THE CORRELATION BETWEEN THE ACTIVE CONCENTRATION OF LABELED AMINO ACIDS BY THE CELLS, AND THEIR INCLUSION IN THE PROTEINS OF TUMORS AND NORMAL TISSUES OF EXPERIMENTAL ANIMALS IN VITRO

K. A. Perevoshchikova

Laboratory of Biochemistry (Head—Prof. I. B. Zbarskii, Scientific Director— Corresponding Member of the AMN SSSR A. I. Savitskii) of the P. A. Herzen State Oncological Institute (Director—Prof. A. N. Novikov), Moscow

(Presented by Active Member of the AMN SSSR A. E. Braunshtein)

Translated from Byulleten' éksperimental'noi biologii i meditsiny Vol. 49, No. 2, pp. 73-77, February, 1960.

Original article submitted February 6, 1959.

Several authors have demonstrated that the concentration of free amino acids is higher in the tissues [16] and erythrocytes [1] than in the blood plasma. It was more recently found that the entry of amino acids into cells is due to a process of active concentration, as opposed to a gradient of concentrations with the consumption of energy [7,12,14].

The inclusion of labeled amino acids in the proteins of tumor sections in vitro is known to be considerably more intense than in the case of normal tissue sections [17]. In homogenates and other acellular preparations made from tumors, this process is no more intense than in analogous preparations made from normal tissue [6].

Since the first stage in the utilizationof amino acids for protein synthesis in their accumulation by the cell, one can propose that the rate of protein synthesis and the growth of tissues in the organism should depend on the ability of the cells to concentrate amino acids. Because tumor cells (ascitic cells of Ehrlich's cancer) possess a heightened ability to accumulate amino acids [11], I. B. Zbarskii has expressed the hypothesis that the greater inclusion of labeled amino acids in tumor cell proteins in vitro can be explained by the increased ability of these cells to accumulate amino acids [2].

In this work, in order to prove this hypothesis, we investigated in vitro the correlation between the concentration of radioactive amino acids by ascitic cells, and by sections of experimental tumors and normal tissues, and the inclusion of such amino acids in the cell proteins.

METHOD

We studied the concentration of glycine-1-C^{14} methionine-S^{35} lysine-1-C^{14} and tyrosine-1-C^{14} by the cells of Ehrlich's ascitic mouse cancer, sarcoma M-1, and rat's ascitic hepatoma.*

The normal tissue used for purposes of comparison was that of rat liver. Two-hundred mg sections were incubated in Robinson's medium with glucose [15] in Warburg's flasks at 37.8° for 30 minutes in an oxygen atmosphere. The radioactive amino acid was added to the flasks in an amount of 30,000-70,000 impulses per 2 ml of the incubation medium.

After incubation, the sections were briefly dried with a filter paper, weighed, ground up, and precipitated with trichloracetic acid in a final concentration of 2.5%. The free amino acids were extracted by thrice extracting the sections with 2.5% trichloracetic acid. The deproteinized incubation medium and the trichloracetic filtrates were neutralized and reduced to a specific volume, from which the sample for radioactivity determination was taken — in the case of glycine, quantitative determination, according to Alexander's method [4], was also done. After the extraction of the free proteins, the proteins of the sections were processed by the standard method [3]. The radioactivity of theproteins was calculated on a "B" type apparatus per every 10 mg protein. The results were expressed as the number of impulses per minute for a specific volume of the incubation medium and for an equal volume of the original tissue.

RESULTS

Table 1 gives the ratio of the radioactivity of the protein-free filtrates of cells or sections to the radioactivity of the incubation medium. As the data in the table indicate, the cells of the experimental tumors concentrated amino acids much more actively than did the cells of the normal liver. This ability of the cells depended on the amino acid employed as well as on the type of experimental tissue. Under these conditions,

* This strain was kindly made available to us by the Laboratory of Strains of the AMN SSSR Institute of Experimental Cancer Pathology and Therapy.

TABLE 1. Concentration of Glycine-C^{14}, Methionine-S^{35}, Lysine-C^{14} and Tyrosine-C^{14} in vitro by Tumor and Liver Cells from Rats and Mice. Ratio of number of impulses per minute in a protein-free filtrate of cells or sections to the number of impulses per minute in an equivalent volume of the incubation medium (30-minute incubation at 37.8° in Robinson's medium. Average values from 7-15 experiments)

Tumor	Intracellular glycine-C^{14} / Glycine-C^{14} in incubation medium	Intracellular methionine-S^{35} / Methionine-S^{35} in incubation medium	Intracellular lysine-C^{14} / Lysine-C^{14} in incubation medium	Intracellular tyrosine-C^{14} / Tyrosine-C^{14} incubation medium
Rat's sarcoma M-1	4.1 ± 0.30	3.0	2.0 ± 0.08	2.3 ± 0.20
Ehrlich's ascitic mouse sarcoma	8.5 ± 1.70	—	3.1	2.9
Rat's ascitic sarcoma	3.3	—	2.1	—
Rat liver	1.3 ± 0.24	1.4	0.8 ± 0.14	1.3 ± 0.10
Mouse liver	1.3	—	—	—

TABLE 2. Correlation Between the Active Concentration of Amino Acids and Their Inclusion in the Proteins of Rat's Sarcoma M-1 and Rat Liver

Amino acids used	Number of experiments	Radioactivity of free amino acids in tumor cells / Radioactivity of free amino acids in liver cells	Number of experiments	Radioactivity of tumor proteins / Radioactivity of liver proteins
Glycine-C^{14}	14	2.9 ± 0.26	15	2.8 ± 0.29
Methionine-S^{35}	5	2.0	5	1.7
Tyrosine-C^{14}	9	1.8 ± 0.26	10	6.3 ± 0.75
Lysine-C^{14}	9	2.0 ± 0.17	9	9.0 ± 1.5

glycine was most actively concentrated by the cells of Ehrlich's ascitic mouse cancer (an average of 8 1/2 times).

In some of the experiments, the concentration of glycine inside the ascitic cancer cells was 20 times higher than the content of this amino acid in the incubation medium. The variations in the concentrating activity of the cells were probably due to disregarded biological factors. Comparing the individual experiments gave us reason to suppose that the stage of the tumor's development if of essential importance.

The concentration of lysine and tyrosine by ascitic cells of Ehrlich's cancer is less intense than that of glycine, but less variable. In almost all the experiments, the concentration of tyrosine and lysine within the cancerous cells was 2-3 times higher than the concentration of these substances in the medium.

The effect observed with the sections of solid tumors was similar. For example, the ratio of the glycine concentration in the cells of sarcoma M-1 to that in the incubation medium was 4.1. In individual experiments, this ratio reached 8-11. The use of Alexander's method for quantitative determination of glycine produced results which coincided with the data obtained from the determination of radioactivity; in the succeeding experiments, therefore, we used only the isotope method. The concentration of lysine-C^{14} and tyrosine-C^{14} in the cells of sarcoma M-1 was twice that in the incubation medium. The concentration of the amino acids in the cells of rat's ascitic hepatoma was 2-3 times higher than in the external environment. Therefore, the tumor cells in the experimental animals were extremely active in regard to the concentration of amino acids. Moreover, the concentration of the experimental labeled amino acids in the rat and mouse liver cells was only 1.3 times higher than their concentration in the medium. The concentration of lysine-C^{14} in the liver cells was even lower than in the incubation medium.

A comparison of the concentrating activity of the cells with the inclusion of amino acids in the cells' proteins (Table 2) will show that the rate of glycine-C^{14} and mehtionine-S^{35} inclusion in the proteins of sarcoma M-1 and rat liver was practically proportional to the ability of these cells to concentrate these amino acids. In fact the ratio of the radioactivity of the free amino acids of the tumor to that of the liver was almost identical to the ratio between the radioactivity figure for the proteins of these cells. These ratios were 2.9 and 2.8 respectively for glycine and 2.0 and 1.7 for methionine (see Table 2). The individual experiments as well as the average values showed this conformity. In one of the experiments, for example, the ratio of the active concentration of amino acids by the cells of a sarcoma M-1 to

their concentration by the liver cells equalled 4.4, while the radioactivity of the tumor proteins was 4 1/2 times greater than that of the liver proteins.

The concentration of tyrosine-C^{14} was an average of 1.8 times higher in the tumor cells than in the liver cells. The rate of tyrosine inclusion in the tumor proteins was 6 times higher than the inclusion of this amino acid in the liver proteins. In this case, as in the case of lysine (for which these figures were, respectively, 2 and 9), with the relatively high concentration of these amino acids by the tumor cells, their inclusion in the protein as compared with the liver cells was considerably higher than their concentratability. In other words, under the experimental conditions described, the ability of the cells to actively concentrate tyrosine and lysine was not proportional to the rate of the inclusion of these amino acids in the tissue proteins.

According to the literature date [see 7,9,10,11], tumor and embryonic tissues have an extremely pronounced ability to actively concentrate amino acids.

Since the first stage in protein synthesis is the utilization of amino acids by the cells, the ability of tumor cells to accumulate amino acids extremely actively could be the main reason for the intensified protein synthesis typical of malignant neoplasms. Until recently, however, tumor cells, particularly ascitic cells of Ehrlich's cancer, have only been used as a convient object on which to study the mechanism of active concentration.

Our experiments showed that cells of sections of sarcoma M-1 and rat's ascitic hepatoma, as well as ascitic tumor cells, possess a greater ability in experiments in vitro than liver cells for the active concentration of amino acids. These data are in accord with the research of Miller and Burke [see 5], which demonstrated that the level of amino acids in the liver cells is higher after the development of a true hepatoma than during the precancer period.

The high concentration of amino acids by tumors is evidently connected with the rate of protein synthesis. In fact, cells of tissues in which the processes of protein synthesis are extremely intense are especially active in the concentration of amino acids. In this respect, the fact that there is a higher level of amino acids in embryonic tissues than in the maternal tissues is particularly interesting. For example, the concentration of glycine in the skeletal muscles of a guinea pig fetus [see 7] is three times higher than that in the muscles of the maternal organism.

Neoplastic cells concentrate amino acids especially actively. In our experiments, the glycine uptake by the ascitic cells of Ehrlich's cancer was an average of 8.5 times (in some experiments, 15-20 times) the uptake by the incubation medium, while the uptake by the cells of sarcoma M-1 was up to 7-8 times (an average of 4 times) the uptake by the medium. The accumulation of the other amino acids by these cells (lysine-C^{14}, tyrosine-C^{14} methionine-S^{35}) was somewhat less intense. The extraordinarily intense inclusion of amino acids in the proteins of tumor cells is in keeping with their high concentration activity. In the case of glycine-C^{14} and methionine-S^{35}, we observed a direct proportionality between these processes. Loftfield and Eigner [13] discovered a similar relation in their research on the synthesis of ferritin in rat's liver carried out in vivo with the aid of leucine-C^{14}.

The increase in the radioactivity of the whole liver protein in this work was proportional to the increase in the activity of the intracellular amino acids. The lack of a proportion between the ratios showing the rate of amino acid inclusion in the protein and the active concentration of these acids by tumor and normal cells observed in the experiments with tyrosine and lysine still does not exclude the possibility that there is a more complex relationship between these processes.

Therefore, cells of sarcoma M-1, rat's ascitic hepatoma, and Ehrlich's ascitic mouse cancer actively concentrate glycine-C^{14}, methionine-S^{35}, tyrosine-C^{14} and lysine-C^{14}. The capacity of the tumor cells for the active concentration of amino acids was 2-8 times greater than that of the liver cells.

The ratio of the inclusion rate of labeled glycine and methionine in the proteins of slices of sarcoma M-1 to the inclusion rate ot these amino acids in the liver proteins equalled the ratio of the uptake of these amino acids by the cells of the tissues named. In the case of labeled tyrosine and lysine, the relative intensity of inclusion in the tumor protein was considerably higher than the relative ability of the tumor cells to concentrate these amino acids.

We regard the results obtained as confirmation of the hypothesis that the increased inclusion of amino acids in tumor proteins in vitro is largely due to the increased ability of tumor cells to concentrate amino acids.

SUMMARY

It is shown that the cells of sarcoma M-1, ascitic hepatoma of rats, and Ehrlich's ascitic cancer of mice actively concentrate glycine-C^{14}, methionine-S^{35}, tyrosine-C^{14}, and lysine-C^{14}. The uptake of amino acids by tumor cells is 2-8 times higher than the uptake by the liver cells.

The ratio of the incorporation rate of labeled glycine and methionine in the proteins of sarcoma M-1 slices to the incorporation rate in the liver proteins in equal to the ratio of the uptake of the corresponding free amino acids by the cells of the mentioned tissues. In the case of labeled tyrosine and lysine, the relative rate of incorporation in the tumor protein is considerably higher than the relative ability of the tumor cells to concentrate these amino acids. The results obtained are regarded as confirmation of the hypothesis that the increased inclusion of amino acids in vitro in tumor proteins is largely due to the increased ability of tumor cells to concentrate amino acids.

LITERATURE CITED

[1] I. B. Zbarskii and N. N. Demin, The Role of Erythrocytes in Protein Metabolism [In Russina] (Moscow, 1949).

[2] I. B. Zbarskii, "Tumor proteins and their metabolism", in: Essential Problems of Modern Biochemistry (Vol. 1, The Biochemistry of Proteins) [In Russian]. (1959) p. 233.

[3] I. B. Zbarskii and K. A. Perevoshchikova, Biokhimiya 22, 1-2, 295 (1957).*

[4] B. Alexander, G. Landwehr, and A. M. Seligman, J. Biol. Chem. 160, 51 (1945).

[5] W. T. Burke and L. L. Miller, Feder. Proc. 14, 187 (1955).

[6] P. N. Campbell and O. Greengard, Biochem. 66, 47 (1957).

[7] H. N. Christensen and J. A. Streicher, J. Biol. Chem. 175, 95 (1948).

[8] H. N. Christensen, J. A. Streicher and R. L. Eblinger, J. Biol. Chem. 172, 515 (1948).

[9] H. N. Christensen, T. R. Riggs, H. Fischer et al., J. Biol. Chem. 198, 1 (1952).

[10] H. N. Christensen, B. Hess, and T. R. Riggs, Cancer Pres. 14, 124 (1954).

[11] H. N. Christensen in: Symposium on Amino Acid Metabolism (Baltimore, 1955), p. 63.

[12] H. N. Christensen, T. R. Riggs, A. J. Aspen et al., Ann. New York, Acad. Sci. 63,983 (1956).

[13] R. B. Loftfield, E. A. Eigner, J. Biol. Chem. 231, 925 (1958).

[14] T. R. Riggs and L. M. Walker, J. Biol. Chem. 233, 132 (1958).

[15] J. R. Robinson, Biochem. J. 45, 68 (1949).

[16] D. D. Van Slyke and G. M. Meyer, J. Biol. Chem. 16, 197 (1913-1914).

[17] P. C. Zamechnik, I. D. Frantz, R. B. Loftfield et al., J. Biol. Chem. 175, 299 (1948).