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 twofold 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 Blood was withdrawn via the vena cava; liver fractions were taken from the left lateral and medial lobes; and muscle samples were taken from the posterior femoral muscle.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 B_6 were excluded from the basal medium for alanine, and all samples assayed for alanine were irradiated with ultraviolet light to destroy any B_6 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; *Leuconostoc mesenteroides* P-60 for aspartic acid, glycine, lysine, phenylalanine, serine, and tyrosine; *Streptococcus faecalis* for threonine; *Lactobacillus fermenti* for methionine; *Lactobacillus brevi* for proline; *Lactobacillus arabinosis* for glutamic acid

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[1] H. M. Chemical Company, Los Angeles, Calif.

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and tryptophan; and *Leuconostoc 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 × 100 cm. column of Dowex-50 (in the sodium

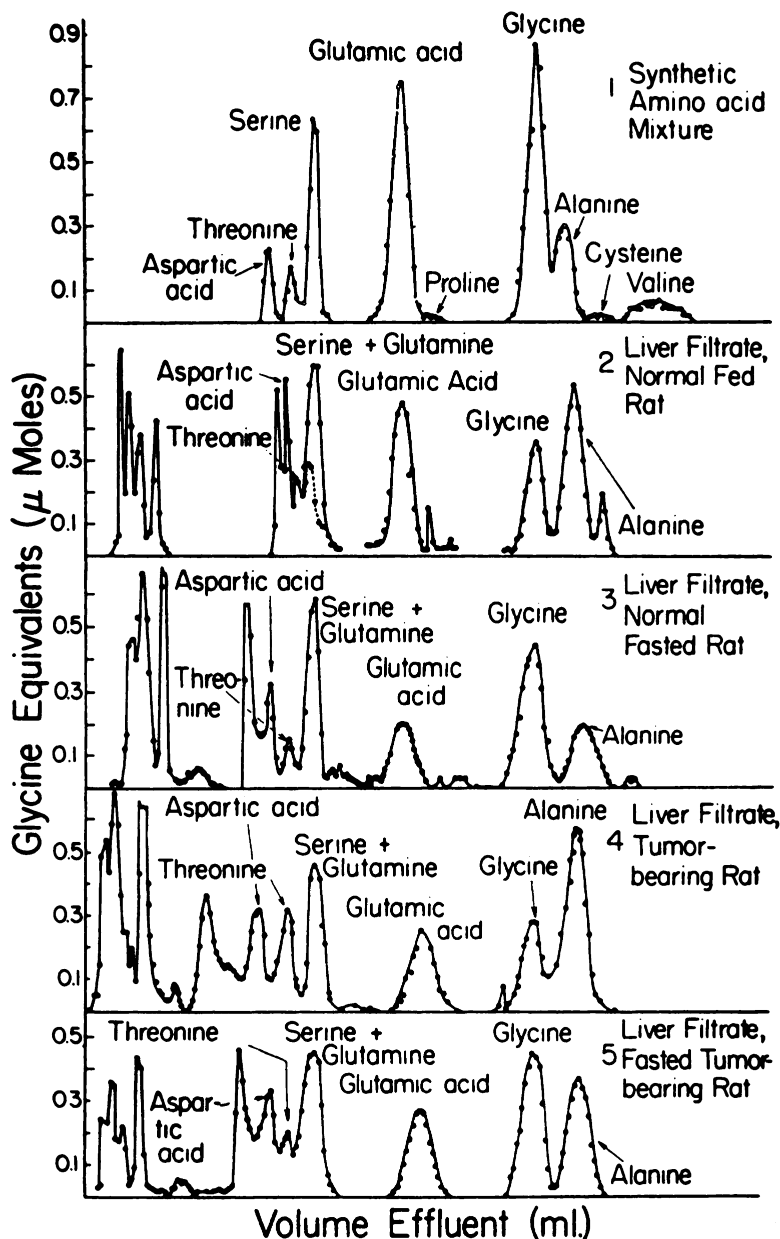

CHART 1.—Ninhydrin-positive components of liver tissue filtrates eluted from a 0.7 × 100 cm. column of Dowex-50 (Na-form) with 0.2 N sodium citrate buffer at pH 3.4. Curve 1 is a synthetic amino acid mixture approximating the microbiological analysis of 1 gm. of liver from a normal fed rat. Curves 2–5 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.

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form). Slight variations in elution volumes between peaks (due to slight variations in resin column heights) have been adjusted for more easy comparison of parallel analyses.

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.

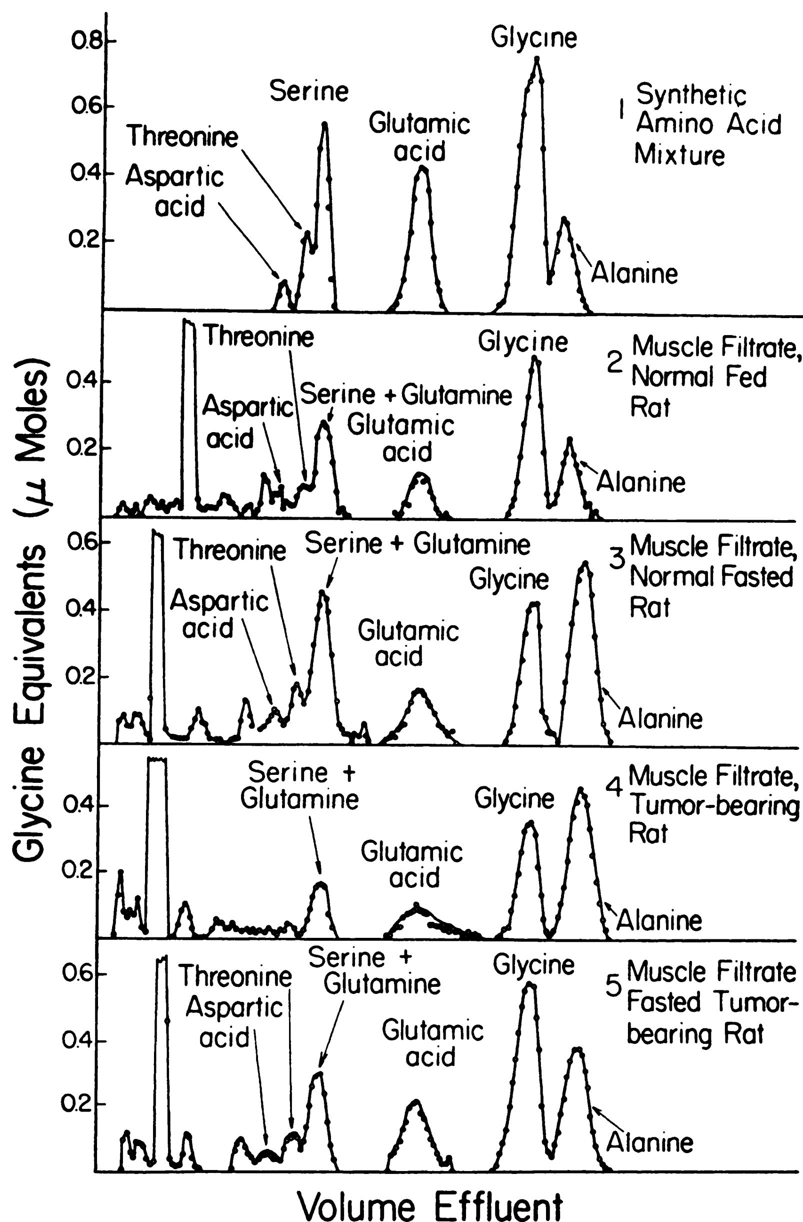

CHART 2.—Ninhydrin-positive components of muscle tissue filtrates eluted from a 0.7 × 100 cm. column of Dowex-50 (Na-form) with 0.2 N sodium citrate buffer at pH 3.4. Curve 1 is a synthetic amino acid mixture approximating the microbiological analysis of 1.6 gm. muscle from a normal fed rat. Curves 2–5 are filtrates from 1.6-gm. fresh muscle samples from (2) fed normal rat, (3) fasted normal rat, (4) fed tumor-bearing rat, and (5) fasted tumor-bearing rat.

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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

Amino acid	Liver, μM/100 gm			Plasma, μM/100 ml			Muscle, μM/100 gm		
	Fed	Fasted	Tumor-bearing	Fed	Fasted	Tumor-bearing	Fed	Fasted	Tumor-bearing
Alanine	150* ± 26	*84*† ± 17	*344* ± 20	25 ± .7	23 ± 0	28 ± .3	121 ± 31	119 ± 17	153 ± 29
Arginine	28 ± 6	32 ± 6	26 ± 2	22 ± 1.4	22 ± 2.6	16 ± 2.0	48.2 ± 7	46.5 ± 8.6	*71* ± 1.2
Aspartic	103 ± 4	127 ± 7	111 ± 2	0	0	0	17 ± .4	22 ± 1.4	18 ± .8
Cysteine	187 ± 21	164 ± 20	154 ± 0.2	12 ± 1.0	10 ± 2.1	9 ± 4.2	24 ± 4.2	22 ± .8	35 ± .6
Glutamic	(577)‡ ± 83	(517) ± 134	(678) ± 54	30 ± 2.9	35 ± 6.3	34 ± 1.5	200 ± 18	197 ± 9.5	223 ± 12
Glycine	455 ± 47	591 ± 84	607 ± 121	26 ± .8	31 ± .1	28 ± 4.3	517 ± 117	478 ± 1	436 ± 44
Histidine	59 ± 1	46 ± 9	*74* ± 4	10.0 ± 2.4	7.9 ± 2.4	7.4 ± .8	89 ± 6.4	86 ± 7.8	80 ± 14
Isoleucine	47 ± 8	35 ± 6	*31* ± 2	10.8 ± 2.4	10.1 ± 1.7	7.3 ± 0	22 ± 3.6	15 ± 2.0	18 ± 2.1
Leucine	81 ± 4	*57* ± 6	*55* ± 1	17 ± 1.8	16 ± 1.4	*11* ± .4	25 ± 2.0	22 ± 1.2	24 ± 1.2
Lysine	112 ± 8	*58* ± 11	*82* ± 8	33 ± 5.8	31 ± 10.9	31 ± 3.8	140 ± 8.9	*68* ± 5.3	*90* ± 5.5
Methionine	13 ± 0	15 ± 1	11 ± 1	3.6 ± .6	3.8 ± .6	4.2 ± .9	7.0 ± .9	9.8 ± .7	9.4 ± .4
Phenylalanine	26 ± 4	22 ± 1	25 ± 5	5.3 ± .7	6.8 ± .4	7.2 ± .8	8.9 ± .9	11.3 ± .3	13.2 ± 3.0
Proline	50 ± 5	39 ± 10	49 ± 4	19 ± 1.4	12 ± 1.6	15 ± 2.6	54 ± 6.5	40 ± 5.4	43 ± 3.2
Serine	(260) ± 25	(117) ± 15	(128) ± 1	32 ± 1.2	40 ± 1.7	30 ± 4.8	(135) ± 7.6	(75) ± 6.7	(77) ± 6.7
Threonine	52 ± 2	57 ± 3	*74* ± 5	20 ± .6	20§	18 ± 2.2	60 ± .8	59 ± 11	*71* ± 5.8
Tryptophan	12 ± 1.4	12 ± .9	10 ± 1.1	6.3 ± 1.3	6.1 ± 1.1	6.5 ± .5	3.5 ± .3	*7.1* ± 1.5	4.7 ± .3
Tyrosine	(30) ± 2.1	(22) ± 3.5	(16) ± .1	8.9 ± .3	7.0 ± .2	8.2 ± .4	16 ± 1.0	19 ± 1.6	18 ± 1.6
Valine	70 ± .6	*54* ± 6.2	*45* ± 3.8	19 ± 1.7	15 ± .4	14 ± 1.9	(34) ± 8.7	(27) ± 2.8	(36) ± 4.2
Total	23.13	19.25	25.19	3.00	2.96	2.77	15.21	13.24	15.65

* 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.

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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 twofold differences; arginine and alanine which show fivefold 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 of 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 concentration 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 mixes, 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 μM/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

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per cent for amino acids in the same concentration ranges as the lowest in our rat tissues. However, resin column analysis offers a graphic 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 tissue 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 organism.

The effects of a rapidly growing cancer must include a demand for amino acids to make possible 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 increased alanine level through the conversion of lactate through pyruvate to alanine. The decreased 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), Henderson *et al.* (4), and Williams *et al.* (16). These investigators have studied the effects of such stresses 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 periods of hours or days. These authors reported glycine values for plasma only, and reported no results 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 principally in the liver levels of lysine, leucine, threonine, valine, and histidine, while muscle and plasma levels remain more constant.

In summary, the present results show no pattern of change in the free amino acid levels of tissues 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 microbiological assay and ion exchange chromatography technics.

2. No pattern of change in the amino acid profile of these tissues was found which was characteristic for the tumor-bearing animal.

3. Those changes in amino acid levels were generally interpreted to be similar to those induced by other states of stress, and may be related to general increased energy demands of the tumor-bearing organism, rather than to specific demands of protein synthesis.

REFERENCES

1. Clark, D. G. G.; Cliffton, E. E.; and Newton, B. L. Antiproteolytic Activity of Serum with Particular Reference to Its Changes in the Presence and Considerations of Its Use for Detection of Malignant Neoplasia. Proc. Soc. Exper. Biol. & Med., **69**:276–79, 1948.
2. Fenninger, L. D., and Mider, G. B. Advances in Cancer Research, **2**:229–53. New York: Academic Press, Inc., 1954.
3. Henderson, L. M.; Brickson, W. L.; and Snell, E. E. A Micromethod for the Microbiological Determination of Amino Acids. J. Biol. Chem., **172**:31–38, 1948.
4. Henderson, L. M.; Schurr, P. E.; and Elvehjem, C. A. The Influence of Fasting and Nitrogen Deprivation on the Concentrations of Free Amino Acids in Rat Plasma. J. Biol. Chem., **177**:815–23, 1949.

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5. HENDERSON, L. M., and SNELL, E. E. A Uniform Medium for the Determination of Amino Acids with Various Microorganisms. J. Biol. Chem., **172**:15–29, 1948.
6. HUGGINS, C. Serum Proteins in Cancer. Cancer Research, **9**:321–27, 1949.
7. HUGGINS, C.; CLEVELAND, A. S.; and JENSEN, E. V. Thermal Coagulation of Serum in Diagnosis. J.A.M.A. **143**:11–15, 1950.
8. MIDER, G. B. Some Aspects of Nitrogen and Energy Metabolism in Cancerous Subjects: *A Review*. Cancer Research, **11**:821–29, 1951.
9. MOORE, S., and STEIN, W. H. Chromatography of Amino Acids on Sulfonated Polystyrene Resins. J. Biol. Chem., **192**:663–81, 1951.
10. SAUBERLICH, H. E., and BAUMANN, C. A. A Microbiological Determination of Alanine. J. Biol. Chem., **177**: 545–51, 1949.
11. SCHURR, P. E.; THOMPSON, H. T.; HENDERSON, L. M.; and ELVEHJEM, C. A. A Method for the Determination of Free Amino Acids in Rat Organs and Tissues. J. Biol. Chem., **182**: 29–37, 1950.
12. SCHURR, P. E.; THOMPSON, H. T.; HENDERSON, L. M.; WILLIAMS, J. N., JR.; and ELVEHJEM, C. A. The Determination of Free Amino Acids in Rat Tissues. J. Biol. Chem., **182**:39–45, 1950.
13. SHERMAN, C. D.; MORTON, J. J.; and MIDER, G. B. Potential Sources of Tumor Nitrogen. Cancer Research, **10**:374–78, 1950.
14. STEIN, W. H. A Chromatographic Investigation of the Amino Acid Constituents of Normal Urine. J. Biol. Chem., **201**:45–58, 1953.
15. THOMPSON, H. T.; SCHURR, P. E.; HENDERSON, L. M.; and ELVEHJEM, C. A. Influence of Fasting and Nitrogen Deprivation on the Concentrations of Free Amino Acids in Rat Tissues. J. Biol. Chem., **182**:47–53, 1950.
16. WILLIAMS, J. N., JR.; SCHURR, P. E.; and ELVEHJEM, C. A. The Influence of Chilling and Exercise on Free Amino Acid Concentrations in Rat Tissues. J. Biol. Chem., **182**: 55–59, 1950.
17. WISS, O., Untersuchungen über die freien Aminosäuren in der Leber bei verschiedener Ernährung. Helv. Chim. Acta, **32**:1345–51, 1949.

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