purified components requires a variety of fairly complicated procedures that can be confusing to persons whose chief interest is in practical applications. In Finland (1975) the question arose “What one substrate can be used to measure all the cellulase components?” Dr. L. G. Petterson [4] opted for cellotetraose because it is acted on by all known members of the cellulse complex. Dr. G. Halliwel [5] decided on cotton because only a complete cellulase will hydrolyze it. So we had the choice of the most susceptible substrate or the most resistant, but both are unsatisfactory for a practical assay. Cellotetraose is not available commercially, but would have to be prepared by the investigator, a major research effort in its own right. Cotton is so slowly hydrolyzed that meaningful assays require 24 hr. Finally, neither cotton nor cellotetraose is representative of a realistic substrate.

In the early studies on cellulase the available enzyme preparations would scarcely hydrolyze insoluble cellulose although they often broke down soluble derivatives such as carboxymethyl cellulose readily. This was because they consisted chiefly of endo β glucanases (C₃) and lacked the exo β glucanases (C₄). This is still true for cellulose preparations derived from organisms like Aspergillus niger or from plant extracts. For such cellulases carboxymethyl cellulose is used as a substrate (Table II).

Since the action on CMC is only linear to about 12% conversion due to interference by substituents (Fig. 2), the units per milliliter were defined as the inverse of the dilution to give 0.4 or 0.5 mg/ml of reducing sugar as glucose with 0.5% carboxymethyl cellulose as the substrate in first a 1 hr and later a 30 min assay. These units were of course arbitrary, but they are quantitative and can readily and preferably be converted to standard units according to the International Union of Biochemistry (i.e., 1 unit equals 1 μmol of product per minute) (Table II). A more serious deficiency is that the quantity of reducing sugars produced (and so the unit values) will be greatly affected by the particular sample of CMC used. The rate of hydrolysis is affected by both chain length and degree of substitution. Similar endo β1,4 glucanase assays can be developed for other cellulase derivatives such as cellulose sulfate, but the unit values will not be directly comparable. Since carboxymethyl cellulose (CMC) is soluble, it is readily hydrolyzed. Trichoderma viride cultures will yield 50 to 150 CMC units/ml with a specific activity of about 100 units/mg of protein. Other organisms such as Pestalotiopsis westerdijkii may yield as much as 400 CMC units/ml of culture filtrate.

For a practical measurement of saccharifying cellulase measurement of endo β glucanase (or of any other single component) is

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**TABLE II**

Endo β Glucanase (C₃) Assay

<table>
<thead>
<tr>
<th>Reference</th>
<th>Enzyme ml</th>
<th>CMC ml</th>
<th>CMC % in Assay</th>
<th>Time Minutes</th>
<th>1 Glucose mg/ml</th>
<th>1 Glucose total mg</th>
<th>Int. units</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>1.0</td>
<td>9.0</td>
<td>0.5</td>
<td>60</td>
<td>0.4</td>
<td>4.0</td>
<td>0.37</td>
</tr>
<tr>
<td>b</td>
<td>0.5</td>
<td>4.5</td>
<td>0.5</td>
<td>60</td>
<td>0.4</td>
<td>2.0</td>
<td>0.37</td>
</tr>
<tr>
<td>c</td>
<td>0.5</td>
<td>5.5</td>
<td>0.5</td>
<td>50</td>
<td>0.5</td>
<td>0.5</td>
<td>0.185</td>
</tr>
</tbody>
</table>

*Substrate = carboxymethyl cellulose, degree of substitution 0.5. Temperature = 50°C. Product = reducing sugar as glucose. Buffer = 0.05 M citrate pH 4.8. Calculations: 1 IU = 1 μmol of glucose/min (0.18 mg/min). 1 mg glucose/0.18 x 60 = 0.0925 units (1 hr) or 1 mg glucose/0.18 x 30 = 0.185 units (0.5 hr).

a 4.0 mg glucose × 0.0925/1.0 ml enzyme = 0.37 IU/C₃ unit.

b 2.0 mg glucose × 0.0925/0.5 ml enzyme = 0.37 IU/C₃ unit.

c 0.5 mg glucose × 0.185/0.5 ml enzyme = 0.185 IU/C₃ unit.

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**Fig. 2.** Effect of enzyme concentration and of temperature on hydrolysis of carboxymethyl cellulose and filter paper by T. viride cellulase. Culture filtrate of QM6a grown in cellulose, 12 C₃ units, 0.2 filter paper units/ml. Filter paper activity (1.0 ml) 1.74, ● - - - ○, 40°C; ○ — — , 50°C, Δ — — Δ, 60°C.