II. Tolerance to Injection of Amino Acids*

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The effect of a transplantable neoplasm on the free amino acid concentrations of certain tissues of the host was reported in a previous paper (8). No pattern of change was found which might be considered characteristic of the neoplastic process. The observed changes in the levels of the different amino acids were interpreted to be similar to those induced by other states of stress.

It seemed possible that more intrinsic differences, if they exist, could be detected by taxing the capacity of the animals to metabolize amino acids. Consequently, for this purpose, time curves of amino acid concentrations were determined following the intraperitoneal injection of massive doses of certain amino acids. The chosen amino acids were those which were known to be converted to other amino acids. This course was undertaken to discover possible influences of neoplasms on the established interrelationships of amino acid metabolism.

Because of the inherent variability in the responses of individual animals, the data reported here represent trends and do not give a final and fixed picture of the effects of flooding the organism with amino acids. It was not feasible to carry out experiments on a sufficiently large number of animals to derive adequate statistical norms for the control and tumor-bearing rats. In addition, there was observed an unexplained response in the microbiological assay methods for glutamic acid which poses difficulties of interpretation.

MATERIALS AND METHODS

Tissues for analysis were taken from normal and tumor-bearing Sprague-Dawley male rats weighing 250–300 gm. Tumor-bearing rats were used at 7–9 days after the unilateral subcutaneous transplantation of a Walker carcinosarcoma 256. Animals were fasted for 16–18 hours, with free access to water, before the injection of the L-amino acid at 0.56 gm/kg body weight, and were sacrificed at intervals of 11–15 hours after injection.

Tissues were excised as previously described (8) and deproteinized by the tungstic acid precipitation procedure of Schurr et al. (9). Tissue filtrates from two or three animals were pooled for amino acid analysis by microbiological assay (MBA) or by column chromatography as previously described (9). Each curve in Charts 1, 3, and 4, showing a change in the level of an individual amino acid with time, is the average of three to five series of MBA determinations, in which tissue filtrates from each time interval from 0 to 6–15 hours after injection were analyzed concurrently. This was done to achieve the greatest reliability for the MBA technic applied to tissue filtrates, i.e., for the determination of relative rather than absolute values (8).

An exchange resin column analysis, also used to check MBA results for specific tissue samples as indicated in the charts, was performed according to the procedure of Moore and Stein (6).

RESULTS

Significant alterations in concentrations were produced by the injected amino acids on other metabolically interrelated amino acids. No changes were produced in the levels of unrelated amino acids, such as leucine, phenylalanine, or tryptophan, and they are not considered further here.

It is to be noted that the shapes of the concentration-time curves are quite similar for all four of the amino acids employed in these experiments (Charts 1–4). The concentration maxima for the injected amino acids were reached in 15–30 minutes, and the level returned to approximately normal in about 2 hours. The coincidence in the curves suggests that the absorption from the peritoneum was essentially the same for the normal and for the tumor-bearing rats; otherwise, one would expect an overlapping of the curves from the two types of animals.
Only with glycine was there a suggestion that the presence of a tumor may lead to a reduction in the maximum concentration attained by the injected amino acid. If this is real, it may be indicative of a more rapid metabolic turnover of this amino acid in the tumor-bearing host.

Another general effect noted throughout was a marked negative dip in the curves for glutamic acid. This dip appeared to be characteristic of microbiological assays for glutamic acid following injection of amino acids which were not direct glutamate precursors. The explanation for this phenomenon is not apparent at present.

Results characteristic of each individual amino acid are considered below.

**Glycine.**—With glycine it was noted that the increase in muscle was considerably higher than that in the liver. This is consistent with reports on the accumulation of this amino acid in skeletal muscle (5).

The increase in glycine was accompanied, in particular, by a rise in alanine in all tissues and by an initial rise in glutamate and aspartate in liver, followed by a drop to below normal. In muscle only the negative response for glutamate was noted.

The serine levels were not determined by microbiological analysis, but determination by column chromatography showed that the serine levels paralleled the glycine changes. From this it may be inferred that the increase in alanine results from the conversion of glycine to serine, followed in turn by the conversion of this to pyruvate and then to alanine. The influx of pyruvate into

![Glycine Injection Chart](https://cancerres.aacrjournals.org)
the tricarboxylic acid cycle could also account for the increases in the glutamate and aspartate in the liver.

**Serine.**—Serine could not be determined by microbiological assay. The results of experiments analyzed chromatographically are shown in Chart 2. The curves are comparable to those for glycine. Of interest is the rapid and extensive conversion of serine to alanine in the liver, presumably through pyruvate. The conversion to glycine is seen to be much more gradual.

The curves obtained on the tumor indicate that the serine is metabolized by the tumor poorly, if at all, by the pathway of pyruvic acid to alanine and glutamic acid. Since no serine deaminase has been found in tumor tissue, this is understandable.

**Threonine.**—A pertinent observation concerning the threonine curves (Chart 3) is the increase in the glycine of liver and of muscle in the control, but not in the tumor-bearing rats. Formation of glycine from threonine\(^1\) has been reported by a number of investigators (2–4). The enzyme catalyzing this reaction has been found only in liver; therefore, the high content of glycine in the muscle is rather unexpected. The absence of a rise in the level of glycine in tumor-bearing animals is in agreement with the previous suggestion of a more rapid utilization of glycine.

\[ \text{Serine Injection} \]

![Chart 2](chart.png)

**Chart 2.**—Concentration-time curves of amino acids following intraperitoneal injection of serine

Proline.—The differences in the responses of individual animals is well illustrated by proline, since in one experiment a peak increase in proline concentration of over 11 μ moles/gm was noted in the liver of the normals, while in another experiment the increase was only 6 μ moles/gm. Proline can be converted to glutamate and

\(^1\) Unpublished experiments of Karasek and Greenberg suggest that glycine is formed only from allothreonine by liver enzyme preparations. However, all commercial preparations of threonine tested were found to contain allothreonine, which would also be true in the present case.
arginine through ornithine. No change was observed in the arginine, but the microbiological assays showed increases in the liver glutamate of both the normal and tumor-bearing rats and in the muscle of the tumor-bearing but not in that of the normal animals. A corresponding rise in aspartate was noted in the liver of the tumor-bearing group.

However, when the microbiological assay data were checked by column chromatography, such pronounced increases as shown in the chart were not found. One may conclude that the organism used for assay responded to something in the tissue filtrate other than free glutamic acid.¹

DISCUSSION

The amino acid concentration-time curves obtained in blood plasma, liver, and muscle following the intraperitoneal injection of amino acids do not permit definite conclusions with regard to

¹ This could not be glutamine, since this had been converted to the inactive pyrrolidonecarboxylic acid before assay. The material responsible for the response may be some bound form of glutamic acid, as suggested by the following observations: (a) hydrolysis of the filtrates liberated much more glutamate than could be derived from the glutamine present; and (b) column chromatography of filtrates from proline-injected animals demonstrated a range of ninhydrin-positive material emerging in the eluate region that would be expected to include glutamic acid peptides or bound glutamate of an acidic nature.

![Chart 3](https://example.com/chart3.png)

**Chart 3.**—Concentration-time curves of amino acids following intraperitoneal injection of threonine

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the effect of a tumor on the amino acid metabolism of the host. There are suggestions of an increased rate of metabolism for several of the injected amino acids, glycine in particular. This indicated increase in glycine metabolism supports previous observations of increased incorporation of glycine-\(^{14}\)C into tissue proteins (7) and into liver deoxyribonucleic acid purines (11) in the tumor-bearing animal.

Since adrenal hormones have been shown to exert an inhibitory effect on tumor growth (10), it is instructive to compare this work with that of Awapara and Kit (1) on the utilization of L-alanine injected into normal and adrenalecto-

**Proline Injection**

![Proline Injection Diagram]

**TABLE 1**

<table>
<thead>
<tr>
<th>Series</th>
<th>Liver Weight (gm.)</th>
<th>Range (gm.)</th>
<th>Threonine Dehydrase Activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Activity/gm Protein</td>
<td>Total Activity/ Livers</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Normal†</td>
<td>7.9</td>
<td>7.2–8.5</td>
<td>1.8–2.3</td>
</tr>
<tr>
<td>Tumor-bearing‡</td>
<td>12.5</td>
<td>9.3–18.3</td>
<td>1.9–1.9</td>
</tr>
<tr>
<td>Normal</td>
<td>10.3</td>
<td>10.0–10.6</td>
<td>2.9</td>
</tr>
<tr>
<td>Tumor-bearing</td>
<td>13.8</td>
<td>10.9–15.8</td>
<td>2.1</td>
</tr>
</tbody>
</table>

*Threonine dehydrase activity determined on liver homogenates and measured in \(\mu\) moles of \(\alpha\)-ketobutyrate formed/half-hour incubation at 37°C.
†Three Sprague-Dawley female rats (200–250 gm/group).
‡Walker carcinosarcoma 256 tumors. One rat in Series I carried this tumor in the ascitic form; all others carried well-developed solid subcutaneous transplants.
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They found a decreased deamination of alanine to pyruvate in the adrenalectomized rat, concomitant with decreased utilization of the glutamate formed from alanine. As might be expected, this is in direct contrast to our observation on serine injection, i.e., an apparently unimpaired conversion of serine to alanine (via pyruvate) and an increased utilization of the glutamate subsequently formed in the tumor-bearing animal.

However, these data are only suggestive of a lowered metabolism of the Krebs cycle amino acids following adrenalectomy and of an increased metabolism of these amino acids in neoplasia. The reason for this inconclusiveness may be that the injection of a large dose of an amino acid was not a sufficiently sensitive method to detect the differences sought; a more direct approach may have to be utilized, namely, assaying the content of the various enzymes concerned with a given metabolic pathway.

The value of this more direct method is illustrated by the data on the enzyme threonine dehydrase, which hydrolytically deaminates this amino acid (Table 1). The data in Table 1 reveal a decrease of about one-third in this enzyme per unit weight of liver in the tumor-bearing rats. However, when considered in terms of the total mass of the liver, which is increased in the tumor-bearing animals, there was no consistent difference in the total enzyme activity. These results are suggestive of a disturbed metabolism of this amino acid, compensated by a hypertrophy of the liver under the stress of the cancer. The specific activity of the corresponding enzyme which deaminates serine (serine dehydrase), on the other hand, was found to be unaltered. Attempts to determine the activity of the enzyme system in the liver which converts glycine to serine were unsuccessful.

SUMMARY

1. Time curves of free amino acid concentrations of liver, muscle, and plasma were determined following the intraperitoneal injection of massive doses of selected amino acids into normal and tumor-bearing rats.

2. Significant alterations in concentrations were produced by the injected amino acids (glycine, serine, threonine, and proline) on the other amino acids with which they are metabolically interrelated.

3. The data suggest an increased rate of metabolism of several of the amino acids, particularly glycine, in the tumor-bearing animal.

4. An altered threonine dehydrase level was demonstrated in the livers of tumor-bearing rats; serine dehydrase levels remained unaltered. Such data suggest that assay of tissue enzyme levels might be a more sensitive method for the detection of a derangement in amino acid metabolism in the tumor-bearing host.

ACKNOWLEDGMENTS

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REFERENCES


Tumor-Host Relationships: II. Tolerance to Injection of Amino Acids

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