Tumor Host Relationships

I. Effects on Free Amino Acid Concentrations of Certain Tissues*

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One of the major effects of the presence of a neoplasm on the host is a disturbance of the protein metabolism (8, 13). Efforts have been made to take advantage of this for diagnostic purposes by determining some particular change in the content or the properties of the plasma proteins (1, 6, 7). These efforts have had a very limited success, because it was found that the observed effects did not develop early, nor were they specifically related to the neoplastic process.

The present investigation on the effect of cancer on the free amino acid concentrations of blood plasma and tissues was undertaken for the two-fold purpose of gaining further information on the derangement in protein metabolism induced by neoplasms, and also in the hope that observed changes might be of such a character as to offer promise of diagnostic usefulness. The latter hope was not realized. The investigation was made feasible by the development in recent years of more sensitive methods for the analytical determination of most of the individual amino acids, i.e., by microbiological assay (MBA) and by ion exchange column chromatography.

MATERIALS AND METHODS

Animals.—The analyses were performed on tissues of normal and tumor-bearing Slonaker male rats weighing between 230 and 280 gm. The Walker carcinoma 256 was transplanted unilaterally by the trocar method, and the animals were sacrificed at 7—9 days after transplanting, when the tumor weighed 2—5 gm. but cachectic symptoms were not yet apparent. The normal and tumor-bearing rats were allowed access to the stock diet of Purina Laboratory Chow and water ad libitum up to the time of sacrifice. The fasted rats were given water only for 20 hours prior to sacrifice.

The animals were sacrificed under anesthesia induced by nembutal injection (4 mg/100 gm body weight), supplemented with ether if necessary. Blood was withdrawn via the vena cava; liver fractions were taken from the left lateral and medial lobes; and muscle samples were taken from the posterior femoral muscle.

Animals were sacrificed in triplicate, and approximately equal amounts of the same tissue from each animal were deproteinized by the tungstic acid precipitation procedure of Schurr et al. (11). Replicate filtrates were pooled and stored frozen until analysis.

Amino acid determinations.—The basal media and procedure for most of the MBA analyses were essentially those of Henderson and Snell (5), with the following modifications: all forms of vitamin B6 were excluded from the basal medium for alanine, and all samples assayed for alanine were irradiated with ultraviolet light to destroy any B6 present (10); glutamic acid samples were autoclaved for 20 minutes at 20 lb. to render glutamine inactive; reticulogen was added to all media for L. citrovorum (10); and commercial basal media mixes were used for the cysteine, proline, and aspartic acid assays.1 Determinations were run on sample levels of 0, 0.05, 0.10, 0.15, 0.20, and 0.25 ml/tube/assay, with the Cannon Automatic Dispensor-Titrator (3).

The organisms for each of the assays were the following: Lactobacillus delbrueckii 3 for arginine, histidine, isoleucine, leucine, and valine; Leucomostoc mesenteroides P-60 for aspartic acid, glycine, lysine, phenylalanine, serine, and tyrosine; Streptococcus faecalis for threonine; Lactobacillus fermenti for methionine; Lactobacillus brevis for proline; Lactobacillus arabinosis for glutamic acid.

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and tryptophan; and Leucosostoc citrovorum 8081 for alanine and cysteine.

All ion exchange resin column analyses were performed according to the procedure of Moore and Stein (9); amino acid concentrations are expressed as glycine equivalents, based on the ninhydrin color produced relative to a standard millimolar glycine solution.

The curves shown in Charts 1 and 2 are proportional to samples of 1 gm. of fresh liver or 1.6 gm. of fresh muscle, chromatographed on a 0.7 X 100 cm. column of Dowex-50 (in the sodium form) with 0.2 N sodium citrate buffer at pH 3.4. Curve 1 is a synthetic amino acid mixture approximating the microbiological analysis of 1 gm. of liver from a normal fed rat. Curves 2-5 are filtrates from 1-gm. fresh liver samples from (2) fed normal rat, (3) fasted normal rat, (4) fed tumor-bearing rat, and (5) fasted tumor-bearing rat.

The broken line serine peak in Curve 2 is from a hydrolyzed sample of filtrate from a normal fed rat and represents a minimum serine value; i.e., minus glutamine and any serine decomposed in hydrolysis.

Distances between peaks have been slightly adjusted for easy comparison of similar peaks among the different samples.
RESULTS

Microbiological assay results.—Results of the assay analyses of the liver, muscle, and plasma filtrates are recorded in Table 1, as the averages ± the mean deviations. Each value is the average of from two to six separate assays, with five sample levels per assay. Those assays which consistently showed “drift” are averaged for comparative purposes only and are shown in italics. “Drift” is a directed nonproportional response at different sample levels, and indicates response by the assay organism to constituents in the sample other than the amino acids used in the standard. Within a group of replicate analyses, isolated assays showing drift were eliminated from the assays averaged.
Those assays which showed abnormally high or low results (usually indicating mutation of the organism) were also excluded.

For most of the amino acids, the analyses from two or three different preparations of pooled tissue filtrates have been averaged. Only in the case of methionine did we find a real difference among different pooled filtrate preparations, whereby one preparation gave a high methionine level in the plasma but not in liver or muscle preparations from the same animals.

An attempt was made to run simultaneously a complete cross-section for each amino acid of the three types of each three tissues analyzed. This was not always possible, but each assay used in the averages reported has been cross-checked for relative reliability by running concurrently assays for at least two or three other tissue samples.

**Comparison of the present MBA values with those in the literature.**—According to the data of Schurr and co-workers (12), the free amino acid levels of plasma, liver, and muscle of the Holtzmann albino strain normal male rat (210–250 gm. weight) are of the same order of magnitude as our values for the Slonaker albino strain (230–280 gm. weight) for all the amino acids studied by both groups except arginine and threonine. These workers report very low arginine levels in liver; however, this may be due to arginase activity, since their liver samples were not boiled immediately on excision; their arginine levels in other tissues were comparable to ours. Our threonine levels are consistently lower in all tissues, which may be a func-

### TABLE 1

**FREE AMINO ACID CONTENT OF LIVER, PLASMA, AND MUSCLE TISSUES IN FED, FASTED, AND TUMOR-BEARING RATS AS DETERMINED BY MICROBIOLOGICAL ASSAY**

<table>
<thead>
<tr>
<th>AMINO ACID</th>
<th>LIVER, µM/100 gm</th>
<th>PLASMA, µM/100 ml</th>
<th>MUSCLE, µM/100 gm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed</td>
<td>Fasted</td>
<td>Tumor-bearing</td>
</tr>
<tr>
<td>Alanine</td>
<td>150*</td>
<td>85†</td>
<td>344</td>
</tr>
<tr>
<td></td>
<td>± 22</td>
<td>± 17</td>
<td>± 20</td>
</tr>
<tr>
<td>Arginine</td>
<td>26</td>
<td>26</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>± 6</td>
<td>± 6</td>
<td>± 2</td>
</tr>
<tr>
<td>Aspartic</td>
<td>108</td>
<td>107</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td>± 4</td>
<td>± 7</td>
<td>± 2</td>
</tr>
<tr>
<td>Cysteine</td>
<td>187</td>
<td>164</td>
<td>154</td>
</tr>
<tr>
<td></td>
<td>± 21</td>
<td>± 20</td>
<td>± 0.2</td>
</tr>
<tr>
<td>Glutamic</td>
<td>(577)‡</td>
<td>(517)</td>
<td>(678)</td>
</tr>
<tr>
<td></td>
<td>± 193</td>
<td>± 194</td>
<td>± 54</td>
</tr>
<tr>
<td>Glycine</td>
<td>455</td>
<td>591</td>
<td>607</td>
</tr>
<tr>
<td></td>
<td>± 47</td>
<td>± 54</td>
<td>± 121</td>
</tr>
<tr>
<td>Histidine</td>
<td>59</td>
<td>56</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>± 1</td>
<td>± 9</td>
<td>± 4</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>37</td>
<td>35</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>± 8</td>
<td>± 6</td>
<td>± 2</td>
</tr>
<tr>
<td>Leucine</td>
<td>81</td>
<td>57</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>± 4</td>
<td>± 6</td>
<td>± 1</td>
</tr>
<tr>
<td>Lysine</td>
<td>112</td>
<td>58</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>± 8</td>
<td>± 11</td>
<td>± 8</td>
</tr>
<tr>
<td>Methionine</td>
<td>12</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>± 0</td>
<td>± 1</td>
<td>± 1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>20</td>
<td>22</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>± 4</td>
<td>± 1</td>
<td>± 5</td>
</tr>
<tr>
<td>Proline</td>
<td>50</td>
<td>59</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>± 5</td>
<td>± 10</td>
<td>± 6</td>
</tr>
<tr>
<td>Serine</td>
<td>(200)</td>
<td>(171)</td>
<td>(208)</td>
</tr>
<tr>
<td></td>
<td>± 25</td>
<td>± 15</td>
<td>± 1</td>
</tr>
<tr>
<td>Threonine</td>
<td>52</td>
<td>57</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>± 2</td>
<td>± 5</td>
<td>± 6</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>12</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>± 1.4</td>
<td>± 1.1</td>
<td>± 1</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>(30)</td>
<td>(32)</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>± 2.1</td>
<td>± 5.5</td>
<td>± 1</td>
</tr>
<tr>
<td>Valine</td>
<td>70</td>
<td>54</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>± 6</td>
<td>± 6.2</td>
<td>± 3.8</td>
</tr>
</tbody>
</table>

* All values are given as averages ± mean deviation of from two to six replicate assays.
† Values in italics are those which appear to fall outside the range for the corresponding tissue of the normal fed rat.
‡ Values in parentheses are shown for comparative purposes only. These assays consistently showed "drift" effects.
§ One assay only.
tion of the strain of rat or of the assay organism. Schurr et al. and other workers have not reported MBA values for aspartic and glutamic acids, alanine, glycine, serine, or cysteine.

Wiss (17) has reported values for nineteen free amino acids (including hydroxyproline) in liver of fasted rats, using MBA technics supplemented with chemical analyses for alanine and glycine. He did not give the weight range or strain of rats used; he fasted them for 3 days. Despite these differences in experimental conditions there is still agreement in the order of magnitude between his values and ours except for glycine, lysine, threonine, valine, and tyrosine, which show two-fold differences; arginine and alanine which show five-fold differences; and cysteine and tryptophan with ten- and 30-fold differences. Because of possible differences in animals and significant differences in length of fasting times it is not profitable to compare further the data of Wiss and those reported here.

Comparison of MBA and ion exchange values.—In view of the inherent difficulties in analyzing tissue extracts by microbiological methods, an attempt was made to check the order of magnitude among the levels of some of the more critical amino acids by means of ion exchange chromatography. In our hands, this technic has not been reproducible enough to pick up small changes in concentrations within one tissue type, or to determine values for amino acids present in low concentrations. It has been a valuable tool, however, in determining the validity of our organism responses with tissue filtrates in such rarely reported assays as alanine, glycine, glutamic acid, aspartic acid, and serine.

To avoid the errors incurred in reporting absolute recoveries, column elution curves are shown in Charts 1 and 2 for various tissue filtrates along with curves for synthetic amino acid mixtures approximating the microbiological analyses for normal liver and muscle. With the standard amino acid mix, variations up to 10 or 15 per cent were found among replicate runs; therefore, only differences among samples exceeding this variation were considered significant.

Identity and homogeneity of the major peaks of Charts 1 and 2 were checked by two-dimensional paper chromatography of the isolated peak fractions, pooled and deionized according to the method of Stein (14).

From the curves of Charts 1 and 2, MBA values for glutamic acid, glycine, and perhaps alanine appear to be questionable. Glutamic acid and glycine assays are high, indicating organism response to other components in the tissue filtrates. Alanine assays on liver appear to be low; but relative variations among normal, fasted, and tumor-bearing livers appear to be comparable by MBA or column analysis. In addition, individual variations seemed greatest for alanine and glycine with samples from replicate animals analyzed on the column.

Serine MBA values are obviously invalid because of pronounced “drift.” Paper chromatography has also shown the “serine” peak of column analyses of tissue filtrates to contain a second major component, which has been identified as glutamine. Column analysis of the hydrolyzed “serine” peak of normal fed rat liver has yielded two peaks corresponding to serine and glutamic acid in a ratio of 1:2.5. Serine values for liver filtrates, determined by column analyses of hydrolyzed samples, are in the range of 40–50 µg/100 gm, showing that the MBA values are too high.

Column analyses of tissues of fasted tumor-bearing animals.—As an added check on the reliability of the differences found between normal and tumor-bearing animals, samples of tissues of fasted tumor-bearing animals were analyzed chromatographically, as shown in curves 5 of Charts 1 and 2. Here, in both liver and muscle, the amino acid profile apparently indicates a blending of the effects of the two states of stress.

DISCUSSION

Evaluation of technics.—This work sharply emphasizes the difficulties involved in using MBA as a quantitative measure of the free amino acid concentrations in nonfractionated biological fluids. Inconsistent responses by the organisms cannot be eliminated in such mixtures of natural metabolites, where the effects of amino acid-vitamin interrelationships, amino acid antagonisms, and peptide utilization may all be active. Further, variation in response levels to replicate samples, and to the same samples at different times, make any statistical treatment of the results impossible. This variation in response level has been noted by Thompson et al. (15) in studying the effects of fasting on tissue amino acids.

However, MBA is unique and more reliable in showing relative differences among tissues. The reported results indicate that stresses, like fasting and the presence of a neoplasm, change the profile of amino acids and “amino acid-like constituents” from that of the normal animal.

The difficulties of ion exchange resin column analysis are also appreciable for this type of work. Stein (14), in using his own technic for the analysis of amino acids in urine, reported errors of 10–30
per cent for amino acids in the same concentra-
tion ranges as the lowest in our rat tissues. How-
ever, resin column analysis offers a graphic meth-
method for showing gross changes in ninhydrin-positive
tissue components, as is demonstrated by changes in
the “forefraction” profile between normal and
tumor-bearing or fasted liver. This forefraction
contains ninhydrin-positive components of tis-
sue filtrates other than the amino acids reported,
and is being studied further.

**Discussion of results**.—It is apparent that the
neoplastic process, like a short period of fast, does
not lead to a characteristic pattern of change in
the free amino acid concentrations within an or-
ganism.

The effects of a rapidly growing cancer must
include a demand for amino acids to make possi-
ble a high rate of protein synthesis. One might
anticipate that this would affect the amino acid
levels of other tissues in the organism, particularly
in the case of the essential amino acids, for which
no machinery of synthesis exists. However, no
such pattern of change is found. Such effects as
are observed may be related to special metabolic
reactions characteristic of given tissues.

Thus, it might be postulated that an increased
rate of glycolysis (and lactate production) in the
tumor-bearing animal could account for the in-
creased alanine level through the conversion of
lactate through pyruvate to alanine. The de-
creased glutamic acid level may be a result of its
participation in the transamination of pyruvate
and/or in response to a general increased energy
demand of the organism via the citric acid cycle.

Glycine determinations show wide variations
even among replicate animals, which is not totally
unexpected, owing to the numerous functions of
glycine. Column results indicate, in general, an
increased level in fasted animals, and a decreased
level in tumor-bearing animals. The latter may be
in response to demands of purine synthesis in the
neoplastic process or in response to a more general
increased energy demand.

Changes in the levels of the other amino acids
present in lower concentrations may be compared
with the results of Thompson *et al.* (15), Hender-
sdon *et al.* (4), and Williams *et al.* (16). These in-
vestigators have studied the effects of such stress-
es as fasting, nitrogen deprivation, chilling,
and exercise on the free amino acid concentrations
of rat tissues. They showed that the magnitude
and direction of a change in a given amino acid
concentration, following fasting and nitrogen
deprivation, can vary greatly with time over peri-
ods of hours or days. These authors reported gly-
cine values for plasma only, and reported no re-
sults for alanine, serine, aspartic acid, or glutamic
acid. However, in general, their fasting and stress
conditions produced changes in the levels of the
same amino acids as observed in the tissues of the
fasted and tumor-bearing rats reported here. Thus,
these stresses apparently evoke responses prin-
cipally in the liver levels of lysine, leucine,
lysine, valine, and histidine, while muscle and
plasma levels remain more constant.

In summary, the present results show no pat-
tern of change in the free amino acid levels of tis-
sues of tumor-bearing animals which might be
considered characteristic of the neoplastic process.
That these results are not indicative of turnover
rates is evident.

However, it is pertinent to note that the
changes which are most evident are in levels of
those amino acids—alanine, glycine, and glutamic
acid—which could conceivably be mobilized to
supply energy demands via the citric acid cycle.
This agrees with the work indicating an over-all
increased energy expenditure in the tumor-bearing
host, as reviewed recently by Fenninger and
Mider (2).

**SUMMARY**

1. The levels of eighteen amino acids have been
studied in liver, muscle, and plasma of normal,
fasted, and tumor-bearing rats, according to micro-
biological assay and ion exchange chromatography
technics.
2. No pattern of change in the amino acid pro-
file of these tissues was found which was charac-
teristic for the tumor-bearing animal.
3. Those changes in amino acid levels were
generally interpreted to be similar to those in-
duced by other states of stress, and may be re-
lated to general increased energy demands of the
tumor-bearing organism, rather than to specific
demands of protein synthesis.

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