II. BIOPHYSICAL CHEMISTRY*

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RESEARCH OBJECTIVES AND PRELIMINARY STUDIES

This project is concerned with the elucidation of the mechanism of physiologically important reactions, primarily through the use of kinetic methods. Very fast reactions involving purified enzymes and simpler model systems will be investigated with the use of several different techniques.

A stopped-flow apparatus[1] permits the study of reactions occurring in times as fast as milliseconds. This method simply involves rapid mixing of the reactants in a specially designed chamber and observation of the mixed solution after the flow has been stopped suddenly. The reaction progress is followed through changes either in optical absorption or optical rotation.

Reactions occurring in even shorter times will be investigated by utilizing recently developed relaxation techniques.[2,3] The principle of these methods is to disturb chemical equilibria by rapidly changing some external parameter (e.g., temperature, pressure) and studying the subsequent re-establishment of equilibrium. A temperature-jump apparatus, which raises the temperature of a reaction mixture 8°C in approximately 5 μsec, has been constructed. This temperature jump is accomplished by charging a condenser to 30,000 volts and then discharging it rapidly through the reaction mixture. Changes in optical absorption and rotation are followed with photomultipliers and associated electronic equipment.

The accessible time scale can be extended to 10^{-9} sec by measurement of ultrasonic attenuation in equilibrium reaction mixtures. The ultrasonic wave acts as a pressure wave in aqueous solution, and thus perturbs pressure-dependent chemical equilibria. The result is the occurrence of maxima in the attenuation at frequencies that are related in a known way to the formal rate constants of a given mechanism.

These techniques will permit the study of elementary steps in chemical reactions, and thus provide some details of the molecular mechanism. Also, rates of fast conformational changes in macromolecules can be measured and related to chemical processes. Preliminary experiments have already been carried out and the results will be presented in a future report.

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References

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