ACTIVITY OF ALANINE AND ASPARTATE AMINOTRANSFERASES IN TRANSPLANTABLE TISSUE CULTURES

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In connection with the widespread use of transplantable cell culture in various branches of medical biology the problem of their stability, the constancy of their morphological and biological properties, has become of particular importance. It is, therefore, interesting to investigate the nitrogen metabolism of such tissue cultures. There are few references to this subject in general in the literature, and this is particularly true of the study of the velocity of enzymic transamination from L-alanine and L-aspartate to α-ketoglutarate.

In the present investigation, the velocity of the transamination processes catalyzed by aspartate-2-hydroxyglutarate aminotransferase (2.6.1.1, aspartate amino transferase, I.C.E.) and alanine-2-hydroxyglutarate amino transferase (2.6.1.2, alanine amino transferase, I.C.E.) was studied in the cells of certain transplantable cultures in the course of their growth.

EXPERIMENTAL METHOD

Experiments were carried out on the cells of a transplantable culture of monkey's kidney tissue (MK, 32-36 passages) and of human bone marrow from a patient with cancer (Detroit, 94-96 passages). The tissues were cultivated in a nutrient medium of a composition described in an earlier paper [9].

The enzyme preparation for investigation of the level of alanine and aspartate amino transferase activity was a 10% homogenate of 2-day and 7-day tissue cultures prepared in 0.15 M phosphate buffer (pH 7.4) from the cell residue. The activity of the alanine and aspartate aminotransferases were determined by the chemical method of Umbreit and co-workers, as modified by T. S. Paskhina [10] and expressed in colorimetric units per gram of cell residue. The experimental results were subjected to statistical analysis [11].

EXPERIMENTAL RESULTS

It is clear from Table 1 that the lines of transplantable cells may be divided into three groups depending on the velocity of enzymic transamination from L-alanine to α-ketoglutarate. One of these lines (Detroit-6) was characterized by high alanine aminotransferase activity in the 2-day culture, falling significantly by the 7th day. Another line (Fl) showed absence of the active form of alanine aminotransferase, but with the passage of time the velocity of enzymic transamination from L-alanine to α-ketoglutarate increased. This cannot be explained by nonenzymic transamination, for this was completely ruled out in these experimental conditions. Finally, in the MK culture throughout the period of growth no activities of glutamate — alanine transaminase could be detected, although additional experiments were carried out with 20-30% tissue homogenates prepared in tris buffer.

Analysis of the results for the velocity of transamination catalyzed by aspartate aminotransferase (Table 1) also showed that the activity of this enzyme was relatively small in the Detroit-6 cells in the first days of cultivation, but by the 7th day it increased by 40.2% compared with the activity of the 2-dav culture. Meanwhile in the Fl cells, the velocity of enzymic transamination from L-aspartate to α-ketoglutarate was stable and remained unchanged during growth of the culture for 7 days. In the MK culture

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TABLE 1. Activity of Transamination Enzymes in Transplantable Cell Lines in the Course of Growth

Cell culture	Age of culture (in days)	No. of expts.	Alanine aminotransferase		Aspartate aminotransferase		
			$\bar{x}\pm S$	t_{05}	$\bar{x}\pm S$	t_{05}	r
M1	2	10	0		720±10,7		0
	7	10	0		496±12,8	20,4[1]	0
F1	2	10	0		672+19,2		0
	7	10	172,0±22,6		680+26,3	0,62	+0,14
Detroit-6	2	10	832,0±10,4		296±7,9		−0,22
	7	10	388,0±34,7	11,5[1]	119,2±4,1	19,1[1]	−0,01

*Aspartate aminotransferase.

the activity of glutamate — aspartate transaminase was 68% lower in the 7-day culture than in the 2-day culture. The dynamics of the change in activity of alanine and aspartate aminotransferases thus differed in pattern for the same culture. This was confirmed by comparing the coefficient of correlation (Table 1).

The results showing the dynamics of alanine and aspartate aminotransferase activity are important for understanding the biology of cells cultivated in vitro. It is generally considered that removal from the organism and cultivation in vitro must be accompanied by changes in the whole biology of the cells, and by the radical modification of the fundamental morphological, physiological, and biochemical properties.

It must be remembered that in certain characteristics considerable differences are present in the metabolism of primary explanted cultures and of transplantable cell lines in which adaptation to prolonged existence in vitro has taken place [6]. The data for the activity of alanine aminotrasnferase in transplantable monkey's kidney cells and for aspartate aminotransferase in transplantable human amnion cells obtained by the authors confirm the above hypothesis and agree well with data for the dynamics of the glycogen content in the transplantable cultures [8].

The reason for the absence of a decrease in activity of the transamination enzymes in the early stages of cultivation of the cells may evidently be the high level of mitotic activity in the cells of the transplantable lines and the high degree of adaptation of these cells to the conditions of existence, as shown in particular by the absence of a period of habituation [5]. This is confirmed by results obtained by other authors [4, 7]. In the present experiments, the activity of the transamination enzymes was not dependent on the number of passages. This is shown by the small scatter of the figures within the series, the small value of the mean error, and the high value of Student's criterion within the variants series of 2- and 7-day cultures.

The concept of adaptation of transplantable cell lines to prolonged existence in vitro does not exclude essential fluctuations in the activity of the enzymes in these cultures, as may clearly be seen by the example of the change in alanine aminotransferase activity in the Fl cells and in aspartatate aminostransferase activity in the MK cells. These fluctuations are evidently dependent on the level of mitotic activity. Probably the decrease in activity of these enzymes during the development of transplantable cell cultures may coincide with the lag period [16, 18].

Finally, the absence of alanine aminotransferase activity in the transplantable kidney culture by comparison with the parenchymatous kidney cells evidently demonstrate simplification of the metabolism and dedifferentiation of the cells in culture, as is shown by disappearance or intensification of the activity of other enzymes [12, 17].

In conclusion, the character of the change in alanine and aspartate aminotransferase activity in the Detroit-6 transplantable cells must be examined more closely, for these changes do not concern the hypothesis of Cohen and co-workers [14], although they show good agreement with the results obtained by other investigators [11, 13, 16]. The present results, together with those obtained by the authors cited above, confirm the views of A. E. Braunshtein [3], who considers that the reversibility of the transamination reaction may create conditions facilitating the formation of large quantities of glutamic acid and, consequently, facilitating protein synthesis. Admittedly, the link between transamination processes and protein synthesis, like the specific participation of glutamic acid in protein biosynthesis, has not yet been fully explained.

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