Alterations in Enzymes of Amino Acid Catabolism in Livers of Rats Bearing the Morris 7800 Hepatoma

Guglielmo de Rosa and Henry C. Pitot

ABSTRACT

The activities of the amino acid-catabolizing enzymes, serine dehydratase (EC 4.2.1.16), ornithine aminotransferase (EC 2.6.1.13), tyrosine aminotransferase (EC 2.6.1.5), and alanine aminotransferase (EC 2.6.1.12), were measured in the livers of rats bearing the Morris 7800 hepatoma for varying periods. A relatively rapid decrease in the activities of hepatic serine dehydratase and ornithine aminotransferase beginning within 1 week after inoculation of the tumor into the host was observed. The levels of tyrosine and alanine aminotransferases remained comparable to that in livers of non-tumor-bearing animals for approximately 4 weeks after the initial tumor transplantation.

The induction of serine dehydratase and tyrosine aminotransferase by glucagon was studied in these tumor-bearing animals. The response of serine dehydratase to this hormone decreased progressively with time after inoculation of the neoplasm. By 30 days after tumor inoculation, no response of this enzyme to glucagon administration was elicited. Tyrosine aminotransferase in these rats, on the other hand, was still inducible at least 5-fold at 35 days after tumor transplantation. By 14 days of tumor growth, ornithine aminotransferase activity was decreased in kidney and muscle as well as liver, although no changes in alanine aminotransferase activity in these tissues of the host could be detected. These results lend further support to the concept that highly differentiated hepatocellular carcinomas induce selective effects in host tissues, reflected by alterations in the levels of these four enzymes of amino acid catabolism.

INTRODUCTION

Generalized effects resulting from the presence of neoplasms in the host organism have been demonstrated in both humans and experimental animals. Alterations of plasma components such as proteins (28) and lipoproteins (1), as well as altered glucose tolerance (3), have been reported in cancer patients. In experimental animals organic and biochemical alterations occur in tissues not directly involved in the neoplastic transformation. Enlargement of the liver and spleen (2, 19, 31), together with an increase in both nucleic acid synthesis and content of these tissues, has been observed (17). Changes in the lipid composition of the liver have also been reported (32).

In addition to these general changes, considerable evidence of enzyme alterations occurring in almost every tissue of the host has been reported. Lundholm et al. (15) and Scherstén et al. (24) reported an increase of lysosomal enzymes in skeletal muscle and in the liver and kidney of tumor-bearing patients, with a decreased incorporation of amino acids into protein and an increased fractional rate of protein degradation in muscle. The literature on enzyme changes occurring in the host liver of tumor-bearing animals has recently been reviewed (9, 14). There is some indication that the observed alterations of the enzymatic pattern in host livers are related to the degree of differentiation of the growing neoplasm. In animals bearing the rapidly growing AH130 hepatoma, tyrosine aminotransferase in the host liver is increased, as compared with the level of the enzyme in the liver of the normal animal (26). On the other hand, no changes were observed in the activity of this enzyme in animals bearing a slowly growing hepatoma. Similar behavior has been reported by Hunt et al. (12) for the mitochondrial form of α-glycerophosphate dehydrogenase. A normal level of activity of this enzyme was observed in rats bearing the Morris 7800 hepatoma, while the activity of the dehydrogenase was above normal in the livers of rats bearing the rapidly growing Novikoff hepatoma.

Both the general effects on whole organism and on organ metabolism by the neoplasm may be the result of the competition of the tumor with the host tissues for specific nutrients, or the neoplastic cells may release specific factors affecting directly or indirectly the metabolic mechanisms in host cells, especially those involved in the regulation of nucleic acid and protein synthesis. Tryptophan oxygenase (EC 1.13.1.12) can be induced by its substrate in host livers, but its response to hormones appears to be impaired in this respect, compared with normal livers (4). The diurnal variation of tyrosine aminotransferase, together with the response of the liver of some tumor-bearing animals (11). On the other hand, the adaptive response of this enzyme and of serine dehydratase to different dietary treatments has been previously shown to be qualitatively similar in host liver and livers of control animals (23).

These 2 mechanisms indicated above are not mutually exclusive; both are probably operating in the host organism, as suggested by the study of Tryfiates et al. (30), which demonstrated the cooperative interaction of hormonal balance and vitamin B₆ availability in determining the metabolic alterations in the host liver. The studies reported in this paper are directed at attempting to distinguish between the primary reactions of the host to the presence...
of viable, replicating neoplastic tissue and those changes occurring secondary to degenerative processes that ensue in the neoplastic tissue or in the organs of a tumor-bearing animal as death is approached. This study was undertaken to determine the stage of growth of an implanted hepatocellular carcinoma at which the first metabolic changes in enzymes of amino acid catabolism in the liver were detectable and the evolution of such changes in those tissues, as reflected by alterations in the activity of amino acid-catabolizing enzymes and their responsiveness to environmental stimuli.

MATERIALS AND METHODS

Animals. Adult male Buffalo rats, weighing 150 to 200 g, were fed ad libitum a commercial diet and housed in a room lighted between 8 p.m. and 8 a.m. and dark from 8 a.m. to 8 p.m. After at least 1 week of acclimatization, the Morris 7800 hepatoma was inoculated s.c. in both posterior limbs (see below). The night before sacrifice (light period for the animals), food was withdrawn, but drinking water remained available to the animals. For elimination of possible influences of diurnal oscillations on enzyme activities, all animals were sacrificed between 10:30 and 11:30 a.m.

For transplantation the tumors, after excision, were trimmed of any visible connective, adipose, and necrotic material, and the remaining solid tissue was minced in chilled "suspension medium" (Swim's-77 medium; Grand Island Biological Company, Grand Island, N. Y.). An aliquot of 0.2 ml of the resulting suspension was inoculated s.c. with a 15-gauge needle. At intervals after inoculation groups of 3 rats each were sacrificed by decapitation and exsanguination. Samples of tissue were promptly removed. Control rats were treated with 0.25 ml of the suspension medium.

Preparation of Tissues. Samples were frozen in liquid nitrogen immediately after excision and were kept frozen at −70°C until ready to be used. Generally the same day the tissues were processed in the following way: the frozen tissue was crushed; approximately 1 g was weighed and immersed in an aliquot of homogenizing buffer (0.1 M Tris-HCl, 10−3 M dithiothreitol, 10−4 M EDTA, and 10−4 M pyridoxal PO₄, pH 8.0) sufficient to give a ratio of tissue to buffer of 1/3 (w/v). After being thawed the tissue suspension was homogenized in a glass homogenizer with a tight-fitting pestle. Samples of the whole homogenate were frozen at −70°C and saved for the determination of ornithine aminotransferase activity. The remaining homogenate was centrifuged at 150,000 × g for 60 min. The floating layer of fat was discarded, and the supernatant fluid was immediately assayed for alanine aminotransferase and serine dehydratase activities. Aliquots of the supernate were frozen at −70°C and assayed for tyrosine aminotransferase activity generally on the next day.

Enzyme Assays. Tyrosine aminotransferase activity was measured by an automated combination unit (22). The activity of ornithine aminotransferase was determined by the procedure described by Peraino and Pitot (20). Alanine aminotransferase activity was measured by the interrupted assay system described by de Rosa and Swick (7). For the determination of serine dehydratase activity, a similar assay was developed: aliquots of the 150,000 × g supernatant (100 to 200 µl) were incubated for 4 min at 30°C in the presence of 2 ml of 0.1 M L-serine in 0.15 M Tris-HCl buffer containing 10−3 M dithiothreitol and 10−4 M pyridoxal at pH 8.1. The reaction was stopped by the addition of 0.2 ml of chilled 30% trichloroacetic acid. The precipitate was removed by centrifugation, and an aliquot of the clear supernatant was used for the spectrophotometric determination of pyruvate in the presence of NADH and excess lactate dehydrogenase in a 0.2 M K₂HPO₄ solution. The molar extinction coefficient for NADH was taken to be 6.22 × 10³. The results are expressed as µmol of product formed per min per g of tissue, wet weight.

RESULTS

The activities of serine dehydratase, tyrosine aminotransferase, ornithine aminotransferase, and alanine aminotransferase in the livers of rats bearing Morris hepatoma 7800 at various day intervals after tumor transplantation may be seen in Charts 1 and 2. To obtain the control or initial value of enzyme activity in host livers at Time 0, we sacrificed a group of animals immediately after inoculation with the tumor. The points obtained at subsequent 5-day intervals were from animals randomly selected from the pool inoculated at Time 0.

As has been reported previously (21, 29), the presence of Morris hepatoma 7800 had a striking influence on the activity of serine dehydratase (Chart 1). As early as 5 days after inoculation of the tumor, serine dehydratase activity had decreased slightly. Thereafter, a relatively rapid decay in the activity of the enzyme occurred, such that, by 15 days after transplantation, serine dehydratase activity was significantly below that of the Time 0 control, exhibiting only about 50% of the original activity of the enzyme. The liver itself was grossly normal. Fifteen days later the activity of this enzyme was only barely detectable in the host liver, and it was undetectable thereafter.

In agreement with other studies in which different neoplasms were used (9), the activity of ornithine aminotransferase also decayed rather rapidly in the host liver as tumor growth progressed. As can be seen from Chart 2, the decay...
in the activity of this enzyme exhibited a plateau between 10 and 25 days after tumor inoculation. Subsequently, there was a rapid linear decay in the activity of the enzyme in the host liver. On the other hand, the behavior of the 2 soluble aminotransferases was significantly different from that observed for the mitochondrial ornithine aminotransferase or for serine dehydratase. Both alanine aminotransferase and tyrosine aminotransferase were significantly increased over the Time 0 control 15 days after transplantation of the Morris 7800 hepatomas into the host. However, the activity of the enzyme tended to decrease slowly for the next 10 days and then precipitously subsequent to 30 days after tumor inoculation. Thirty-five days after transplantation the rats usually exhibited a terminal cachexia (6) with significant loss of weight, fatty metamorphosis of the liver, and tumor necrosis. Even at this advanced state, however, the activities of the soluble aminotransferases were between 30 and 40% of the initial control values.

The response of tyrosine aminotransferase and serine dehydratase of the host liver to the s.c. injection of glucagon (1 mg/100 g) 8 hr prior to sacrifice was investigated to determine whether the response of these enzymes to hormonal regulatory stimuli was altered in the host liver during the 5-week period subsequent to inoculation of the tumor. Both of these enzymes in normal liver have been reported to be induced by the administration of glucagon at these dosages (10, 13). As can be seen from the data in Chart 3, the responses to the administration of glucagon of these 2 enzymes are distinctly different in the tumor-bearing host. There is a progressive decrease in the inducibility of serine dehydratase in the host liver by glucagon after the inoculation of the tumor. Twenty-five days subsequent to tumor transplantation and thereafter, no induction of serine dehydratase by glucagon was evident in the host liver. On the other hand, at 10 days after inoculation of the tumor the response of tyrosine aminotransferase to glucagon was significantly enhanced over control values, although by 20 days postinoculation and thereafter the inducibility of this enzyme was about 30 to 50% lower than was that in control normal liver.

To investigate the possibility that, during the growth of Morris hepatoma 7800, metabolic alteration of the enzymes might occur in tissues other than liver, we determined the levels of ornithine aminotransferase and alanine aminotransferase in muscle and kidney 15 days after tumor implantation (Table 1). This interval between tumor inoculation and sacrifice of the host was selected as the shortest period giving the most consistent metabolic changes in the host liver without any visible degenerative alterations in either liver or hepatoma. As can be seen from the table, the activity of ornithine aminotransferase is consistently and significantly lower in these tissues of tumor-bearing animals than in normal controls. No significant changes were observed in the levels of alanine aminotransferase when normal and host tissues were compared. Not shown in the table is a further decrease of ornithine aminotransferase in muscle and kidney noted at 35 days after tumor inoculation, while alanine aminotransferase activities in these 2 tissues remained unchanged, as did the activity of these enzymes in brain and heart at 15 and 35 days postinoculation. After the 25th postinoculation day, tumor-bearing animals lost total body weight, while the tumors, which weighed 1 to 2 g at the 25th day, doubled or tripled their weight prior to the 35th postinoculation day.

**DISCUSSION**

The influence of an implanted tumor on the level of a number of hepatic enzymes has been described previously (9). In these studies only 1 or, at the most, 3 time intervals after transplantation of the tumor were studied. Such experiments have the disadvantage of not showing the progression of events in the process under study. Some investigations in which the evolution of changes occurring in host tissues has been monitored during the course of
that the increases noted at 15 days in tyrosine and alanine.

The results reported here indicate that several host tissues, especially liver, are affected at very early stages of development of the tumor, as demonstrated by the fact that the basal levels of some enzymes (serine dehydratase and ornithine aminotransferase) appear to be already repressed between 5 and 10 days after the inoculation of a suspension of neoplastic cells into the host animal. The decreased level of these 2 enzymes does not appear to be merely 1 aspect of a generalized depression of host liver function; rather, it probably indicates the occurrence of selective metabolic changes in the host. Such selectivity is indicated by the observation that, at the same time, increases in the levels of other enzymes (tyrosine aminotransferase and alanine aminotransferase) are observed. The different sensitivity of these 2 groups of enzymes to glucagon treatment further supports the hypothesis of selective modification of liver metabolism occurring at this stage.

The observations that alanine aminotransferase activity was not altered in kidney and muscle and that both alanine and ornithine aminotransferase remained at control levels in brain and heart at 35 days of tumor development suggest that the specificity of the regulatory mechanism operating in different tissues is preserved even in the more advanced stages of neoplastic growth. These results, taken together with the effects on amino acid-catabolizing enzymes exhibited in liver (even early after transplantation of the Morris 7800 hepatoma), demonstrate that effects of this neoplasm on host metabolism are a dynamic feature of the overall host-tumor relationship in this and quite likely other host-tumor interactions.

Table 1

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Liver</th>
<th>Kidney</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Host</td>
<td>Control</td>
</tr>
<tr>
<td>AAT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.86 ± 2.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>67.06 ± 7.55</td>
<td>3.97 ± 0.09</td>
</tr>
<tr>
<td>OT</td>
<td>2.83 ± 0.21</td>
<td>1.83 ± 0.29</td>
<td>3.36 ± 0.24</td>
</tr>
</tbody>
</table>

<sup>a</sup> AAT, alanine aminotransferase; OT, ornithine aminotransferase.

<sup>b</sup> Mean ± S.E. of enzyme activity from 3 rats. The liver weights and body weights of control and tumor-bearing animals were comparable at this time.

tumor development have indicated that misleading conclusions can result by limiting the study of host-tumor relationships to observations at only 1 point in time (33).

The use of animals bearing fully developed tumors will, as a consequence, always carry the uncertainty of whether the observed effects are the direct result of the presence of neoplastic cells in the organism or an indication of nonspecific degenerative changes resulting only indirectly from the presence of the neoplasm. Such nonspecific degenerative changes may be the result of necrotic degeneration of neoplastic tissue or of secondary bacterial infection, or they may be by-products of either of these processes.

During the advanced phase of tumor growth, many host tissues, liver in particular, often undergo degenerative changes (6). The use of animals bearing fully developed tumors will, as a consequence, always carry the uncertainty of whether the observed effects are the direct result of the presence of neoplastic cells in the organism or an indication of nonspecific degenerative changes resulting only indirectly from the presence of the neoplasm. Such nonspecific degenerative changes may be the result of necrotic degeneration of neoplastic tissue or of secondary bacterial infection, or they may be by-products of either of these processes.

The results reported here indicate that several host tissues, especially liver, are affected at very early stages of development of the tumor, as demonstrated by the fact that the basal levels of some enzymes (serine dehydratase and ornithine aminotransferase) appear to be already repressed between 5 and 10 days after the inoculation of a suspension of neoplastic cells into the host animal. The decreased level of these 2 enzymes does not appear to be merely 1 aspect of a generalized depression of host liver function; rather, it probably indicates the occurrence of selective metabolic changes in the host. Such selectivity is indicated by the observation that, at the same time, increases in the levels of other enzymes (tyrosine aminotransferase and alanine aminotransferase) are observed. The different sensitivity of these 2 groups of enzymes to glucagon treatment further supports the hypothesis of selective modification of liver metabolism occurring at this stage.

While our results do not give evidence of mechanisms responsible for the early changes in amino acid-catabolizing enzymes in host liver, several possibilities suggest themselves. Since many neoplasms stimulate increased adrenal hormone production in the host (6), it is possible that the increases noted at 15 days in tyrosine and alanine aminotransferases may be due to the increased production of glucocorticoids by the host in response to the neoplasm (10, 25). In a similar manner but through opposite effects, an increase in plasma glucocorticoid concentration could account for the marked suppression of ornithine aminotransferase (18). Furthermore, the fact that changes in the levels of some of these enzymes may be seen as early as 10 to 15 days after tumor transplantation when no neoplastic tissue is grossly visible at the site of inoculation suggests that the observed effects are not secondarily mediated by necrotic tumor or bacterial toxins.

From the experiments reported in this paper, it is possible to divide the time of host-tumor interaction after transplantation into 3 stages, which are quite comparable to those originally described by Mider et al. (16) in rats bearing the transplanted Walker 256 tumor: an early phase, which develops during the first 10 days of the process, when selective alterations occur in tissues; an intermediate period, between 10 and 20 days after tumor transplantation, which could be interpreted as an attempt of the host organism to compensate for the metabolic imbalance evoked by the growing tumor; and a final stage of exhaustion of the compensatory mechanism of the host. The metabolic alterations observed in the host liver in the first days after tumor implantation could be the result either of specific nutritional requirements of the tumor cells [an elevated uptake of amino acids is characteristic of many neoplastic tissues, and a selective affinity for specific amino acids has been demonstrated (5)] or of an aberrant metabolism of the growing neoplastic tissue, which could result in changes in plasma metabolites other than amino acids. Many tumor cells have a predominantly anaerobic metabolism with high rates of lactate production (8). Catabolites might also be produced by the neoplastic cells, which are not present in the plasma of normal animals. In this category could be included the "toxohormones," the presence of which in the plasma of tumor-bearing animals has been postulated by a number of investigators (for review see Ref. 27).

The observations that alanine aminotransferase activity was not altered in kidney and muscle and that both alanine and ornithine aminotransferase remained at control levels in brain and heart at 35 days of tumor development suggest that the specificity of the regulatory mechanism operating in different tissues is preserved even in the more advanced stages of neoplastic growth. These results, taken together with the effects on amino acid-catabolizing enzymes exhibited in liver (even early after transplantation of the Morris 7800 hepatoma), demonstrate that effects of this neoplasm on host metabolism are a dynamic feature of the overall host-tumor relationship in this and quite likely other host-tumor interactions.

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REFERENCES


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