Free Amino Acid Content and Transaminase Activity of Lymphatic Tissues and Lymphosarcomas*

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The observed levels of free amino acids within a tissue reflect the capacity of the tissue to remove these substances from the blood, produce them from other carbon sources, and utilize them in various processes. Each tissue has a characteristic pattern (2, 15). Since the dynamic equilibria which determine the endogenous amino acid concentrations may be disturbed in disease, observation of the pattern of the pathological tissue may provide insight into the nature of the pathological condition itself.

It seemed particularly appropriate to investigate neoplasms and their normal homologs in this connection. Qualitative patterns of a number of neoplasms and normal tissues have already been published by Roberts and Frankel (15). In the present study, quantitative determinations were carried out on four lymphosarcomas, a transplantable methylcholanthrene-induced rat tumor, and normal lymphatic tissues of three animal species.

Since the transaminases seem to regulate the formation and utilization of amino acids from carbohydrate precursors, a cursory survey was also made of these enzymes.

METHODS

Healthy adult rabbits, C3H and Ak mice, and Sprague-Dawley and Wistar rats were used in these experiments. The rat and mouse thymi and spleens were from nontumor-bearing animals. It was necessary to pool two or three mouse spleens and five or six mouse thymi in order to provide enough tissue for an experiment. One animal provided enough tissue for each experiment in the case of the other tissues.

Two tumors were transplanted subcutaneously into each animal, and the tissues were used 12-14 days later in the case of the Gardner lymphosarcoma and 10-12 days later in the case of the other tumors. At this time, each of the mouse tumors weighed about 300-400 mg., and there was no gross evidence of necrosis. Non-neoplastic samples of the rat tumors (600-800 mg.) were taken. A description of the lymphosarcomas, including photographs of microscopic sections has been published by Sugiura and Stock (16, 17).

Fat and mucoid materials were trimmed away, and the tissues were quickly weighed and then homogenized in 20 cc. of 80 per cent ethanol. Centrifuge tubes containing the alcoholic solution were placed in a boiling water bath to complete the coagulation of protein. After centrifugation, the protein precipitate was discarded, and the alcoholic solution was extracted with chloroform (2). The water phase was separated from the chloroform and evaporated to dryness on a steam bath. The residue was taken up in a volume of water equivalent to the weight of the original tissue, and the resulting solution was filtered. Aliquots equivalent to from 30 to 80 mg. (usually 50 mg.) wet weight of tissue were used for chromatography.

Quantitative amino acid determinations were performed by the method of Awapara (3), except for the following modifications as suggested by Fowden (7); (a) after development with water-saturated phenol, the papers were dried at about 35° C. in a stream of air, and residual phenol was removed with ether; (b) the ninhydrin spots were cut from the paper, placed in test tubes, and after the addition of 0.1 cc. of 0.1 M NaOH, these test tubes were stored in an evacuated vacuum desiccator for 8 hours. Additional citrate was included in the Moore and Stein ninhydrin reagent (18) equivalent to the NaOH which had been added. By this procedure, reagent blanks against propanol-water were usually about 0.090 optical density units, and paper blanks about 0.140. Standards at three levels for glutamic, aspartic, glycine, and alanine were run on paper simultaneously with all tissue extract determinations. The optical density of 0.25 μg of glutamic acid was about 0.400.

Transaminases were measured by the method of Awapara and Seale (8), except that incubations were carried out for 30 minutes at 38° C. under N2 in the Dubnoff apparatus. Experiments were terminated by the addition of 5 cc. of ethanol, and the contents of the beakers were transferred to 18-ml centrifuge tubes. Beakers were rinsed with 2 cc. of ethanol, and the combined ethanolic solutions were boiled for 2 minutes to coagulate protein and prepared for chromatography as previously described. Quantitative determinations after chromatography with phenol-water were completed with the previously mentioned modification; an alkali treatment of the paper was used to minimize paper blanks. The determinations were performed in duplicate.

1 The authors are indebted to Dr. K. Sugiura of the Sloan-Kettering Institute for the gift of Ak mice bearing the Patterson and Mecca lymphosarcomas and the Wistar rats bearing the Murphy-Sturm lymphosarcoma. The methylcholanthrene-induced rat tumor was induced at this institution and was diagnosed as a carcinosarcoma.
RESULTS

Distribution of amino acids.—The distribution of free amino acids in various lymphatic tissues and in some lymphosarcomas is shown in Charts 1 and 2. Values are expressed as \( \mu \text{M amino acid/gm wet weight of tissue} \). It should be noted that this form of expression does not correct for the relative cellularity per unit weight of each tissue. The reproducibility from experiment to experiment was relatively good, except in the case of those amino acids present at relatively low concentrations. The amino acid pattern of a given tissue, however, was found to be extremely constant.

As may be observed from the charts, the amino acid patterns of the normal lymphatic tissues resemble one another closely. The lymphosarcomas show characteristic patterns that are different in several respects from the normal lymphatic tissues and also from the carcinosarcoma. Chromatograms of the free amino acids from the lymphosarcomas are similar to the chromatogram of the transplantable mouse lymphosarcoma reported by ...
Roberts and Frankel (15), and the chromatograms from the normal lymphatic tissues resemble that published by Roberts and Frankel for a mouse lymph node.

All five tumors were found to contain high concentrations of alanine. This amino acid exists in normal lymphatic tissues in amounts corresponding to one-fourth of the concentration of glutamic acid, but in the lymphosarcomas the concentration of alanine attains values as high as 8 times the concentration of glutamic acid. The concentration of ethanolamine phosphoric ester is drastically reduced in the Mecca tumor, somewhat diminished in the Gardner and Patterson lymphosarcomas, and relatively lower in the methylcholanthrene-induced tumor and the Murphy-Sturm lymphosarcoma. Proline, which is found only in trace amounts in lymphatic tissues, was clearly detectable in all the tumors studied, when chromatograms were made with the equivalent of 50 mg. wet weight of tissue. Glutamine is usually present in concentrations similar to the concentration of alanine in most tissues but is reduced in the lymphosarcomas. The glycine content of the Mecca, Patterson, and Murphy-Sturm tumors is very high compared to the content of glutamic acid; in the Gardner lymphosarcoma the glycine concentration is about 5 times greater than the concentration of glutamic acid. The concentration of aspartic acid was found to be 4–5 times less than the concentration of glutamic acid in the lymphosarcomas.

A species difference was noted in the case of taurine. This metabolite is relatively low in the lymphatic tissues of the rabbit, but very high in rat and mouse lymphatic tissues. The low content of taurine may be ascribed to the inability of the rabbit to convert cysteine into this compound.

See Chart 1 for abbreviations.
Data obtained in this laboratory indicate that after cysteine is injected into the rabbit it is converted slowly and only in very small amounts into taurine in the liver, whereas in rat liver this conversion occurs rapidly and in substantial amounts (1, 4).

Amino acid pattern of solid and ascites tumor.—The Gardner lymphosarcoma will grow as an ascites tumor in C3H mice. It was of interest to compare the amino acid pattern of the ascites tumor with that of the subcutaneously grown Gardner tumor, which was used in the experiments reported above. Ascites tumors were therefore induced in two mice by intraperitoneal inoculation of tumor cells, and the animals were sacrificed 10 days later. The tumor cells were sedimented by centrifugation at 0° C. and the ascites serum discarded. The cells were extracted with 80 per cent ethanol, and the extract was prepared for chromatography as previously described.

The amino acid pattern of the ascites tumor was found to resemble the solid tumor. Thus, if one assumes that each cc. of sedimented cells weighed 1 gm., the concentrations in μM/gm wet weight for the ascites tumor were: taurine, 6.1; alanine, 5.6; glycine, 5.4; glutamic acid, 2.6; aspartic acid, 0.2; and ethanolamine phosphoric acid, 1.7. The corresponding values for the solid tumor were 9.7, 5.9, 10.1, 2.1, 0.3, and 1.3.

Thus, the concentrations of alanine and glycine were relatively high and aspartate and ethanolamine phosphoric ester relatively low. In addition, proline was readily detectable on the chromatograms, while glutamine was not present.

Transaminase activity.—All the normal lymphatic tissues and tumors possess active transaminases. In Table 1 are given values for some of the transaminase activities measured. The QT values (μl of amino acid formed/mg dry weight/hour) for the glutamic-oxalacetic transaminase was calculated to range from 42 for the appendix (rabbit) to 99 for mouse spleen. Redfield and Barron (14) reported QT values of 24 for appendix slices, while Cohen found a QT of 16 for rat spleen (6). The less active alanine-α-ketoglutaric transaminase was found in all the tissues. Very little alanine was formed, however, in the Gardner lymphosarcoma from pyruvate and glutamate or pyruvate and glutamine. The alanine-oxalacetic transaminase activity may be the summation of the alanine-α-ketoglutaric and the glutamic-oxalacetic transaminase activities. Active transamination between leucine, phenylalanine, and isoleucine with α-ketoglutaric acid was observed in the appendix (rabbit), spleen (rat), and Gardner lymphosarcoma. The values, not shown on the table, ranged from 11 to 89 μM of amino acid formed in 30 min. Further work on these transaminases is in progress.

DISCUSSION

The high concentrations of alanine in the tumors may be partially related to an increased tendency of certain tumors to concentrate amino acids (5). The same might apply to glycine or other amino acids. However, it seems likely that the high alanine concentrations are also attributable to an increased ability of these tissues to form alanine. The following observations support this hypothesis: (a) After tumor cell suspensions were incubated with carbon-14-labeled lactate (10), considerable radioactivity was found in alanine; (b) The incorporation of isotopic lactate into tumor cell protein in vitro (9) is markedly inhibited when pyruvate is added to the medium. This may be interpreted as due to a dilution of isotopic alanine with nonisotopic alanine from the pyruvate.

In addition, it is well known that the glycolysis of tumors is very high. Thus, pyruvate, the precursor of alanine, is rapidly formed in tumors. It was interesting to note, however, that, although appreciable amounts of alanine transaminated with α-ketoglutarate in the tumor homogenates, only a weak glutamine-pyruvate or glutamate-pyruvate transaminase was discernible. It is not clear whether other untested amino acids are the principal amino donors to pyruvate or whether other mechanisms than transamination are involved in alanine formation.

The low concentration of aspartic acid in the lymphosarcomas is of interest, since it was shown by Kit and Greenberg (10) that little radioactivity was to be found in aspartic acid after lymphosarcoma cells were incubated with labeled lactate, although considerable radioactivity was present in the aspartic acid fraction of spleen cells. It is difficult to reconcile the fact that glutamic-oxalacetic transaminase is very active in the tumor cells with the fact that aspartic acid exists in very low concentrations in the same cells. Further inquiry into this problem has revealed that considerable aspartic acid exists in bound form in these tumors. This could be shown by chromatography of the extracts before and after acid hydrolysis. Indeed, a fractionation of a strip chromatogram into six areas revealed that four of the six areas (none of which corresponded to the Rp of free aspartic acid) contained substances which, on hydrolysis, released aspartic acid. The nature of these substances is not known. It is possible, therefore, that the low levels of aspartate imply...
rapid utilization of this amino acid. Aspartic acid or a related compound is a precursor of tissue pyrimidine (11).

The high concentration of glycine and relatively low content of ethanolamine phosphoric ester in the lymphosarcoma may also be related phenomena. Ethanolamine may be formed from glycine by way of serine (8, 12). The multiple functions of glycine in metabolic reactions are well known and necessitate no further comment.

Cohen (6) has reported that transaminase activity decreases during the induction of rat tumors, and it was suggested that rapid growth or a related compound is a precursor of tissue weight of tumor.

The high concentration of glycine and relatively elevated contents of alanine and glycine. Proline was also elevated in these tissues compared to the normal tissues. Diminutions were observed of aspartic, ethanolamine phosphoric ester, and glutamine. Measurements were also made of transaminase activity. The results were discussed in terms of the metabolic reactions which lead to the formation and utilization of these amino acids.

REFERENCES

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