very thick and since cellulose is insoluble, the effective concentration is the available surface. Increasing the effective cellulose concentration by adding more of it or by milling the cellulose will increase the rate of the reaction and also make it more linear to a higher sugar value [10] but this increases the relative contribution of the enzymes acting on the more amorphous portions of the cellulose. We are more

enzyme preparations, giving apparently high but unreal values. A series of dilution curves (Fig. 5) shows the effects of time, enzyme concentration, and cellulose concentration on the FP activity. It is evident that FP activity per unit of enzyme decreases with increasing enzyme concentration, that FP activity per unit of time decreases with increasing time of incubation, and that FP activity increases as cellulose concentration increases (Table IV).

It was obviously time to start using a cellulase unit based on the International system but two problems arose. The first problem was what concentration of cellulose to use in the assay and the second problem was what extent of conversion was required for meaningful results. For a soluble substrate the answers are simple. Substrate level should be high enough that it does not limit the reaction, and the extent of conversion should be slight before depletion of the substrate or product inhibition affects the reaction rate. Because of the low bulk density of cellulose, concentrations greater than about 5% become

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Fig. 4. Measurement of filter paper cellulase units per ml. Follow procedure as outlined in Fig. 3. If filter paper activity for 0.5 ml of enzyme is equal to or less than 2.0, units per ml equal FP activity x 0.185. If the FP activity is greater than 2.0, repeat using diluted enzyme and estimate the ml of enzyme required to give a FP activity of 2.0. Units per ml equals 0.185/ml of enzyme to give a FP activity of 2.0.

Fig. 5. Hydrolysis of filter paper by T. viride cellulase. Effect of assay conditions, 0.5 or 1.0 ml enzyme + 1 ml buffer pH 4.8 + 25, 50, or 100 mg Whatman No. 1 filter paper. Units = µmol of glucose/min based on dilution to give 2 µg of glucose. △——△, 30 min incubation 50°C; O——O, 60 min incubation 50°C; □——□, 120 min incubation 50°C.