A PHOTOMETRIC METHOD FOR THE DETERMINATION OF 
α-AMYLASE IN BLOOD AND URINE, WITH USE OF 
THE STARCH-IODINE COLOR

BY BENJAMIN W. SMITH AND JOSEPH H. ROE

(From the Department of Biochemistry, School of Medicine, George Washington 
University, Washington)

(Received for publication, December 20, 1948)

The use of the starch-iodine color for the estimation of α-amylase appears to be on a sound theoretical basis. Swanson (1) has shown that in the degradation of amylose by α-amylase there is a random attack by the enzyme upon the polysaccharide chain yielding hexose units of varying lengths. Swanson (2) has further observed that chains 4 to 6 glucose units in length give no color with iodine, chains containing 8 to 12 units give a red color, and chains of 30 glucose units, or longer, yield a blue color.

We have developed a starch-iodine method for the determination of α-amylase in serum and urine in which the blue color formed by the reaction of starch with iodine is measured photometrically before and after incubation of soluble starch with material containing the enzyme. The decrease in blue color obtained after the incubation is a measure of the amylase concentration. When appropriate conditions are set up, starch-iodine color values are obtained that are proportional to the amount of enzyme present and to the time of incubation, with use of a fairly wide range of concentration of substrate.

The use of the photoelectric colorimeter removes the limitations inherent in starch-iodine methods in which an end-point is visually selected. In addition to being sound theoretically, the proposed method has certain practical advantages: It requires less work and time than the saccharogenic methods, the procedure is simple and is applicable to the estimation of amylase in blood and urine without change in the basic technique, and it permits the achievement of a high degree of accuracy and precision.

Method

Reagents—

1. Substrate. A 1.2 per cent solution of soluble starch is made up at the time of use. Weigh accurately 1.2 gm. of Merck's soluble starch (Lintner). Suspend this in about 10 cc. of distilled water in a 100 cc. volumetric flask. Make up to slightly under volume with boiling dis-
tilled water. Place the flask in a boiling water bath for 3 minutes and make up to volume. Allow the starch and bath to cool to 90° and hold at that temperature during pipetting.

2. Phosphate buffer, pH 7.2 (Myers, Free, and Rosinski (3)). Dissolve 7.62 gm. of anhydrous potassium dihydrogen phosphate and 20.45 gm. of disodium hydrogen phosphate in distilled water in a liter flask and make up to volume.

3. 0.5 M sodium chloride.

4. N hydrochloric acid.

5. Iodine reagent. Dissolve 30 gm. of potassium iodide and 3 gm. of iodine in distilled water in a liter flask and make up to volume.

Procedure

Pipette 5 cc. of 1.2 per cent starch solution (60 mg.) at approximately 90°, 3 cc. of phosphate buffer, and 1 cc. of 0.5 M sodium chloride into each of two test-tubes, one labeled A for the digest, and one labeled B for the control with undigested starch. Into a third tube (C), the blank, pipette 5 cc. of distilled water, 3 cc. of phosphate buffer, and 1 cc. of 0.5 M sodium chloride. Place all tubes in a water bath at 37° until they have reached the temperature of the water bath. To Tube A (for the digest) add 1 cc. of enzyme solution (serum, plasma, or urine). Keep all tubes in the water bath for exactly 30 minutes. Promptly add 2 cc. of N hydrochloric acid to each tube. This brings the pH below 2, a step that stops amylase action in the digest tube and prevents action of the enzyme next added to the control tube. Add 1 cc. of enzyme solution to Tubes B (control) and C (blank) and mix thoroughly. Pipette 2 cc. of each of these reaction mixtures into appropriately labeled 500 cc. volumetric flasks containing about 400 cc. of distilled water and 5 cc. of N hydrochloric acid. Add 1 cc. of iodine reagent to each flask and make up to volume. The resulting blue solutions are decanted into cuvettes and read in a photoelectric colorimeter at a wave-length of 620 mµ. The colorimeter is set at 100, or the null point, with solution from Tube C. The latter usually reads 99.75 on the Evelyn colorimeter against distilled water; hence distilled water may be used for the colorimeter setting without materially affecting the results. Solution from Tube B gives the iodine color value without amylase action and solution from Tube A gives the value after enzyme action.

Calculations—Let

\[ D = 2 - \log G = \text{optical density} \]

\[ \frac{(D \text{ of control}) - (D \text{ of digest})}{(D \text{ of control})} \times 60 = \text{mg. of starch hydrolyzed} \]

The amylase unit is defined as the amount of enzyme that under the conditions of this procedure, with 60 mg. of starch present, will hydro-
lyze 10 mg. of starch in 30 minutes to a stage at which no color is given with iodine at 620 m\(\mu\). The definition of this amylase unit was established to make the unit conform as closely as possible to the units of methods in general use.

For plasma or serum the calculation is

\[
\frac{(D \text{ of control}) - (D \text{ of digest})}{(D \text{ of control})} \times \frac{60}{10} \times 100 = \text{amylase units per 100 cc.}
\]

**DISCUSSION**

As shown in Fig. 1, the blue color developed by this procedure has been found to conform to the Beer-Lambert law throughout the range of concentration of starch used. The region of maximum absorption of the color was found to be between 600 and 620 m\(\mu\) (Fig. 2, Curve B). We have adopted 620 m\(\mu\) as the most desirable wave-length for readings for several reasons: The absorption due to the iodine reagent alone is negligible at 620 m\(\mu\) (Fig. 2, Curve C); Hanes and Cattle (4) and Swanson (1) have shown that as \(\alpha\)-amylase hydrolyzes amylose, producing shorter chains of hexose units, the range of maximum absorption of the starch-iodine color shifts towards the lower wave-lengths. Our observations are in accord with the work of these authors, as shown by the absorption curve for starch hydrolyzed by amylase (Fig. 2, Curve A). The use of the higher wave-length reduces to a minimum the possibility of interference by the short chain products of hydrolysis.

Starch and iodine concentrations were selected to give a range and
PHOTOMETRIC DETERMINATION OF AMYLASE

Flexibility well suited to clinical use. The technique outlined permits the accurate determination of amylase concentrations in the blood up to 500 units per 100 cc. For greater concentrations of enzyme the serum should be diluted. Marked changes in temperature are known to affect the intensity of the starch-iodine color. At room temperatures, however, the color produced is stable.

The starch used in these experiments was Merck's soluble starch.

**Fig. 2.** The absorption curves of the colors used. Curves A and B, the color obtained by treatment of hydrolyzed and unhydrolyzed soluble starch, respectively, with iodine reagent; Curve C, the iodine reagent only.

**Table I**

Comparison of Ten Starches with Use of Urine As Source of Enzyme

With the exception of the first two samples all starches were made soluble by the method of Small (5). The amylase values are averages of three determinations.

<table>
<thead>
<tr>
<th>Starch</th>
<th>Amylase units per 100 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merck soluble, Lot 42196</td>
<td>183.2</td>
</tr>
<tr>
<td>&quot; &quot; &quot; 42477</td>
<td>198.0</td>
</tr>
<tr>
<td>Potato, laboratory Preparation 1</td>
<td>214.2</td>
</tr>
<tr>
<td>&quot; &quot; &quot; 2</td>
<td>196.4</td>
</tr>
<tr>
<td>Corn, commercial, extracted 48 hrs. with dioxane</td>
<td>162.0</td>
</tr>
<tr>
<td>&quot; not extracted</td>
<td>150.7</td>
</tr>
<tr>
<td>Rice*</td>
<td>109.6</td>
</tr>
<tr>
<td>Sago palm*</td>
<td>110.2</td>
</tr>
<tr>
<td>Sweet potato, laboratory preparation</td>
<td>153.0</td>
</tr>
<tr>
<td>Repetition of 1st starch after 48 hrs.</td>
<td>178.8</td>
</tr>
<tr>
<td>Potato, Merck</td>
<td>165.4</td>
</tr>
</tbody>
</table>

* Kindly furnished to us by Dr. C. S. Hudson of the National Institutes of Health.
This starch was chosen because it is readily available commercially and requires no special treatment before use. It was expected that starches from different sources would not give the same results with this procedure due to variations in composition. A series of determinations was made upon ten starches of different types and also upon different preparations of the same starch. The enzyme solution used was human urine. Table I shows the results of these experiments. The first four starches tested, which were of Irish potato origin, gave comparable results. Corn-starch gave higher values after extraction with dioxane. Irish potato starch gave the highest values of all starches examined. The last sample of starch in Table I was raw starch from a commercial source which was made soluble by the method of Small (5). No explanation is obvious for the low value obtained with this starch as compared with other Irish potato starches.

We have adopted the phosphate buffer used by Myers, Free, and Rosinski (3). We found this buffer satisfactory for serum and urine. The HCl-NaCl buffer used by Somogyi (6) appears adequate for serum in lower ranges of values but at high enzyme concentrations the phosphate buffer gave higher values, as shown in Fig. 3. The lower activity observed with the HCl-NaCl buffer is more marked in urine.

A series of experiments was performed to study further the validity of the procedure. In the first experiment rabbit serum was used as the enzyme source. The amount of enzyme present was held constant and the starch content of the digest varied from 12 to 120 mg. The results
### Table II

**Effect of Variation of Starch Concentration on Enzyme Hydrolysis**

<table>
<thead>
<tr>
<th>Amount of starch in digest</th>
<th>Amylase units per 100 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg.</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>83.2</td>
</tr>
<tr>
<td>24</td>
<td>83.7</td>
</tr>
<tr>
<td>48</td>
<td>88.8</td>
</tr>
<tr>
<td>72</td>
<td>82.6</td>
</tr>
<tr>
<td>120</td>
<td>86.8</td>
</tr>
</tbody>
</table>

### Table III

**Effect of Serial Dilution of Enzyme on Hydrolysis of Starch**

60 mg. of starch were used in each tube.

<table>
<thead>
<tr>
<th>Enzyme solution</th>
<th>Urine amylase units per 100 cc.</th>
<th>Serum amylase units per 100 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>73.2</td>
<td>106.9</td>
</tr>
<tr>
<td>0.4</td>
<td>142.8</td>
<td>198.5</td>
</tr>
<tr>
<td>0.6</td>
<td>218.0</td>
<td>303.6</td>
</tr>
<tr>
<td>0.8</td>
<td>288.6</td>
<td>400.0</td>
</tr>
<tr>
<td>1.0</td>
<td>377.4</td>
<td>478.8</td>
</tr>
</tbody>
</table>

### Table IV

**Amylase Values of Fifteen Human Sera Determined by Authors' and Somogyi Methods, Showing Relation Between Units of Two Procedures**

<table>
<thead>
<tr>
<th>Authors' method units per 100 cc.</th>
<th>Somogyi method units per 100 cc.</th>
<th>Somogyi unit Authors' unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>82.3</td>
<td>91.3</td>
<td>1.11</td>
</tr>
<tr>
<td>74.8</td>
<td>78.9</td>
<td>1.05</td>
</tr>
<tr>
<td>63.1</td>
<td>69.7</td>
<td>1.10</td>
</tr>
<tr>
<td>90.0</td>
<td>105.9</td>
<td>1.18</td>
</tr>
<tr>
<td>136.6</td>
<td>138.9</td>
<td>1.02</td>
</tr>
<tr>
<td>61.2</td>
<td>66.1</td>
<td>1.08</td>
</tr>
<tr>
<td>57.8</td>
<td>68.2</td>
<td>1.18</td>
</tr>
<tr>
<td>25.2</td>
<td>32.0</td>
<td>1.28</td>
</tr>
<tr>
<td>45.0</td>
<td>47.1</td>
<td>1.05</td>
</tr>
<tr>
<td>88.0</td>
<td>76.3</td>
<td>1.12</td>
</tr>
<tr>
<td>49.4</td>
<td>52.2</td>
<td>1.05</td>
</tr>
<tr>
<td>62.4</td>
<td>71.4</td>
<td>1.14</td>
</tr>
<tr>
<td>96.9</td>
<td>106.6</td>
<td>1.10</td>
</tr>
<tr>
<td>90.0</td>
<td>90.8</td>
<td>1.01</td>
</tr>
<tr>
<td>64.9</td>
<td>70.1</td>
<td>1.08</td>
</tr>
</tbody>
</table>

Average ....................... 1.10
(Table II) showed practically no variation over the range of concentra-
tion of substrate tested.

A second experiment was performed with use of a constant amount
of starch with serial dilutions of enzyme solution. Both serum and
urine were used as a source of enzyme. The results, as shown in Table
III, indicate a satisfactory proportionality between enzyme concentra-
tion and the values obtained.

In a third experiment the relation of time of hydrolysis to amylase
activity was studied. Enzyme and starch concentrations were held
constant and the time of incubation was varied. Fig. 4 shows the re-
sults of this experiment. The curve obtained shows a straight line rela-
tionship during the 1st hour of hydrolysis, with some loss of activity
between 1 and 2.5 hours of incubation. These results demonstrated
the validity of the use of the 30 minute incubation period.

To show the relation of the authors' amylase unit to the unit of the
Somogyi method, which is in wide-spread use, amylase determinations
by both methods were made on the sera from fifteen human subjects.
The results are recorded in Table IV. The amylase values by the Somo-
gyi method are about 10 per cent higher than those obtained by our pro-
cedure. For practical purposes values by the two methods may be con-
sidered directly comparable.

**SUMMARY**

An amyloclastic method for the determination of α-amylase in blood
and urine has been developed. The method makes use of the difference
in the intensity of the color produced with iodine by a measured amount
of soluble starch before and after hydrolysis by the enzyme. The
method is rapid and has a high degree of accuracy.

**BIBLIOGRAPHY**

A PHOTOMETRIC METHOD FOR THE DETERMINATION OF α-AMYLASE IN BLOOD AND URINE, WITH USE OF THE STARCH-IODINE COLOR
Benjamin W. Smith and Joseph H. Roe


Access the most updated version of this article at http://www.jbc.org/content/179/1/53.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/179/1/53.citation.full.html#ref-list-1