In the case of PBG the helix coil transition theory describes the appropriate mechanism for the transition process. For our purposes it is sufficient to extend the results of Applequist,[8] who made use of the model of Zimm and Bragg[14] for long chains, to describe the pressure dependence on the fraction f of hydrogen-bonded carbonyls at the midpoint of the transition

$$\left(\frac{\partial f}{\partial P}\right)_{T, f = 1/2} = + \frac{\Delta V_m{}^{\circ}}{4RT_r \sqrt{\sigma}} \qquad (3)$$

where $\Delta V_m{}^{\circ}$ is the standard state volume increase upon disruption of 1 mole of hydrogen-bonded carbonyl groups, T_r is the temperature at the center of the transition, and σ is the parameter which represents the equilibrium for the formation of an interruption in a sequence of bonds by a process which maintains a constant number of bonds. An equation analogous to eq. 3 is given by Applequist for the temperature dependence as

$$\left(\frac{\partial f}{\partial T}\right)_{T, f = 1/2} = \frac{-\Delta H_m{}^{\circ}}{4RT_r{}^2 \sqrt{\sigma}} \qquad (4)$$

where we define $\Delta H_m{}^{\circ}$ as the standard state enthalpy of transition for disruption of 1 mole of hydrogen-bonded carbonyl groups. For internal consistency the Δ terms in eq. 3 and 4 have been defined according to the reaction expressed by eq. 1. Applequist uses the reverse equation and describes the enthalpy for the reaction of formation of hydrogen bonds. The two equations may be combined to yield an equation which has the familiar form of the Clapeyron equation

$$\left(\frac{\partial P}{\partial T}\right)_f = \frac{\Delta H_m{}^{\circ}}{T \Delta V_m{}^{\circ}} \qquad (5)$$

Equation 5 actually applies for any constant helical content fraction f, but if $f = 1/2$ then $T = T_r$.

Results and Discussion

Some preliminary attempts were made to induce the entire transitions of ribonuclease and PBG isothermally by application of pressure, but the entire transition could not be covered for either case within our range of pressures to 1500 atm. Consequently, reversible thermal transitions were examined at various fixed pressures. We shall describe the results as they apply to each material in the next two sections. In the following results it is helpful to remember that an increase in temperature promotes unfolding for ribonuclease but folding for PBG.

Ribonuclease A. A 5.491 wt.-vol. % solution of ribonuclease A was studied at 589 mμ. Studies on less concentrated solutions of ribonuclease yielded similar results but with less precision.

Reversible thermal transitions were observed at atmospheric pressure, 680, 1020, and 1360 atm. Transition temperatures (where $f = 1/2$) were found to be 46.0, 44.4, 43.5, and 40.7° for the above pressures, respectively.

The effect of pressure on the thermal denaturations is seen in Figure 1 which illustrates the change in observed rotation with temperatures at various pressures. The filled symbols represent values obtained for decreasing temperatures and substantiate the reversibility of the transition. At low temperatures a significant increase in the optical rotation was found with increase of pressure. This effect is possibly due to solvent effects. The effect of pressure on the unfolded form from 1 to 681 atm. may be due to small apparatus changes or to denaturation. These latter two effects are insignificant if relative properties are examined as we shall do in studying the fraction of denaturation as a function of temperature and pressure. Figure 2 shows the effect of temperature on undenatured fraction at various pressures. The undenatured fraction, f, is assumed to be determined by the fractional change of the optical rotation upon temperature.

The results of the effect of pressure on the undenatured fraction at various temperatures are shown in Figure 3. The slopes at the center of the transition, $(\partial f/\partial P)_{f = 1/2}$, have values of -3.5×10^{-4} and -3.0×10^{-4}/atm. for 42.5 and 45.0°, respectively. On the basis of an independent r-step denaturation mechanism, eq. 2 yields values of -36 and -31 ml./mole for 42.5
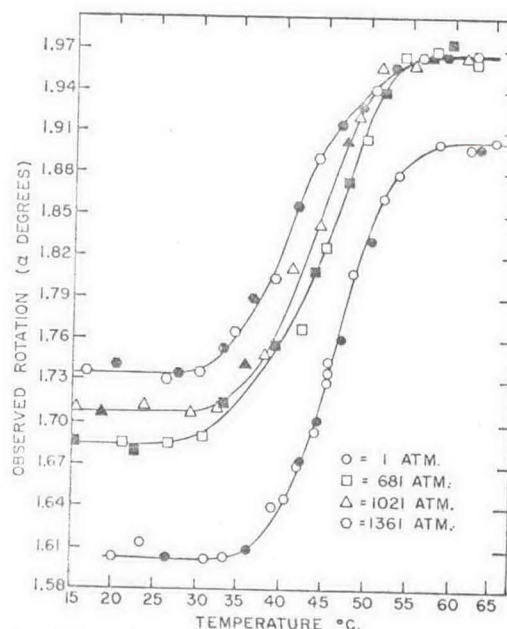
Figure 1. Effect of temperature on observed rotation at 589 mμ and various pressures for a 5.491 wt.-vol. %, pH 2.80 buffer solution of ribonuclease A.