effect of starvation on mice with transplantable tumors of different days of growth were performed.

A control series of 12 normal weanling C57 black mice were kept in individual cages. They received water *ad libitum* but no food. The animals were sacrificed in groups of 3 at 0, 1, 2, and 3 days, respectively. Studies beyond 72 hr were not performed for it is known that approximately 20% of the animals will die on the 4th day.

An equal number of weanling C57 black mice who had been inoculated with 0.1 ml of a tumor homogenate were also individually housed. They received no food but water *ad libitum*. They were sacrificed in identical manner.

Two other groups of 12 animals were inoculated with a transplantable tumor. The tumor was allowed to grow for 5 days in 1 group and 10 days in another group before starvation and sequential sacrifice.

In all of these experiments, the animals were weighed daily and at sacrifice, the weight of the tumor, the weight of the liver, and weight of the whole animal were recorded. The presence of the tumor was histologically confirmed.

LIVER AND SERUM LIPID EXTRACTION. Either 1 ml of serum or approximately 1 gm of liver was homogenized in 50 ml of chloroform-methanol (2:1 volume for volume) for 3 min in a micro-Waring Blendor, filtered through Whatman No. 3 paper under slight vacuum, and washed with 25 ml of the above solvent. To the filtrate was added 0.2 volume of 0.75% NaCl. The mixture was stirred and the aqueous layer was separated by centrifugation (2). The aqueous layer was aspirated and 4 successive washes with chloroform-methanol-0.75% NaCl (3:48:47) by volume were performed. The total lipid extract was evaporated to dryness under nitrogen and taken up in light petroleum ether. Insoluble material was removed and the extract was concentrated to a small volume (10 ml/gm of liver or 10 ml/ml of serum).

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THIN LAYER CHROMATOGRAPHY OF NEUTRAL LIPIDS. Thin layer plates were prepared by mixing a slurry of silica gel G and water 1:2 and spreading an even layer 0.25 mm thick on 20- X 20-cm glass plates using a mechanical spreader. The plates were dried at 100°C for 1 hr before use.

Standard solutions of approximately 0.4 mg/ml of triolein and tripalmitin in chloroform were prepared. Various amounts of this standard (from 0.2 to 16 µg) were spotted on silicic acid plates and developed in an ascending manner using n-hexane-diethyl ether-glacial acetic acid, 80/20/3, v/v/v for 40 min.

Standard triglycerides, for thin layer chromatography were obtained from Applied Science Laboratories, State College, Pennsylvania. Silicic acid and organic chemicals of analytic reagent grade were received from Mallinckrodt Chemical Works. Silica gel G for thin layer chromatography was obtained from Research Specialties Company.

The plates were sprayed with chromic sulfuric acid (a saturated solution of K₂Cr₂O₇ in 87% H₂SO₄) and heated at 180°C for 35 min. After cooling, the charred spots were measured on a Photovolt Densitometer. As reported by Mangold (4) and Privett et al. (5), the areas under the densitometer curves are directly proportional to the amount of sample. Privett and Blank (6) recommend the use of chromic-sulfuric acid instead of 50% sulfuric acid as the charring agent. They found that unsaturation per se had no effect on the yield of carbon. A standard curve for triglycerides was obtained for each plate.

Results

Chart 1 is a graph of weights of the tumor mass from 0 to 20 days. It is apparent that the transplanting inoculum remains small for approximately 10 days; thereafter, the volume and weight of the tumor increase extremely rapidly. Histologically during the first few days after inoculation, the transplant is a central core of necrotic cells, debris with only a few peripherally located viable tumor cells. By the 4th day, solid nests of tumor with a pattern typical of an ependymoma appear. The tumor becomes readily palpable approximately 10 days after inoculum and weighs approximately 1.0 gm. Grossly, the tumor is smooth, lobulated, gray-white, and not infiltrating. The lesion grows subcutaneously in an expanding manner. When the tumor exceeds 1 cm in diameter, spontaneous areas of necrosis and cystic degeneration occur.

In Chart 2, the results of the serial triglyceride analysis of liver in relationship to days of tumor growth are given. Within 48 hr, the hepatic triglycerides fell to approximately 2-3 mg/gm of tissue. However, during the next 2 days, there was a sharp rise in triglyceride levels which did not return to the previous low level until the 7th day. Thereafter, with rapid growth of the tumor, the low levels of triglyceride in the liver persisted throughout the remainder of the 20 days of observation.

The nonesterified fatty acids (NEFA) and triglyceride serum levels in pooled samples from 10-day-old tumor-bearing mice are given in Table 1.

**TABLE 1**

<table>
<thead>
<tr>
<th>Starvation (hr)</th>
<th>Control (mg/ml serum)</th>
<th>Tumor—10 day (mg/ml serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>0.52</td>
<td>0.56</td>
</tr>
<tr>
<td>48</td>
<td>0.26</td>
<td>0.71</td>
</tr>
</tbody>
</table>

* NEFA, nonesterified free fatty acid.

![Chart 1](chart1.png)

**Chart 1.** The relationship of the weight of the tumor to the days of growth. Each point is a mean of 3 and its S.D.

![Chart 2](chart2.png)

**Chart 2.** The relationship of hepatic triglyceride concentrations to the days of tumor growth. Each point is a mean of 3 and its S.D.

<table>
<thead>
<tr>
<th>MOUSE HEPATIC TRIGLYCERIDES, mg/gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAYS OF TUMOR GROWTH</td>
</tr>
<tr>
<td>AFTER INOCULATION</td>
</tr>
</tbody>
</table>

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**Figure 3.** Poststarvation hepatic triglycerides of control and subcutaneous tumor-bearing (C57) black mice (10-day-old transplant). Each point is a mean of 3 and its S.D.

**Figure 4.** Poststarvation hepatic triglycerides in glioma-bearing C57 black mice. Each point is a mean of 3 and its S.D.

The normal control weanlings on starvation showed continuous rise in the hepatic triglycerides during the 1st and 2nd days. On the 3rd day, the hepatic lipids fell (Chart 3).

The effect of immediate starvation on C57 mice who have received 0.1 ml of tumor homogenate from day 0 to 24 hr (Chart 4) is similar to the control mice for the first 24 hrs. However, after 24 hr the tumor-bearing mice demonstrated a fall in the hepatic triglycerides.

Animals bearing 5- and 10-day-old tumor transplants on starvation showed a transient rise in triglycerides of very low amplitude and a rapid return to the previous low levels (Charts 4, 5).

When the total weight of the mouse controls and tumor-bearing animals were plotted for the periods of starvation, no significant changes were apparent (Chart 5). When the weight of the liver of animals with tumors that were 0, 5, and 10 days old at the initiation of the starvation were compared with the controls, no significant changes were noted (Chart 6).

**Discussion**

The initial fall of the hepatic triglycerides after the inoculation of the tumor homogenate (Chart 3) may be related to a cellular product which was within this homogenate. The total wet weight of the tumor was less than 0.1 gm, and histologically the transplant showed only a small number of viable cells. Apparently some factor was present in the homogenate which, on a humoral basis, initiated the sharp reduction in hepatic triglycerides. The mechanism of the subsequent triglyceride rise which occurred over the next few days is not clear.

Furthermore, beyond the 7th day the depression of the triglycerides remained relatively stable in spite of the rapid enlargement of the total tumor mass. When compared to normal hepatic triglyceride levels, the decrease of hepatic triglycerides was significant (Student t test: P < 0.001).
When weanling mice were starved, during the first 48 hr a sharp rise in both the serum and hepatic triglycerides was observed. This response has been previously described and was due to increased lypolysis in subcutaneous fat and other stores which resulted in a rise in serum free fatty acids and triglycerides and associated increased triglyceride storage in the liver (3). However, by 72 hr the hepatic triglycerides had returned to original base levels. When the hepatic triglyceride levels after 24 or more hr of starvation in control animals or in the 0-day-old transplant-bearing animal were compared with 5- and 10-day-old tumor transplant animals there was a consistent significant decrease (Student t test: P < 0.001) (Chart 5).

In tumor-bearing animals in which the tumor was 10, 5, and 0 days old, the hepatic lipid patterns after starvation were similar to the controls, but they were diminished in time and amplitude. The quantitative hepatic lipid patterns in tumor-bearing mice following starvation can be related to the combined effects of sequential alterations directly associated with the transplantation in conjunction with the sequential effects of starvation.

Recently, Devlin and Costa reported (1) that the s.c. injection of Krebs II cancer in male Swiss mice resulted in a 30% depletion in body fat after 5 days. Furthermore, no significant difference in respiration and oxidative phosphorylation in isolated liver mitochondria from control and tumor-bearing mice 5 days postinoculation was observed. However, the rise in serum triglycerides and nonesterified free fatty acids after 48 hr of starvation in mice bearing a 10-day-old tumor was present when the hepatic triglycerides were low. These serum lipid findings contrast with the control levels and suggest a block in triglyceride synthesis in the liver. The mechanism of alteration in the hepatic triglycerides at any particular time in the growth of the tumor may be related to the triglyceride utilization of the tumor, to secondary homeostatic responses of the host, and possibly to humoral factors of the tumor influencing triglyceride synthesis in the host.

References
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