ELECTROPHORETIC STUDIES OF SONIC EXTRACTS OF 

PROTEUS VULGARIS

I. EFFECT OF GROWTH ENVIRONMENT ON ELECTROPHORETIC PATTERNS

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The analytical electrophoretic pattern of the buffer-soluble proteins extracted from bacterial cells is both characteristic and descriptive of the organism (Hess et al., 1957; Wagman et al., 1958; Rodenberg, 1958). Further, a relatively large fraction of the material of the cell can be studied by the electrophoretic method. Hess et al. (1957) using successive extractive procedures on three strains of *Streptococcus pyogenes* obtained electrophoretic data on as much as 84 per cent of the dry weight of the cell and Rodenberg (1958) reported electrophoretic data on *Proteus vulgaris* strain OX-19 which were descriptive of 70 per cent of the total cellular nitrogen.

It was the object of this study to determine whether altered environmental situations would produce sufficiently large qualitative or quantitative differences in the soluble proteins of the cell so that they might be detected electrophoretically. In part, some of these studies were reported previously (Rodenberg, 1957) and Wagman et al. (1958) found some corroborative results.

MATERIALS AND METHODS

The organism used for these experiments was *P. vulgaris* strain OX-19. The cells used as standard material were grown at 37 ± 1°C in a medium containing glucose, 0.1 per cent; yeast extract, 1 per cent; tryptone, 1 per cent; and K2HPO4, 0.2 per cent (hereafter called “S” broth). The culture was carried on agar slants of the same medium and was readied for electrophoretic study as follows: a small amount of agar slant culture was used as the inoculum for a tube of broth; after 6 to 12 hr incubation, 1 loopful of broth was used to inoculate a second broth tube; after 12 hr incubation, 1 ml was used as the inoculum for 25 ml of broth contained in a 250-ml Erlenmeyer flask. This flask and all subsequent cultures were shaken for aeration on a New Brunswick rotary shaker. After 12 hr, a second flask containing 25 ml of medium was inoculated with 1 ml of medium from the first, and after 12 hr incubation, 1 ml amounts of medium from the second flask were used to inoculate one or more 2-L Erlenmeyer flasks containing 1 L of medium. The contents of the large flasks were harvested following 12 hr incubation.

When the effect of different media was studied, brain heart infusion (Difco) and nutrient broth (Difco) were inoculated from the “S” agar slant and thereafter all transfers described above were made in the test medium.

In the experiment at 27 ± 2°C, all cultures beyond the slant culture were incubated at that temperature.

For the experiment to determine the effect of reduced aeration, transfer from the second broth tube was made to a 250-ml Erlenmeyer flask containing sufficient media to bring the surface of the broth within the neck portion of the flask, thereby reducing the available surface area. A 1-ml transfer was made after 12 hr incubation to a similar flask, and after 12 hr incubation, 1 ml of the second flask was used as the inoculum for a 6-L round bottom flask containing 5.5 L of medium. None of these cultures was agitated during incubation.

The harvest, wash, and sonic extraction of the cells were as previously described (Rodenberg, 1958). The extract was clarified by centrifugation at 18,400 × G for 15 min and the supernatant was centrifuged at 18,400 × G for 110 min.

The buffer used for wash, extraction, dialysis, and electrophoresis was a 0.1 ionic strength phosphate buffer prepared at pH 7 (Miller and Golder, 1950). Dialysis was carried out in the cold for 24 to 48 hr during which time three complete changes of buffer were made. The total volume

1 Aided by a grant (G-722) from the National Science Foundation.
of buffer used for dialysis was 4 to 6 L. Following
dialysis and immediately prior to electrophoretic
examination, the nondialyzable material was
centrifuged at 4600 × G for 15 min.

The electrophoretic mobilities were calculated
in the conventional manner. The areas under
the peaks were described according to the sug-
gestions of Svedberg and Pederson (1940), and
were determined by planimetric measurements
of approximately 3.5 × enlarged tracings of
photographs (Moore and White, 1948).

RESULTS AND DISCUSSION

Standard patterns. The conditions for the
growth and extraction of cells to be used as a
standard are described above. The electrophoretic
patterns shown in figure 1 are typical of such
extracts when the nondialyzable nitrogen con-
centration is approximately 5 mg per ml. When
the protein concentration is increased, three
new ascending components and another descend-
ing component become detectable. All of the
components observed at increased protein con-
centration are faster-moving than those shown
in figure 1 and are described in table 2.

The analyses at high protein concentration
have also shown that some of the normal com-
ponents (A, F, and e; figure 1) are composed of
at least two constituent peaks with similar
mobilities.

Evaluation of the effects of altered environ-
ments requires that the control situation be
reproducible. Although the control patterns show
an over-all similarity, the individual components
which make up the patterns may vary with
respect to mobility and area. These variations
may be the result of differences in protein con-

**Table 1**

**Analytical data on electrophoretic components in sonic extracts of Proteus vulgaris strain OX-19**

<table>
<thead>
<tr>
<th>Ascending Limb Components</th>
<th>Descending Limb Components</th>
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<tbody>
<tr>
<td><strong>A</strong></td>
<td><strong>a</strong></td>
</tr>
<tr>
<td><strong>B</strong></td>
<td><strong>b</strong></td>
</tr>
<tr>
<td><strong>C</strong></td>
<td><strong>c</strong></td>
</tr>
<tr>
<td><strong>D</strong></td>
<td><strong>d</strong></td>
</tr>
<tr>
<td><strong>E</strong></td>
<td><strong>e</strong></td>
</tr>
<tr>
<td><strong>F</strong></td>
<td></td>
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<tr>
<td><strong>G</strong></td>
<td></td>
</tr>
<tr>
<td><strong>H</strong></td>
<td></td>
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</tbody>
</table>

| Electrophoretic mobility ($\times 10^{-5}$ cm$^2$ Volts$^{-1}$ sec$^{-1}$) | 0.33 ± 0.09 | 0.45 ± 0.11 | 0.38 ± 0.05 | 0.52 ± 0.33 | 0.30 ± 0.20 | 0.33 ± 0.31 | 0.40 ± 0.34 |
| Component size (% of total pattern area) | 7.34 ± 2.11 | 9.47 ± 2.83 | 7.37 ± 3.05 | 31.25 ± 3.05 | 10.8 ± 0.68 | 21.7 ± 4.38 | 5.83 ± 1.70 |

* These data were obtained from six independently grown cell crops. All cells were grown for 12 hr
  at 37 C in "S" medium with aeration. All values given are mean values ± the 95 per cent confidence
  interval.

† The components b, c, and d were usually large and overlapping to the extent that individual com-
  ponent areas could not be measured with accuracy.
Comparison of areas of ascending limb electrophoretic components in extracts of cells of Proteus vulgaris strain OX-19 grown with and without aeration*

<table>
<thead>
<tr>
<th>Component</th>
<th>Per Cent of Total Area</th>
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<tbody>
<tr>
<td></td>
<td>Aerated culture</td>
</tr>
<tr>
<td>A</td>
<td>6.4</td>
</tr>
<tr>
<td>B</td>
<td>8.8</td>
</tr>
<tr>
<td>C</td>
<td>6.1</td>
</tr>
<tr>
<td>D + E</td>
<td>44.1</td>
</tr>
<tr>
<td>F</td>
<td>20.9</td>
</tr>
<tr>
<td>G + H</td>
<td>9.2</td>
</tr>
<tr>
<td>I†</td>
<td>2.4</td>
</tr>
<tr>
<td>J†</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* While the areas of the components of the two runs are directly comparable, data for them were obtained from photographs made at an earlier time than usual so that faster moving components (I, J, and K) could be recorded. Therefore, areas of slower moving components in these data could not be determined with the accuracy of those shown in Table 1 and some of the normal components determined at a later time (D, E, and G + H) were not separated.

† The mean values for the mobilities of these components from seven runs in which they were detected were as follows: I, -11.7; J, -13.2; and K, -15, all $\times 10^{-5}$ cm² V⁻¹ sec⁻¹. When these components were detectable, an additional component with mobility of $-18.5 \times 10^{-5}$ cm² V⁻¹ sec⁻¹ was also found in descending limb patterns.

Because the descending limb patterns were similar in both extracts and component A was undetectable in one of the ascending patterns, it seems likely that electrophoretic component a in the descending limb patterns is represented by components A and B in ascending patterns. Component a withdrawn from the descending limb of the electrophoretic cell and examined spectrophotometrically gives the absorption spectrum characteristic of a nucleoprotein containing about 3 per cent nucleic acid.

The biological activities of the molecules associated with a particular electrophoretic component are, at present, unknown and it is

![Electrophoretic Mobility](https://example.com/electrophoretic-mobility.png)
therefore impossible to relate these data to chemical events within the cells. However, the data show that the organisms' response to the altered environment is a complex one, involving alteration in the quantities of at least three different molecules. Inasmuch as each of the three electrophoretically distinct components may be composed of molecules with different capabilities, this pattern of response is potentially quite complex.

Effect of growth medium on electrophoretic patterns of cell extracts. Most studies of adaptive enzyme formation have been based upon the response of the organism to a new chemically known substrate (Stanier, 1951). It was considered likely, therefore, that the use of different complex media would be productive of numerous differences in the electrophoretic patterns. Although the patterns obtained in this study (figure 2) show some large differences, these are quantitative ones. In descending limb patterns, component e with mobility between -7 and $-8 \times 10^{-3}$ cm$^2$ V$^{-1}$ sec$^{-1}$ varied most conspicuously. In the cell extract from cells grown in brain heart infusion broth, the area of component e was a greater part of the total area, and in the nutrient broth grown cell extract, a smaller part of the total than in the standard cell extract.

The ascending limb patterns (figure 2) show a great many differences. Although most of these differences are quantitative, some differences in electrophoretic mobilities are apparent. Until these ascending limb components have received further study, and because the mobilities of the descending components are very similar, differences in their mobilities will be regarded as the result of protein-protein interaction.

The data show that the relative amounts of each of the components D, E, F, G, and H present in the extracts is subject to considerable variation. The fact that these five components amount to approximately 75 per cent of the total area in each of the three extracts studied is further evidence of the complexity of adaptive change.

In a separate series of experiments in which the sugar concentration of "S" broth was either 0, 0.1, 0.5, or 1 per cent, the cell-free extracts gave very similar electrophoretic patterns. However, an increase in the relative concentration of components G + H and e were noted at the higher sugar concentration. The pH of the media prior to growth was 7 and following growth it was from 7.1 at zero sugar concentration to 5.6 at 1 per cent sugar concentration. If the media had been strongly buffered at pH 7 or if the organisms had been grown at a single sugar concentration in media strongly buffered at various levels of acidity or alkalinity, the effect of sugar concentration and the effect of pH might have been more evident.

Effect of incubation temperature on the electrophoretic patterns of cell extracts. A 10 degree difference in temperature of incubation provided cells whose electrophoretic patterns were strikingly different. The patterns of cells grown at 27 C, shown in figure 3, were made from an extract approximately 1.5 times as concentrated as the extract for cells grown at 37 C so that the
components present in small amount in the former might be detectable. The leading slope of the large descending-limb-component (27 C) shows irregularity in width and light intensity indicative of heterogeneity. Although in some analyses the individual components which make up the large descending component may become more distinct, they never become fully separated. The ascending-limb pattern of cells grown at 27 C likewise show the greatest part of the total area within a single component. The large component as well as the other smaller components have mobilities only slightly dissimilar to those of components in the extracts from cells grown at 37 C. These slight differences in mobilities are, as previously described, probably the result of protein-protein interaction which would very likely occur in such quantitatively different complex protein solutions. The influence of incubation temperature on growth rates offers some additional problems to reliable interpretation of the data and as a result, this problem is being further pursued.

This unique opportunity to characterize a large fraction of a complete organism has shown that the adaptive response may be extremely complex. Although P. vulgaris may show greater sensitivity to the environment than other unicellular organisms, the data suggest that in the numerous studies of single proteins extracted from microorganisms, precise control of environmental conditions may be necessary in order to obtain yields of a consistent value or in conformity with the reports of others.

SUMMARY

The effect of aeration, different media, and altered temperature as incubation conditions for cultures of Proteus vulgaris strain OX-19 on the buffer-soluble, nondialyzable of cell-free extracts was determined by electrophoretic analysis. The data show complex quantitative differences in response to changes in the environment.

REFERENCES


