RAPID MIXING AND SAMPLING TECHNIQUES IN BIOCHEMISTRY

Edited by

BRITTON CHANCE
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A SYMPOSIUM
of the
INTERNATIONAL
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BIOCHEMISTRY

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University of Pennsylvania

School of Medicine

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Preface

As the application of rapid reaction techniques to biochemical problems has grown by leaps and bounds, it is no more than appropriate that a symposium be devoted specifically to this topic. We are by no means the first to recognize its importance; in fact, the 1934 meeting of the Royal Society of London, attended by the pioneers in this field—Hartridge, Roughton, and Millikan—outlined many of the basic points we will consider here (1). Hartridge, indeed, pointed out four methods for displacing a chemical system into an unstable state which may in themselves furnish an excellent outline for our deliberations. Twenty years later, the Faraday Society held a general discussion on the topic of rapid reactions in general (2), and, in 1959, Eigen organized a most effective meeting on fast reactions at Hahnenklee (3). Just this year, Caldin has produced a book of fast reactions in solution (4).

The present meeting, however, has the honor to be the first to be organized under the auspices of the International Union of Biochemistry. Like its predecessors, it brings together nearly all those who are active in the field today. It is the purpose of this gathering to discuss and evaluate currently available apparatuses for rapid mixing and sampling, and to point to the limitations which nature sets to their performance and to those which technology can extend or overcome.

Our topic falls naturally into two sections. The first covers rapid physical methods for displacing a biochemical system from its equilibrium or steady state level, with the simultaneous physical measurement of the extent of the biochemical reaction due to this perturbation; the second deals with methods by which the reaction may be quenched, or samples withdrawn from the reacting system, with great rapidity for subsequent measurement of the extent of reaction.

B. Chance and Q. H. Gibson have organized the first session under the heading "Rapid Flow Methods," with particular emphasis on techniques for rapid and/or efficient mixing as the primary means of perturbation, although photolysis and temperature-jump techniques are also considered. R. H. Eisenhardt and K. K. Lonberg-Holm have directed the attention of the second session, "Rapid Stopping and Sampling Techniques," to the sudden arrest of chemical reactions, and to methods for rapid sampling of stationary liquid volumes, or of tissues and organs by quick freezing.

Since one of our principal aims was to provide for the fastest possible publication, much of this volume has not been as fully edited as is usually

implied in the term. The emphasis of the symposium being upon experimental developments, we have, with the authors' consent, removed some of the more theoretical material to appendices. Otherwise, a majority of the manuscripts stand essentially as they were received from the authors. We wish to express our thanks to them, to Academic Press, and to our hard-working and loyal (and most of the time even cheerful) assistants, Lillian S. L. Chance and Jan Bright, for the preparation and production of this volume. It presents, indeed, a "rapid sampling" of the field; it is our hope that it has not been subject to excessively "rapid mixing."

THE EDITORS

August 27, 1964

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INTRODUCTORY REMARKS

B. Chance

Flow methods generally derive time resolution by rapid movement from one point to another in a closed tube, as, for example, in the pioneer experiment of Rutherford (1) on the lifetime of an excited state of a gas. For the study of biochemical reactions, the flow of liquids is employed, and most of the discussion of this session has to do with methods for rapidly generating a state of non-equilibrium in biochemical systems. The Hartridge-Roughton rapid mixing technique is the most important of these, and, indeed, stands as a tribute to their genius. With it, a great variety of chemical species may be studied over wide concentration ranges.

Hartridge and Roughton also considered perturbation by photolysis and temperature change (2); in these methods, rapid mixing is required only to establish particular chemical conditions in the flow tube. It may confidently be predicted that both these techniques will receive great impetus from the development of laser light sources of extraordinary intensity and short duration. Physical methods for the evaluation of chemical change in the flow tube are also of key importance; spectrophotometry, fluorometry, EPR, and many other techniques are suitable and will be considered here.

It is fitting that this session be dedicated to the memory of G.A. Millikan, the first American to contribute extensively to the development of the continuous flow method for studying biological reactions. His penetrating analysis of flow methods (3) affords, nearly thirty years later, a sound basis for the evaluation of today's equipment.

Professor F.J.W. Roughton is well known and held dear by all of us. It is he who, through his ingenious initial work with Hartridge and by his continuing interest in the development and stimulation of the younger workers in this field--among them Millikan--deserves the first place of honor on our program today. It is not only for his outstanding contributions to the field of rapid reactions in general, but for personal reasons as well, that it gives me great pleasure to present to you my thesis supervisor, friend, and colleague, F.J.W. Roughton.

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THE ORIGIN OF THE HARTRIDGE-ROUGHTON RAPID REACTION VELOCITY METHOD

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Dr. Chance, ladies and gentlemen, may I begin by saying on behalf of the participants how grateful we all are to you, sir, and to your wife and our other Philadelphia friends for arranging and inviting us to this conference. I am sure that we are all at one in feeling morally certain that this will be a very fruitful, useful, and successful conference. I, myself, am especially happy to be here today for several reasons, of which I will mention but two. It is naturally a delight to me to take part in a meeting at Philadelphia at which several of the local pundits are such old friends and intimate research partners of mine (as Britton Chance, Quentin Gibson, and for all too short a time, Robert Davies). Much of my scientific time during the past quarter of a century has indeed been spent in the company of one or the other of these people. Together we have worked and played on many of these subjects which are right up the street of this conference. One of the best ways of keeping young, as has often been said, is to remain alert and active in making new and more youthful friends. May I have the opportunity today and, I hope, tomorrow, of doing just this and imbibing wisdom, not only from my old and tried friends, but also from new colleagues whom I have not had the privilege of meeting before and who, I am sure, will teach me much of what is now going on in a field in which I was once a pioneer, but in which I can now claim, without false modesty, to be rather a back number?

Some of you here today took part in a very successful conference on an allied subject which our friend, Dr. Eigen, organized in the Harz Mountains five years ago. At that conference I had likewise the honor of being one of the opening speakers, and chose as my subject then the origin of the Hartridge-Roughton rapid reaction method and its application to the kinetics of hemoglobin in the intact red blood corpuscle. I am delighted that our Chairman has added the name of Rutherford to those of ours this morning, for the

late Ernest Rutherford was rather easily the greatest scientist I have known intimately. Five years ago I laid much more stress upon the results than upon the history of the method. Very little as to the latter did, in fact, appear in the subsequent published proceedings of the 1959 conference, so that there is something of a gap in the historical record; this morning, if you will bear with me, I should like to take the opportunity of trying to plug that gap.

Hamilton Hartridge was the first of what I might call a series of human enzymes with whom I have been in a sense a co-enzyme during the past 45 years. Before World War I, while I was still a school-boy, Hartridge had already leapt into fame by the invention of his cunning reversion spectroscope, an instrument by which he was enabled to measure about 10 times more accurately than heretofore the position of absorption bands in the visible spectrum. The question of whether the human lung has the power of pumping oxygen actively into the blood from the air was a very controversial subject in respiration physiology at that time, and Hartridge's reversion spectroscope was a peculiarly apt instrument for shedding new light on that problem. He was able, with this instrument, to measure the mean position of absorption bands in the visible spectrum to an accuracy of about $\pm$ 1 Å unit. When oxygen in combination with hemoglobin is replaced with CO, there is a shift in the absorption bands of about 60 Å in the case of mammalian hemoglobin. By measuring the position of the absorption bands in a mixture of COHb and O_2Hb it is, conversely, possible from calibration curves to estimate the proportion of the two compounds in the mixture.

During World War I, Hartridge's almost diabolical technical ingenuity was harnessed to solve problems of a different kind. It was not until about 1919 that he returned to his first baby, the reversion spectroscope. Stimulated by the basic and remarkably pregnant discovery of J. S. Haldane at Oxford that the displacement of oxygen from hemoglobin by CO is photochemically reversible, Hartridge shone strong beams of light on a dilute solution of COHb in water containing dissolved oxygen: the CO came off, the oxygen in solution replaced the CO in combination with hemoglobin, the shift of 60 Å units occurred in the visible band, and this could be watched with great ease through his reversion spectroscope. On reducing the intensity of the light, the bands reverted to the position corresponding to those of COHb. This last demonstration, which could be repeated ad lib, was of course, nothing more nor less than primitive flash photolysis.

Origins of the Rapid Flow Method

Although Hartridge was, as I have said, diabolically ingenious on the technical side, he was the first to admit that he was much less at home when it came to the physico-chemical interpretation of the results. Accordingly, he began at that point to look out for a partner on the theoretical side. By great good luck for me, I happened to be around and about at that very moment. My background, such as it was, in physical chemistry and physiology was almost entirely on paper, and it seemed indeed that there were genuine possibilities of a successful combination in which each partner would fill in the deficiencies of his opposite number. It was, in fact, not long before our British colleagues came to compliment each of us on the remarkable way in which we complemented each other.

Much the most exciting of Hartridge's preliminary investigations were his attempts to measure the actual rate at which the absorption bands moved from the oxyhemoglobin position to that of COHb during the period of recovery in the dark from the effects of the bright light. These experiments held out, for the first time, some hope of obtaining the velocity constants of a fairly rapid reaction of hemoglobin with dissolved gases. I say a "fairly rapid reaction" because there were at this time no such pieces of equipment as photocells, cathode ray oscilloscopes, CAT's, and the like. We had to make out as best we could with our own naked eyes, and thus were limited to processes with half-times of at least 1 second.

This particular replacement reaction, CO + O_2·hemoglobin → CO·hemoglobin + O_2, therefore could not be followed at a temperature above 15°. As physiologists, we naturally wanted to go to the body temperature, i.e., 37° C. Accordingly, I turned the heat on Hartridge to change the technique so that time scales of 0.1 second instead of 1 second could be covered. With his usual speed, he came up with the reply, almost over night; his answer was to combine flash photolysis with the continuous flow principle.

The set-up is shown in Figure 1. We prepared a solution containing 75 per cent COHb and 25 per cent O_2Hb in a large Marriotte bottle. From there the solution flowed under gravity up a vertical tube, where it was illuminated with a beam of light from a carbon arc, which was a strong light source for those days, although not so nowadays. The photochemical displacement occurred as the fluid ran up the light tube, but when it passed thence into an observation tube in semi-darkness, the reverse process set in, and could be followed by observations on the moving fluid with the reversion

spectroscope at various points along the observation tube.

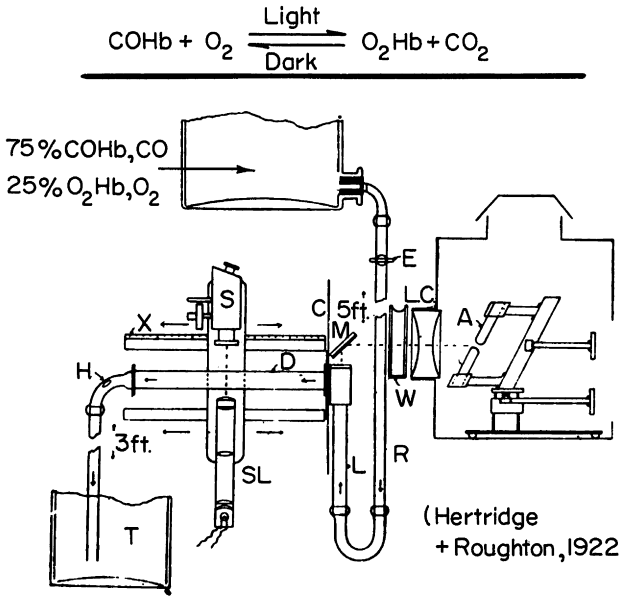

Figure 1. Flash photolysis flow apparatus. L, exposed to light; D, kept in dark; A, arc lamp consuming 30-40 amperes; LC, lantern condenser; W, water cooling trough; M, silver mirror; B, 20-liter bottle; T, tank; H, small hole in rubber connection to admit air at this point; S, reversion spectroscope; SL, illuminating system of reversion spectroscope. (Hartridge and Roughton, Proc. Roy. Soc. B, 94, 336 (1923).

Hartridge, without knowing it, was doing again in essence what Rutherford had done a quarter of a century before. Rutherford led a stream of gas past a source of uranium oxide which caused the gas to ionize, and then followed the recombination of gaseous ions by observing the saturation current at different points along the observation tube with the aid of electrodes and electrometers. So Hartridge's arc light corresponded to Rutherford's uranium oxide, whereas the reversion spectroscope corresponded to the electrometer.

In this fashion, we were able to measure times down to