# GLYCOGEN CONTENT AND SUCCINATE DEHYDROGENASE ACTIVITY IN HEP 2 CELLS INFECTED WITH RNA-CONTAINING COXSACKIE B3 VIRUS

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A cytophotometric investigation of the glycogen concentration and succinate dehydrogenase activity in HEP 2 cells under control conditions and after infection with Coxsackie B3 virus showed that the glycogen concentration in the infected cells 30 min and 1 h after infection was lower, and 48 h after infection higher, than in control cells at the same times. Succinate dehydrogenase activity in the infected cells was higher 6 h after infection and lower 24 and 36 h after infection than in control cells.

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The object of this investigation was to determine the glycogen concentration and succinate dehydrogenase activity cytophotometrically in HEP 2 cells infected with Coxsackie B3 virus.

Such an investigation can shed considerable light on the pathogenesis of virus infections at the cell level. Data in the literature on this problem are incomplete and contradictory [2-5].

## EXPERIMENTAL METHOD

Experiments were carried out on HEP 2 cells grown on slides in medium No. 199 with 10% bovine serum. Infection took place on the 3rd day of growth of the culture, when a continuous layer had been formed, by replacing the growth medium by medium No. 199 without serum and containing Coxsackie B3 virus, strain Nancy, in a dose of 1000 $CPD_{50}$/ml. In the control the medium was changed but without addition of the virus. The infected culture was investigated 30 min, and 1, 2, 4, 6, 8, 12, 24, 36, and 48 h after infection. Samples of the control culture were investigated at the same time. For determination of polysaccharide the cells were fixed for 20 min in 10% alcohol-formalin. Glycogen was detected by A. L. Shabadash's method. Succinate dehydrogenase activity was determined by Hirono's modification of the method of Seligman and Rutenburg. From each replica 30 cells were investigated under standard conditions by a photographic variant of consecutive cytophotometry [1]. The areas of the cells were measured by planimetry. The results were subjected to statistical analysis. Parallel determinations were made of the titer of the virus in the culture fluid.

## EXPERIMENTAL RESULTS

The results are shown in Table 1. The decrease in glycogen concentration and increase in succinate dehydrogenase activity in the cells of the experimental and control cultures during the first hour after the experiment began were evidently connected with a nonspecific reaction of the cells to changes in the external environment. The more intensive decrease in glycogen concentration and higher activity of the enzyme in the infected cells than in the control culture may be attributed to the fact that the medium containing virus was a more powerful (pathogenic) stimulus than medium without virus. It can be assumed that the primary reaction of the cells to a change in the external environment is associated with considerable expenditure of energy, and this is reflected in utilization of glycogen and increased activity of the oxidative enzyme.

The subsequent decrease in glycogen concentration (between 4 and 6 h) and the extremely low glycogen concentration between 6 and 12 h after infection and change of the medium in both cultures, accompanied at certain times by an increase in succinate dehydrogenase activity, can be explained as follows.

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TABLE 1. Glycogen Concentration and Succinate Dehydrogenase Activity in HEP 2 Cells under Normal Conditions and after Infection with Coxsackie B3 Virus (M ± m)

| Time (in h) after infection and change of medium | | Glycogen | Succinate dehydrogenase activity |
|---|---|---|---|
| Infected culture | ½ | 2,10±0,16 | 2,04±0,22 |
| | 1 | 1,33±0,11 | 3,02±0,24 |
| | 2 | 1,69±0,14 | 2,04±0,22 |
| | 4 | 1,82±0,15 | 2,22±0,27 |
| | 6 | Glycogen concentration extremely low and not determinable cytophotometrically | 3,33±0,34 |
| | 8 | | 3,01±0,33 |
| | 12 | | 2,58±0,26 |
| | 24 | 2,37±0,15 | 2,20±0,22 |
| | 36 | 2,90±0,27 | 1,24±0,13 |
| | 48 | 4,94±0,31 | — |
| Uninfected culture | ½ | 3,02±0,22 | 2,15±0,19 |
| | 1 | 2,24±0,14 | 3,49±0,35 |
| | 2 | 1,99±0,12 | 2,23±0,19 |
| | 4 | 1,95±0,15 | 2,82±0,28 |
| | 6 | Glycogen concentration extremely low and not determinable cytophotometrically | 1,86±0,18 |
| | 8 | | 2,40±0,36 |
| | 12 | | 3,18±0,31 |
| | 24 | 2,90±0,23 | 3,11±0,35 |
| | 36 | 2,59±0,21 | 3,30±0,29 |
| | 48 | 3,16±0,26 | — |

In the cells of the control culture at this time, because of adaptive responses to new conditions of existence and the need for synthesis of new RNA molecules, as a result of the expenditure of energy, a large proportion of the carbohydrate reserves of the cells is used up.

The low glycogen concentration in the cells of the infected culture (between 6 and 12 h), associated with increased activity of the enzyme 6 h after infection, can be explained by reproduction of the virus, which is also associated with expenditure of energy.

Accumulation of glycogen in the cells of the control culture between 24 and 48 h after changing the medium, coinciding with high succinate dehydrogenase activity, is explained on the basis that cells of the control culture, having become adapted to the new nutrient medium, accumulate an important biological component (glycogen) in connection with the raised level of oxidative processes in the cells. The result of adaptation of the cells to the new nutrient medium is thus not merely accumulation of glycogen in them, but also a higher level of succinate dehydrogenase activity in the adapted cells.

The sharp increase in glycogen content and accompanying fall in succinate dehydrogenase activity in the infected cells in the late stages of infection (between 24 and 48 h after infection) is explained by degenerative changes associated with lowering of the intensity of oxidative processes, and with the reduced consumption and pathological deposition of polysaccharide in the infected cells. This suggests that depression of oxidative processes in the infected cells is one of the causes of their death.

The approximately equal glycogen content in the cells of the control and experimental cultures in the period of virus reproduction (the first 12 h after infection) suggests that the level of energy expenditure of the infected cells in the period of virus synthesis and of the control cells at the corresponding period does not differ significantly. The results also indicate that in the period of virus reproduction the cells maintain a high level of oxidative processes.

Our results show good agreement with those of the biochemical investigations of Kovacs and coworkers [6], who showed that the level of glucose consumption by tissue culture cells under normal conditions and after infection with enteroviruses does not differ significantly.

## LITERATURE CITED


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