practically lacking[3] while the frequencies of types $Rh_1$ and $Rh_1Rh_2$ are high. In fact, if the single Rh-negative individual and the single individual of type Rh are discounted as possibly due to racial admixture, then the distribution in our series of Chinese reduces itself to a two-factor scheme, quite similar, for example, to the distribution of the M-N types among American Indians.

The application of simplified gene frequency formulæ calculated from the frequencies of classes U, V, UV, and W supplies further support for the hypothesis of multiple allelic genes. However, the family and statistical data are still inadequate to test the theory satisfactorily with regard to the rarer types, Rh′, Rh″, and Rh′Rh″.

3 Levine and Wong found only 0.7% Rh-negative individuals among 150 Chinese (*Am. J. Obst. and Gyn.*, 1943, **45**, 832).

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## Comparison of Dextran Synthesis by Leuconostoc Enzyme with Starch Synthesis by Potato Phosphorylase.*

EDWARD J. HEHRE.

*From the Department of Bacteriology and Immunology, Cornell University Medical College, New York City.*

Earlier papers[1,2] described the synthesis from sucrose of a serologically reactive dextran by cell-free enzymes obtained from a species of streptococcus-like bacteria, *Leuconostoc mesenteroides*. Some evidence was obtained which indicated that the course of the synthesis follows the equation:

$$n \text{ sucrose} \rightarrow \underset{\textit{dextran}}{(\text{glucose anhydride})_n} + n \text{ fructose} \quad (1)$$

However, that mode of dextran formation was not proved and, indeed, analogy with the enzymatic syntheses of glycogen and starch would suggest the possibility that glucose-l-phosphate might participate as an intermediate substance in the conversion of sucrose to dextran. That possibility was made more significant by a recent report[3] that suspensions of *Leuconostoc mesenteroides* can phosphorolyse sucrose with the production of glucose-l-phosphate. If glucose-l-phosphate does serve as an intermediate substance in the formation of dextran, the synthesis of that polysaccharide by the bacterial enzyme would fall in the same class of reactions as the syntheses of glycogen and starch by the phosphorylases of other biological origin.

In order to get experimental data on this question, a comparison was made between leuconostoc enzyme and potato phosphorylase in respect to their action upon sucrose and upon glucose-l-phosphate. The leuconostoc enzyme was obtained from sucrose broth cultures of the strain used in previous studies; it was prepared by an improved method which will be described in another paper, and was of much greater potency than any of the preparations utilized in the earlier studies. The potato phosphorylase was prepared according to the first steps in the procedure described by Green and Stumpf.[4]

A set of 3 test mixtures was prepared with each enzyme. The first consisted of equal parts of enzyme solution and of 0.2 M sucrose in 0.1 M acetate buffer, pH 5.6; the second

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1 Hehre, E. J., *Science*, 1941, **93**, 237.

2 Hehre, E. J., and Sugg, J. Y., *J. Exp. Med.*, 1942, **75**, 339.

3 Kagan, B. O., Latker, S. N., and Zfasman, E. M., *Biokhimiya*, 1942, **7**, 93.

4 Green, D. E., and Stumpf, P. K., *J. Biol. Chem.*, 1942, **142**, 355.

TABLE I.
Comparison of Leuconostoc Extract and Potato Phosphorylase in Respect to Action upon Sucrose and upon Glucose-1-phosphate.

| Source of enzyme | Substrate | Properties of test mixtures incubated 4 hours at 25°C: Appearance | Precipitate with 1.25 vol. alcohol | Serological reactivity | Color with iodine | Reducing sugar mg/ml | Inorganic phosphate mg/ml |
|---|---|---|---|---|---|---|---|
| Leuconostoc | sucrose | opalescent | ++++ | 20,000 | yellow | 6.2 | <0.001* |
| | glucose-1-phosphate | clear | 0 | 40 | " | 0.1 | 0.03 |
| | buffer control | " | 0 | 40 | " | 0.1 | <0.001* |
| Potato | sucrose | " | ± | 0 | " | 0.3 | 0.07 |
| | glucose-1-phosphate | cloudy | ++++ | 0 | blue-black | 0.3 | 6.4 |
| | buffer control | clear | ± | 0 | yellow | 0.3 | 0.07 |

* No phosphate was detected by the Fiske-Subbarow[6] procedure or by the Deniges[7] method, either before or after the mixture had been digested with sulfuric and nitric acids.

comprised equal parts of enzyme and of 0.2 M glucose-1-phosphate in 0.1 M acetate buffer, pH 5.6; and the third or control mixture consisted of equal parts of enzyme and of the buffer solution. All the test mixtures were incubated for 4 hours at 25°C and were then examined for opalescence or cloudiness and for amount of precipitate formed upon addition of 1.25 volumes of alcohol; each mixture was also tested for reactivity with type 2 antipneumococcus serum, color with iodine, reducing sugars[5] and inorganic phosphate.[6] The data are summarized in Table I.

*Results.* It is evident (Table I) that the leuconostoc enzyme, when incubated with sucrose, caused the formation of abundant amounts of dextran together with the liberation of free reducing sugar (fructose); and that the potato phosphorylase, when incubated with glucose-1-phosphate, caused the formation of abundant amounts of starch together with the liberation of inorganic phosphate. In contrast, the leuconostoc enzyme had no demonstrable action upon glucose-1-phosphate and the potato phosphorylase had none upon sucrose. The lack of reactivity with glucose-1-phosphate on the part of the leuconostoc enzyme which was so reactive with sucrose is good evidence that glucose-1-phosphate is not an intermediate substrate in the enzymatic synthesis of dextran from sucrose. The absence of any appreciable amount of phosphate in the system which formed abundant dextran suggests that the enzymatic synthesis of that polysaccharide from sucrose does not require the mediation of any phosphorylated sugar substrate, but occurs by a direct condensation of the sucrose in the way indicated by Equation 1.

Though the enzymes operate upon different substrates and yield chemically different products, the synthesis of dextran by leuconostoc enzyme does have a fundamental point of similarity to the syntheses of starch and glycogen by the various phosphorylases. That is, in each instance the substrate (sucrose or glucose-1-phosphate) contains the basic unit of the final polymer product in the form of a glycoside radical that is exceedingly easily split off by acids. The same relationship obtains in the enzymatic synthesis of levan, because here also the basic unit (fructose anhydride) is contained as a glycoside radical in the substrates (sucrose and raffinose) from which the polymer product is derived.[8-10]

*Summary.* Comparison with starch synthesis by potato phosphorylase indicates that dextran synthesis from sucrose by leuconostoc enzyme does not require the mediation of any phosphorylated sugar.

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