Activities of Various Aminotransferases in Tumor-bearing Rats

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SUMMARY

This communication reports changes in the activities of tyrosine aminotransferase (EC 2.6.1.5, L-tyrosine:2-oxoglutarate aminotransferase), alanine aminotransferase (EC 2.6.1.2, L-alanine:2-oxoglutarate aminotransferase), and aspartate aminotransferase (EC 2.6.1.1, L-aspartate:2-oxoglutarate aminotransferase) in tumor-bearing rats. From the results and studies on various analogous conditions, the metabolism of tumor-bearing animals is discussed. It was found that the activities of aminotransferases of liver cell sap show a biphasic change—first decreasing and then increasing during the tumor-bearing process—and that the raised activities are reduced before death. It is suggested from our studies that, although there seems to be a continued internal protein deficiency (hypoproteinosis) from an early stage of tumor bearing, there is a hyperfunction of the hypophyseal-adrenal cortical system, perhaps induced by the increased internal utilization of glucose (by the tumor) in the advanced stage, and a sudden decrease in adrenal function prior to death of the animal.

A preliminary report on the changes in various enzymes in the tumor-bearing rats has appeared (19).

This communication reports studies on (a) the enzymatic pattern in the liver during the tumor-bearing process and (b) the regulatory mechanisms that control these enzymatic patterns in the tumor-bearing animal. With enzymatic activity used as an index, the dynamic changes in these activities under various conditions were examined, and the regulatory mechanisms of tumor-bearing animals are discussed on the basis of the findings, that is, the viewpoint of "enzymatic biology."

It is necessary to consider tumor bearing in relation to time, because the metabolism of tumor bearers changes during this process. To discuss the metabolic regulatory mechanisms in tumor-bearing animals, various analogous conditions, i.e., pregnancy, a nonprotein (high carbohydrate) diet, a low carbohydrate (high protein) diet, and fasting, were examined from the standpoint of protein metabolism and hypophyseal-adrenocortical function.

It is well known that liver tyrosine aminotransferase is a readily inducible enzyme (1, 5, 8–11, 13–15, 25). Therefore, to clarify the tumor-host relationship, the liver tyrosine aminotransferase activities in the tumor-bearing animals were studied.

It is well known that the liver alanine and aspartate aminotransferases are distributed in the mitochondrial fraction and in the supernatant fraction, and so are so-called isoymes (2, 6, 7). To study the dynamics of the isoymes in tumor-bearing animals, especially in relation to their metabolic functions, the liver alanine and aspartate aminotransferase activities were examined during the tumor-bearing process and in other conditions.

In addition to these biochemical studies, morphologic studies were also made.

MATERIALS AND METHODS

Male albino rats (Donryu strain), weighing about 100 gm., and ascites tumor cells, strain AH130, maintained in this laboratory, were used throughout this work. Tumor bearing was started by intraperitoneal implantation of 1 ml. of ascites, containing approximately 2.5 × 10⁸ cells of strain AH130. Tumor-bearing animals died between the 10th and 14th days. In studies on pregnancy, female albino rats (Donryu strain) weighing approximately 250 gm. were used. Tumor-bearing and pregnant rats were fed ad libitum on commercial rat food, purchased from Oriental Yeast Co., Ltd., Japan. In nutritional experiments, synthetic foods were given ad libitum. The content of the nonprotein diet used was as follows: 28.34 per cent sucrose, 58 per cent dextrin, 2 per cent cellulose powder (purchased from Toyo-Roshi Co., Ltd., Japan), 4 per cent salt mixture, 5 per cent vitamin mixture, and 2 per cent oil, all of which were purchased from Tanabe Essential Amino Acids Research Foundation, Japan, and 0.66 per cent choline chloride. The content of the high protein diet used was as follows: 85 per cent milk casein, 4 per cent starch, 4 per cent salt mixture, 5 per cent vitamin mixture, and 2 per cent oil. Usually five to ten rats were used in each experiment.

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† Numbers and nomenclature assigned by the Commission on Enzymes of the International Union of Biochemistry.
and the results were expressed as mean values. Animals were sacrificed by cervical dislocation between 10 and 12 A.M. and perfused intravenously to remove residual hepatic blood with ice-cold 0.9 per cent saline in situ. Then the liver and kidneys were removed and washed with the same saline to remove adherent ascites.

Enzymes were assayed as follows. To obtain tyrosine aminotransferase, the liver was homogenized in a Potter-Elvehjem glass homogenizer with 9 volumes of 0.005 N NaOH in 0.14 M KCl for 2 minutes. The homogenate was centrifuged for 20 minutes at 13,000 × g and the supernatant fraction was used as the enzyme solution. Before use, the enzyme was preincubated with 30 μmoles of pyridoxal phosphate. The assay system for tyrosine aminotransferase contained 200 μmoles of tris(hydroxymethyl)aminomethane (Tris) buffer, pH 8.0, 30 μmoles of α-ketoglutarate, 12 μmoles of tyrosine, 10 μmoles of diethyldithiocarbamate (DDC), and 0.5 ml. of enzyme. The final volume was adjusted to 3 ml. Incubation was performed at 37°C for 10 min. The p-hydroxyphenylpyruvate formed was measured spectrophotometrically at 310 μm as its borate complex with 1 M borate in 2 M arsenate, pH 6.5 (12). The specific activity of tyrosine aminotransferase was expressed as μmoles of p-hydroxyphenylpyruvate produced/minute/mg of protein. To obtain alanine and aspartate aminotransferases, the liver was homogenized in 9 volumes of 0.25 M sucrose in a Potter-Elvehjem glass homogenizer and fractionated by differential centrifugation. The alanine aminotransferase activity was assayed by a modification of the method of Friedemann and Haugen (7). The aspartate aminotransferase activity was assayed by the same procedure as the alanine aminotransferase after decarboxylation of oxaloacetate with aniline citrate (6). The assay system for alanine aminotransferase contained 60 μmoles of Tris buffer, pH 8.0 (for mitochondrial) and pH 8.6 (for supernatant), 10 μmoles of α-ketoglutarate, 10 μmoles of L-alanine, 50 μmoles of glutathione, and 0.2 ml. of enzyme. That for aspartate aminotransferase contained 60 μmoles of Tris buffer, pH 8.6, 10 μmoles of α-ketoglutarate, 10 μmoles of L-aspartate, and 0.05 ml. of enzyme (preincubated with 0.05 μmole of pyridoxal phosphate at 37°C for 10 min. before use). In both cases, the final volume was adjusted to 1 ml. Incubation was performed at 37°C for 10 min. in the former and 15 min. in the latter case. The specific activity is expressed as μmoles of pyruvate (alanine aminotransferase) or oxaloacetate (aspartate aminotransferase) produced/min/mg of protein. Spectrophotometric measurements were carried out in a Beckman model DU spectrophotometer.

Adrenalectomy was performed bilaterally by the retroperitoneal approach after the animals had been anesthetized with ether. Hydrocortisone (Schering) was used as the glucocorticoid preparation.

To study the morphology of the adrenal glands of the rats, these were stained by hematoxylin-eosin and Sudan III.

RESULTS

Growth of ascites tumor cells, strain AH130.—The strain of AH130 cells used throughout this study was implanted intraperitoneally and the cells were grown as shown in Chart 1.

Ratio of liver weight to carcass weight.—Chart 2 shows that the ratio was slightly elevated in the advanced stage (6—9 days) and decreased in the late stage (11—14 days).

Enzymatic activities in tumor-bearing rats.—As shown in Charts 3—5, although the mitochondrial aminotransferase activities did not change, the supernatant aminotransferase activities showed marked changes during the
and we have confirmed, that glucocorticoid induces these liver aminotransferase activities in both normal and tumor-bearing rats (Chart 7). Moreover, it was found that the reduced levels of these soluble aminotransferase activities in the liver increased even in the protein-deficient state on administration of glucocorticoid (Chart 6) and that the increased levels of these enzymatic activities were reduced by adrenalectomy when animals were fed on a diet deficient in carbohydrate (Table 1) or were fasted (Table 2). It was also found that the

tumor-bearing process, i.e., an early decrease, then an increase, and finally a decrease.

Enzymatic activities of rats fed on a nonprotein diet.—The liver aminotransferase activities decreased in the supernatant fraction, whereas they did not change in the mitochondrial fraction (Chart 6).

Enzymatic activities of rats fed on a sugar-deficient diet.—When the rats were fed on a 85 per cent casein diet, the liver supernatant aminotransferase activities increased but the mitochondrial isozymes hardly changed in activity (Table 1).

Enzymatic activities in fasting rats.—As shown in Table 2, the liver soluble aminotransferase activities increased during fasting (external malnutrition), although the mitochondrial isozymes remained at almost the normal level.

Morphologic studies on the adrenal glands.—It has been reported, and we have also found, that hypertrophy of the adrenal glands (Table 3) and atrophy of the thymus occur in the advanced stage of the tumor-bearing process. As shown in Figures 1–8, the zona fasciculata of the adrenal cortex was hypertrophied, whereas the zona glomerulosa almost disappeared (Figs. 1–4), and the sudanophil droplets of the zona fasciculata became fine and almost disappeared (Figs. 5–8) in tumor-bearing animals (9 days after tumor implantation). There were no tumor metastases in the adrenal glands.

Effect of adrenal glucocorticoid on the soluble aminotransferases.—It has been stated (1, 4, 5, 8–11, 13–15, 23, 25),

TABLE 1

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Nonoperated</th>
<th>Sham-operated</th>
<th>Adrenalectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine aminotransferase</td>
<td>0.082</td>
<td>0.07</td>
<td>0.022</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>0.67</td>
<td>0.63</td>
<td>0.42</td>
</tr>
<tr>
<td>Supernatant</td>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>1.8</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td>4.9</td>
<td>4.5</td>
<td>3.8</td>
</tr>
<tr>
<td>Supernatant</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CHART 3.—Liver tyrosine aminotransferase activities in tumor-bearing rats. Enzymatic activities are expressed as μmoles of p-hydroxyphenylpyruvate produced/min/mg of protein. Points represent the means, and vertical lines on each point indicate the range of variation. Final point is the value obtained from the animals before death.

CHART 4.—Liver alanine aminotransferase activities in tumor-bearing rats. Enzymatic activities are expressed as μmoles of pyruvate produced/min/mg of protein. —, enzymatic activity of supernatant fraction (GPTₔ); —0---, that of mitochondrial fraction (GPTₐ). Points represent the means, and vertical lines on each point indicate the range of variation. Final point is the value obtained from the animals before death.
TABLE 3

WEIGHT OF ADRENAL GLANDS IN VARIOUS CONDITIONS

Male albino rats weighing 100 gm. were used. Normal control and tumor-bearing rats were fed on a commercial rat food (Oriental Yeast Co., Ltd., Japan) ad libitum. Tumor-bearers were used 9 days after intraperitoneal implantation of AH130 cells. Fasting rats were given nothing except 0.9 per cent saline for 5 days. Sugar or protein deficiency was introduced to normal rats fed on a high protein or nonprotein diet for 7 days, respectively. Values represent the means of the bilateral two glands of at least ten animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Adrenal glands (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>22.8</td>
</tr>
<tr>
<td>Protein-deficient</td>
<td>22.4</td>
</tr>
<tr>
<td>Sugar-deficient</td>
<td>38.0</td>
</tr>
<tr>
<td>Fasting</td>
<td>43.2</td>
</tr>
<tr>
<td>Tumor-bearing</td>
<td>49.4</td>
</tr>
</tbody>
</table>

TABLE 4

ENZYMATIC ACTIVITIES AFTER ADRENALECTOMY IN TUMOR-BEARING RATS

Tumor-bearers, 8 days after tumor implantation, were subjected to operation 24 hr. before sacrifice. Values represent the mean specific activities of ten animals. The specific activities are expressed in the same way as those of Table 1.

<table>
<thead>
<tr>
<th>Enzyme and Treatment</th>
<th>Normal control</th>
<th>Tumor-bearing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-operated</td>
<td>Sham-operated</td>
</tr>
<tr>
<td>Tyrosine aminotransferase</td>
<td>0.013</td>
<td>0.041</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>0.40</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td>2.6</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>1.4</td>
<td>1.4</td>
</tr>
</tbody>
</table>

* Hydrocortisone (Schering) was injected intraperitoneally immediately and 8 hr. after operation and 4 hr. before sacrifice (5 mg/100 gm of body weight).

increased levels of these aminotransferase activities in the supernatant of the liver in the advanced stage of tumor bearing were reduced after adrenalectomy and maintained on administration of glucocorticoid following adrenalectomy (Table 4).

Enzymatic activities in pregnant rats.—As shown in Table 5, there was no change in the tyrosine aminotransferase activity and a slight decrease in the soluble alanine and aspartate aminotransferase activities, but no enzyme increased in pregnancy.

DISCUSSION

Since Greenstein first reported a decrease in liver catalase activity in tumor-bearing animals, there have
been various reports on enzymatic activities in tumor-bearing animals. Moreover, Mider reported that the nitrogen balance of rats bearing specific tumors changes from positive to negative during tumor growth and that the ratio of tumor weight to body weight influences the nitrogen balance (16, 24). This means that the metabolism of animals apparently changes in the course of tumor bearing, so that it is necessary to study tumor bearing in the light of the "process." There have been few reports on studies from this standpoint, although, as already shown in this communication, it is very important. Indeed, as shown in Charts 3–5, liver soluble aminotransferase activities showed successively a decrease, an increase, and finally a decrease during the tumor-bearing process. It is interesting in comparing Charts 1 and 3–5 that the decrease in aminotransferase activities during the first 3 days covers the period during which the tumor cells did not increase in number. The great increases in aminotransferase activities parallel the increase in cell number of the tumor but apparently continue for a few days after rapid increase in number of cells has ceased. Then the sudden drop in enzymatic activities appears, apparently when the animals are moribund. Of course there were no tumor metastases in the livers morphologically. Therefore the modified enzymatic levels reflect exclusively the alterations in hepatic enzymes per se. Moreover it is believed that the major factor causing these changes in aminotransferase activities is "apoprotein," because the intraperitoneal administration of pyridoxine (5 mg/100 gm. of body weight) had no effect on the decreased aminotransferase activities in the early stage and also because the addition of pyridoxal phosphate (50 μg/enzymatic assay system) to a preparation with decreased alanine aminotransferase activity did not restore the activity in vitro.

It is said that there is decreased albumin with increased globulin in the plasma of tumor-bearing animals (17). Therefore, there is a hypoalbuminosis in these animals. Indeed, it was found that the contents of liver soluble aminotransferases decreased when animals were fed on a nonprotein diet to develop a state of "external (dietary)" hypoalbuminosis (Chart 6). Therefore, it seems that there is an "internal (metabolic)" hypoalbuminosis from an early stage in tumor-bearing animals. This was confirmed and reported from other studies made in our laboratory (21). However, it was also found that these enzymatic activities did not increase in animals with hypoalbuminosis only (Chart 6), and it is emphasized that there must be an additional factor involved in the advanced stage of tumor bearing.

As shown in Table 2, the activities of these liver soluble


table 5

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Normal</th>
<th>Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine aminotransferase</td>
<td>0.013</td>
<td>0.014</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supernatant</td>
<td>0.38</td>
<td>0.29</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supernatant</td>
<td>2.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>1.5</td>
<td>1.4</td>
</tr>
</tbody>
</table>
of tumor bearing were reduced after adrenalectomy. Therefore, the increase in the activities of these liver enzymes in the advanced stage seems to be controlled biochemically by the adrenal cortical hormone (glucocorticoid). Hypertrophy of the adrenal glands (zona fasciculata) was seen in the advanced stage of tumor bearing as well as in the fasting condition and also in animals fed on a high protein-low carbohydrate diet (Table 3 and Figs. 1–8). Since these morphologic findings are similar to those seen when there is hypersecretion of ACTH, hypophyseo-adrenocortical function must be increased in the advanced stage of tumor bearing. From these data and discussion, it is emphasized again that the modified hepatic enzyme levels are due to the tumor-induced adrenal hypertrophy.

In pregnant rats, as shown in Table 5, there was no increase in enzyme activity in the liver soluble fraction, whereas there was an increase in the advanced stage of tumor bearing. The fetus is a rapidly growing organism, and the mother ("host") provides it with synthetic materials and sources of energy. It has been reported that various enzymes which decrease in protein deficiency and in the tumor-bearing process also have decreased activities in pregnancy (21). Possibly, therefore, although there may be an internal hypoproteinosis in both pregnancy and the tumor-bearing process, the metabolism of tumor-bearing animals in the advanced stage is not comparable to that of pregnant animals. Because it is thought, as has been reported (20), that the content of various aminotransferases of tumor cells is not equal to that of fetus liver cells, the metabolism of these two types of cells is not the same. There does not seem to be the same degree of utilization and uptake of an energy source (glucose). There is apparently an increased tendency to waste blood glucose and liver glycogen in the advanced stage of tumor bearing (18). It is known that in tumor cells, as first reported by Warburg (29), there is increased glycolysis with decreased respiration (3, 26–28). This means that in rapidly dividing tumor cells the demand for a large energy supply is mainly supported by glycolysis, a very inefficient procedure; thus it is believed that there is an increased tendency to degrade glucose in the tumor cells and for them to take up sugar from the blood. It is considered that the changes in the blood constituents may stimulate the metabolic regulatory mechanism of the host, and its metabolism must be adapted accordingly to maintain the homeostasis of blood. Because the tumor cell traps (blood) glucose as well as nitrogen, the host seems to defend itself by glucocorticoid in the advanced

![Chart 7.—Effect of glucocorticoid on enzymatic activities in tumor-bearing rats. Hydrocortisone (5 mg/100 gm of body weight) was injected intraperitoneally 5 hr. before sacrifice (○). Hydrocortisone was not given to the controls (□—□). Values represent the means of ten animals, and vertical lines on each point indicate the range of variation.](chart7.png)

*Fig. 1.*—Normal control.
*Fig. 2.*—Tumor-bearing.
*Fig. 3.*—Fasting.
*Fig. 4.*—Sugar-deficient.
Figs. 5-8.—Morphology of the adrenal cortex of rats in various conditions. Sudan III, X 100. The sudanophil droplets of the zona fasciculata became fine and almost disappeared in the tumor bearer (9 days after tumor implantation).

Fig. 5.—Normal control.
Fig. 6.—Tumor-bearing.
Fig. 7.—Fasting.
Fig. 8.—Sugar-deficient.
stage of tumor bearing. Indeed, it was found that hypophyseo-adrenocortical function was apparently stimulated in the advanced stage of tumor bearing, as described above, and that the plasma corticosterone level was high, as reported elsewhere (22). Moreover, as shown in Chart 2, the ratio of liver to carcass weight increased slightly in the advanced stage of tumor bearing. This appears to support the above idea, because it is well known that the nitrogen content of the carcass decreases and that of the liver increases on administration of glucocorticoid; i.e., nitrogen is transported from the carcass to the liver by the action of glucocorticoid, resulting in hypertrophy of the liver and emaciation of the body. Thus, this control mechanism, although it regulates the metabolism of the host and maintains the homeostasis of blood and fluid, enhances the pathologic features of tumor bearing and pushes the host toward cachexia. In the late stage there seem to be metabolic disturbances due to sudden injury and decrease in hypophyseo-adrenocortical function, as shown by the decrease in liver soluble aminotransferase activities.

It is very interesting that the changes of liver enzymatic activities were observed only in the soluble fraction and that the mitochondrial aminotransferases showed little change. It has been said that the mitochondrial aminotransferases are related to the tricarboxylic acid cycle and thus to energy metabolism (6, 7). Therefore, it is suggested that the respiration of the host liver cells is maintained at almost the normal level during the tumor-bearing process.

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