PHYSIOLOGICAL YOUTH AS AN IMPORTANT FACTOR IN ADAPTIVE ENZYME FORMATION

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It has been known for many years that bacteria, cultivated upon a specific substrate, may not ferment other materials unless cell multiplication occurs. Karström (1930) observed that cells grown in the presence of one sugar, then suspended in solutions of other sugars under conditions preventing multiplication, could not ferment the new sugars for long periods of time, if at all. Stephenson and Strickland (1933) showed that there was no natural selection in the case of formate adaptation by Escherichia coli and that the formation of the enzyme can occur while no cell division is taking place. Stephenson and Yudkin (1936) observed that top yeasts can adaptively ferment galactose without cell multiplication but no adaptation could be obtained with cultures incapable of growth. They state, however, that a study of their total and viable counts suggests that it is not the viable cells alone which are capable of adaptation. Stephenson and Gale (1937) found that the galactozymase of Escherichia coli was formed only when growth occurred in the presence of the specific substrate. This adaptation was proportional to the number of cells which had divided in the presence of the galactose.

The following experiments are the result of a search for the factors controlling the mechanism of adaptation. Old cells adapt themselves slowly if at all (Karström 1930) and young, growing cultures produce the new enzyme easily. Therefore, experiments were designed to test cells of various ages and to determine

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the stage of growth during which new enzymes are most easily manufactured.

METHODS

All of the experiments were performed with a culture of *Streptococcus lactis* (No. 125) which ferments all of the sugars characteristic of that organism when grown in nutrient media containing the various carbohydrates. The cells were grown in a medium consisting of 0.5 per cent peptone, 0.5 per cent tryptone, 1.5 per cent phosphate buffer at pH 7.0 and 0.5 per cent of the chosen carbohydrate (Rahn, Hegarty and Deuel, 1938). When the culture was at the desired age, the cells were centrifuged and resuspended in a solution containing 0.5 per cent peptone, 2.0 per cent phosphate buffer at pH 7.0 and 2.0 per cent of the carbohydrate to be tested. A heavy suspension of cells was used. With full-grown cultures, the cells from 250 cc. of medium were resuspended in 50 cc. of a 2 per cent buffer solution, and with growing cultures the ratio was changed accordingly. Frequent plate counts and Petroff-Hausser counts (Knaysi, 1935) showed no indications of growth in any of these suspensions. The rate of fermentation was determined by titration of 5.0 cc. samples with N/10 NaOH. If the cells were difficult to obtain, 1.0 cc. samples were titrated with N/18 NaOH, using a micro-burette.

Preliminary experiments showed that the enzymes attacking glucose, fructose and mannose were constitutive, i.e., produced under all circumstances, while the enzymes attacking all of the other sugars were adaptive, according to the terminology of Karström (1930).

A 12-hour old culture in glucose medium was centrifuged, and the cells resuspended in the buffer solution. One-third of this contained 2 per cent glucose, one-third 2 per cent sucrose, and one-third 2 per cent sucrose + 0.2 per cent glucose. Glucose was fermented from the start. No measurable acidity was produced from sucrose within 5 hours. The cells in sucrose + 0.2 per cent glucose produced 0.18 per cent lactic acid within the first hour, utilizing the glucose, but no further fermentation occurred until after 5 hours. The initiation of fermentation of one sugar had no effect upon acid production from other sugars.
A series of experiments was designed to determine the effect of the age of the culture upon adaptation. The cells were grown in glucose-tryptone broth, centrifuged out after various periods of incubation, and placed into buffered solutions of glucose and sucrose. Table 1 shows the gradual development of lactic acid from glucose and sucrose by cells centrifuged at various ages of the culture. All of the cells, regardless of age of the culture, fermented glucose from the start at rapid rates. The cells centrifuged when the culture was 2 hours old started fermentation in the sucrose medium earlier than cells obtained when the culture was older and adaptation required more time as the age of the culture increased.

A more extensive study was made of adaptation to galactose. 13.5 liters of glucose-tryptone broth were inoculated with 1.5 l. of a 36-hour culture of *Streptococcus lactis*. At the start and after every hour, samples of this culture were centrifuged and the cells resuspended in buffer plus glucose or galactose. The curves of these fermentations are shown in figure 1. Glucose was fermented from the start by cells of all ages. The number of cells per cubic centimeter of suspension could not be kept the same. The suspensions from the 3- and 4-hour old cultures contained about twice as many organisms per cubic centimeter as the 1- and 2-hour suspensions. The fermenting capacity per cell

<table>
<thead>
<tr>
<th>AGE OF CELLS</th>
<th>SUGAR</th>
<th>TIME IN HOURS</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hours</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>2</td>
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</tr>
<tr>
<td>2</td>
<td>Sucrose</td>
<td>.00</td>
</tr>
<tr>
<td>12</td>
<td>Glucose</td>
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<tr>
<td>12</td>
<td>Sucrose</td>
<td>.00</td>
</tr>
<tr>
<td>24</td>
<td>Glucose</td>
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<tr>
<td>24</td>
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<tr>
<td>48</td>
<td>Glucose</td>
<td>.04</td>
</tr>
<tr>
<td>48</td>
<td>Sucrose</td>
<td>.00</td>
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</tbody>
</table>

Table 1

Per cent lactic acid formed from glucose and sucrose by glucose-grown cells of various ages
per hour was $14.5 \times 10^{-10}$ mgm. glucose for the 1 hour cells and had dropped to $9.3 \times 10^{-10}$ mgm. when the culture was 4 hours old. These facts account for the differences in the rate of glucose fermentation.

Quite different are the fermentation curves for galactose. The cells collected when the culture was 1 hour old started fermentation soon after resuspension while the 4-hour cells required 9 hours for adaptation. Cells collected after longer periods of incubation (not shown on the graph) did not produce any acid for 13 hours. The growth curve of the original culture supplying the cells for this experiment (fig. 2) showed that after 1 hour, the cells were just coming out of the lag phase. It is evident that as the culture passes from the stage of physiological youth into the logarithmic phase, adaptation becomes slower. Plate counts and Petroff-Hauser counts were made of all suspensions at frequent intervals and no increases in the numbers of cells could be detected.

![Graph showing fermentation curves for glucose and galactose with adaptation to galactose by glucose-grown cells.]
Two other sets of experiments were performed in the same way, but with different sugars. In one, the cells were grown in glucose, and resuspended in buffer plus glucose, galactose, lactose, or sucrose. In the other experiment, the cells were grown in maltose, and tested in glucose, maltose, and sucrose. In general, the results were the same as those obtained before, as may be seen from table 2. Glucose-grown cells fermented glucose from the start. They showed rapid adaptation to galactose when obtained from a physiologically young culture, but required long initiation times when the culture was older. In lactose, the delay was 8 hours, with cells obtained from a physiologically young culture, and 20 hours, with cells from older cultures. The glucose-grown cells in sucrose showed no delay when the culture was just coming out of the lag phase. A growth curve showed that this culture had a 2-hour lag phase and therefore the cells centrifuged after 2 and 3 hours were physiologically young. The cells from older cultures exhibited a short delay.
In another experiment there was an adaptation time of 5.5 hours before glucose-grown cells produced acid from maltose.

The maltose-grown cells from cultures of all ages showed no delay in either glucose or maltose. This is not unexpected as the organisms had produced the adaptive maltase during growth in the original maltose culture, and the glucose enzyme is constitutive. Maltose-grown cells in sucrose required a short adaptation period when old, but showed no delay during physiological youth. It is interesting that maltose-grown cells from older cultures initiated fermentation in sucrose more rapidly than similar cells grown in glucose.

**TABLE 2**

*Hours delay before adaptation occurs by cells of various ages grown in glucose and maltose*

<table>
<thead>
<tr>
<th>AGE OF CELLS</th>
<th>GROWN IN GLUCOSE</th>
<th>GROWN IN MALTOSE</th>
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<tbody>
<tr>
<td></td>
<td>Glucose</td>
<td>Galactose</td>
</tr>
<tr>
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<td>18</td>
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</tr>
<tr>
<td>10</td>
<td>0</td>
<td>13</td>
</tr>
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</table>

In every case where it was necessary for the organisms to adapt themselves to a new carbohydrate, the cells obtained from physiologically young cultures accomplished this much more rapidly than cells from a later stage of growth.

**DISCUSSION**

These experiments have shown that at the end of the lag phase, before the maximal growth rate or the logarithmic phase is reached, the cells are most adaptable to new types of food. During the logarithmic phase, while the rate of growth remains constant, adaptability decreases rapidly.
At this stage of early development, the cells are quite different from cells of slightly older cultures as was first shown by Sherman and Albus (1923) who termed them physiologically young. Later Bayne-Jones and Rhee (1928), Walker, Winslow, Huntington and Mooney (1934), Mooney and Winslow (1935) Huntington and Winslow (1937) and others have shown that the rate of metabolism per cell (production of heat, CO₂, NH₃, oxygen consumption, etc.) is maximal just before the logarithmic growth rate is reached.

Hershey and Bronfenbrenner (1938) state that "correlations of viable counts, centrifugeable nitrogen and turbidity, with oxygen consumption, indicate that the increased metabolism during the early portion of the growth period is quantitatively referable to increased average size of cell," which is not in complete agreement with the work of Huntington and Winslow (1937). While all these properties represent only quantitative changes, the case of adaptive enzymes is also a qualitative one, and therefore more significant, and in a class by itself.

It seems quite noteworthy that at the stage of rejuvenation of the old cells (Sherman and Albus, 1924), when the cell shows the most intensive metabolic activity, with distinct morphological changes (Henrici, 1928), the cell is also most ready for physiological changes and adaptations.

CONCLUSIONS

Cells from a mature culture of Streptococcus lactis in glucose-tryptone broth can not attack galactose, lactose, sucrose or maltose at once, unless they have been allowed to multiply in the presence of that sugar.

If cells are obtained from a glucose culture while still in the stage of physiological youth, they may attack these sugars either at once or with a short delay, even under conditions preventing proliferation.

The most rapid adaptation to new sugars is always observed with cells from cultures just coming out of the lag phase, during the period of physiological youth. During the logarithmic phase, adaptability decreases rapidly and continuously.
The ease of adaptation and the rate of loss of adaptability during the ageing of a culture varies greatly with each sugar.

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REFERENCES


HENRICI, A. T. 1928 Morphologic Variation and the Rate of Growth of Bacteria. Baltimore, Maryland.


