occasionally under pressure, but with the dilute enzyme and concentrated sucrose the lag was sometimes scarcely perceptible. For analysis, the rate constant of the reaction was calculated from the straight-line portion of the curve, between 60 and 120 min., in a plot of the natural logarithm of the percentage of the initial amount of sucrose remaining against time in seconds.

To carry out the reaction under pressure, the solution of sucrose and enzyme was transferred, immediately after mixing, to a test tube that could be closed with a rubber stopper in a manner leaving no air space between the stopper and solution. The tube was placed in a water-filled, steel pressure chamber, previously equilibrated at the desired temperature. The chamber was then attached to a hydraulic pump and pressure applied. These operations generally required about $2\frac{1}{2}$ min. while a somewhat less interval of time, $1\frac{1}{2}$ min., was required to remove the tube and add the sodium hydroxide after releasing the pressure. Thus a slight error, difficult to avoid, was introduced in the observed reaction rates under pressure, owing to the relatively short time that the reaction proceeded at normal pressure.

The following buffers were used, in final concentrations as indicated: pH 0.96, 0.1 *N* hydrochloric acid (no buffer); pH 1.5 to 3.0, glycine–hydrochloric acid, 0.02 *M*; pH 3.0 to 4.5, acetic acid–sodium acetate, 0.02 *M* (in some experiments, 0.1 *M*); pH 5.0 to 7.5, potassium dihydrogen phosphate–sodium monohydrogen phosphate, 0.02 *M*.

RESULTS OF EXPERIMENTS

The relation between enzyme concentration and rate of hydrolysis was studied at 30°C., pH 7.03–7.05, under normal and 7000 pounds pressure. At enzyme concentrations of 1 per cent or less the rate is proportional to the amount of enzyme, during the logarithmic period of the reaction, as illustrated by the data in figure 1. This figure also shows that, under these conditions of temperature and pH, the net rate is uniformly increased by pressure, and a similar increase was noted with an enzyme preparation from other sources (Pfanstiehl).

The time course of hydrolysis at 30°C., under normal and increased pressures and at two hydrogen-ion concentrations, is illustrated in figure 2. The influence of pH is indicated in figure 3, and of temperature in figure 4. The observed dependence of the pressure effect on temperature and pH was anticipated by analogy with the results of earlier studies on different systems, referred to above. Thus, the acceleration in rate by pressure becomes more pronounced as the medium is made more alkaline, or as the temperature is raised. It also becomes pronounced in the range of pH acid to that of maximum rate. The influence of temperature and of pressure on the alkaline side of the pH optimum was studied in more detail, in an effort to analyze the volume changes, as described shortly.

The work of Chase, Reppert, and Ruch (6) has shown that the inactivation of invertase at 50°C. in acid solution is reversible on cooling. The present results indicate that the temperature and pH inactivations may be considerably reversed by pressure, e. g., at 40°C. and pH 7.03–7.07 the rate of hydrolysis is increased 250 per cent by applying 680 atm. pressure (figure 4). The conclusion