Transaminase Activities of Liver Tumors and Serum

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SUMMARY

The glutamic oxalacetic transaminase (GOT) activities of five transplanted malignant liver tumors were compared, as well as that of mammary adenocarcinoma 6388, the Walker carcinosarcoma 256, and the livers and sera of the host rats. Bile and urine were analyzed in some additional experiments with Hepatoma 5123.

The GOT activity of Hepatoma 5123, unlike that of the Dunning, the Novikoff, the Morris 3683, and the Sidransky ethionine-induced hepatomas, was greater than that of liver. The livers of the host animals had approximately the same transaminase activities as livers of tumor-free animals of the same ages. No strain or sex differences were observed.

An elevation in serum GOT was observed in rats bearing the transplanted tumors. The amount of elevation was sometimes insignificant with the Novikoff tumor and was moderate with the other tumors studied, except with Hepatoma 5123, which was associated with an elevation of 300–4000 per cent of normal. The degree of elevation was dependent upon the specific tumor type, but within the range of increase for the type, serum GOT activity increased proportionally with the weight of the transplant with 5123, and also with the Novikoff and the Sidransky liver tumors. Tumorectomy of 5123 was followed by a decrease to a normal level of serum GOT in 48 hours.

Urine and bile contained very little GOT activity compared with that of serum. GOT activity was increased in these two excretory fluids with increased serum GOT, but the amount of increased activity found accounted for little of the activity that disappeared from the blood following tumorectomy of 5123, or following the intraperitoneal injection of a GOT-rich tumor extract.

The high GOT activity in the slowly growing Hepatoma 5123, and the very low activity in the Novikoff, the fastest growing of the tumors examined, is in accord with an earlier observation of Cohen et al. that there is an inverse relation of GOT activity to rate of growth of tissues, including liver.
oxalacetic acid transaminase activity in malignant hepatomas which arose from normal livers of known transaminase activity during the feeding of 4-dimethylaminoazobenzene to rats. Transaminase activity in livers from the rats fed the carcinogen for varying lengths of time up to 300 days decreased with time to about one-third the initial value in the resulting tumor tissue.

The glutamic-oxalacetic transaminase activities of five transplanted malignant liver tumors of the rat have been compared, as well as that of a mammary tumor, a carcinosarcoma, and the liver, and serum or plasma of the host animals. In exploring the fate of the transaminase activity found associated with the growth of one of the hepatomas, bile and urine have also been analyzed.

MATERIALS AND METHODS

Analytical methods.—The method of Tonhazy, White, and Umbreit (23) was used to determine the glutamic-aspartic transaminase activity of tissues and that of Karmen (9) as modified by Steinberg, Baldwin, and Ostrow (30) for the assay of glutamic-oxalacetic transaminase of serum, whole blood, bile, and urine. Both methods measure the glutamic-oxalacetic transaminase activity (GOT). For tissues, the reaction between aspartate and a-ketoglutarate with the production of oxalacetate is stopped after 10 minutes with trichloroacetic acid, which precipitates all protein. After the oxidation of oxalacetate to pyruvate by means of aniline citrate, the pyruvate is converted to its dinitrophenylhydrazone, which is extracted in toluene and measured colorimetrically in alkaline alcoholic solution at a wave-length of 505 m\u2013. In the method used for blood, oxalacetate is also produced quantitatively by the enzyme activity with aspartate and a-ketoglutarate in the substrate. This reaction is coupled with a malic dehydrogenase reaction in the presence of reduced diphosphopyridine nucleotide (DPNH). Oxalacetate is reduced to malate, and the reaction is followed by measuring the decrease in the characteristic light absorption peak of DPNH at 340 m\u2013, where DPN has virtually no absorption. A Beckman Model DU Photoelectric Spectrophotometer was used. With both methods duplicate or triplicate analyses were made at two to four different dilutions.

Total nitrogen was measured by a micro-Kjeldahl method (6), and dry weights of liver and tumor tissue were determined by heating the quickly minced fresh tissue for 24 hours in an oven at 107 ± 3° C., followed by powdering and drying to constant weight in a desiccator over sulfuric acid.

Biologic materials.—Young adult inbred rats, male or female, of the Buffalo, Irish (A × C), Fischer, and Osborne-Mendel strains and Sprague-Dawley and Holtzman stocks were used with and without tumor transplants. Analyses were made of liver tumors: Morris 5123 (15), Morris 3683 (13), the Novikoff (16),1 the Dunning (5), and the Sidransky,1 and of Walker carcinosarcoma 256 (21),2 and Morris mammary tumor 6338.3 Hepatoma 5123 of transplant generations 19–21 and sublines B, C, and D was used for many of the studies. Comparisons were made with groups of at least two or three rats bearing the other types of tumor, and of two or three control rats of each of the strains used as hosts to the tumor transplants. Intraperitoneal, subcutaneous, and intramuscular transplants were analyzed. Grossly apparent necrotic areas of tumor tissue were discarded. For many of the studies the 5123 liver tumors were transplanted into the ovaries or kidneys of female rats following a “host-isolated transplant” technic recently reported (8).4 The ovarian and kidney transplants originated from a subcutaneous transplant of the sixteenth generation before any sublines were established.

Portions of two lobes of each liver and two pieces of each tumor, and sometimes one kidney, were homogenized in concentrations of 0.03125 to 1.0 per cent in 0.1 m potassium phosphate buffer, pH 7.4. The tissues and homogenates were kept ice-cold and analyzed at once.

At the time of killing blood was withdrawn from the abdominal aorta of the animal under mild ether anesthesia. In some cases blood samples were taken repeatedly from the jugular vein with a syringe rinsed with heparin (0.5 mg/ml saline). When blood samples from the tumor and from the liver were desired, they were collected from the vein leading from the ovary in which the transplant was growing and from one of the hepatic veins, respectively. For comparison of serum GOT and the GOT of whole defibrinated blood, 0.5 ml. of the blood sample was hemolyzed by introduction into 9.5 ml. of distilled water at the time of collection. The remainder of the blood was allowed to clot in the refrigerator, and the serum was removed after centrifugation. The refrigerated, hemolyzed whole blood was centrifuged after 24–48

1 We are grateful to Dr. Mary E. Mauer of this laboratory for rats bearing the Novikoff tumor and to Dr. H. Sidransky of the Laboratory of Pathology of the National Cancer Institute for hepatoma transplants from the third and seventh generations of an ethionine-induced tumor, and for rats bearing primary liver tumors induced by ethionine.

2 Carried for many years at N.C.I. in Sprague-Dawley rats.

3 Induced in a Buffalo rat by feeding N-2-fluroenylaceta-

4 We acknowledge, with appreciation, the technical assistance of Mrs. Flora Grantham in many of these studies.
hours to remove fibrin which had precipitated. An approximation of the amount of GOT activity in 1 ml. of blood exclusive of the plasma was made by use of the following calculation:

$$\text{GOT of 1 ml. defibrinated, hemolyzed blood}$$

$$- \left[ \frac{\text{GOT of 1 ml. plasma}}{100 - \text{hematocrit value}} \right] \times 100$$

To study the fate in rats of injected transaminase activity, phosphate buffer homogenates of 12.5 or 25 per cent concentrations of Hepatoma 5123 were centrifuged under refrigeration for 30 minutes at 3000 r.p.m. The supernatant layers used for intraperitoneal injection into rats were measured for GOT activity by the method used for serum. The blood plasma of injected rats was analyzed at intervals for GOT activity, and in some cases the animals were kept in all-glass metabolism cages designed to separate the urine and feces so that the urine could be collected for analysis. Other rats were hepatectomized subtotally to ascertain the effect of a reduced amount of liver upon the disappearance from the blood of increased concentrations of GOT resulting from the intraperitoneal injection of tumor 5123 extracts. The effect of tumorectomy on the level of serum GOT was investigated after surgical removal of the kidney containing the transplant of Hepatoma 5123.

Bile was collected from two normal Buffalo rats through fistulas, and its effect on serum GOT was determined in vitro following addition of bile to serum, and also in vivo following single or repeated intraperitoneal injections of bile into rats. Bile was analyzed after collection at intervals by fistula from a Buffalo rat bearing a 21-gm. healthy-appearing, intramuscular transplant of Hepatoma 5123. The GOT of bile was determined before and at intervals following the intraperitoneal injection of a GOT-rich tumor extract. The effect of surgical removal of kidney transplants of Hepatoma 5123 upon the GOT of bile and of serum was also determined.

RESULTS

Nitrogen and moisture contents of tissues analyzed for GOT.—Table 1 presents the data for total nitrogen and moisture contents of the tumor tissues

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Strain</th>
<th>Sex</th>
<th>No. Rats</th>
<th>Tumor Nitrogen (per cent)</th>
<th>Tumor Moisture (per cent)</th>
<th>Liver Nitrogen (per cent)</th>
<th>Liver Moisture (per cent)</th>
<th>Ratio of Tumor Nitrogen to Liver Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>All</td>
<td>♂♂</td>
<td>8</td>
<td>10.58 ± 0.19</td>
<td>69.4 ± 0.19</td>
<td>10.58 ± 0.19</td>
<td>69.4 ± 0.19</td>
<td>106 ± 52.7</td>
</tr>
<tr>
<td>Morris mammary tumor 6338</td>
<td>Buffalo</td>
<td>♂♂</td>
<td>2</td>
<td>11.24 ± 0.31</td>
<td>85.8 ± 0.38</td>
<td>10.6 ± 0.46</td>
<td>71.0 ± 0.15</td>
<td>106 ± 52.7</td>
</tr>
<tr>
<td>Dunning hepatoma</td>
<td>Fischer</td>
<td>♀♀</td>
<td>2</td>
<td>11.5 ± 0.43</td>
<td>85.9 ± 0.43</td>
<td>12.5 ± 0.60</td>
<td>77.3 ± 0.48</td>
<td>116 ± 72.5</td>
</tr>
<tr>
<td>Walker carcinosarcoma 256</td>
<td>Sprague-Dawley</td>
<td>♀♀</td>
<td>2</td>
<td>12.96 ± 0.31</td>
<td>82.9 ± 0.35</td>
<td>11.7 ± 0.32</td>
<td>74.5 ± 0.11</td>
<td>111 ± 74.4</td>
</tr>
<tr>
<td>Novikoff hepatoma</td>
<td>Holtzman</td>
<td>♀♀</td>
<td>5</td>
<td>12.5 ± 0.51</td>
<td>82.2 ± 0.06</td>
<td>11.4 ± 0.58</td>
<td>71.5 ± 0.41</td>
<td>110 ± 60.6</td>
</tr>
<tr>
<td>Morris hepatoma 3688</td>
<td>Irish (A×C)</td>
<td>♂♂</td>
<td>3</td>
<td>13.3 ± 0.71</td>
<td>83.1 ± 0.63</td>
<td>10.9 ± 0.83</td>
<td>71.0 ± 2.00</td>
<td>122 ± 83.5</td>
</tr>
<tr>
<td>Morris hepatoma 5123</td>
<td>Buffalo</td>
<td>♂♂</td>
<td>16</td>
<td>12.8 ± 0.33</td>
<td>79.2 ± 0.26</td>
<td>11.0 ± 0.16</td>
<td>71.45 ± 0.29</td>
<td>116 ± 84.1</td>
</tr>
<tr>
<td>Sidransky hepatoma</td>
<td>Osborne Mendel</td>
<td>♀♀</td>
<td>2</td>
<td>12.98 ± 0.99</td>
<td>78.0 ± 1.4</td>
<td>11.1 ± 0.44</td>
<td>71.8 ± 0.14</td>
<td>117 ± 93.3</td>
</tr>
</tbody>
</table>

* Nitrogen determinations were made on the liver and tumor homogenates that were analyzed for transaminase activity. For moisture, quickly minced samples of tissues were used. Values are expressed as averages ± standard error (12).
and of the livers of the normal rats and of the rats bearing the various tumors that were analyzed for GOT. With two exceptions the moisture contents of the livers of tumor-bearing rats were only slightly greater than those of the livers of normal rats of the same strains. The livers of normal rats of the Sprague-Dawley stock and the Fischer strains had the same moisture content as the livers of normal rats of other strains, but the livers of the two Sprague-Dawley rats bearing the Walker tumor and of two Fischer rats bearing the Dunning hepatoma had significantly higher moisture contents than the livers of the tumor-free rats. The moisture content of tumor tissue from all types of tumors was greater than that of the livers of the host rats, having values varying from 78 to 86 per cent compared with 71–77 per cent for liver.

The nitrogen content of liver tissue was greater than that of tumor on a wet weight basis, but, calculated for dry weights, tumor tissue had a significantly higher nitrogen content than the liver tissue of the host. There was rarely any difference found in the nitrogen or moisture content of the same type of tumor grown subcutaneously, intramuscularly, intraperitoneally, and, in the case of Hepatoma 5123, transplanted in the ovary or in the kidney.

GOT activity of tumors and of liver.—In Chart 1 is shown the low concentration of GOT activity in all but one of the different types of tumors analyzed compared with normal liver. Hepatoma 5123 contained 2–5 times as much activity as normal liver. No differences were found relative to the different transplant sublines or generations or to the sites of transplantation. Livers of rats bearing the tumor transplants had approximately the same activity as livers of normal rats. The relative concentrations of transaminase activity of the various tumors were the same on the basis of wet weight, total nitrogen, or dry weight.

GOT of blood.—Either anesthesia of 15 minutes’ duration was found to have little if any effect on the GOT concentration of serum or plasma. No stimulating effect of food restriction on serum GOT was observed as a result of pair-feeding normal rats, for 10 days, the amount of food ingested by rats of the same age bearing Hepatoma 5123. The pair-fed normal rats lost ~5 gm. each. The serum GOT activities of the tumor rats were 146 and 152 units/ml, whereas the sera of normal rats fed ad libitum contained 171 and 191 units/ml. When all food was withheld for 3 days, with water available, two adult normal rats lost 26 and 28 gm., compared with two control fed rats with body weights unchanged and increased by 3 gm., respectively. No effect of the 3-day period of inanition was observed on the serum GOT. Heparin does not affect the GOT of serum according to Steinberg et al. (20). Analysis of samples of plasma obtained by using a syringe rinsed in the heparin solution, and of sera from blood collected from the same rats, demonstrated that there was no significant difference detectable in the concentrations of GOT. A similarity in the GOT contents of plasma and serum has been reported (20).

Chart 2 shows that the serum GOT was elevated

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**Chart 1**—Transaminase activity of liver and of transplanted tumors. Unit: 1 μmole of pyruvate/hour/mg dry tissue at 37.5°C. The numbers in parentheses indicate the numbers of rats in each group. The brackets show standard errors.

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Transaminase Activity (units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOV, Novikoff hepatoma</td>
<td>5123 (16)</td>
</tr>
<tr>
<td>DUN, Dunning hepatoma</td>
<td>6338 (3)</td>
</tr>
<tr>
<td>SID, Sidransky hepatoma</td>
<td>3683 (2)</td>
</tr>
<tr>
<td>WAL, Walker carcinosarcoma 256</td>
<td>6338 (7)</td>
</tr>
<tr>
<td>LIV, Livers of normal rats of all strains used for tumor transplants</td>
<td>5123 (33)</td>
</tr>
<tr>
<td>LIV-I, Livers of tumor-bearing rats</td>
<td>5123</td>
</tr>
</tbody>
</table>

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**Chart 2**—Transaminase activity of sera or plasma of rats with and without tumor transplants. Transaminase unit: the amount of enzyme (GOT) that will cause the reading at 340 μm to decrease at the rate of 0.001 O.D. units per minute per centimeter light path at 25°C under the described conditions. Average values, with range of standard error in brackets. Number of rats in each group in parentheses.
to some extent in rats bearing transplants of all types of tumors studied compared with that of normal rats of the same strain. There was much variation in the amount of elevation in GOT with the different tumors, the Novikoff and Sidransky hepatomas having insignificant effects and Hepatoma 5123 resulting in an average serum GOT 8 times as great as that of tumor-free rats. The wide differences in the degree of elevation of serum GOT with tumors of different origins could not be attributed to the size of the transplants. However, within the limits of the effect of a specific tumor type, the amount of GOT in the serum appeared to be roughly proportional to the amount of tumor tissue present. Table 2 shows the comparative in-

fluence of extremely large, firm transplants of Sidransky, Novikoff, and 5123 liver tumors. Table 3 shows the relation between plasma GOT and the weights of Hepatoma 5123 transplants in individual rats. This table also shows the variation found in the concentration of transaminase activity in different transplants of the same tumor. Investigation of the distribution of GOT activity between the plasma and the nonplasma fraction of defibrinated hemolyzed blood demonstrated that the increased activity of the blood associated with the presence of tumor 5123 in the rat was located in the serum or plasma only.

As seen in Table 4, in five of eight rats the concentration of GOT was greater in blood coming from the tumor than in the general venous circulation of the rat or in blood collected concurrently from the liver. The effect of tumorectomy in lowering the plasma GOT is seen in Table 5.

### Table 2

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Serum GOT* (units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>141 ± 3</td>
</tr>
<tr>
<td>Sidransky</td>
<td>47± ± 42</td>
</tr>
<tr>
<td>Novikoff</td>
<td>53 ± 62</td>
</tr>
<tr>
<td>Morris 5123</td>
<td>5044 ± 800</td>
</tr>
</tbody>
</table>

* Average ± standard error (12). At least three analyses at two different dilutions of serum were used for each sample.

† Subcutaneous tumor transplants weighed 36 and 80 gm. each.

‡ Tumors weighed 12–21 gm. each; two animals bearing subcutaneous transplants of the Novikoff and three bearing intramuscular transplants of Hepatoma 5123.

### Table 3

<table>
<thead>
<tr>
<th>Site and age (days)</th>
<th>Weight* (gm.)</th>
<th>Plasma GOT† (units/ml)</th>
<th>Tumor tissue glutamic-aspartic transaminase† (units/mg dry wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovary</td>
<td>1.0</td>
<td>190</td>
<td>1988</td>
</tr>
<tr>
<td>&quot; 31</td>
<td>1.1</td>
<td>265</td>
<td>1994</td>
</tr>
<tr>
<td>&quot; 32</td>
<td>1.5</td>
<td>630</td>
<td>2638</td>
</tr>
<tr>
<td>&quot; 36</td>
<td>1.9</td>
<td>528</td>
<td>5390‡</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.3</td>
<td>185</td>
<td>4190</td>
</tr>
<tr>
<td>&quot; 38</td>
<td>2.4</td>
<td>146</td>
<td>2491</td>
</tr>
<tr>
<td>Average and S.E.‡</td>
<td>1.67 ± 0.26</td>
<td>274 ± 70</td>
<td>3256 ± 661</td>
</tr>
<tr>
<td>Ovary</td>
<td>42</td>
<td>2.4</td>
<td>531</td>
</tr>
<tr>
<td>&quot; 35</td>
<td>2.6</td>
<td>659</td>
<td>2849</td>
</tr>
<tr>
<td>&quot; 45</td>
<td>2.7</td>
<td>856</td>
<td>2460</td>
</tr>
<tr>
<td>&quot; 32</td>
<td>2.9</td>
<td>480</td>
<td>4006</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.4</td>
<td>708</td>
<td>3598</td>
</tr>
<tr>
<td>Ovary</td>
<td>3.7</td>
<td>796</td>
<td>3583</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.2</td>
<td>620</td>
<td>2382</td>
</tr>
<tr>
<td>&quot; 29</td>
<td>4.3</td>
<td>776</td>
<td>1971</td>
</tr>
<tr>
<td>Average and S.E.‡</td>
<td>3.27 ± 0.23</td>
<td>688 ± 43</td>
<td>2994 ± 339</td>
</tr>
<tr>
<td>Kidney</td>
<td>46</td>
<td>5.0</td>
<td>2705</td>
</tr>
<tr>
<td>Ovary</td>
<td>37</td>
<td>5.5</td>
<td>1911</td>
</tr>
<tr>
<td>Kidney</td>
<td>5.6</td>
<td>1170</td>
<td>2306</td>
</tr>
<tr>
<td>&quot; 42</td>
<td>6.8</td>
<td>2570</td>
<td>2306</td>
</tr>
<tr>
<td>&quot; 42</td>
<td>7.2</td>
<td>2712</td>
<td>2306</td>
</tr>
<tr>
<td>&quot; 42</td>
<td>8.4</td>
<td>1343</td>
<td>2306</td>
</tr>
<tr>
<td>Average and S.E.‡</td>
<td>6.41 ± 0.57</td>
<td>2031 ± 257</td>
<td>2626 ± 370</td>
</tr>
<tr>
<td>Intramuscular, 90</td>
<td>21</td>
<td>4739‡</td>
<td></td>
</tr>
<tr>
<td>&quot; 90</td>
<td>21</td>
<td>6401§</td>
<td></td>
</tr>
<tr>
<td>&quot; 100</td>
<td>11</td>
<td>4000‡</td>
<td></td>
</tr>
<tr>
<td>Average and S.E.‡</td>
<td>17.6 ± 3.3</td>
<td>5044 ± 800</td>
<td></td>
</tr>
</tbody>
</table>

* Accurate weights of tumors were difficult to determine because of variation in the amount of fibrous and connective tissue which surrounded the transplants.

† One unit of glutamic-oxalacetic transaminase (GOT) is the amount of enzyme that will cause the reading at 340 m/μ to decrease at the rate of 0.001 O.D. (optical density) units per minute per centimeter light path at 25° C. under the described conditions. Beckman DU spectrophotometer was used.

‡ A unit of glutamic-aspartic transaminase (GOT) in tissues is expressed in terms of a Q 10/t unit, or µl. of CO2 (or pyruvate) liberated per mg. dry wt. of tissue per hour based upon a 10-min. incubation period at 37° C. Necrotic areas of tumor tissue, if seen by gross examination, were discarded. Animals bearing transplants with extensive areas of necrosis were not included.

§ These rats were severely jaundiced, as indicated by a deep green-yellow color of the serum.

# Average for group and standard error (12).
Chart 3 demonstrates the increased GOT activity of the plasma of rats at 6 hours, the decrease in activity at 24 or 30 hr., and the return to the level of normal rats at 48 and 54 hours following intraperitoneal administration of GOT-rich supernatant solutions of centrifuged homogenates of Hepatoma 5123. In the second group of seven rats (designated II) in which both intact and hepatectomized animals were used, the injected tumor extract contained less transaminase activity than that used in the first group of three intact rats (I), and the level of plasma GOT at 6 hours was considerably less than in those of the first group. Subtotal hepatectomy, performed 24 hours before the administration of the tumor GOT, did not prevent the rapid disappearance of the increased GOT activity of plasma. Moreover, hepatectomy alone resulted in a temporary increase in plasma GOT activity. In this experiment the increase in plasma GOT associated with hepatectomy was approximately the same as that observed in the intact rats given injections of GOT activity from the tumor. The length of time for the decrease in plasma GOT to a normal level was approximately the same. The hepatectomized rats given injections of the same amount of GOT had about twice as much activity in the plasma at 6 hours as those rats that were hepatectomized only. This level was approximately the sum of the increased GOT following hepatectomy and the increase observed in intact rats following the injection of tumor GOT. The

**TABLE 4**

**COMPARISON OF GOT OF BLOOD PLASMA FROM THE GENERAL CIRCULATION OF THE RAT, FROM THE LIVER, AND FROM HEPATOMA 5123**

<table>
<thead>
<tr>
<th>AGE AND WEIGHT OF TUMOR†</th>
<th>TRANSAMINASE ACTIVITY OF SERUM‡</th>
<th>From general venous circulation</th>
<th>From tumor§</th>
<th>From liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days (gm.)</td>
<td>(units/ml)</td>
<td>(units/ml)</td>
<td>(units/ml)</td>
<td></td>
</tr>
<tr>
<td>(35) 0.95</td>
<td>190</td>
<td>280</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(31) 1.07</td>
<td>955</td>
<td>964</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(45) 2.70</td>
<td>623</td>
<td>838</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(42) 2.44</td>
<td>531</td>
<td>681</td>
<td>666</td>
<td></td>
</tr>
<tr>
<td>(35) 2.60</td>
<td>650</td>
<td>1247</td>
<td>661</td>
<td></td>
</tr>
<tr>
<td>(35) 2.90</td>
<td>489</td>
<td>655</td>
<td>448</td>
<td></td>
</tr>
<tr>
<td>(37) 3.70</td>
<td>796</td>
<td>932</td>
<td>835</td>
<td></td>
</tr>
<tr>
<td>(37) 5.30</td>
<td>1911</td>
<td>1817</td>
<td>1891</td>
<td></td>
</tr>
</tbody>
</table>

* Each tumor was transplanted into one ovary of a rat.
† Weights of tumors are approximate as a result of variation in the amount of fibrin and connective tissue which enveloped each transplant.
‡ Systemic blood was taken from the jugular vein, tumor blood from the ovarian vein, and liver blood from one of the hepatic veins.
§ The values given in italics represent those rats in which the concentration of GOT was greater in blood from the tumor than in the general venous circulation.

**TABLE 5**

**EFFECT OF HEPATOMA NO. 5123 UPON GOT OF PLASMA OF HOST RAT**

<table>
<thead>
<tr>
<th>GOT OF PLASMA</th>
<th>TIME OF TUMORECTOMY*</th>
<th>DAYS AFTER TUMORECTOMY</th>
<th>UNITS GOT PER ML PLASMA AS PERCENT OF AVERAGE OF NORMAL RATS</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEFORE TRANSPLANTATION OF TUMOR INTO ONE KIDNEY (units/ml)</td>
<td>days</td>
<td>units/ml</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>5536</td>
<td>1</td>
<td>546</td>
</tr>
<tr>
<td>118</td>
<td>927</td>
<td>1</td>
<td>214</td>
</tr>
<tr>
<td>115</td>
<td>1334</td>
<td>1</td>
<td>145</td>
</tr>
<tr>
<td>137</td>
<td>935</td>
<td>2</td>
<td>174</td>
</tr>
<tr>
<td>164</td>
<td>6850</td>
<td>2</td>
<td>70</td>
</tr>
<tr>
<td>137</td>
<td>810</td>
<td>7</td>
<td>90</td>
</tr>
</tbody>
</table>

* Transplants were 39-49 days old.

**CHART 3:** Disappearance of GOT from blood plasma of intact or hepatectomized rats. GOT: glutamic-oxalacetic transaminase. The supernatant buffer solution of a centrifuged homogenate of Hepatoma 5123 was injected intraperitoneally 24 hours after subtotal hepatectomy. Rats in group I received 130,000 units, and those in group II received 32,270 units of tumor GOT.
GOT activity of the supernatant layer, and of a 1:80 buffer dilution of the supernatant layer of a centrifuged tumor homogenate used for injection into rats, had not decreased after refrigeration at 4°C for 10 days. However, incubation at 39°C of an aliquot of the supernatant and of a 1:10 dilution of the supernatant layer without a preservative led to a loss of 90 per cent and 50 per cent, respectively, of the activity of GOT in 24 hours, followed by a much more gradual loss, so that 7 and 40 per cent of the original activity were present after 48 hours and 7 and 32 per cent, respectively, after 72 hours of incubation. During the first 24 hours of incubation the concentrated supernatant following tumorectomy the 24-hour urinary output of GOT of the first two rats had decreased to 31 and 167 units, respectively.

**GOT of bile.**—Rats bearing 10- to 21-gm. transplants of Hepatoma 5123 were invariably found to have bile-contaminated serum as indicated by a deep-greenish-yellow color and positive furfural tests for bile salts. Bile contamination of the serum was seen also in rats bearing 26- and 80-gm. transplants of ethionine-induced hepatomas. Rats with extensive primary liver tumors induced by ethionine feeding were also jaundiced. Bile from normal rats had a very low concentration of GOT activity, similar in concentration to that of normal rat

| G0T Activity | Increased GOT Activity in Plasma of Rat After Injection | GOT in Urine
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hr. 24 hr. 140 hr.</td>
<td></td>
</tr>
<tr>
<td>(units)</td>
<td>(per cent of inj.)</td>
<td>(units/hr)</td>
</tr>
<tr>
<td>40,000</td>
<td>11 0 0</td>
<td>2.7 3.0 3.0</td>
</tr>
<tr>
<td>40,000</td>
<td>11 0 0</td>
<td>2.6 3.5 3.5</td>
</tr>
<tr>
<td>60,000</td>
<td>19 0 0</td>
<td>9.5 3.2 14.0</td>
</tr>
</tbody>
</table>

*G0T: Glutamic-oxalacetic transaminase activity.
† The supernatant fraction of a centrifuged homogenate of Hepatoma 5123 was injected intraperitoneally.
‡ It is assumed that each rat contained 10 ml plasma.
§ Assays were made of 24-hour samples of urine for 4 days and finally of a 44-hour sample.
† Later experiments demonstrated that the increased GOT activity of plasma at 6 hours accounted for 17–30 per cent of the GOT administered.
∥ The urine of this rat was sometimes contaminated with feces.

**GOT of urine.**—The data in Table 6 indicate that the GOT activity that disappears from the blood cannot be accounted for quantitatively in the urine. Control experiments demonstrated that the enzyme activity of the supernatant buffer solution from the Hepatoma 5123 homogenate, when added to rat urine and refrigerated for 2 weeks, was completely stable. Two of three rats bearing large kidney transplants of Hepatoma 5123 showed increased urinary output of GOT daily during three days in metabolism cages. The 24-hour urinary excretion of GOT varied from 1078 to 3100 units compared with 25–44 units for tumor-free control rats. The third rat, with an equally large tumor, showed no greater urinary excretion of GOT than did normal control rats. On the 4th day urine. The addition of normal bile to serum in vitro had only an additive effect on the GOT activity of the serum. Moreover, GOT of the sera of rats was not influenced by the intraperitoneal injection of 1 ml., 3 ml., or six repeated hourly doses of 1 ml. of normal bile. The intraperitoneal injection of a GOT-rich Hepatoma 5123 extract caused a sixfold increase in the level of GOT in bile collected between 4 and 28 hours, and the presence of a large, healthy-appearing 21-gm. intramuscular transplant of this hepatoma was associated with a GOT level of the bile of the host rat 50 times that of the average tumor-free rat. However, the bile from one of two bile fistula rats bearing 7-week-old, 6-gm. transplants of Hepatoma 5123 in one kidney showed no elevation in GOT activity. The bile of the second animal, collected from 0 to 7 hours and 7 to 24 hours after introduction
of the fistula, contained twice and 4 times, respectively, as much activity as the average low 15 units per ml. of the tumor-free rats. Following tumorectomy the bile of both rats collected at intervals during 29 hours had the low GOT activity characteristic of tumor-free rats.

**DISCUSSION**

With the exception of Hepatoma 5123, the transplanted tumors analyzed—hepatomas, a mammary carcinoma, and a carcinosarcoma—were quantitatively similar in their GOT activity, having values less than half that of normal liver or of liver of the tumor-bearing rat. A close resemblance in the enzymatic patterns of different types of tumors has been reported by Greenstein (7) and by other investigators. Recently Angeletti, Moore, and Suntzeff (3), reporting analogous observations, concurred with the suggestion that the similarity in enzyme behavior of different tumors may be due to the tendency of neoplastic tissues to approach a common metabolic type.

Hepatoma 5123 is a transplantable liver carcinoma which was induced in inbred, female, Buffalo strain rats by feeding N-2-fluorenylphthalamic acid. Tumors developed some months after completion of a 9.9-month feeding period (15). This tumor grows slowly and metastasizes to the lung. The fourteenth- to sixteenth-generation transplants were found to retain many of the morphological characteristics found in liver and in the primary tumor.

Potter et al. (19), Pitot (17), and G. M. Tompkins (5) observed significant differences in certain enzyme activities of tumor 5123 compared with other liver tumors of the rat, including the Novikoff, Dunning L-C-18, primary and transplanted ethionine-induced, and the Morris 3683. Hepatoma 5123 was the only liver tumor found to resemble normal liver in the possession of some seventeen enzyme activities (18). It was found to have less tryptophan peroxidase activity than liver and primary ethionine-induced tumors, and in contrast to almost all the other enzymes studied the threonine dehydrase activity of 5123 was about 40 times that of the livers of animals bearing the neoplasm (17).

GOT resembles threonine dehydrase in its greater activity in Hepatoma 5123 tissue than in liver. Among the transplanted tumors analyzed for GOT activity, the Novikoff liver tumor, which was the fastest growing of all, had the lowest activity; Hepatoma 5123, the most slowly growing, had the highest activity; and the other tumors were intermediate in their growth rates and in enzyme activity. This finding is consistent with the observation made earlier by Cohen et al. (4) that there is an inverse relation of GOT activity to rate of growth of tissues, including livers. Experiments in which pyridoxal phosphate was added to the substrate without any significant increase in activity indicated that the low GOT activity of the Novikoff hepatoma was not due to a deficiency in this coenzyme. Preliminary studies of their free amino acid contents disclosed no qualitative differences in the amino acids identified in two-dimensional paper chromatography of the supernatant fractions of centrifuged 80 per cent alcohol homogenates of host livers, Novikoff, and 5123 hepatomas.

Although all the tumors studied were associated with some elevation of serum GOT, the rats bearing the fastest growing tumor, the Novikoff, had the lowest plasma or serum activity of any of the tumor-bearing rats. Rats carrying Hepatoma 5123, the most slowly growing of any of the tumors, had the highest serum GOT. The degree of elevation in serum GOT was dependent upon the type of tumor present, but within the limits of activity for each tumor type with rats bearing the Novikoff, transplants of the ethionine-induced, or Hepatoma 5123, the serum GOT increased with increased amounts of tumor tissue present.

Koj and associates (11) reported the distribution of GOT in human blood as 2 per cent in serum or plasma, 5 per cent in leukocytes, 13 per cent in platelets, and 80 per cent in erythrocytes. Correcting the values obtained for GOT of defibrinated, hemolyzed blood for the GOT of the plasma of ten normal rats, an average of 91.8 ± 0.8 (S.E.) per cent of the total blood GOT was accounted for in the nonplasma portion and 8.0 ± 3.4 per cent in the plasma. The data indicated further that most, if not all, of the increased GOT of the blood associated with the presence of Hepatoma 5123 was present in the serum or plasma.

Since the GOT of liver from Hepatoma 5123-bearing rats was the same as that from control tumor-free rats and since tumorectomy resulted in a rapid decrease in serum GOT to the low values of normal rats, it seems probable that the source of the elevated serum transaminase was the tumor. This conclusion is supported by the fact that some animals had higher GOT in the plasma of blood collected from the "host-isolated" tumor than in that from the liver or from the general circulation. That tumor blood was not always higher in activity than systemic blood from the same animal may be due to an accumulation of GOT activity in the blood resulting from its slow elimination.

Increased GOT of serum has frequently been associated with muscle damage, as with cardiovascu-
lar infarct, and with liver damage, with a concept of leakage of the enzyme from the damaged tissue, as reviewed by Udenfriend et al. (24). Tisza and Tényi (22) have suggested, however, that the increase associated with damaged liver may actually be the result of a regenerative process following the damage. An increase in both liver and serum GOT of the hepatectomized rat at 5 days, and a return to normal values at 31 days, led them to conclude that newly formed cells or regenerating rat liver produce more GOT than normal liver cells. However, in our experiments the elevation in serum GOT, following removal of two-thirds of the liver, did not occur immediately after surgery, but was greater at 6 than at 24 hours and had returned approximately to normal by 48 hours. Regenerating liver, 14 and 16 days after heptectomy, had the same GOT activity as well as moisture and nitrogen contents as liver of the average intact rat.

With the available data there is no way to know whether transaminase passes continuously from intact cells of the tumor to the blood or exclusively from tumor cells that are breaking down. With Hepatoma 5123 the average increase in GOT activity of the plasma was directly proportional to the average weight of the transplanted tumors in groups averaging from 1.6 to 10 gm. wet weight. Further increases in tumor weight resulted in little additional elevation of serum GOT. Although increased necrosis may be expected to accompany increased size, no evidence was found to suggest that the serum GOT was increased as a result of necrosis in the tumors. In the first studies with Hepatoma 5123 extensive areas of necrosis were found and discarded in some of the tumors, and in those experiments the serum GOT was lower than with healthy transplants of the same size.

Excretion by the kidneys and bile seems to account for very little of the GOT that disappears from the serum during 48 hours following the intraperitoneal administration of the enzyme-rich extract from tumor 5123. Bile from tumor-free rats, like that of the urine, has low GOT activity, but the bile GOT was elevated when the serum GOT was very high. Bile-contaminated sera of rats carrying Hepatoma 5123 and of rats with primary ethionine-induced liver tumors always had exceptionally high GOT activities. Normal bile had no effect on serum GOT activity in vitro or in vitro. Bile may be secreted by the cells of tumor 5123, but no significant amount was detected in tumor homogenates by the furfural test for bile salts. The jaundiced condition of rats having extensive amounts of ethionine-induced primary liver tumors may have been due to blockage of the bile duct, or to damage to liver cells. Both of these conditions have been found to result in an elevated serum GOT activity (1, 24).

Elevated serum GOT has been associated with several pathologic conditions in addition to damaged heart muscle and liver (1). Since this enzyme is present in other tissues such as skeletal muscle, brain, kidney, testes, lung, and spleen (24), its concentration in serum could conceivably be increased as a result of damage to these tissues. Ujházy et al. (25) observed an elevated serum GOT level when Brown-Pearce tumor was introduced through a vessel that led to growth of transplants in the liver, whereas the same suspensions, introduced into a marginal vein, resulted in an equal amount of tumor transplant growing in the lungs, but the tumor in the lungs did not result in elevation of the serum GOT. In the present experiments the increase in serum transaminase activity may be due to invasion of the muscle and to the kidney in transplants to those tissues, but the possibility would appear to be remote that the same would be true with subcutaneous, intraperitoneal, and “host-isolated” ovarian transplants that were associated with equally high serum GOT levels.

In addition to some cases of jaundice and some toxemias of pregnancy, elevated serum GOT has been observed in monkeys under conditions of non-specific stress (24). Since so many different pathologic conditions have been found to be associated with an elevation in GOT activity of the serum, little use of the test could be expected in the diagnosis of cancer. Moreover, with the exception of the GOT-rich Hepatoma 5123, 4 gm. or more of the transplanted tumor tissue were needed to cause a significant increase in serum GOT of the rat. That amount of tumor is at least 1/50th the body weight of the animal, an unreasonable proportion for usefulness in diagnosis.

The high GOT activity of Hepatoma 5123 may be due to a predominance of those liver cells that synthesize the enzyme. The quantitative differences in GOT activities of the several hepatomas analyzed do not appear to be related to “large” and “small” cell types, as discussed by Pitot and Potter with relation to other enzyme activities of these tumors, since the Novikoff, Dunning, and Hepatoma 3683 resemble one another in the concentrations of transaminase activity but not in cell type as classified by Pitot (17). The elevation in serum GOT found with all the types of transplanted tumors examined could be explained by the presence in the body of an additional tissue that synthesizes the enzyme, if the enzyme passes continuously into the blood on its way to be excreted or destroyed. However, the results of GOT studies elicit a number of queries. What role does
this enzyme activity have in vitro? If the transaminase activity makes possible the synthesis of some amino acids needed for protein synthesis, why is there more activity in slowly growing than in rapidly growing tissues? Is the finding of more activity in a slowly growing tissue due, perhaps, to the synthesis by the tissue of more of the enzyme than is needed so that the enzyme is free and the activity measurable, whereas the activity in a rapidly growing tissue may be unmeasurable because the enzyme is tied up in catalytic reactions? Are damaged cells the only source of elevated serum GOT activity? Where is the enzyme destroyed or inactivated or excreted? Some of these questions may be raised also for other enzyme activities, for much more seems to be known of the kinetics of enzyme activities in vitro than of their synthesis, behavior, and fate in vivo.

REFERENCES

Transaminase Activities of Liver Tumors and Serum


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