Succinic Dehydrogenase Activity in HeLa Cells Infected with Newcastle Disease Virus

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SUMMARY

Changes in succinic dehydrogenase activity of HeLa cells infected with Newcastle disease virus (NDV) were studied cytochemically and quantitatively. A decrease in enzymatic activity preceded morphologic changes. Succinic dehydrogenase activity decreased 56 per cent 6 hours after inoculation with the virus. There was a decrease in the number of cells in the prophase and an increase in the number of cells in the telophase of NDV-treated cells at 6 and 9 hours, but the metaphase and anaphase were unaltered. There was also a significant drop in mitotic activity at 6 hours.

With the methods employed, no evaluation of viral effect could be made in 6 hours on cells in the interphase unless the succinic dehydrogenase activity was determined.

The capacity of certain viruses to destroy cells in tissue cultures stimulated investigations in virus-host cell relationship and accompanying biochemical alterations. Experiments with carbon-14 suggest that tumors possess the enzymes of the Krebs citric acid cycle (8). More specifically, extracts of HeLa cells contained the enzymes of the cycle (1). Since these enzymes are important for supplying energy requirements, the following study was made to determine the progressive changes in succinic dehydrogenase activity in HeLa cells infected with Newcastle disease virus. Enzymatic changes were correlated with morphologic changes, mitotic activity, and distribution of Feulgen-positive material.

MATERIALS AND METHODS

The California strain of Newcastle disease virus (NDV) was used. This virus was obtained from the American Type Culture Collection and had passed through chicken eggs 38 times by this group and 8 times in this laboratory. The virus was propagated in the allantoic sacs of 9- to 10-day-old chick embryos by an infecting inoculum of 0.1 ml. of a 10^-4 dilution of stock virus. The inoculated eggs were incubated at 37° ± 1° C. for 30-36 hours, and the allantoic fluid was harvested after the eggs were chilled 1-2 hours. The experiment was carried out with a single pool of virus. Aliquots were stored in sealed glass ampoules at -70° C. The infectivity titer was calculated by the method of Reed and Meunch (6). The EID_{50} was 8.5-9.0/0.1 ml, and the hemagglutination titer was 1:1000 at 4° C.

Stock cultures of HeLa (Gey) cells were grown and maintained in 8-ounce glass bottles containing 10 ml. of medium. The medium consisted of 30 per cent human serum, 60 per cent synthetic medium 199, and 10 per cent tryptose phosphate broth. The medium contained 0.1 mg/ml of streptomycin and 100 units/ml of penicillin. Occasionally a few drops of 10 per cent sodium bicarbonate were added to adjust the pH to 7.2. Each bottle was inoculated with 50,000-75,000 cells/ml of medium. For cytochemical study 16 X 125 mm. screw cap tubes each containing a 6 X 40 mm. coverslip were seeded with 75,000 cells in 1.5 ml. of medium incubated in a stationary position at a 5° incline at 37° C.

Tubes were inoculated with 0.2 ml. undiluted NDV or normal allantoic fluid (NAF). Succinic dehydrogenase (S.D.) activity was demonstrated by the method of Seligman and Rutenburg (7) which was adapted for monolayer tissue culture preparations (3). The method incorporates the admixture of specific substrate into agar prior to the overlaying of the cells. The agar-substrate mixture consisted of 2.0 ml. of 1.5 per cent agar containing 10 per cent glycerine, 0.2 ml. of 0.4
per cent neotetrazolium, and 0.5 ml. of 0.2 M sodium succinate. Distilled water replaced the succinate in the controls. A coverslip with HeLa cells was inverted on a slide containing the agar-substrate mixture. The preparation was placed immediately in a dry-ice chamber for 3 minutes to destroy endogenous activity. Slides were then placed in a 37° C incubator for 1-2 hours.

The succinic dehydrogenase activity was determined quantitatively by a method similar to that for xanthine oxidase (2). Three determinations for succinic dehydrogenase activity were made on cells inoculated with NDV and NAF at 0, 2, 6, and 9 hours. The pool of cells from seven bottles were used at 6 and 9 hours of the NDV-treated cells. The pooling of cells from seven bottles was necessitated by the decrease in the number of cells due to viral infection. Determinations of the NDV-treated cells at 0 and 2 hours and the NAF-inoculated cells at 0, 2, 6, and 9 hours were accomplished by pooling the cells from four bottles. Bottles of 7- to 10-day-old HeLa cells containing approximately 10-12 × 10⁶ cells per bottle were inoculated with 2.5 ml. of NDV (approximately 2,500 infectious particles per cell). Controls were inoculated with 2.5 ml. NAF. The medium was then removed, and the bottles were placed in a dry-ice chamber for 3 minutes to minimize endogenous activity. Cells were scraped from the wall of the bottles, suspended in 5.0 ml. of phosphate buffer, pH 7.4, and counted in the white cell chamber of a hemocytometer. Cells were added to test tubes (5-10 × 10⁶ cells/tube). To each tube 1.0 ml. of 0.4 per cent neotetrazolium and 1.0 ml. of 0.2 M sodium succinate was added. Distilled water was used in place of the succinate for the controls. After the removal of air by evacuation for 5 minutes, the tubes were incubated at 37° C. for 3 hours. They were centrifuged at 2000 r.p.m. for 10 minutes, and the supernatant was discarded. To extract the formazan from the cells, 1.5 ml. glacial acetic acid was added, followed by 4.5 ml. of toluene. Optical density was read in a spectrophotometer at 495 mμ. Densities were compared with standards of known amounts of neotetrazolium which were reduced by an excess amount of ammonium sulfide. Values for cellular activity were expressed as gamma of neotetrazolium reduced by 10 × 10⁶ HeLa cells.

For the Feulgen reaction, cells were fixed in Carnoy's solution for 3 minutes, hydrolyzed in 1 N hydrochloric acid for 8 minutes, and placed into Schiff's reagent for 90-120 minutes. After being bleached with 1.0 per cent sodium metabisulfite, the preparation was counterstained with 0.01 per cent fast green in 95 per cent alcohol for 15-30 seconds. Unhydrolyzed preparations served as controls.

Two test and one control slide were used for the histochemical determination of succinic dehydrogenase activity at 0, 2, 6, and 9 hours for NDV- and NAF-treated cells. Similarly, two test coverslips and one control coverslip were allocated for Feulgen reaction for NDV- and NAF-inoculated cells at the mentioned intervals of time. To determine the percentage of cells in different stages of mitosis, in the Feulgen preparations, 5000 cells per slide were counted. Mitotic rate was determined by counting 1000 cells per slide. One coverslip for NDV and one for NAF sufficed for hematoxylin preparations at 0, 2, 6, and 9 hours. Repetition of the experiment gave a total of 98 slides. Results from the two runs were in agreement, and the results were totaled.

### Table 1

**The Effect of Newcastle Disease Virus on Cell Division**

<table>
<thead>
<tr>
<th>Mitotic Phase</th>
<th>0 Hours</th>
<th>2 Hours</th>
<th>6 Hours</th>
<th>9 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninoculated</td>
<td>NDV</td>
<td>NAF</td>
<td>P&lt;sub&gt;t&lt;/sub&gt;</td>
</tr>
<tr>
<td><strong>Time after inoculation</strong></td>
<td>(per cent)</td>
<td>(per cent)</td>
<td>(per cent)</td>
<td>(per cent)</td>
</tr>
<tr>
<td>Prophase</td>
<td>92 ± 3.3</td>
<td>27 ± 7.7</td>
<td>29 ± 2.6</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>Metaphase</td>
<td>46 ± 6.4</td>
<td>52 ± 4.2</td>
<td>51 ± 4.2</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>Anaphase</td>
<td>5 ± 1.4</td>
<td>1.4 ± 1.4</td>
<td>5 ± 2.2</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>Telophase</td>
<td>17 ± 2.2</td>
<td>17 ± 18</td>
<td>15 ± 3.9</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>Mitotic rate</td>
<td>6.5 ± 0.7</td>
<td>5.5 ± 0.7</td>
<td>5.6 ± 0.6</td>
<td>&gt;0.01</td>
</tr>
</tbody>
</table>

* There was no significant difference (P > 0.01) when the uninoculated cells of zero hours were compared with the NDV- and NAF-inoculated cultures at 2 hours.

† P = probability based on student's "t" test.

‡ Standard deviation.
RESULTS

Cell walls and nuclei were intact at the time of viral inoculation. The 2- and 6-hour samples were similar to the zero hour except that in a few cells some disintegration of the cytoplasmic wall was evident in both the NDV- and NAF-inoculated cultures. Isolated instances of karyorrhexis and pyknosis were noted. These minor changes were considered to be nonspecific and attributed to a period of adjustment by the cells to the addition of a foreign substance to the medium. At 9 hours there was extensive degeneration of cells in the NDV-inoculated tubes, whereas those inoculated with NAF were similar to the zero hour cultures. The fragmentation of the cells seen in the Feulgen stains coincide with those in hematoxylin and eosin preparations described above. Pyknosis and conglomeration of nuclear Feulgen-positive material were evident at 9 hours in the NDV-inoculated cells, but not in the controls.

An indication of cellular activity after virus infection was obtained from the mitotic rate (Table 1). The mitotic rate for the NDV-inoculated cells was 6.5 per cent at zero hour, 5.5 per cent at 2 hours, 4 per cent in 6 hours, and 3 per cent in 9 hours. With NAF the rate was 5.6 per cent, 5.6 per cent, and 5 per cent at 2, 6, and 9 hours, respectively. Thus, the effect of NDV on nuclear division was first noticed at 6 hours after inoculation.

There was no statistical difference in the percentage of cells in the prophase, metaphase, anaphase, and telophase of the virus-treated cells when compared with the NAF-inoculated cells after 2 hours. At 6 hours a mitotic shift was noted. The percentage of dividing cells in the metaphase and anaphase was unaltered, but a significant departure from the controls was seen in the prophase and telophase of the NDV-inoculated cultures. At this time, 19 per cent of the dividing cells with NDV were in the prophase, as compared with 34 per cent of the NAF-inoculated cells. An increase to 28 per cent was noted in the telophase of virus-treated cells, in contrast to 17 per cent in the telophase for NAF-treated cells (Table 1). Similar changes were seen in the prophase and the telophase at 9 hours.

Succinic dehydrogenase activity was 4+ prior to and immediately after inoculation with NDV or NAF. Nearly every cell contained numerous particles of precipitated formazan. The particles were strongly purple. After 2 hours, no histochemical changes were seen in either the NDV- or NAF-inoculated cultures. The amount of precipitate in the NDV-inoculated cells was markedly reduced by 6 hours. A few cells did not contain purple granules but stained red and were vacuolated. By 9 hours fewer cells were present in the NDV-inoculated preparations. Approximately half this number of cells contained occasional small granules, while the remainder were red to yellow without precipitate. In contrast, tubes inoculated with NAF showed a strong 4+ activity throughout the 9 hours.

Quantitatively, the succinic dehydrogenase activity at zero hours was 27 ± 3 µg/10 X 10^6 cells. By 2 hours, the activity in the NDV-inoculated tubes was 21 ± 5 µg., and in the NAF-inoculated tubes it was 28 ± 4 µg. By 6 hours, it dropped to 12 ± 3 µg. in the infected tubes, but was 26 ± 3 µg. in the NAF-inoculated tubes. By 9 hours, the activity in the NDV-infected tubes dropped to 7 ± 2 µg., whereas the activity in the NAF tubes was 27 ± 3 µg/10 X 10^6 cells (Chart 1).

DISCUSSION

When HeLa cells were infected with NDV, a reduction of succinic dehydrogenase activity preceded cytologic changes. It is not known whether the decrease in succinic dehydrogenase activity is caused directly by NDV or is a secondary manifestation of other metabolic disturbances in the cell. In the latter case, morphologic changes usually precede a decrease in succinic dehydrogenase activity. This is seen in aging tissue cultures and when there are changes in the optimum hy-
hydrogen ion concentration of the medium. This is also seen in vivo when certain bacterial toxins affect tissues. For example, Mishukova and Lebedeva (4) noted no diminution of respiratory enzyme efficiency in the myocardium in spite of structural changes produced by diphtheria toxin. Since HeLa cells infected with NDV do not follow this pattern it would seem that diminution in the succinic dehydrogenase activity is related more directly to NDV infection.

Although the reduction of succinic dehydrogenase activity and its accompanying color changes cannot be interpreted definitely as a reaction specific to NDV, such changes may find value as an indication of cell disturbance in that they can be observed sooner than cytologic changes. Quantitatively, the changes in the succinic dehydrogenase level paralleled those seen histochemically in that significant changes were noted at 6 hours, whereas the NAF-treated cells retained their normal activity level.

In the Feulgen preparations, the cells inoculated with NDV showed a drop in percentage of dividing cells in the prophase and an increase in the telophase at 6 and 9 hours. This suggests that NDV inhibits the initiation of cell division but permits cells already dividing to proceed to their final stage. Orsi et al. (5) found a similar reaction with West Nile Virus and Ehrlich ascites tumor in vivo experiments. Our cytochemical studies showed that a decrease in succinic dehydrogenase activity preceded nuclear changes observed in the Feulgen-stained preparations. No evaluation of viral effect could be made at 6 hours on cells in the interphase unless the succinic dehydrogenase activity was determined.

REFERENCES
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