Depression of Alanine Transaminase Activity in the Liver of Rats Bearing Walker Carcinoma 256*

HOMER R. HARDING,† FRED ROSEN, AND CHARLES A. NICHOL

(Department of Experimental Therapeutics, Roswell Park Memorial Institute, State of New York Department of Health, Buffalo, New York)

SUMMARY

Alanine transaminase activity in the liver of adult rats (350–450 gm.) fell progressively from the 10th through the 30th day of growth of Walker carcinoma 256. The enzyme activity decreased to a level about 30 per cent of that found in comparable rats of the same age without tumors. Loss of enzyme activity occurred concurrently, with a gradual loss of carcass weight. Treatment with cortisol (4 mg/kg, administered during the first 10 days of tumor growth) increased the activity of alanine transaminase in liver 2.5-fold, but the same dose did not alter transaminase activity when the tumors were large (days 20 through 30). The activity of this transaminase could be raised to normal in the presence of large tumors by feeding a diet containing 75 per cent casein. When this high-protein diet was fed, treatment with cortisol (4 mg/kg) increased alanine transaminase activity fourfold. Tyrosine transaminase and tryptophan pyrrolase differed from alanine transaminase in that both of these enzymes were increased four- to fivefold by cortisol (10 mg/kg) in the livers of rats with established Walker tumors.

MATERIALS AND METHODS

Male rats of the Holtzman strain were used in these studies. The Walker carcinoma 256 which has been maintained in our laboratory for several years was obtained through the courtesy of Dr. K. Sugiura of the Sloan-Kettering Institute. The tumors were cut into 1-mm. cubes and were implanted subcutaneously by trocar into the right lateral flank of adult male rats weighing 380–450 gm. Sarcoma 180, which has been carried in Swiss mice (HaICR) in this laboratory for several years, was obtained originally from the Sloan-Kettering Institute. Lymphoid tumors P-388 and P-1798 were supplied through the courtesy of Dr. M. Potter of the National Institutes of Health. The recipient animals for the P-388 tumor were DBA/2 mice and, for the P-1798 tumor, CDF1 (BALB/c X DBA/2) mice. All tumors used were grown subcutaneously.

The pelleted stock diet (Teklad) and water were supplied ad libitum. The purified diet containing 75 per cent casein, prepared as described elsewhere (34), was also fed ad libitum.

Tissue homogenates were prepared by methods described previously (34). Protein was measured by the modified Folin method described by Lowry et al. (25). Alanine- and aspartate-α-ketoglutarate transaminase activities were measured by procedures (34) similar to those described by Wróblewski and La Du (37) except that, instead of measuring the oxidation of DPNH spectrophotometrically, DPN was determined by a fluorometric method.

* This work was supported in part by a research grant, CA-05671, from the National Cancer Institute of the United States Public Health Service.
† Present address: Sterling-Winthrop Research Institute, Rensselaer, New York.

Received for publication February 10, 1964.
procedure developed by Lowry and co-workers (24). Tyrosine-α-ketoglutarate transaminase activity was determined by a modification (33) of the method of Canellakis and Cohen (4), and tryptophan pyrrolase activity was determined by the method described by Knox (20).

RESULTS

The rate of growth of the Walker carcinoma 256 in adult rats was comparable initially to that observed in young animals. However, after 10 days the tumors grew more rapidly in adult rats than in immature animals. After 25–30 days the adult animals bearing tumors (about 100 gm.) were markedly cachectic, and about 25 per cent of the rats succumbed. Carcass weight (total body minus tumor weight) of the adult tumor-bearing rat was maintained only for the first 10 days after transplantation (Chart 1).

During tumor growth progressive loss in carcass weight occurred until, after 30 days, carcass weights were 60 gm. less than at the start of the experiment. Rats without tumors gained about 20 gm. during the same period.

Alanine and aspartate transaminase activities were determined in liver and tumor during the period of tumor growth. The level of aspartate transaminase in the Walker tumor was about the same in tumors grown for 10 or 30 days. Alanine transaminase activity in liver fell gradually after the 10th day (Chart 2) until, between the 25th and 30th day, the activity of this enzyme was more than 70 per cent below the level in rats without tumors. In contrast to alanine transaminase, liver aspartate transaminase activity in tumor-bearing rats did not vary significantly and remained at normal levels throughout the experiment.

Alanine transaminase activity in rat liver may be enhanced by administering glucocorticoids or by feeding a high-protein diet (34). During the first 10 days of tumor growth daily treatment with cortisol (4 mg/kg) elevated alanine transaminase activity in liver 2.5-fold and inhibited tumor growth by 68 per cent (Experiment 1, Table 1). Increasing the dose of cortisol to 10 mg/kg increased enzyme activity almost fourfold and resulted in 90 per cent inhibition of the tumor. Feeding a 75 per cent casein diet during the first 10 days of tumor growth resulted in only a slight increase in hepatic transaminase activity, although the growth of the Walker tumor was inhibited by 80 per cent. Combining daily administration of the smaller dose of cortisol with the high protein feeding did not enhance the transaminase response to cortisol, although tumor inhibition was greater than that obtained with only the 4 mg/kg dose of cortisol.

The daily administration of cortisol (4 mg/kg) to animals bearing established tumors did not significantly
TABLE 1
RESPONSE OF LIVER ALANINE TRANSAMINASE TO TREATMENT WITH CORTISOL OR FEEDING A HIGH PROTEIN DIET

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver weight (gm.)</th>
<th>Alanine transaminase activity</th>
<th>Tumor weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total liver</td>
<td>Per gm protein</td>
</tr>
<tr>
<td>None</td>
<td>11.6 ± 1.7</td>
<td>35.6 ± 6.1</td>
<td>18.2 ± 3.9</td>
</tr>
<tr>
<td>Cortisol, 4 mg/kg x 10 days</td>
<td>11.9 ± 0.5</td>
<td>94.8 ± 12.7</td>
<td>46.2 ± 7.4</td>
</tr>
<tr>
<td>Cortisol, 10 mg/kg x 10 days</td>
<td>12.1 ± 1.1</td>
<td>140.0 ± 15.0</td>
<td>68.7 ± 6.5</td>
</tr>
<tr>
<td>75% Casein x 10 days</td>
<td>13.4 ± 0.9</td>
<td>58.6 ± 5.6</td>
<td>26.5 ± 4.1</td>
</tr>
<tr>
<td>75% Casein + cortisol, 4 mg/kg x 10 days</td>
<td>12.6 ± 1.9</td>
<td>112.1 ± 14.3</td>
<td>48.7 ± 4.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver weight (gm.)</th>
<th>Alanine transaminase activity</th>
<th>Tumor weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total liver</td>
<td>Per gm protein</td>
</tr>
<tr>
<td>None</td>
<td>15.7 ± 0.1</td>
<td>87.3 ± 7.4</td>
<td>34.5 ± 3.9</td>
</tr>
<tr>
<td>Cortisol, 4 mg/kg x 5 days</td>
<td>14.7 ± 3.0</td>
<td>67.6 ± 6.5</td>
<td>29.7 ± 3.5</td>
</tr>
<tr>
<td>75% Casein x 5 days</td>
<td>12.3 ± 2.4</td>
<td>48.0 ± 9.5</td>
<td>22.3 ± 2.6</td>
</tr>
</tbody>
</table>

* mMoles of substrate utilized/hr; mean values ± standard deviation are given. Livers were obtained 11 and 30 days following transplantation of Walker tumor.

Exp. 1: five rats per group; cortisol administered daily, days 1 through 10.
Exp. 2: five rats per group; cortisol administered daily, days 20 through 29.
Exp. 3: eight rats per group; cortisol administered daily, days 25 through 29.

TABLE 2
LIVER TRANSAMINASE AND TRYPTOPHAN PYRROLASE ACTIVITY IN ADULT RATS BEARING WALKER CARCINOMA 256

Results are expressed as mmoles of substrate utilized per gram protein per hour.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Transaminase activity</th>
<th>Tryptophan pyrrolase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alanine</td>
<td>Aspartate</td>
</tr>
<tr>
<td>None</td>
<td>24.4 ± 5.4†</td>
<td>110.0 ± 5.4</td>
</tr>
<tr>
<td>Cortisol, 10 mg/kg*</td>
<td>56.6 ± 6.0</td>
<td>99.3 ± 8.0</td>
</tr>
<tr>
<td>None</td>
<td>11.2 ± 2.6</td>
<td>99.0 ± 16.1</td>
</tr>
<tr>
<td>Cortisol, 10 mg/kg*</td>
<td>49.8 ± 9.4</td>
<td>130.0 ± 17.1</td>
</tr>
</tbody>
</table>

* Cortisol treatment was started on day 15 and continued for 10 days; the animals were sacrificed on day 25, 5 hours after the last injection.
† Average values of five rats per group ± standard deviation.

enhance alanine transaminase activity in liver nor appreciably alter tumor weights measured on day 30 (Experiment 2, Table 1). The larger dose of cortisol (10 mg/kg) administered during the last 10 days of tumor growth did elevate liver transaminase activity almost fourfold and inhibited tumor growth by more than 50 per cent. However, the level of enzyme activity attained was less than half that found in the liver of rats treated during the first 10 days of tumor growth. Feeding the high-protein diet for 10 days during the final phase of tumor growth restored alanine transaminase activity in liver to normal levels. The administration of 4 mg/kg of cortisol to the animals fed the high-protein diet increased the level of enzyme activity in liver to that obtained with 10 mg/kg of cortisol. However, feeding the 75 per cent casein diet alone or in combination with the administration of 4 mg/kg of cortisol did not affect final tumor size. Comparable results were obtained when the high-protein diet was fed or the animals were treated with cortisol for 5 days beginning on the 25th day of tumor growth (Experiment 3, Table 1).

The response of three other amino acid-metabolizing enzymes was compared with that of alanine transaminase in rats bearing the Walker tumor. Two of these enzymes, tyrosine transaminase and tryptophan pyrrolase, show an adaptive response to their amino acid substrates and to cortisol (21, 23). The activity of both enzymes can be markedly increased within 5 hours following an intraperitoneal injection of cortisol. The third, aspartate transaminase, is not appreciably altered by cortisol administration. On the 25th day of tumor growth, tyrosine and aspartate transaminase and tryptophan pyrrolase activities in liver were comparable to the levels of activity found in animals without tumor, whereas hepatic alanine transaminase levels were still elevated.
transaminase showed the characteristic depression in activity (Table 2).

The induction of these enzymes was studied in tumor-bearing rats given injections subcutaneously of 10 mg/kg of cortisol for 9 days; on day 10 this dose was given intraperitoneally, and the animals were sacrificed 5 hours later. This schedule of treatment with cortisol increased tyrosine transaminase and tryptophan pyrrolase activities fourfold in the liver of rats with the Walker tumor (Table 2). These results were comparable to those obtained in tumor-free rats given cortisol in which liver tyrosine transaminase and tryptophan pyrrolase activities were enhanced 3.7- and fivefold, respectively, following similar treatment. Aspartic acid transaminase did not respond to cortisol in the liver of the rats either with or without tumor.

The relationship between hepatic alanine transaminase levels and the growth of other transplantable neoplasms in rats and mice is shown in Table 3. Alanine transaminase activity was slightly increased in rats bearing the Murphy-Sturm lymphosarcoma for 24 days; the average weight of these tumors was 50 gm. The growth of two tumors in mice was associated with a depression of liver alanine transaminase activity. CDF1 mice bearing the cortisol-sensitive line of the lymphosarcoma P-1798 and Swiss mice carrying Sarcoma 180 both had levels of enzyme activity in liver about one-third lower than that of the control animals. However, alanine transaminase activity was unaltered in the liver of DBA mice during the growth of the P-388 tumor.

**DISCUSSION**

Goodlad and Clark (8) have also observed that alanine transaminase activity was decreased in the liver of rats bearing the Walker tumor. They related this effect to an increased capacity of tumor-bearing rats to inactivate corticosterone (7). However, other factors may be involved, since alanine transaminase activity was also reduced in the livers of pregnant and in partially hepatectomized animals.1 Similar to results in the experiments with the tumor-bearing rats, alanine transaminase in partially hepatectomized animals was refractory to induction by treatment with small amounts of cortisol, and in pregnant rats this enzyme in liver is unresponsive to doses of cortisol as high as 100 mg/kg. In each of these conditions the growth of new tissues requires amino acids for protein synthesis. Mider (26, 27), LePage et al. (22), and others (13) have demonstrated that tumors act as a "nitrogen trap," whereby amino acids incorporated into tumor protein exchange very slowly with the body pool of amino acids. The depression in alanine transaminase activity and the failure to respond to the small dose of cortisol (4 mg/kg) suggested that the utilization of amino acids by the tumor might deplete the liver of the protein precursors required for enzyme synthesis. The gradual reduction in alanine transaminase activity in the livers of the tumor-bearing rats was associated with some loss in carcass weight. This metabolic change differs from that which occurs when loss of body weight is associated with starvation. The utilization of tissue proteins when food is withheld is accompanied by an enhanced rate of gluconeogenesis, and, under such conditions, the activity of alanine transaminase in liver is increased several-fold (30).

Treatment with cortisol or feeding a purified diet containing 75 per cent casein was previously found to inhibit the growth of the Walker tumor and to increase the ac-
tivity of alanine transaminase in this neoplasm (32). In the present experiments, hepatic alanine transaminase activity was elevated to normal levels by feeding the 75 per cent protein diet for 10 days. This effect of the high-protein diet may be related to an enlarged amino acid pool in liver that would allow for the synthesis of this enzyme. This concept was supported by the finding that a dose of cortisol, ineffective alone in the tumor-bearing rat, increased liver alanine transaminase activity when given concurrently with the 75 per cent casein diet. Since casein replaces part of the sucrose in this diet, an increase in the rate of gluconeogenesis may influence the level of this enzyme.

In contrast to alanine transaminase, aspartic acid and tyrosine transaminase, as well as tryptophan pyrrolase, did not decrease in liver as tumor growth progressed. It has been reported that tryptophan pyrrolase activity in liver varies during the growth of tumors in mice and rats (15, 19, 36), whereas hepatic levels of tyrosine transaminase have been shown to increase in rats bearing large Walker tumors (6). There are reports that the adrenal glands are larger than normal in mice bearing Sarcoma 180 (28, 35) and in rats bearing Jensen sarcoma (12), Walker carcinoma 256 (1, 2, 12), and the Murphy-Sturm lymphosarcoma (28, 29). The increase in tyrosine transaminase activity was attributed to increased corticosterone production caused by the stress induced by the tumor. However, this explanation may not be adequate, since Δ4-steroid dehydrogenase, an enzyme which inactivates corticosterone, is increased in activity in the liver of rats bearing the Walker tumor (7), and plasma corticosterone levels were not consistently elevated during the growth of Sarcoma 180 in mice (14).

Both tryptophan pyrrolase and tyrosine transaminase can be increased by cortisol treatment, and, in contrast to alanine transaminase, these inducible enzymes respond equally well to cortisol during the early and final stages of tumor growth. The response of these enzymes to cortisol might be related to their relatively short induction periods, 4–6 hours compared with 24 hours for alanine transaminase (33). Thus, whereas an enhanced rate of protein catabolism, maintained in liver for several hours following a single dose of cortisol, would be sufficient to induce a response of tyrosine transaminase or tryptophan pyrrolase, a more sustained period of catabolism and prolonged high levels of the steroid would be required for the induction of alanine transaminase in liver. Aspartate transaminase does not change with steroid treatment or adrenalectomy, and the lack of change in the specific activity of this enzyme is consistent with other observations (3, 5, 11).

The progressive growth of Walker carcinoma 256 resulted in a marked fall in liver alanine transaminase activity, whereas a similar but lesser effect occurred with lymphosarcoma P-1798 and Sarcoma 180. No depression of enzyme activity occurred in the cases of the Murphy-Sturm lymphosarcoma or the P-388 tumor (Table 3). In each of the tumor-host systems studied the animals were killed and the livers assayed for the enzyme when tumor growth was far advanced. Although marked losses in body weight and cachexia were observed in rats bearing either the Walker or Murphy-Sturm tumor, hepatic alanine transaminase activity was depressed only in animals bearing the Walker tumor. Thus, factors other than cachexia must be involved in the fall in activity of a specific enzyme. It is probable that the age of the animal as well as the growth pattern of the tumor are important factors conditioning the observed effect. Further studies are required to determine whether a depression in liver alanine transaminase activity occurs during the time of rapid growth of certain neoplasms but not during the period when tumor growth is slow or has reached a plateau. The factors which maintain the high activity of this transaminase in older rats may be more readily affected by tumor growth than those which regulate the level of this enzyme in the liver of young rats. The depression in alanine transaminase in animals bearing the Walker tumor was seen in mature animals in which the basal activity of this enzyme in liver is several-fold higher than it is in the immature rat.

The livers of tumor-bearing animals have been studied for changes in the activity of a number of enzymes. The decrease in catalase activity in the livers of rats and mice bearing a variety of tumors has been well established (9). The fall in the activity of this enzyme occurs at a gradual rate during the growth of the tumor and returns to normal when the tumor is extirpated (10). These changes in the activity of catalase in the liver have been attributed to the release of a specific and characteristic substance, toxohormone, from tumor cells (26, 27). More recently, Kampschmidt and co-workers (16–18) have related the decrease in the activity of this enzyme to the release of bacterial endotoxin by the tumor. The varied response of alanine transaminase to cortisol and high-protein feeding suggests that this enzyme may reflect changes in the metabolism of the host induced by the tumor. The consistent decrease in the activity of alanine transaminase associated with the rapid growth of the Walker tumor and also during the period of rapid fetal growth or regeneration of the liver suggests that the change in activity of this enzyme is related to the diversion of amino acids for protein synthesis.

ACKNOWLEDGMENTS

The technical assistance of Jo Ellen Budnick, Louis Budnick, and Phyllis Roswick is gratefully acknowledged.

REFERENCES

Depression of Alanine Transaminase Activity in the Liver of Rats Bearing Walker Carcinoma 256

Homer R. Harding, Fred Rosen and Charles A. Nichol

Cancer Res 1964;24:1318-1323.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/24/8/1318

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.