TUMOR – HOST RELATIONS IN ^{14}C-THIAMINE UTILIZATION

Yu. M. Ostrovskii,* R. V. Trebukhina, and G. N. Mikhal'tsevich

UDC 577.161.11:577.124.25

The content of ^{14}C-thiamine and activity of the thiamine-dependent enzyme transketolase (TK), and the thiamine diphosphate (TDP) content were studied in mouse tissues during the development of Erhlich's ascites tumor. The TDP concentration in the liver of animals with tumors fell steadily for 10 days after inoculation, and the TDP level in the tumor itself fell more steeply toward the terminal period of tumor growth (10th day). Meanwhile a sharp deficiency of coenzyme was found in the tumor, but there was an excess of apoenzyme forms of TK. The increasing thiamine deficiency in the tumor placed a greater strain on thiamine metabolism and led to the appearance of competitive relations between tumor and host, which were manifested most clearly against a background of hypovitaminosis.

KEY WORDS: thiamine; transketolase; Ehrlich's ascites tumor.

Development of tumors in animals is accompanied by a decrease in the concentration of thiamine and its coenzyme form thiamine diphosphate (TDP) in the host's tissues [1, 6, 7]. Changes in the tissue thiamine reserves may be due to a disturbance of absorption or to increased excretion of the vitamin with the urine or to a disturbance of its metabolism during malignant growth. The mechanisms of absorption of the vitamin and its penetration into the tissues, like the metabolic pathways of thiamine itself under conditions of malignant growth have not yet been fully explained. The investigations into this problem have so far been concentrated on the study of the level of the vitamin in the tissues and its excretion with the urine. No investigations with labeled thiamine have yet been undertaken, so that the possibility of formation of depots of vitamin B_1 or the dynamics of its renewal in the tissues of animals with tumors has not yet been explained.

The object of the present investigation was to study tumor-host relations in vitamin B_1 utilization by administration of ^{14}C-thiamine to animals with an Ehrlich's ascites tumor followed by determination of the label in the tissues, and also activity of the thiamine-dependent enzyme transketolase.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino mice weighing 20-22 g, kept on the normal animal house diet. Ehrlich's ascites tumor was inoculated in a dose of 1.5×10^6 cells intraperitoneally from a donor mouse on the 8th day of tumor growth. The animals were decapitated 24 h and on the 1st, 5th, and 10th days after inoculation with the tumor. ^{14}C-Thiamine was injected in a dose equivalent to the daily consumption of thiamine by the animals (10 μg/mouse subcutaneously 24 h before decapitation). Some of the mice were kept on a synthetic diet [8] free from vitamin B_1, the latter being given in a measured dose by injection depending on the age of the experiment: to completely satisfy the requirement of thiamine – 10 μg, a subnormal dose of thiamine 5 and 2 μg/mouse/day, leading to the development of a state of hypovitaminosis in the animals in the course of two weeks [2]. ^{14}C-Thiamine was injected into these animals in a dose of 1 μg per mouse 24 h before decapitation. The quantity of label was expressed in micrograms percent. Radioactivity was counted on a 4P gas-slow counter of the 2154-1-1M type and on a "Vallak 81000" liquid scintillation counter (from LKB, Sweden). A Walker-256 carcinosarcoma was transplanted into female rats weighing 180-200 g in the form of tumor tissue homogenized with physiological saline (1:10) by subcutaneous injection in a dose of 1 ml into the inguinal region. The material was taken from the donor rats on the 7th day of tumor growth. The animals were decapitated on the 4th, 8th, and 12th days after inoculation of the tumor. Transketolase activity in the

*Corresponding member, Academy of Sciences of the Belorussian SSR.

Department of Regulation of Metabolism, Academy of Sciences of the Belorussian SSR, Grodno. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 12, pp. 708-710, December, 1979. Original article submitted August 16, 1978.

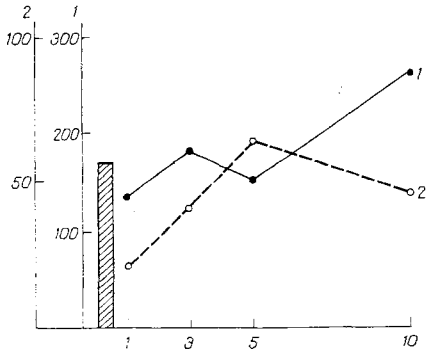

Fig. 1

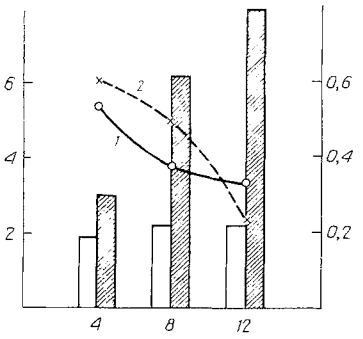

Fig. 2

Fig. 1. Accumulation of ^{14}C-thiamine in liver (1) and tumor cells (2) of mice kept on ordinary animal house diet, during growth of Ehrlich's ascites tumor. Shaded column represents liver of intact mice. Ordinate, content of ^{14}C-thiamine (in g percent); abscissa, time of growth of tumor (in days).

Fig. 2. Content of TDP and apotransketolase activity in liver, erythrocytes, and tumor tissue in animals with Walker-256 carcinosarcoma. Unshaded columns represent content of apotransketolase in erythrocytes; shaded columns the same in tumor cells (in conventional units). Ordinate, TDP content (in μg/g tissue), on left in liver, on right in tumor; abscissa, time of growth of tumor (in days).

erythrocytes, tissue homogenates, and tumor cells was determined by Bruns' method [5] and expressed in micromoles sedoheptulose-7-phosphate per gram tissue per hour. The TDP-stimulating effect was expressed as a percentage of that for the sample treated under identical conditions but without the addition of TDP. The TDP level was determined by an enzymic method [9] and calculated in micrograms per gram tissue.

EXPERIMENTAL RESULTS

The dynamics of distribution of ^{14}C-thiamine is illustrated in Fig. 1. During the first five days the label was taken up more actively by the tumor cells than the liver. This can evidently be explained by the competitive situation arising on the 5th day, when the liver was already beginning to fall behind appreciably with respect to this index. It was at this time that the absolute content of ^{14}C-thiamine in the two types of tissue was closest. Later, when the tumor cells switched to assimilation of other (noncarbohydrate) substrates, the thiamine requirement was reduced and the content of label in the liver increased, whereas in the tumor cells it decreased. The negative correlation between the two indices ($r = -0.63$) can be regarded as a manifestation of competitive relations between the tumor and liver tissue in thiamine assimilation. The increased uptake of labeled thiamine may be due to the metabolic demands of the malignant cells (TDP as a coenzyme of transketolase), the high mitotic activity and need to provide for growth of which are linked with intensive utilization of glucose [4]. Much of it is metabolized via the pentose phosphate pathway in the early stages of growth. At later times of growth (8th-12th day) the tumor cells assimilated more fatty acids [3] and proteins, on account of which the requirements of the "carbohydrate" vitamin was somewhat reduced. Transketolase activity in the tumor cells was reduced by 40% ($P = 0.05$) by this time, in the erythrocytes it was unchanged, and in the liver it was reduced by only 15% (from 257 ± 9.4 to 223 ± 16.7, $P = 0.1$) on the 8th day of tumor growth. There are thus two types of relationships in metabolism and utilization of thiamine by the tumor and host. During the first five days binding of label by liver tissue shows no significant change, whereas the tumor accumulates ^{14}C-thiamine progressively more rapidly (a condition for competitive relations). Later the picture changes qualitatively: the tumor begins to lose ^{14}C-thiamine whereas the liver accumulates it.

Thiamine metabolism was studied in more detail on a different object (Walker-256 carcinosarcoma). The following indices were determined in this series of experiments: the concentration of free thiamine and its coenzyme form (TDP), the TDP-stimulating effect in vitro in the transketolase reaction. The TDP content in the liver of animals with tumors fell steadily during the 10 days after inoculation, whereas the TDP level in the tumor itself fell more steeply as time went on (Fig. 2). Meanwhile a sharp deficiency of coenzyme and excess of apoenzyme forms of transketolase, detected in experiments in vitro on the addition of the specific coenzyme, were observed in the tumor. Similar results were obtained during the investigation of the same

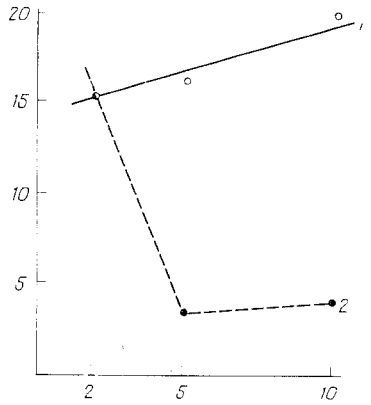

Fig. 3. Binding of ^{14}C-thiamine in liver (1) and tumor cells (2) in mice with Ehrlich's ascites tumor kept on synthetic diet with added vitamins. ^{14}C-Thiamine given in dose of 1 μg per mouse. Ordinate, concentration of ^{14}C-thiamine (in μg %); abscissa, daily dose of thiamine per mouse (in μg).

indices in mice with Ehrlich's ascites tumor. When these relationships are assessed, it will be noted that stimulation of transketolase, characteristic of TDP deficiency in the tumor, increased all the time, reflecting an increase in its deficiency. In the erythrocytes, on the other hand, the picture was relatively stable. The free thiamine (the transport form of the vitamin) level in the liver of the rats fell progressively during growth of the Walker-256 carcinosarcoma: 0.20 ± 0.035 (4th day of tumor growth), 0.14 ± 0.032 (8th day), 0.07 ± 0.022 (12th day); the tumor tissue, however, accumulated thiamine: 0.05 ± 0.017 (8th day), 0.20 ± 0.041 (12th day). Considering the data given earlier on TDP deficiency in the tumor tissue, it can be tentatively suggested that the process of phosphorylation of the vitamin to coenzyme forms was disturbed in it.

On the basis of the data given above it might be expected that the increasing thiamine deficiency in the tumor would place a greater strain on thiamine metabolism, and that the appearance of competitive relations between tumor and host would be most marked in the presence of a small over-all deficiency of the vitamin. Accordingly an additional experiment was carried out on mice kept on optimal, semi-optimal, and deficient diets as regards the supply of the vitamin.

The experimental results are given in Fig. 3. A reduction of the thiamine intake with the diet from 10 to 2 μg daily had the effect that additional administration of labeled thiamine by subcutaneous injection into the animals was accompanied by different distributions of the label in two extreme situations. In mice receiving a higher dose of the vitamin the liver incorporated more label than the tumor. In the presence of marked hypovitaminosis the level of label in the liver and tumor was about equal, i.e., in this situation it could be concluded that the liver and the tumor were equal (and noticeably competitive) consumers of the vitamin.

When the role of vitamin B_1 in tumor growth is assessed it will be recalled that the coenzyme functions of TDP, linked with the supply of energy (pyruvate and ketoglutarate dehydrogenase reaction) are not absolutely essential. Both reactions can be replaced by any other energy-yielding process. The other aspect of the problem – the role of TDP in the transketolase reaction in the pentophosphate cycle – is more important. The enzyme is a factor controlling the formation of ribose-5-phosphate and its utilization, i.e., metabolism of phosphoribosyl pyrophosphate, a precursor of all nucleotides.

Consequently the discovery of competitive relations in the field of factors (vitamins) absolutely essential and irreplacable in metabolic processes is consequently of fundamental importance.

LITERATURE CITED

1. M. Ya. Dzyubko, Vopr. Onkol., No. 6, 67 (1971).
2. L. V. D'yachkova, "The effect of variation in the thiamine intake on the development of experimental tumors," Author's Abstract of Dissertation, Moscow (1970).
3. V. Z. Lankin, Dokl. Akad. Nauk SSSR, Ser. Biol., 211, No. 2, 485 (1973).
4. V. S. Shapot, Priroda, No. 3, 29 (1968).

5. F. H. Bruns, Biochem. Z., 330, 483 (1958).
6. K. H. Kiessling, Exp. Cell Res., 24, 311 (1961).
7. K. H. Kiessling and C. G. Lundquist, Exp. Cell Res., 26, 198 (1962).
8. K. Takahashi, A. Nakamura, and J. Nose, J. Vitamin., 17, 207 (1971).
9. J. Ullrich, Methods in Enzymology, Vol. 18, Academic Press, New York (1970), p. 109.