ACTIVITY OF SOME GLYCOLYSIS ENZYMES IN THE LIQUID PHASE OF TISSUE CULTURES INFECTED WITH VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS

T. L. Miryutova, et al

Army Medical Research Institute of Infectious Diseases
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Authors: T. L. Miryutova, Ye. A. Peshkova, Tomsk Scientific Research Institute of Vaccines and Serums

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The reproduction of Venezuelan equine encephalitis virus in cultures of chick and human embryo fibroblasts is accompanied by changes in the activity of some enzymes of carbohydrate metabolism (glucosophosphatisomerase, aldolase, and lactatedehydrogenase).

The enzymatic changes in the cells were determined by the changes in enzymatic activity in the tissue culture fluids. The experiments performed demonstrated that the activity of glucosophosphatisomerase increases so early as 4 hours after infection of fibroblasts, before cytopathic changes develop in the cells. It should be noted that the intensity of the enzymatic changes reflects the extent of virus reproduction. Lactatedehydrogenase activity also exhibited a statistically significant increase before the appearance of cytopathic changes (24 hours after infection), and was twice as high as the control levels by the time of cell sheet degeneration. Unlike the activity of these two enzymes, the aldolase activity of the medium did not change in advance of cell sheet degeneration and did not increase until after the death of the cells.

References are made in the literature to change in the activity of enzymes in the culture fluid of cells infected with adenoviruses, mixoviruses, and sarcoratoid viruses, as well as viruses of the smallpox group [1,4], but there are no data on the influence of arboviruses. We have studied the activity of three enzymes -- glucosophosphatisomerase, aldolase, and lactatedehydrogenase -- which limit the decomposition of carbohydrates in the initial, intermediate, and final stages of the glycolytic phosphorylation cycle in the dynamics of reproduction of the Venezuelan equine encephalomyelitis (VEE) virus in a cell culture. Previously trypsinized tissues of chick embryo fibroblasts (CEF) and human embryo fibroblasts (HEF) were employed as the cellular model.

Material and Methods. The embryo fibroblast cells were prepared in the conventional manner and inoculated in test tubes with 1 mln/ml. The culture medium employed was 0.5% lactalbumin hydrolyzate in Hanks' solution with 10% bovine serum. After formation of the monolayer (after 24 hours in the case of the CEF cells and after 2 to 3 days in the case of the HEF cells), the medium was poured out and the cells washed off with Hanks' solution and infected with the VEE virus. The virus was in contact with the cells for 30 to 40 minutes at 37°. The infecting dose for the cells of the strains studied ranged from 0.06 to 0.006 CPD_{50}/cell. The substrate

was represented by 0.5% lactalbumin hydrolyzate in Hanks' solution with 2% bovine serum. The control (non-infected tissue) was cultivated in the same medium. The monocellular fluid was collected 4, 8, 24, 48, 72, and 96 hours after infection of the cells. The glucosophosphatisomerase content was determined by the Bodanskiy method as modified by Korovkin [3] and expressed in micromoles of fructoso-6-phosphate fructose formed by the action of 1 ml of culture fluid in 1 hour at 37°. Free D-fructose was used in order to plot the standard calibration curve. The intensity of coloration was measured with an SF-4a spectrophotometer at a wavelength of 490 nm. The amount of aldolase was determined by the Bruns method as modified by Tovarnitskiy and Voluyskaya [3]. The enzyme activity was expressed in micrograms of crystalline enzyme in 1 ml of culture fluid. The calibration curve was plotted on the basis of crystalline aldolase deriving from rabbit muscle.

The lactatedehydrogenase activity was measured with a colorimeter by the method of Chevel and Tovarek [3], use being made of a color filter with a maximum transmission of 563, and was expressed in micrograms of pyruvic acid formed on incubation of 1 ml of culture fluid for 1 hour at 37°. The calibration curve was plotted on the basis of pyruvic acid.

The morphological state of the cell sheet was observed under the optical microscope at the times the samples were taken for analysis. In addition, permanent preparations were produced on cover slips.

The titer of the virus was determined on the basis of its cytopathic effect in test tube CEF cultures, and the results were calculated by the method of Reed and Mentsch.

Results. Infection of the CEF cells with the VEE virus causes statistically reliable increase in glucosophosphatisomerase activity in the culture fluid so early as 4 hours after the infection (Table 1). After 24 hours, observation under the optical microscope and inspection of the stained preparations show the signs of specific degeneration of the fibroblasts to be manifested merely in intensified vacuolization of the cytoplasm and more clearcut limitation of the nucleus in comparison with the control; the enzyme activity continues to increase. The maximum glucosophosphatisomerase activity is observed 48 hours after infection, when the basic mass of the cells degenerates. On the following days (72 and 96 hours) the enzyme activity decreases sharply in the culture fluid. Comparison of the glucosophosphatisomerase activity with the titer of the VEE virus in the pericellular fluid revealed that the maximum enzyme concentration (1.39 micromole) corresponds to the highest activity of the virus (5.0 lg TDC_{50}/ml).

The aldolase activity of the medium, as determined in the nutrient medium washing the cells, is shown by our observations to undergo irregular change. Some authors [2] note that the configuration of the aldolase molecule is such that it with difficulty penetrates the membrane of fibroblastoid cells and not until after destruction of the cell by the action of the virus does it appear in sufficient quantity in the pericelluiar

fluid. It was found on infection of the cells with the VEE virus that the aldolase activity in the medium remains within the range of the control during the first 24 hours; decreases and increases in the activity of the enzyme do not depend on the dose of virus administered and on the time elapsing since infection. After 48 hours, when the completeness of the membrane of the majority of cells has been destroyed, there is a statistically certain increase in the aldolase activity in the culture fluid (Table 2). This is in all probability due to degeneration of the cells along with emergence of the cellular contents into the medium. The aldolase activity in the medium then declines, since the cells cease to form the enzyme, and the enzyme present in the cells is inactivated.

Table 1. Glucosophosphatisomerase activity (mk/mol) in CEF culture fluid infected with VEE virus (M ± m).

1 Время после заражения (в часах)	2 Контрольные клетки	3 Зараженные клетки	P	4 Титр вируса 5 lg $ТЦД_{50}$/мл
4	0,22±0,03	0,50±0,07	0,01	3,9
24	0,56±0,07	0,81±0,06	0,05	3,7
48	0,90±0,09	1,39±0,11	0,01	5,0
72	0,89±0,12	1,19±0,09	0,5	4,2
96	0,94±0,10	0,63±0,11	0,02	1

Key: 1. Time after infection (hrs). 2. Control cells. 3. Infected cells. 4. Virus titer. 5. lg TCD_{50}/ml.

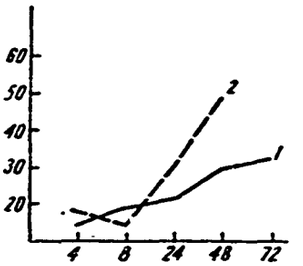
Table 2. Aldolase activity (mkg/ml) in culture fluid of CEF and HEF cells infected with VEE virus (M ± m).

1 Время после заражения вирусом (в часах)	2 ФЭК			3 ФЭЧ			7 Степень ЦПД*
	4 зараженные клетки	5 контрольные клетки	6 титр вируса (в lg $ТЦД_{50}$/мл)	4 зараженные клетки	5 контрольные клетки	6 титр вируса (в lg $ТЦД_{50}$/мл)	
4	1,2±0,02	2,2±0,1	4,3	1,1±0,1	1,2±0,05	3,7	—
8	1,4±0,3	0,4±0,15	4,8	1,6±0,4	0,2±0,1	3,7	—
24	0,6±0,05	2,3±0,2	4,3	1,2±0,5	2,2±0,15	5,3	—
48	4,2±0,04	1,2±0,5	5,1	3,8±0,25	1,6±0,1	5,1	++++
72	1,2±0,08	1,4±0,5	3,7	2,0±0,5	2,6±0,20	3,7	++++

Notation: CPE corresponds to standard.

Key: 1. Time after infection with virus (hrs). 2. CEF. 3. HEF. 4. Infected cells. 5. Control cells. 6. Virus titer (lg TCD_{50}/ml). 7. Degree CPE*.

Our observations indicate that the last enzyme of the glycolytic phosphorylation cycle, lactatedehydrogenase, undergoes extensive change under the influence of the virus. During the first 8 hours after infection a statistically certain decrease in the activity of this enzyme is noted in the culture fluid of the CEF and HEF cells; its activity in the medium then begins to increase, so that 24 hours later it considerably exceeds the control level (see drawing). At this time the cells when observed under the optical microscope exhibit no signs of specific degeneration, although the protoplasm is vacuolated and its granularity has increased. The lactate dehydrogenase activity increases by the time of degeneration of the cell sheet, reaching a level twice as high as the control after 48 hours. Data characterizing the activity of lactatedehydrogenase in the culture fluid of infected HEF cells are presented in Table 3.



Lactatedehydrogenase activity on infection of HEF culture with VEE. 1. Control. 2. Experiment. Vertical axis: ferment activity (mkg/ml pyruvic acid); horizontal axis: time after infection (hours).

Table 3. Activity of lactatedehydrogenase (mkg/ml pyruvic acid) in culture fluid of HEF cells infected with VEE virus (M ± m).

1 Время после заражения вирусом (в часах)	2 Зараженные клетки	3 Контрольные клетки	4 Титр вируса (в lg TCD_{50}/мл)
4	11,1±0,25	10,0±0,2	4,0
8	9,0±0 30	12,5±0,35	4,5
24	23,0±0,15	12,1±0,10	6,0
48	38,2±0,20	14,5±0,17	6,0
72	45,3±0,35	20,0±0,26	5,0

Key: 1. Time after infection with virus (hrs). 2. Infected cells. 3. Control cells. 4. Virus titer (lg TCD_{50}/ml).

Discussion. The experiments conducted demonstrate that infection of CEF and HEF cells with the VEE virus modifies the activity of the enzymes of certain links in the chain of glycolytic phosphorylation -- a process that plays a prominent part in provision of energy for resynthesized virus

particles. Two of the enzymes investigated -- glucosophosphatisomerase and lactatedehydrogenase -- freely penetrate the cell wall, and their activity in the culture fluid undergoes statistically certain change even during the first hours after infection.

The activity of these enzymes assumes considerable values (25% higher than the control for glucosophosphatisomerase and 75-100% for lactatedehydrogenase) 24 hours after infection. This increase is accompanied by a high virus titer (5.5--6.0 TCD_{50}/ml) in the medium, but no specific degeneration of the cell sheet is revealed over this period by observation under the optical microscope. The enzyme activity reaches its maximum value during the subsequent hours, when complete degeneration of the cells takes place.

The aldolase activity of the medium remains at the level of the control values as regards cell sheet degeneration, increasing only after the death of the cells.

The data obtained permit assessment of certain aspects of carbohydrate metabolism on interaction between cell and virus on the basis of the embryo fibroblast and VEE virus model.

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Reproduction of Venesuelan equine encephalomyelitis virus in cultures of chick and human embryo fibroblasts is accompanied by changes in the activity of some enzymes of carbohydrate metabolism (glucosophosphatisomerase, aldolase and lactatedehydrogenase).

The enzymatic changes in the cells were determined by the changes in the enzymatic activity in the tissue culture fluids. The experiments which were carried out demonstrated the activity of glucosophosphatisomerase to increase as early as 4 hours after infection of fibroblasts, before development of cytopathic changes in the cells. It should be noted that the intensity of enzymatic changes reflects the extent of virus reproduction. The activity of lacatedehydrogenase also showed a statistically significant increase before appearance of cytopathic changes (24 hours after infection), and by the time of the cell sheet degeneration (48 hours) doubly exceeded control levels. In contrast to the activity of the above-mentioned enzymes, aldolase activity of the medium did not change before degeneration of the cell sheet and increased only after the death of the cells.