# COMPARATIVE STUDY OF ENZYMES OF TRANSPLANTABLE HUMAN LEUKEMIA CELLS WITH RESISTANCE TO VIRUSES ACQUIRED IN VITRO

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UDC 616.988-097.3-02:616-006.446-008.931]-092.4

Leukemia cells sensitive to Coxsackie and poliomyelitis viruses and cells resistant to these viruses differ in the activity of their glucose-6-phosphate dehydrogenase and NAD-diaphorase isozymes.

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By the action of viruses on transplantable cells of reticular type, V. D. Solov'ev and N. E. Gulevich obtained subcultures resistant to the cytopathic action of these viruses [5]. The resistance acquired by the cells was specific against a definite virus and was transmitted by heredity to several generations of cells, after which it was spontaneously lost. Special investigations failed to reveal the presence of virus, antibodies, or substances of interferon type [4] in the resistant cells. The results of later investigations showed that, regardless of their origin (from human leukemia cells or from monkeys' tonsil cells) or of the virus producing resistance (Coxsackie $B^3$ virus or type I poliomyelitis virus), the resistant subcultures possessed diminished ability to reproduce, diminished activity of several enzymes, and a reduced content of RNA and protein SH-groups [1, 6].

These authors accordingly postulated that the resistance of the cultures studied by them is based on mechanisms similar to those of natural immunity to certain infections, consisting of a temporary repression of the production of certain ingredients essential for entry of the virus into the cell or for its intracellular development. However, the important question of the specificity of this acquired resistance is left open.

The object of the present investigation was to study certain enzymes and proteins contained in extracts of leukemia cells sensitive or resistant to two different viruses by the method of fractionation on polyacrylamide gel.

## EXPERIMENTAL METHOD

Three lines of cells were used in the investigation: original strain L-96 (isolated by Osgood and Brooks from a patient with subacute monocytic leukemia), sensitive to Coxsackie and poliomyelitis viruses, and strain L-41, obtained from it and the resistant to Coxsackie $B^3$ virus, and strain P-123, resistant to type I poliomyelitis virus.* Both strains were highly resistant and tolerated infection exceeding 10 PFU/cell.

The cells were grown in Roux flasks on medium No. 199 with 10% bovine serum. On the 4th-5th day of growth the cells were removed with 0.02% versene solution, washed 2 times with cold physiological saline, and centrifuged in the cold at 1000 rpm. The cells which settled were resuspended in 1 ml distilled water and disintegrated by freezing in a mixture of dry ice and acetone and thawing 3 times, after which they were centrifuged for 30 min at 4500 rpm. The residue was removed. The protein content was determined spec-

* The resistant strains were obtained by N. E. Gulevich and T. Crispin by treating cells of line L-96 with Coxsackie $B^3$ and type I poliomyelitis viruses, and made available to use.

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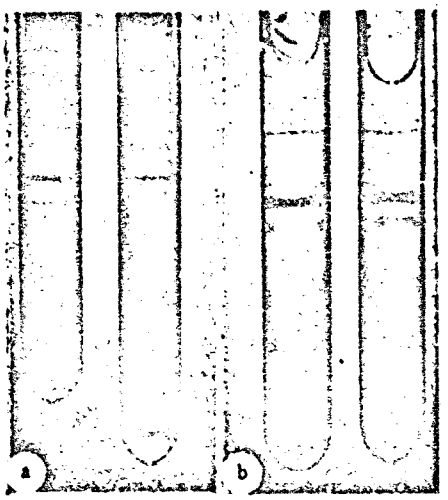


Fig. 1

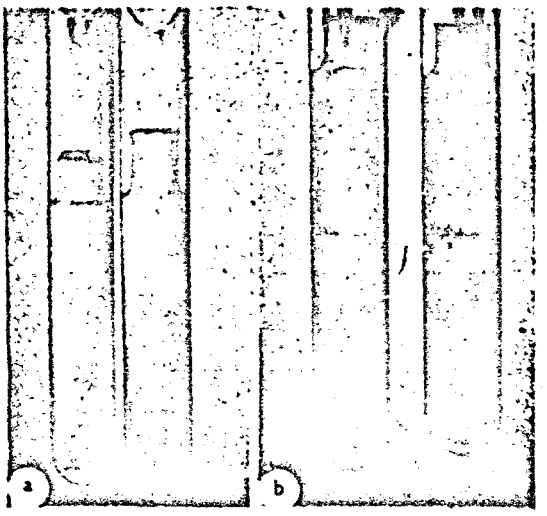


Fig. 2

Fig. 1. Electrophoresis of extracts of sensitive and resistant cells. Isozymes of glucose-6-phosphate dehydrogenase. a) On the left, extract of sensitive L-96 cells, 3 isozymes of the enzyme visible; on the right, extract of L-41 cells resistant to poliomyelitis virus, considerable reduction in intensity of first isozyme visible. Protein (50 μg) applied to each column; b) on the left, original L-96 cells, on the right P-123 cells resistant to poliomyelitis virus, no differences in enzyme activity. Protein (200 μg) applied to each column.

Fig. 2. NAD-diaphorase detected by electrophoresis of cell extracts. a) On the left, extract of sensitive L-96 cells; on the right, extract of cells resistant to Coxsackie $B^3$ virus; b) on the left, extract of sensitive L-96 cells, on the right, extract of cells resistant to poliomyelitis virus.

trophotometrically in the supernatant, the protein concentration in the tubes was equalized, and the extract was mixed with an equal volume of 60% sucrose solution.

Electrophoresis in polyacrylamide gel was carried out by Davis's method [9] in 8 columns measuring 6 × 70 mm, with an initial voltage of 300 V and current 50 mA, for 3 h. Protein was applied to the column at the rate of 50-200 μg in a volume of 0.1 ml. The apparatus for electrophoresis was cooled with dry ice. Glucose-6-phosphate dehydrogenase (G6PD) [2, 8], and NAD-diaphorase (ND) [3, 7] isozymes were determined electrophoretically. In separate series of electrophoretic investigations staining was carried out for protein [9]. The electrophoretic mobility Rf of the isozymes was determined as the ratio between the distances in millimeters moved by the isozyme and the distance moved by the yellow band marking the changing boundary between gel and buffer. All determinations for each strain of cells were made on material from 5-8 flasks, from 2-4 parallel tests being carried out with material from each flask. In each series of experiments simultaneous observations were made on sensitive and resistant cells.

## EXPERIMENTAL RESULTS

Electrophoresis of extracts of the original cells (strain L-96) revealed 3 isozymes of G6PD with Rf values of 0.21 for the first isozyme (the farthest from the starting line), 0.14 for the second, and 0.09 for the third. The 2nd isozyme was the most active in these cells, occupying an area 1-2 mm in width which was stained most intensively. The color appeared in the earliest stages of incubation with the substrate mixture compared with the appearance of color of the other isozymes. The first isozyme likewise gave a wide band, but this was less intensively stained than the second. The 3rd isozyme was the least active, giving a narrow band of pale color. In individual experiments the 1st and 2nd isozymes were split into two.

Some weakening of activity of the 3rd isozyme and a considerable decrease in activity of the first were characteristic of the cells resistant to Coxsackie $B^3$ virus (strain L-41). In some series of experiments the 1st isozyme could not be found. It is interesting to note that activity of the 2nd isozyme was un-

changed in these circumstances (Fig. 1a). With loss of resistance by the L-41 cells, their G6PD activity rose to its level in L-96 cells.

No decrease in activity of the isozymes of G6PD could be observed in the cells resistant to poliomyelitis virus (strain P-123; Fig. 1b). Moreover, in some experiments an increase in the intensity of staining of the 1st isozyme was observed.

Hence, the two resistant strains of cells differed in their G6PD activity. It is interesting to note that the loss of activity of this enzyme in cells resistant to Coxsackie $B^3$ virus was observed mainly in one of the 3 isozymes of the enzyme. That viruses can cause various changes in the isozymes of the same enzyme was demonstrated by the findings of Latner and co-workers [10], who studied changes in lactate dehydrogenase in a culture of monkey's kidney cells infected with poliomyelitis viruses and adenovirus.

During electrophoresis of extracts of the original cells (strain L-96), NAD-diaphorase (ND) appeared as a single, very weakly stained band with a mobility of 0.46. In cells resistant to Coxsackie $B^3$ virus (strain L-41) the ND activity was much higher (Fig. 2a). In 2 experiments we observed the appearance of a 2nd isozyme with mobility 0.37. Loss of specific resistance to virus by the cells was accompanied by weakening of ND activity. Strain P-123, resistant to poliomyelitis virus, also showed increased ND activity compared with the sensitive line L-96 (Fig. 2b). However, in contrast to the cells resistant to Coxsackie virus, no additional isozyme appeared. In earlier histochemical investigations of a number of enzymes in different cell lines resistant to Coxsackie and poliomyelitis viruses, the only change found was a decrease in enzyme activity [1, 6]. The activation of ND – an enzyme of the oxidative cycle – may be one of the signs of the relatively greater differentiation of cells of resistant lines than of the original line L-96, which, like other tumor cells, is characterized by predominance of anaerobic glycolysis over respiration.

When staining for protein was carried out after electrophoresis, more than 20 bands of color of different intensities were found. Comparison of the sensitive and resistant cells showed differences in the intensity of staining of individual proteins. Some bands of resistant cells were weakened, others strengthened. However, the results were not clear enough for definite conclusions to be drawn regarding changes in the properties of proteins in the resistant cells.

Hence, since both strains resistant to viruses were obtained from a single maternal cell line, the change in G6PD activity in the cells resistant to Coxsackie $B^3$ virus and the absence of such changes in cells resistant to poliomyelitis virus suggest that certain stages of metabolic processes in the resistant cells undergo different changes depending on the virus to which these cells are resistant.

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