MEASUREMENT OF AMYLASE ACTIVITY*

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The Nelson photoelectric method for the determination of glucose in biological fluids (1) has proved to be the method of choice in this laboratory and in many others, owing to its reproducibility, stability of color, and excellent agreement with the Beer-Lambert law. Nelson, however, states that the method is unsuitable for the determination of diastatic activity. Since it is convenient and economical for a laboratory running large numbers of glucose and amylase determinations to use the same reagents for reduction and color development, it seemed advisable to attempt to modify the amylase procedure to this end.

The difficulty in using the substrate of the Somogyi amylase method (2) with the Nelson reagents arises from the clouding of the final colored solution by the presence of unhydrolyzed starch. It was reasoned that, if a smaller amount of starch were present, the final solution might be clear. This was realized by diluting the Somogyi substrate after incubation and running the diluted solution through the Nelson procedure. Thus, while the original relative concentrations of substrate and serum which Somogyi found to be optimum were preserved, the final colored solutions were clear in all cases, including the starch blank, and were suitable for photoelectric reading. This final concentration of starch may be attained in a slightly different manner by using only 0.3 per cent starch and 0.2 ml. of serum and omitting the 1:5 dilution after incubation. It is interesting to note in this regard that "micromethods" in current use (2) require the employment of 1 ml. of serum.

Macromethod

Reagents—These are the same reagents which are used in the Somogyi saccharogenic method (2): 1.5 per cent starch paste;1 per cent NaCl con-

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1 The starch paste was prepared in the usual manner by triturating 1.5 gm. of Baker's soluble starch powder "according to Lintner" in a small amount of cold distilled water, pouring the suspension into 100 ml. of boiling distilled water, boiling for a minute, and stoppering the flask before cooling at room temperature. The 0.3 per cent starch paste for the micromethod was prepared in the same manner.

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The procedure involves measuring enzyme activity using a specific set of reagents and conditions. The solution contains 3.0 ml. of 0.1 N HCl; 5 per cent CuSO₄·5H₂O; 6 per cent sodium tungstate.

**Procedure**—5.0 ml. of starch, 2.0 ml. of acidified saline, and 1 ml. of serum are incubated at 40° for 30 minutes in Tube A. A control, Tube C, is incubated containing the same solutions but substituting 1 ml. of water for the serum. A third tube, Tube B, is set up containing 5.0 ml. of water, 1 ml. of serum, and 2.0 ml. of acidified saline. This tube is for the determination of serum glucose and is not incubated. At the end of the 30 minute period 1 ml. of copper sulfate followed by 1 ml. of sodium tungstate is added to each of the three tubes. The tubes are shaken, then centrifuged or filtered. To 1 ml. of each filtrate or supernatant add 4 ml. of water. 1 ml. of each of these diluted filtrates is now subjected to the Nelson procedure. The per cent transmissions are read at 660 mμ, and their values in mg. per cent of glucose are read from a previously standardized table.

**Calculation**—

\[
\text{Glucose equivalents} = A + (B - C)
\]

where \( A, B, \) and \( C \) represent the concentrations in mg. per cent of glucose in the respective tubes and the amylase value is expressed in glucose equivalents.

**Table I**

**Comparison of Methods**

Results expressed as "glucose equivalents," i.e. \( A + (B - C) \).

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>Folin-Wu*</th>
<th>Nelson (micro)</th>
<th>Nelson (macro)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>88</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>142</td>
<td>147</td>
<td></td>
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<td>3</td>
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<td>1162</td>
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<td>9</td>
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<td>58</td>
<td>58</td>
</tr>
<tr>
<td>10†</td>
<td>690</td>
<td>748</td>
<td>701</td>
</tr>
</tbody>
</table>

* Read on a Dubosco colorimeter.
† This was artificially prepared "high" by adding a small amount of saliva to the serum. In this case an additional dilution of the substrate was necessary in all three methods before reduction.

* The Coleman junior spectrophotometer, model 6A, was used for the photometric measurements.
Micromethod

Reagents—0.3 per cent starch paste; NaCl (acidified) (see macromethod) 1 per cent CuSO₄·5H₂O; 1.2 per cent sodium tungstate.

Procedure—The procedure, with the above dilute reagents, is the same as for the macromethod with the following exceptions: (1) use 0.2 ml. of serum and 0.8 ml. of water in Tube A; (2) use 0.2 ml. of serum and 5.8 ml. of water in Tube B; (3) use 1 ml. of the filtrate directly for the reduction without dilution.

Transmissions are read at 660 mµ and the same table of glucose equivalents is used as for the macromethod, since the absolute amounts of reducing substances are the same.

Table II
Reproducibility of Micromethod

Results expressed as "glucose equivalents."

<table>
<thead>
<tr>
<th>Specimen No.</th>
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<th>2nd determination</th>
</tr>
</thead>
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<td>60</td>
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<td>12</td>
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<td>14*</td>
<td>769</td>
<td>763</td>
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<td>15*</td>
<td>860</td>
<td>820</td>
</tr>
<tr>
<td>16*</td>
<td>1650</td>
<td>1630</td>
</tr>
</tbody>
</table>

* Artificial "high" prepared by adding saliva to serum. Dilution of the substrate was necessary before reduction.

Results

Random specimens of human sera submitted for amylase assay were run by both the old method (Folin-Wu reduction) (3) and the method described above. In all cases separate incubations were made. Table I records amylase activity in glucose equivalents. The greatest difference noted between the two methods was 10 per cent.

Reproducibility of Micromethod—Table II records amylase activity on separately incubated samples of identical serum specimens, with use of the micromethod. In seven consecutive determinations, the greatest difference that occurred was less than 5 per cent.

Summary

1. A procedure has been presented for the determination of amylase activity in biological fluids by the Nelson blood glucose method.
2. A procedure for a micromethod for serum amylase assay has been outlined.

BIBLIOGRAPHY

CORRECTION

On page 702, line 3, Table I, and in the equation, line 16, Vol. 135, No. 2, August 1950, read \( A - (B + C) \) for \( A + (B - C) \).
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