In figure 5 the parallel lines show that ΔV is the same at 35° and 40°C., and the slope indicates a volume increase of reaction amounting to approximately 69 cc. per mole in going from the active to the inactive form of the enzyme. This is much greater than the volume change of activation in mutarotation, which is in the opposite direction and which involves molecules smaller by a different order of magnitude than those of the protein enzyme. Moreover, it is very close to the values that have been reported in other cases of protein denaturation: 64.6 cc. for the reversible denaturation of bacterial luminescence at 35°C.; 62.6 cc. for the same system in the presence of alcohol; 71 cc. for the irreversible denaturation of the luminescent system; and roughly 100 cc. for the precipitation of purified human serum globulin at 65°C.

DISCUSSION

The reversible inactivation of protein enzymes revealed in this and the earlier studies is associated with marked increases in entropy, energy, and volume. While these changes may sometimes involve dissociation of primary bonds, they probably represent chiefly an unfolding of the globular protein into more linear, fibrous forms. If this unfolding is carried beyond a certain critical stage, or is accompanied by hydrogen bonding with narcotics such as alcohols, ethers, and similar polar bonds, the enzyme loses, at least temporarily, its power to take the shape appropriate for combination with the molecule to be catalyzed. The unfolding of a hundred or so amino acids is observed to result in a volume increase equivalent to about three-quarters of the volume of one amino acid.

In general, the folded enzyme must change its shape in the activation process to fit the molecule to be catalyzed, unless the active part of the enzyme is at the surface. Thus, bacterial luciferase expands about 50 cc. per mole in going from the normal to the activated state. This indicates a good deal of unfolding. Active invertase, on the other hand, shows practically no volume change in combining with sucrose to form the activated complex. This probably indicates that the active part of invertase is on the surface of the normal native molecule, so that very little change in the shape of the enzyme is necessitated during the activation process. The available data, of course, do not exclude the possibility that an unfolding of the invertase molecule, followed by a refolding to the same volume, takes place, with the result that the net effect of pressure is the same at different pressures.

In addition to the above evidence that enzyme molecules are effective catalysts only when folded in the appropriate way, the relation between the optical configuration of the substrate and the activity of the specific enzyme provides familiar evidence that the catalytic action depends as much on the surface shape and the secondary bonds made with the reactant as on the primary bond structure. Optical isomers have the same primary bonds and yet frequently differ enormously in their reactivity with enzymes. The experiment of Pasteur (24), whereby an optical isomer was separated from a racemic mixture by means of an organism, is a classic example. The difference in reactivity is to be traced to a better fitting of the enzyme with the one than the other of the two isomers.

The pressure effects, indicating an unfolding process in the reactivity of pro-