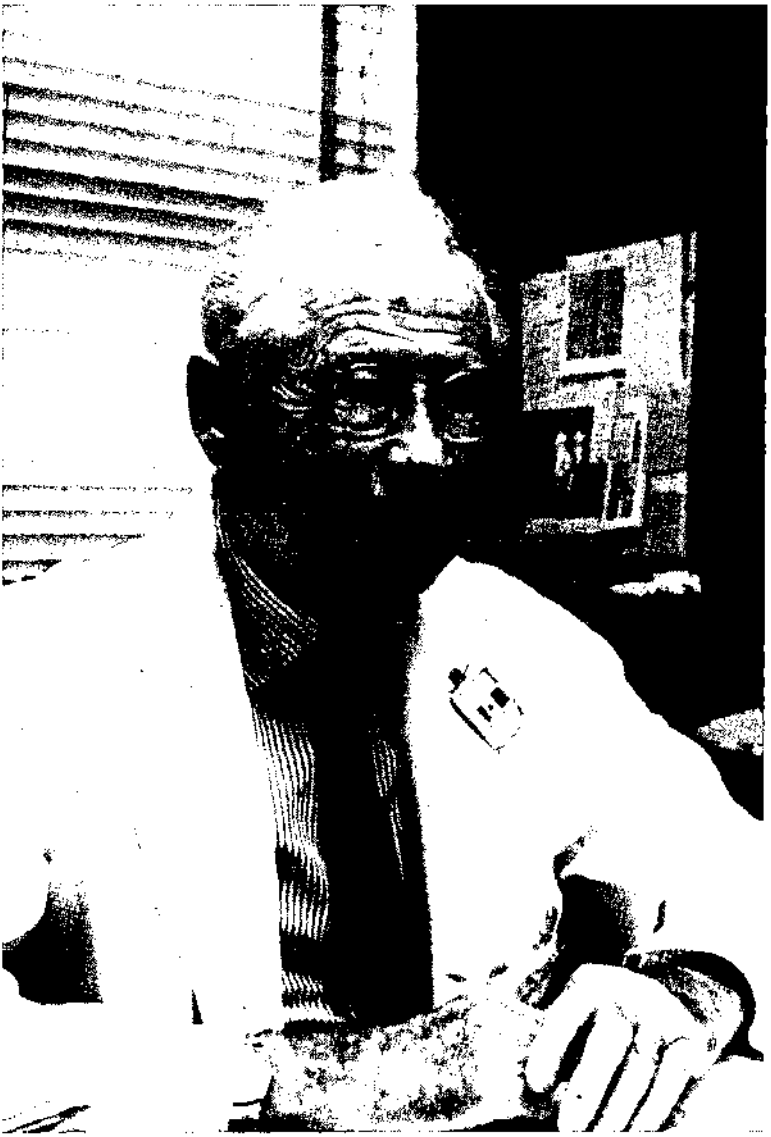

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A BIOCHEMIST'S ANABASIS

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CONTENTS

I felt honored when I was asked to prepare this prefatory chapter, but dismayed when I sat down to write it. In the light of present research activities in biochemistry at the molecular level, my own contributions seem meager and relatively inconsequential. Yet I have had such joy in the laboratory and it has brought me into such close association with other scientists and students, that hopefully I can transmit to others my gratitude for the opportunity I have had to have "Fun in Labs." Though I would not presume to dignify it as ancient history, one might appropriately call it "aging history."

Mine has been, in no way, an unusual life—studded with adventure, spiked with successes, flooded with disappointments. Rather, I have had the good fortune to have many friends throughout this century who have helped me improve my knowledge of the world we live in, and who have been responsible for making a career in research and teaching possible to me.

As I explore my past for facts or figures worthy of recording in a volume of the *Annual Review of Biochemistry,* I feel as the astronauts of Apollo 8 must have felt as they looked from the moon back to the earth, "Do you suppose that anything goes on on that small and insignificant spotted body?" My life has been remarkable only in that I have been asked to write this chapter. I have arranged what follows into those broad scientific subjects that have occupied my laboratory life, rather than as an account of events in chronological sequence. The seven labs in my life were at Michigan (1915–17); Columbia (1918–21); Rockefeller Institute (1921–

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26); Chicago, Physiological Chemistry (1926–28); Chicago, Medicine (1928–35); Harvard (1935–58); Scripps Clinic and Research Foundation (1959–66). If there is a theme, it is, what insight can be gained through studying man as a heterogeneous system with extracellular, intracellular and solid phases.

Preliminaries—1895–1917

I was the firstborn to Otis Luther and Elizabeth Baird Hastings in Dayton, Kentucky, on November 20, 1895. By my father, I was taught that nothing less than my maximum effort was acceptable; from my mother I learned of beauty, helpfulness to others and gratitude for others' help. Our home was paternally Quaker, maternally Presbyterian and familiarity with the Bible was taken for granted. By 1901 we had arrived in Indianapolis, Indiana, with a stopover in Richmond, Indiana, the homeland of my father's many Quaker relatives and friends.

The impetus to become an educated man came from two inspiring Shortridge High School teachers: from Miss Marthens, I learned Latin and Greek; from Miss McClellan, for whom I worked as a laboratory assistant, I learned Zoology. But for them, I would probably never have finished high school, let alone have gone to college. They wouldn't hear of anything else so not wanting to displease them, I enrolled in the University of Michigan on borrowed money. My registration in Chemical Engineering was not an altogether suitable one for me, and when at the end of my second year I was asked by Dr. Floyd E. Bartell, Professor of Physical Chemistry, to be his assistant for the laboratory course in Physical Chemistry, I jumped at the chance. My assistantship carried with it a good-sized lab of my own which adjoined Professor Bartell's laboratory. I made it my home by day and much of the night for most of the next two years.

Whether my appointment was a wise decision on Bartell's part is questionable because being a junior, I had not yet had organic chemistry or the physical chemistry laboratory course. As a result, I almost anesthetized the inhabitants of our floor by distilling ether without an iced receiver. On another occasion I put our bank of large lead storage batteries on charge over a weekend in a closed room without ventilation. The energy released in the inevitable reaction of hydrogen and oxygen was dramatically demonstrated.

Yet Bartell forgave me and I loved this lab of mine so much that I hated to be out of it. It was a time for enjoying the sheer delight of experimenting —unhampered by "pride in paternity or hope of posterity," scientifically speaking. Incidentally, I had learned to measure hydrogen ion concentrations with the bubbling hydrogen electrode as part of my job. In my senior year, Professor Bartell suggested that I work for a Ph.D. in Physical Chemistry on graduation. This seemed to me to be beyond my capabilities, but he said, "You work hard, you are resourceful." So he introduced me to the more productive methods of systematic investigation and suggested that I undertake the study of the permeability properties of collodion membranes.

Thus, in 1916, began an interest which has led me into many strange laboratory adventures. But World War I intervened, and I left this physical chemistry environment for a physiological one. As will be clear from what follows, I have been a biochemist in name only.

Acid-Base Balance and Metabolism

My first paper, presented before the American Physiological Society in Baltimore in 1919 was entitled: "The Effect of Fatigue on the Bicarbonate Content on Blood Plasma" (1). My last appearance before the Federation was in 1962 to deliver a paper entitled: "The Effect of CO_2 Concentration on Carbohydrate Metabolism in Rat Liver" (2). This is an account of what happened in the interim.

Because I could measure hydrogen ion concentrations with a hydrogen electrode, I was employed as an Assistant Sanitary Chemist by the United States Public Health Service in 1917, to study whether acidosis was an accompaniment of physical fatigue (pH terminology had not yet reached chemistry).

After being assigned for a brief period at the Ford Motor Company to the study of urine [sic] of workers engaged in operations requiring different degrees of exertion, I requested transfer to a laboratory where I could study prolonged exercise on animals and obtain blood instead of urine for analysis. This was granted and I was ordered to the Department of Physiology, Columbia University, where I would work under the supervision of Professor Frederic S. Lee, known for his work on fatigue, and Dr. E. L. Scott, a biochemical-minded physiologist. Since Dr. Scott shortly left for active duty in France, I was left pretty much on my own. I had a great deal to learn. However, the staff in the Department of Physiology were all extremely helpful, and in addition, I was generously aided by Dr. C. S. Robinson and Dr. G. E. Cullen who were in D. D. Van Slyke's department at the Hospital of the Rockefeller Institute. Dr. Van Slyke himself also took an interest in the work I was doing because he was then engaged in his classic studies on "Acidosis." So I soon found myself determining the pH and "alkali reserve" of the blood plasma of dogs before, during, and following long exercise on a treadmill. At times, I also studied fatigue in marathon runners, 6-day bike riders and on myself as subject.

Early in my work I measured the "alkali reserve" of plasma by electrometric titration in a McClendon hydrogen electrode vessel. However, it had the disadvantage of not being a closed system, so it could not be used to measure the original pH of CO_2-containing solutions. I got around this by making a vessel with a flanged collar around the top to contain mercury. I then arranged to rotate the vessel horizontally about the fixed platinum electrode which served as a paddle, thus providing stirring of the sample. The electrode, micropipette, and hydrogen inlet and outlet were passed through a stopper in a glass tube, the lower extension of which dipped into the mercury in the doughnut-shaped collar on the electrode vessel. I thus had a mi-

croelectrode in a closed system that permitted me to measure pH or carry out titrations without frothing. This hydrogen electrode vessel was used on numerous occasions in my laboratory until glass electrodes became available.

If I have dwelt overly long on the little known "Hastings Hydrogen Electrode," it is because it played a decisive role in my life. In 1921, as I was completing my work at Columbia University on the "Physiology of Fatigue" for the U.S. Public Health Service (it was published by the PHS as Bulletin 117 and accepted by Columbia as my Ph.D. thesis) (3), I was encouraged by Cullen to write up a description of my electrode and submit it for publication in the *Journal of Biological Chemistry*. I did so and took the manuscript over to Cullen at the Rockefeller Institute. After reading it, he said, "Wait here a minute. I want to show it to Van." When he returned shortly after, he said, "Van wants to see you." And Van Slyke in addition to accepting my three-page paper, said, "Don't make any commitments for next year until you hear from me." That was in March, my paper appeared in May (4), and I was appointed in the fall to the staff of the Hospital of the Rockefeller Institute to succeed Glann Cullen as Van Slyke's first assistant.

Thus began five of the most profitable, productive, and happy years of my life. When I asked Cullen what my main duties would be, he replied: "You will be responsible for accurate pH measurements, and for getting Van out of his office and into the lab regularly." I found that these were relatively easy, but I soon had my hands full executing the experiments which Van planned under the general title of "Gas and Electrolyte Equilibria in Blood."

In order to achieve the accuracy required to determine the CO_2 titration curves of oxygenated and reduced hemoglobin, Van had converted his volumetric gas apparatus into a manometric one. We also devised a satisfactory double tonometer system for equilibrating blood and separating the gas and liquid phases for subsequent analysis. Besides blood, which I drew in large quantitities from the jugular vein of a Rockefeller Institute horse, we needed crystalline, isoelectric, salt-free hemoglobin whose oxygen-combining capacity was undiminished. Dr. Michael Heidelberger, who was a member of Van's department in 1921, undertook to devise a method that would lead to hemoglobin with such qualities. He succeeded magnificently and kept us supplied with all the pure, active horse hemoglobin that we needed for our experiments. Indeed, he was soon engaged in a collaborative research with Karl Landsteiner on the solubilities of hemoglobins of different species. At the same time, he was beginning with Dr. Oswald Avery his now classic work on the type-specific mucopolysaccharides of pneumococci.

We now had the techniques and the material to tackle with accuracy the question of how much shift occurs in the CO_2 titration curves of reduced blood and hemoglobin on oxygenation. From our first experiments in early 1922, we obtained accurately repeatable results which resulted in two papers: one entitled "The Alkali-binding and Buffer Values of Oxyhemoglobin and Reduced Hemoglobin" (5); the other, "The Effect of Oxygenation and

Reduction on the Bicarbonate Content and Buffer Value of Blood" (6). Later, we published a long paper entitled "The Acid Properties of Reduced and Oxygenated Hemoglobin" (7), in which the equivalents of net negative charges and buffer values over the pH range 6.8-7.6 were reported for oxy- and reduced hemoglobin. Also, the apparent shift in the pK of a proton donor group of the hemoglobin molecule upon oxygenation was calculated, and found to be close to the shift predicted by the late L. J. Henderson in 1920 (8).

This, then, seemed to account quantitatively for the effect of oxygenation on the CO_2 carrying power of blood. What was missing from the picture was the extent to which the total CO_2 in solution, which was not HCO_3^- or dissolved CO_2, was present as hemoglobin carbamate. Its existence was not yet known, and it was therefore included in our figures for bicarbonate.

The data obtained on the equivalents of net negative charges of hemoglobin in the physiological pH range formed the basis for reaching an understanding of the distribution of readily diffusible ions between red blood cells and plasma. Since these negative charges were nondiffusible and at cell pH = 7.2 amounted to about 50 meq per kg of cell water in oxygenated blood and about 30 in reduced blood, the Gibbs-Donnan Law would predict an unequal distribution of diffusible anions of definite value, *r*. This led to the equation derived by Van Slyke during a trip to China and published in one of biochemistry's classic papers, "Studies of Gas and Electrolyte Equilibria in the Blood. V. Factors Controlling the Electrolyte and Water Distribution in the Blood," by Van Slyke, Wu & McLean (9). This equation in its general form was:

$$r = \frac{[Cl^-]_c}{[Cl^-]_s} = \frac{[HCO_3^-]_c}{[HCO_3^-]_s} = \frac{[H^+]_s}{[H^+]_c} = 1 - \frac{[BP]_c + [Hb_c]_c - [BP_c]_s}{2([B]_s - [BP]_s)}$$

When actual Cl^- and HCO_3^- distribution ratios were compared with the predicted values for oxygenated blood at pHs 7 to 7.8, the agreement was good and the change in ratios with pH change was excellent. On Van Slyke's return from China, we set about repeating and extending the experiments on oxygenated blood to include distribution ratios in reduced blood over the pH range 7.0 to 7.6. We also made direct determinations of pH on serum and cells in order to calculate values for hydrogen ion activities (10). Briefly, we found that the distribution ratios of H^+, Cl^-, and HCO_3^- changed with pH changes, and with oxygenation, as predicted for both oxygenated and reduced blood. However, the absolute values of the ratio $[HCO_3^-]_c/[HCO_3^-]_s$ was about 20% higher than the ratio $[Cl^-]_c/[Cl^-]_s$ at pH = 7.4. We attributed this discrepancy to a different activity coefficient for HCO_3^- than for Ci^- in red cells. Had we been less under the influence of current physicochemical concepts, we might have reasoned that the Cl^- ratio was probably correct and, assuming that the true HCO_3^- ratio had to equal it, have calculated the amount of cell CO_2 that was bound. Three years later,

Henriques discovered that some cell CO_2 is, indeed, combined with hemoglobin as carbamate. "A little knowledge is a dangerous thing"—if thereby you neglect to use common sense!

However, as I look back on the years I spent in Van Slyke's department, I realize how much Donald Van Slyke has been responsible for the sound groundwork of biochemistry that was laid in the early part of this century. And happily he has continued his leading role during its subsequent growth. Indeed, he is still active at the Brookhaven National Laboratory where he has been since his retirement from the Rockefeller Institute and at this writing is putting finishing touches to a book on microgasometric methods.

To remember what Van Slyke's laboratory was like in the 1920s is to recall that he thought out the attack to be made on problems with care and clarity; that nothing short of the highest accuracy and convincing documentation satisfied him; that he was a leader but never a boss; and that each of his associates was a personal friend who would never think of addressing him except as Van. Biochemistry and medicine are both deeply in his debt for his contributions to them in this century.

Now to return to the specific subject of the acid-base balance of the blood. It was recognized in 1921 but rarely applied in medical practice that one must know two of the three variables of the mass law equation for carbonic acid $[H^+][HCO_3^-]/f \cdot pCO_2 = K$ if one were to characterize adequately the acid-base condition of a patient. At that time, it was customary to determine the CO_2 concentration of the plasma by Van Slyke's gasometric method and, if done at all, the plasma pH by Cullen's 1 ml colorimetric method (later adapted to 0.1 ml plasma samples by Hastings & Sendroy) (11). The CO_2 tension, pCO_2, was calculated from the above equation.

Even so, the methods required several milliliters of blood which necessitated venepuncture, or in rare instances, arterial puncture. The result was that most acid-base data were limited to single or, at most, two or three blood samples from any one patient.

Van Slyke & Cullen had published in 1917 the first paper of Van Slyke's series, "Studies of Acidosis: I. The Bicarbonate Concentration of Blood Plasma. Its Significance and Its Determination as a Measure of Acidosis" (12). This series was to have about two dozen entries. The seventeenth of the series published in 1921 under the title "Normal and Abnormal Variations in the Acid-Base Balance" defined the limits of variability to be found in normal individuals and specified the types of abnormalities in HCO_3^- and pH that are encountered in clinical medicine (13). For all practical purposes, nothing new of importance has been added to the concepts presented in this paper. The primary abnormalities may be (*a*) a CO_2 excess or deficit or (*b*) a proton donor (other than CO_2) excess or deficit. Mixed abnormalities of the two classes are frequent. In CO_2 excess or deficit, the red cell hemoglobin is the primary blood buffer being titrated. In the case of other proton donors (e.g., lactic acid) both the hemoglobin and the blood HCO_3^- are titrated. The pH and HCO_3^- in this case will be determined by the CO_2

tension and controlled by the respiratory system. In chronic acid-base abnormalities the kidneys are of course important in the restoration of the normal acid-base state.

Realizing these implications, Van Slyke and I collected together what data we had, and could find in the literature, on blood pH and $[HCO_3^-]$ in acidosis not due to CO_2 accumulation. Our objective was to see whether the body, in adjusting to and ridding itself of an excess of protons, tried to keep the plasma pH as closely as possible to pH = 7.4 or the blood CO_2 tension close to 40 mm Hg. Unfortunately, there was not much good data of this kind on arterial blood, but there was enough for us to decide that the body strikes a compromise between the two—it seemed to be interested in keeping both of these parameters as nearly normal as possible.

So I left the Institute for a professorship at the University of Chicago in 1926 with this unanswered question on my mind. Shortly after I arrived there, I had as a graduate student, the late Professor Henry N. Harkins, who undertook to determine in anesthesized dogs the pathway taken by the acid-base balance of the arterial blood after the intravenous injection of HCl in rather large amounts. When the data were plotted logarithmically on triaxial coordinate paper, it was clearly apparent that the path taken by the acid-base balance of blood in recovery from acute HCl acidosis was much closer to an iso-CO_2 tension line than to an iso-pH line. However, since these were anesthetized dogs and the acidosis was produced quickly by intravenous injection, the question of man's response to slowly produced acidosis remained unsettled.

At this point, I had the good fortune to have another graduate student, Nathan W. Shock, who met the challenge of this question. Since we needed to obtain data on numerous samples of arterial blood from each individual, Shock first developed a method that permitted the determination of the CO_2, pH, and hematocrit on the same 0.1 ml of cutaneous blood, obtained by a finger prick. A comparison of the oxygen saturation and acid-base balance of cutaneous and arterial blood showed no significant differences.

After Shock had determined the variations to be expected in pH, $[HCO_3^-]$ and pCO_2 in 56 normal individuals, he proceeded to study the pathways of acid-base displacement and of recovery in 38 experiments on 7 subjects (14). Metabolic acidosis was produced by the ingestion of NH_4Cl, metabolic alkalosis by $NaHCO_3$, respiratory acidosis by rebreathing into a spirometer allowing CO_2 to accumulate slowly, respiratory alkalosis by overventilation until the blood CO_2 tension reached 20 mm or below. We again plotted the results on triaxial coordinate paper. The displacement and recovery paths of CO_2 excess were not appreciably different from those predicted from the titration of blood with CO_2 in vitro. (These were of course acute experiments that were over in half an hour, and did not last long enough for kidney involvement.) The displacement path of CO_2 deficit also followed the in vitro CO_2 titration curve closely. However, the return pathway was displaced toward the path of acid excess due to the entrance of

lactic acid into the bloodstream, if the subject had overventilated to the point of tetany.

In the subjects who had ingested NH_4Cl, the path of acid-base displacement from normal took from 2 to 3 hr to reach a maximum and tended to follow a constant CO_2 tension line. Recovery took several hours and the acid-base path was either along an iso-CO_2 tension line or much closer to such a line than it was to an iso-pH line. In the experiment with $NaHCO_3$ ingestion, the same thing happened—the displacement path was along an iso-CO_2 tension line, the recovery path along a line closer to a constant CO_2 tension than to a constant pH. These four types of acid-base displacement were carried out on each of our subjects and involved 40–50 blood samples from each subject (15). This could hardly have been undertaken had Shock not devised the micro method.

I have dwelt at length on these experiments because they are the basis for the statement which I have frequently made that "The body is more interested in maintaining a constant CO_2 tension of the arterial blood than in maintaining a constant pH." However, such a statement has largely fallen on deaf ears and in spite of abundant evidence to the contrary, it is taught and widely believed that the body responds to acids and alkalis by either blowing off or retaining CO_2 until it brings the pH of the arterial blood as close to 7.40 as possible.

Of course, one encounters many pathological states (such as severe diabetic acidosis) in which the pH may be normal and the HCO_3^- and pCO_2 low, but this is the result of a combination of overventilation plus metabolic acidosis. If one corrects the metabolic acidosis of diabetes with alkalis or insulin, he finds he is left with a respiratory alkalosis from which it takes the patient a day or two to recover.

As the years have worn on and the evidence for the desire of the body to maintain a constant CO_2 tension has been ignored, I have come to the conclusion that it is easy to accept homeostasis with respect to pH because all enzyme activity and enzyme reactions are pH sensitive. But because we have been without a biochemical reason, it is not so easy to accept homeostasis with respect to CO_2 tension, except insofar as changes in it bring about changes in pH indirectly.

Again serendipity took a hand. After a lapse of some years, I returned to full-time laboratory work in 1959 at the Scripps Clinic and Research Foundation in La Jolla, California. As a rehabilitating project, I first undertook to repeat some experiments done by Dr. John M. Buchanan in my Harvard laboratory. This was to determine the effects of changing pH from 7.0 to 7.8 in a bicarbonate:CO_2 buffered medium, on the synthesis of glycogen from glucose by liver slices in vitro. For good measure, we also carried out the same experiment in a phosphate-buffered medium and in a tris-buffered medium, both without bicarbonate plus CO_2. We confirmed Buchanan's results in the bicarbonate medium but got no glycogen synthesis in the media without CO_2. This result led us to wonder whether the concentration of CO_2 in

the intracellular environment could play a metabolic role independent of its influence on intracellular pH. As a result, experiments were carried out over the years 1960–1966 with, successively, Drs. Dowdle, Mahowald, Fanestil, and Longmore to further document and explore this possibility.

The concentration of total CO_2 in our incubation medium was varied from a low value of about 10 m*M* to a high value of about 40 m*M* with the CO_2 tension so adjusted as to maintain a constant pH of 7.40 ± 0.05. In other words, we varied the CO_2 tension and the HCO_3^- concentration over a fourfold range, which was numerically equivalent to varying the pH from 7.0 to 7.6 ($[H^+]$ from 1×10^{-7} to 2.5×10^{-8}). We also studied the effect of changing pH between 7.0 and 7.6, both by varying the HCO_3^- concentration and by varying the CO_2 tension, independently. The important question of the extent to which the intracellular environment of the liver slices paralleled the $[HCO_3^-]$ and the pH of the incubation medium was determined by Dr. Fanestil who found that under the conditions of our experiments the two follow each other closely, because liver cells in vitro exchange ions rapidly.

We found that increasing the CO_2 concentration from 10 m*M* to 40 m*M*, at pH = 7.4 increased the glycogen synthesized by 60%. This turned out to be as great as the increase in glycogen synthesized when we held CO_2 tension constant and varied $[HCO_3^-]$ or vice versa, so that the pH rose from 7.0 to 7.6. Thus we concluded that $[CO_2]$, either as HCO_3^- ion or dissolved CO_2 molecules, exerted an enhancing effect on one or more steps between glucose and glycogen in rat liver in vitro (16). The questions of how and where this "CO_2 effect" occurred became the primary subject of my remaining laboratory activity.

For example, we found that the "CO_2 concentration effect" was equally as large when fructose was substituted for glucose; and with glycerol as substrate, it was even greater. With pyruvate as the substrate, there was good glycogen synthesis, but no increase due to increased CO_2 concentration. This made it unlikely that the "CO_2 effect" operated at the pyruvate carboxylation step. We narrowed the possibilities by finding that the first step of glucose and glycerol phosphorylation by ATP was stimulated by increased CO_2 concentration to about the same degree that glycogen synthesis was increased. It was also found that ATPase activity of liver mitochondria was markedly affected by the CO_2 concentration at constant pH (17). This is as far as we went in my laboratory with our search for a specific biochemical explanation of the effect of CO_2 concentration on carbohydrate metabolism.

Whether this involvement of CO_2 concentration, either as $[HCO_3^-]$ or CO_2 molecules, independent of their effect on environmental pH, will provide the reason for the body's attempt to maintain a constant CO_2 tension, remains for the future. For myself, I am pleased that some biochemical clues have turned up that point in that direction. If its only result were to be that biochemists will not be content to study enzymes and biologically sig-

nificant reactions in exclusively non-CO_2 containing environments, much more biological relevance will attach to their work.

Although much of our effort was expended on the effect of CO_2 concentration on carbohydrate metabolism, Longmore and I also looked into the effect of CO_2 on long-chain fatty acid synthesis from acetate (18). We knew in advance the chemical role that CO_2 plays, through carboxylation of acetyl-CoA. But whether variation in the concentration of CO_2 at constant pH, within the physiological limits of 10 and 40 m*M*, would have any influence on the rate of synthesis by liver in vitro, presented a challenge. Here, we found an even greater CO_2 concentration effect than we found on glycogen synthesis. The rate of long-chain fatty acid synthesis was four- to fivefold higher in the 40 m*M* CO_2 media than in the 10 m*M* media, pH being constant. And this was true for both the triglyceride and phospholipid fatty acids—but not for the cholesterol synthesized where carboxylation of acetate plays no significant role. Comparable experiments in which the pH was varied by varying the CO_2 tension at constant HCO_3^- concentration resulted in no change in the rate of fatty acid synthesis—whereas varying the pH by changing the HCO_3^- concentration at constant CO_2 tension gave results like those obtained by varying CO_2 at constant pH. This implicated the HCO_3^- as the species controlling the carboxylation reaction, under our experimental conditions.

Though these experiments added nothing new to our knowledge of the chemistry of fatty acid synthesis, they came as somewhat of a surprise in showing that changes in bicarbonate ion concentration over such a narrow range can be limiting and controlling on the rate of fatty acid synthesis in liver.

This concludes my experience with acid-base balance and metabolism. The measurement of the acid-base balance of the blood can now be done on very small samples; pH determinations can be done with a glass electrode on 25 μl amounts. Van Slyke's new micromanometric technique can be accurately performed on the same amount, pCO_2 values can be measured directly. A variety of nomograms are available for the conversion of the data to pCO_2 and buffer base (i.e., the concentration in equivalents per liter of the proton acceptors, HCO_3^- plus other titratable blood buffers, primarily hemoglobin). The interpretation of the deviations in pH, pCO_2, and buffer base from normal values in the interest of diagnosis and treatment of patients is now known and widely applied. Remaining for future investigation are the metabolic consequences at the biochemical level of the commonly occurring acid-base abnormalities. This is a fertile ground for exploration by biochemists, physiologists and clinicians. Had I not had the opportunity to work intimately with clinicians on their problems from 1921 to 1935, I doubt that I would have ever worked on these problems that have occupied so much of my laboratory life. Hopefully, the body's equal concern for the preservation of homeostasis with respect to CO_2 tension, as well as pH, will no longer be neglected (19).

Electrolytes, Isotopes, and Metabolism

I suppose my interest in electrolytes and their transport through membranes began at Michigan when I began to study the rate of transfer of water and ions across collodion membranes. It continued during my study of fatigue at Columbia, our study of blood as a physicochemical system at the Rockefeller Institute and our studies of water and electrolytes in tissues at Chicago and Harvard, and it played a determining part in our studies of intermediary metabolism in liver at Harvard and La Jolla.

When Dr. Lillian Eichelberger joined me at Chicago in the study of the water and electrolytes of skeletal tissue, I fully expected to be able to directly apply my knowledge of ion distribution gained through my experience with blood. As it turned out, nothing could have been more naive. We wasted most of our first year in experiments that were relevant only to dying muscle. Then we decided to revise our approach entirely, and work on the assumption that cells are not normally freely permeable to inorganic cations or anions as long as the muscle is well supplied with O_2. Though this assumption would not be justified on those terms today, it served us in good stead at the time. We also adopted the habit of expressing our results in terms of units of fat-free tissue. This was fortunate because our results could thereby be compared from one animal to another. We were now able to calculate the extracellular fluid mass, by equating it with the chloride space. From this we could estimate the mass of intracellular phase in the muscle and the intracellular concentration of potassium, sodium, and water.

After making a study of the mass and electrolyte composition of the extracellular and intracellular phases of the abdominal muscles of normal dogs, we studied the effects of experimentally produced edema and dehydration, and of acidosis and alkalosis, both metabolic and respiratory, on these parameters (*20–22*).

In 1935 I went to Harvard where Dr. Jeanne Manery extended these studies to many other tissues, the late Dr. William Wallace showed that muscle cells are not freely permeable to bicarbonate ions, and Dr. Oliver Lowry made a study of the effect of aging on mass and composition of the phases of muscle, heart, liver, kidney, and brain (*23*).

It was a direct result of Lowry's experiments with liver that directed our attention to the role that the composition of the intracellular ionic environment plays in metabolism.

In 1941 we had been trying, along with other laboratories, to achieve a net synthesis of glycogen from glucose by incubating liver slices in an incubation medium simulating extracellular fluids—but without success. We always ended up with less glycogen than we started with. Then Lowry showed me his results on incubating liver and muscle in an extracellular-type medium. The liver had rapidly exchanged its high intracellular potassium for extracellular sodium, whereas the muscle had done so relatively slowly The thought occurred: perhaps some step or steps in glycogenesis or glyco-

genolysis were influenced by whether the enzymatic ionic environment was predominantly potassium or sodium. This led to a series of comparisons by John Buchanan of glycogen synthesis by liver, in vitro, incubated in an extracellular-type medium and in an intracellular-type medium, containing K^+ and Mg^{++} at their concentrations in liver cells. This resulted in finding net glycogen synthesis in the K-Mg cation medium but none in the predominating Na cation medium (24). Later it developed that this result occurred partly because of the stimulating effect of sodium ions on glycogenolysis—but at the time, it provided us with an experimental system that made possible the study of factors affecting carbohydrate metabolism of liver, in vitro.

Though my 23 years at Harvard were so filled with teaching and administrative activities, both intramural and extramural, that I seldom got into my laboratory for extended lab work, there was always something new going on there. A steady procession of able graduate students and postdoctoral fellows, under the watchful eye of my assistant, Frances Nesbett, kept the place humming. Perhaps our greatest excitement occurred when we undertook the study of intermediary metabolism, using isotopically labeled substances.

This started with a suggestion made in 1938 to Professor George Kistiakowsky and me by Dr. J. B. Conant who was then President of Harvard University. It concerned the then unsettled question of how lactic acid was converted to glycogen. Since the Harvard cyclotron was expected to be operative soon, we proposed to prepare ^{11}C-lactate, administer it to a rat, and isolate and count the glycogen. This was done—but not without difficulty. Dr. A. K. Solomon, who had had previous cyclotron experience, would provide the physics knowhow, Dr. R. D. Cramer would provide the synthetic organic chemistry skills, and Dr. F. W. Klemperer, Dr. Birgit Vennesland, and I would do the biochemistry. John M. Buchanan also joined us shortly after we were under way. The reason for the need of so many hands was the 20 min half-life of ^{11}C, which gave us a scant 6 hr between the time the boron oxide target containing the ^{11}C came from the cyclotron and the time when there was nothing left to count. In the interim, carboxyl-labeled lactic acid had to be synthesized, purified, neutralized, and administered to a rat; time had to be allowed for synthesis of liver glycogen and its isolation and purification; finally, the glycogen had to be counted. Everything had to be done on a tight time schedule with no hitches, else we had nothing to show for our efforts.

The results were exciting from our first successful experiment onward in that we found that less than one-tenth as many of the lactate molecules appeared in glycogen as one would have concluded from strictly chemical analysis (25, 26). But our greatest surprise came when we found in the course of a control experiment with unlabeled lactate and $NaH^{11}CO_3$ that the glycogen contained labeled carbon that had been administered as HCO_3^-. This was hard to believe in mammals, and not until Dr. Vennesland succeeded in purifying, hydrolyzing, and making the glucosazone without loss

of activity, did we accept it as a fact (*27*). It is hard today to understand our skepticism in view of the already demonstrated Wood-Werkman reaction—but such is the tenacity with which man holds on to his so-called verities.

Jack Buchanan extended our work with lactic acid to the study of ^{11}C-labeled acetic, propionic, and butyric acids. Then the Harvard cyclotron went to war and our use of isotopes in further investigations of intermediary metabolism were suspended until carbon 14 with its more humane half-life of 5000 years became available.

This proved to be a powerful tool to explore the alternate metabolic pathways of carbohydrate metabolism in liver, muscle, and heart, and factors such as ions and hormones that quantitatively modify these pathways. About three dozen papers were published from our Harvard laboratory between 1947 and 1958 embodying our findings on these subjects, but in only four instances did I feel that my participation in the work justified my name appearing as the senior author. Whereas I rejoiced in the accomplishments of my younger associates, I envied them their opportunity to be the first to know whether the experiment had been a success or a failure. Citations of their work will be found in (*28–30*).

My interest in the relation of intracellular cations to metabolism did not end when I left Harvard for La Jolla. There, all of our liver slice experiments on carbohydrate metabolism were done in a high-potassium, high-magnesium medium and when we undertook the study of fatty acid synthesis from acetate, we used the same type of incubation medium. When we compared the effectiveness of this so-called "intracellular cation medium" with a typical extracellular, high-sodium medium, we again encountered striking differences: the former medium gave much greater fatty acid synthesis in both triglycerides and phospholipids than did the latter medium. Though this difference was partly due to the potassium, a large part of it was due to the high intracellular magnesium (*31*). At this point (1966), my laboratory life terminated and the question of what constitutes the optimum intracellular concentration of magnesium ions in liver and how it is maintained remains for future investigators to determine.

One theme has been ever present in my mind in my experiments: the biological unit is the cell plus its extracellular environment of proper mass and composition. This holds for associations of cells in tissues, organs or whole organisms. Though one may estimate that a single cell, living in the sea and without specialized organ systems, has at its disposal one million times its mass of extracellular fluid, man and mammals in general require but 20% of their cell mass as extracellular fluid. Part of this reduction in extracellular mass is made possible by the evolution of hemoglobin which, through its efficient transport of O_2 and CO_2, permits a reduction in mass by two orders of magnitude. The remaining reduction in extracellular fluid mass has been achieved through highly specialized organ systems such as the respiratory, cardiovascular, gastrointestinal, endocrine, excretory, and ner-

vous systems. Together, these may be viewed as equivalent to an extracellular fluid mass of four orders of magnitude. The basic biological unit and its environmental requirements for normal metabolic activity remain fundamentally the same, whether as single cells or multicellular organisms.

> So lives the heterogeneous man
> Product of ingenious plan,
> And hard though biochemists try
> He still defies their prying eye. (32)

Calcium and Calcification

The study of calcium and its biological ramifications has been a recurring interest ever since Henry Alexander Murray, then a recent Columbia M.D., proposed to me in 1919 that we join forces and "settle" the argument about what happens chemically in the blood plasma of dogs after removal of their parathyroid glands. It was known that they get tetany and die, and it had been reported by MacCallum and his associates years earlier that whole blood calcium concentrations were lower than normal during parathyroid tetany (33). This work had been confirmed by Howland & Marriott, but disputed by some other investigators (34).

We decided that what was needed were measurements of the calcium concentration of the blood serum (not the whole blood) before and at frequent intervals after thyroparathyroidectomy. Also, because it had been claimed that the tetany may have resulted from an alkalosis, we determined the pH and CO_2 of the plasma on the same blood samples. For good measure, we also determined the blood sugar, which had also been reported to drop after parathyroidectomy. We literally lived with the animals—night and day—so that we could obtain blood samples at the height of their tetanic attacks, which for some reason seemed to come at 3 A.M. For me it had to be an extracurricular activity.

The results were cleancut—the serum calcium began to fall within 5 hr after parathyroid removal and when it reached 6 or 7 mg calcium per 100 ml of serum, an attack of tetany occurred. It continued to fall to a minimum of 5 mg per 100 ml, where it remained until death some days later. No changes were found in blood sugar, pH, or CO_2 so hypoglycemia and alkalosis could be ruled out as contributing to the tetany. Our results were published in 1921 (35). Even though my role in this work was secondary, it inoculated me with the "calcium" virus" from which I have never recovered.

While I was at the Rockefeller Institute, any work that I did on calcium problems was again secondary to my principal activity, which was to get on with the description of the blood as a physicochemical system. So when Cecil D. Murray, better known as Mike, and a Fellow in Van Slyke's department in 1923–1924, suggested that we work on why bone calcified, our experiments were again extracurricular. My colleague, Dr. Julius Sendroy, joined us in this undertaking.

Mike Murray had become impressed with the work of Brønsted of Co-

penhagen on the solubility of difficultly soluble salts and proposed that we apply this physicochemical approach to the system, bone salt plasma. Our hope was to answer two questions: What is the calcium ion concentration in plasma and other body fluids? Is the blood plasma in equilibrium, supersaturated, or undersaturated with respect to bone salt?

So Murray, Sendroy, and I undertook to settle these questions by doing a few experiments "on the side." As it turned out, it grew into almost a full-time job for Sendroy after Murray left in 1924, and continued so until 1926. The results were finally published in three papers in 1927 (36, 37). The first was on "The Solubility of Calcium Carbonate," the second on the "Solubility of Tertiary Calcium Phosphate," and the third on the "Solubility of Calcium Carbonate and Tertiary Calcium Phosphate in Salt Solutions of Varying Ionic Strength and in Blood Plasma." We were not the only ones trying to use this physicochemical approach to explain calcification. Holt, La Mer & Chown of Columbia were simultaneously carrying out similar experiments with tertiary calcium phosphate, only, as the solid phase (38). When we learned that we had the same objectives, we compared notes only to find that we were reaching opposite conclusions. From our experiments on the equilibrium of serum with powdered $CaCO_3$ we had tentatively concluded that body fluids were probably in equilibrium with bone because the serum Ca did not change. On the other hand, from their experiments with $Ca_3(PO_4)_2$, they were of the opinion that serum was supersaturated with respect to bone because the serum Ca decreased after long equilibration. This led us to repeat and extend their experiments with $Ca_3(PO_3)_2$ before our publication. Needless to say, neither of us settled the problem of calcification, because of the irrelevancy of our experiments. We did, however, provide some new physicochemical data that extended the applicability of the Debye-Huckel theory to weak electrolytes of biological interest, and to the effect of ionic strength on the solubility products of calcite (later, also, of aragonite) and of tertiary calcium phosphate (in reality an apatite).

Before starting our equilibration experiments with $CaCO_3$, we realized that we would need to know how pK_1 and pK_2 of H_2CO_3 varied as a function of ionic strength at 38°C, in order to calculate the activity of $CO_3^=$ from pH and CO_2 measurements, under physiological conditions. Sendroy and I took time off to do this and found that $pK_1' = 6.33 - 0.5\sqrt{\mu}$ and $pK_2' = 10.22 - 1.1\sqrt{\mu}$, up to the physiological ionic strength of $\mu = 0.160$. This was published in the *Journal of Biological Chemistry* in 1925 (39). It is the publication of which I am proudest, and perhaps the least known.

A word of explanation on why we first chose to equilibrate blood serum with calcium carbonate. It was simply because textbooks of that day said that bone salt was a mixture of 15% $CaCO_3$ and 85% $Ca_3(PO_4)_2$. There were also those who said that the calcium phosphate portion might be a mixture of $CaHPO_4$ and $Ca_3(PO_4)_2$. The reason for such conclusions was that all we had in 1924 were gross analyses of bone for CO_2, Ca, and inorganic phosphate. Since Murray, Sendroy, and I accepted as fact the statement that

$CaCO_3$ existed in bone as such, we deliberately decided to use it as our solid phase because we felt we were on firmer ground in calculating $CO_3^=$ activities than we would be in calculating $PO_4^=$ activities. As it turned out, Sendroy and I also studied the effect of ionic strength at 38°C on pK_1', pK_2', and pK_3' of H_3PO_4 before we were through.

In retrospect, it is hard to understand our naiveté in undertaking such exacting and elaborate experiments without first knowing what the solid phase of bone really is, and what the calcium ion concentration of serum is. Answers to both these questions were to come later after I was at the University of Chicago. Both answers came through serendipity.

First, regarding the solid phase of bone. One day at lunch, in 1928, I happened to sit next to a friend in the physics department, Jared Morse, who was studying crystal structure through X-ray diffraction. "Why do you look so depressed, Baird?" he asked. "You'd be depressed, too, if you'd just read that blood serum is undersaturated with the bone salt," I replied. I went on: "We think it's in equilibrium. Another group thinks it's supersaturated. Today's paper completes the possibilities. But none of us knows what the solid phase really is. It isn't crystalline." "Don't be absurd," Jared said. "Every solid's got structure to X rays. Bring some of your bone powder over this afternoon and we'll see what it is."

Thus began a collaboration with Jared Morse and H. H. Roseberry (one of his students) that quite quickly dispelled the idea that the bone salt is a mechanical mixture of $CaCO_3$ and $Ca_3(PO_4)_2$, or that there was a detectable amount of $CaHPO_4$. All that we ever got on our films of X-ray diffraction patterns was evidence that the bone salt is an apatite—presumably a carbonate apatite (40). Taylor & Sheard were making similar observations at the Mayo Foundation at the same time and reached the same conclusions we did. Had we both followed the foreign literature more closely, we would have known that deJong had anticipated us both (41). However, the important thing was that now we had a better idea of what the bone salt is—and that neither we who had shaken serum with $CaCO_3$ nor those who had shaken it with $Ca_3(PO_4)_2$ or $CaHPO_4$ had obtained data of physiological relevance. As of the present writing, arguments still go on as to whether the $CO_3^=$ is in, or on the hydroxyapatite crystal.

Now that we had the solid phase more or less settled, there remained the question of the calcium ion concentration of plasma. We had pretty good evidence in our 1926 $CaCO_3$ paper that the concentration of total calcium in body fluids was linearly related to their protein concentration. Indeed, we had an empirical equation that expressed the relationship:

$$[Ca] \text{ mmoles/kg } H_2O = 0.014 \text{ [protein] g/K}_gH_2O + 1.4$$

But we were so convinced that something in addition to serum proteins was involved that we belittled this relation. As it turned out later, 1.4, the intercept at zero protein concentration, is close to the actual $[Ca^{++}]$ of serum. We missed its significance at the time, however, and it wasn't until 1932 that

serendipity gave us another chance to settle this question. This time it was Dr. Franklin C. McLean who joined me and solved this problem.

It came about this way: McLean had brought me to the University of Chicago in 1926 as one of the new full-time medical school staff. In 1932 when I knew that he was giving up his administrative position as Director of Clinics, I lured him into my Department of Medicine laboratory to help me set up a rabbit heart perfusion, which he had learned to do in Otto Loewi's lab. Once set up, we moved it into a small 38°C laboratory where we proceeded to study the effect of Ca^{++}, K^{+}, and H^{+} on the electrocardiogram and mechanical work of the perfused heart. We were just beginning to get new and quite interesting results when, through an administrative decision, our experiments were transferred to the Department of Physiology. The only trouble was that Physiology had no 38° room, so we could not continue our experiments with the rabbit heart. McLean remembered that Straub had shown in 1912 that contraction of the frog heart was sensitive to small changes in calcium concentrations in perfusion fluids (42). "Why not continue our ion studies on the frog heart and forget about the ECG? The frog heart doesn't need a 38° room." So that's how it came about that we were able to determine the calcium ion concentration of mammalian blood serum under normal conditions, in hypo- and hyperparathyroidism, and in hypo- and hyperproteinemia. It was McLean who came in one day with the idea that perhaps the relation between calcium ions and plasma proteins was really just the dissociation of a partially ionized calcium protein salt (now regarded as chelated calcium) and that the mass law would quantitatively describe the relationship. When put to the test, this proved to be the case, and took the form:

$$\frac{[Ca^{++}] \times [\text{protein}^{=}]}{[\text{Ca proteinate}]} = K = 10^{-2.22}$$

We prepared a nomogram from which one could read off the $[Ca^{++}]$, providing one knew the total calcium and protein concentration of serum. This has found wide use in clinical medicine, because the $[Ca^{++}]$ is the only chemical species of serum calcium that has biological significance (43, 44).

Franklin McLean continued to study calcium metabolism and the physiology of bone until his death at age 80 in 1968. He became so well known and internationally honored for his work in this field that his prior important contributions to medical education as the administrator and guiding hand at the Peking Union Medical College (1916–1923) and at the University of Chicago Medical School (1923–1932) were almost forgotten.

So, finally, ten years after Murray, Sendroy and I naively undertook to settle with a few experiments whether or not blood plasma was in equilibrium with the inorganic bone salt, the necessary information about the state of calcium in the solid phase and the state of calcium in the liquid phase was in hand. But even if we had had it, I doubt that we would have materially advanced our understanding of how calcification occurs by our approach.

The simple laws of physical chemistry are inadequate by themselves to account for calcification and for Ca^{++} homeostasis.

As the years have passed, we have learned to accept that serum and other accessible extracellular body fluids have calcium ion concentrations that are remarkably constant at 1.2–1.3 mmoles per liter. If it drops much below this value, the parathyroid hormone brings it back; if it rises above this value, the recently discovered ultimobranchial cell hormone, calcitonin, lowers it to normal. The actions of these hormones have nothing to do with the solubility products of $CaCO_3$, $Ca_3(PO_4)_2$, $CaHPO_4$, or hydroxyapatite. As far as $CaHPO_4$ is concerned, body fluids are undersaturated; as far as $Ca_3(PO_4)_2$ or hydroxyapatite are concerned, they are supersaturated and would remain so unless they were seeded. The most intriguing development in recent years has been the evidence presented by Dr. Melvin Glimcher that collagen has a spatial structure such that it can serve to initiate hydroxyapatite crystal formation (45). Crystals so formed do not grow indefinitely until equilibrium is established, but are limited to a few molecular units because their surfaces become distorted by adsorption of other ions such as $CO_3^{=}$, Na^{+}, and $citrate^{\equiv}$. To get more hydroxyapatite formation requires a new collagen nidus. Whether this view of calcification will stand the test of time remains to be seen. At all events, the adventures of calcium in the body are still my favorite extracurricular subject.

With this I conclude my account of my primary laboratory adventures. There are numerous others on other subjects which I had in company with students and colleagues at home and abroad, which brought me much pleasure and new knowledge in the doing. Were I to relate all those that we explored together, this chapter would far exceed the bounds allotted to it. My indebtedness is especially great for the privilege of working in the laboratories of Otto Warburg (1925), Hsien Wu (1930), K. Linderstrom-Lang (1939, 1950), Sir Rudolph Peters (1952), Sir Hugh Ennor (1962), and R. A. Phillips (1962). Each of these experiences broadened my biochemical outlook and resulted in life-long friendships which have enriched my life. And so, I close this brief and personal view of the liquid and solid phases of "Man: a Heterogeneous System."

Extracurricular Activities

Though I have never held a full-time government position except during the years 1917–1921, I have had the privilege of part-time service with several agencies. The one that had the greatest consequences for me was my appointment by President Roosevelt in June 1941 to the Committee on Medical Research (CMR) of the newly established Office of Scientific Research and Development (OSRD). Dr. Vannevar Bush was the director of OSRD. The late Professor A. N. Richards of the University of Pennsylvania was Chairman of our Committee; other civilian members were Dr. Lewis Weed, Dean and anatomist of Johns Hopkins, and Dr. A. R. Dochez, bacteriologist

of Columbia. The representatives of the Service were Brig. Gen. J. S. Simmons for the Army, Rear Admiral Harold Smith for the Navy, and Dr. R. E. Dyer for the Public Health Service.

Our Committee met regularly in Washington from Wednesday through Friday each week for four and a half years. My colleague Professor Eric G. Ball served as Administrative Head of our Harvard department through most of this period, with great wisdom and effectiveness. For his wartime contributions and for his continuous, effective, unselfish service to Harvard and to our department of Biological chemistry ever since 1940, he can never be adequately repaid.

The mission of CMR was to mobilize civilian medical research resources for the solution of medical problems of importance to the armed forces. Because the Division of Medical Sciences of the National Research Council, under the chairmanship of Dr. Weed, was already organized for wartime problems, it became the primary advisory body to our Committee. Dr. Chester S. Keefer was appointed our Medical Administrator and under him were a number of divisions, each headed by an experienced medical scientist. The problems that confronted us at the beginning were the usual military ones of improved treatment of burns, wounds, and shock. We also needed better antimalarials, anti-infectious agents, protection against chemical warfare agents, better insect repellents and insecticides, and more knowledge about the physiological hazards encountered by fliers at high altitudes. Contracts were let to civilian scientists, mostly at universities, to obtain answers to these questions in the shortest possible time. This marked the birth of the "crash program" approach with organized teams of investigators in civilian institutions to obtain answers to specific medical problems in the shortest possible time.

In certain specific instances, such as penicillin, plasma fractionation, blood preservation, antimalarials, and DDT, this approach was highly successful. But in each instance the basic research had already been done, and what was really accomplished was more rapid development to the point of availability and usefulness.

When the war was over and it was time to terminate the activities of OSRD as a government agency, the Committee on Medical Research had over 200 active contracts. Except in a few instances, these were all transferred to the National Institutes of Health of the Public Health Service. These became the nucleus of the large and effective extramural program of the NIH which exists today. Without such government support, the postwar growth of the medical sciences in general, and biochemistry in particular, might never have occurred, or would at best have been much delayed. Quite as important as the support of research programs have been the training and fellowship programs. These have given us the qualified research personnel in our medical schools and research institutions that have made their growth and effectiveness possible.

It was my privilege to serve as a member of four of the Councils of the

National Institutes of Health: Cancer, Health Advisory, Arthritis and Metabolic Diseases, and Heart. This experience during the period of rapid expansion of the extramural program of the NIH gave me the opportunity to evaluate it as it grew. From the outset, the administration of NIH emphasized that the program should be that which the nation's scientists identified as worthy of support. Decisions on the scientific merit of applications have been made by the peers of the applicants. It is to the credit and glory of the Public Health Service that this principle of operation has persisted in spite of the growth of the research program to 200 times its size 22 years ago. The advances in the medical sciences made since World War II bear adequate testimony to the wisdom and power of this method of administration. The tax dollar of the public has never been better spent.

During the postwar years, in addition to my service for the Public Health Service, I was a member of the committee on Biology and Medicine of the AEC, of the Scientific Advisory Board of the Walter Reed Army Institute for Research, a trustee of Associated Universities, Inc., and of a number of other committees that had responsibilities for medical research and teaching. For a time, the deficit in scientific personnel was our greatest concern and I made the development of graduate and postgraduate education opportunities my primary objective. Today, in spite of great increases in the number of fellowships and traineeships at all educational levels, the need exceeds the supply. The trained scientist is one of our country's most valuable assets and we cannot afford to neglect his production.

Though my personal research participation suffered from all these extramural activities, I have never regretted the time I devoted to them. I had had no responsibilities except those of research until I was 40, and it was high time that I paid my debt for this privilege.

Moscow, 1944.—In January 1944, I went to Russia for the Committee on Medical Research of OSRD, for the purpose of initiating an exchange of medical research information between Russian medical scientists and their American, British, and Canadian counterparts. The late Lord Florey accompanied me and we had as colleagues Dr. M. B. Shimkin from the National Institutes of Health and Dr. A. G. Sanders of Oxford University. We spent a month in Moscow and met, in all, about 100 Russian medical scientists, many of them in their laboratories. The reception we received was both open and friendly, and we returned with the impression that as far as the medical research subjects we discussed were concerned, there was little reticence about open exchange of information. What reticence we encountered was on subjects on which they were either not working or on which they had done little.

It was there that we first made the acquaintance of Academician Engelhardt who demonstrated his actomyosin filaments with ATPase activity on which he and his wife were then working. On another occasion, we met Professor V. Parnas of Lwow, the progenitor of biochemistry's phospha-

gens and a great teacher of biochemists. He was present on our visit to the Blood Research Institute in Moscow, when I displayed the preparations of plasma protein fractions packaged for their respective uses: concentrated plasma albumin, fibrinogen, thrombin, fibrin film and fibrin foam, gamma globulin, concentrated hemagglutinins, etc. These were the so-called Cohn fractions, the separation of which had been developed by the late Professor Edwin J. Cohn and his colleagues at Harvard. He had undertaken this fractionation of human plasma proteins at the request of our government and had accomplished it expeditiously because of his thorough study of the physical-chemical properties of amino acids, peptides, and proteins over a score of years—studies which to many had seemed to be without practical value. As I concluded my presentation, Professor Parnas remarked with emphasis, "Ah, there is nothing so practical as sound fundamental work." The importance of Edwin Cohn's basic studies on proteins could receive no higher accolade.

Shortly after the war, Shimkin and I published in *Science* (46) an abridged account of our 1944 trip and closed with the statement:

> Soviet scientists are progressing soundly along many lines also being developed by our investigators. Both they and we would profit by greater familiarity with the work going forward in both countries.

As a step in this direction of exchange of information, we arranged to subscribe to 20 American medical journals until the end of the war and have them delivered through the Embassy to the Central State Medical Library. They in turn agreed to reciprocate in kind. I am pleased to say that both of these commitments were carried out. However, we were unsuccessful in arranging for any further exchange of scientists to either country—only long after the war was over were our hopes for exchange visits by scientists realized.

This trip was carried out in the midst of the war and took altogether three months to complete. It was my only venture into diplomacy. My memories of it remain those of friendly frankness on the part of the Russian scientists and the universality of the approaches to the problems we discussed. My one regret is that the relations between our two countries were such for a number of years that their scientists and ours could not work together toward solutions to problems of benefit to men everywhere.

My Latter Days

When the time came in 1966 for me to "retire" for the second time I moved to the Department of Neurosciences of the University of California at San Diego, with my quarters temporarily at the Scripps Institution of Oceanography. My neuroscience and oceanographic associates have opened my eyes to two new worlds which I had previously never known. It is a wonderfully stimulating experience to be confronted with two such widely different and challenging frontiers. Were I just beginning my research I would be hard put to make a choice between them. Though my active labo-

ratory days seem to be over, I find daily excitement and stimulation through being sometimes among colleagues to whom all aspects and forms of life in the sea are challenges, and sometimes with those whose concern is with that highest evolutionary development, the nervous system. Although I cannot aspire to being a neuroscientist myself, I find that the principles that I have learned through my study of blood, bone, muscle, and liver are applicable to the study of that wondrous medium of communication.

I even have the occasional opportunity of teaching medical and oceanographic students. This makes these twilight days full and rewarding. As I review my adventures over half a century, I prize those years which gave me full time for research, but I value, even more, those years in which teaching others was intermingled with my laboratory life. Were I asked to evaluate my preference I would without hesitation choose and recommend a career of teaching and research. Both benefit by being equal responsibilities; one without the other tends toward sterility.

My final foreign university experience occurred because my new office at the Scripps Institution of Oceanography would not accommodate my books and journals. At the suggestion of my Scripps Clinic colleague, Dr. Youssef Hatefi, who had served as Provost of Pahlavi University, Shiraz, Iran, I presented most of my small library to their new medical school. Though I miss them daily, I am comforted by the knowledge that, though dated, they fill a need and find constant use in their new home. The books and journals were installed in a room easily accessible to the staff and students of the basic medical sciences as a small working library. In 1967, Mrs. Hastings and I spent a month in Shiraz as the guests of the University where I gave lectures to the medical students and to the staff and where the Shah was present for the opening of the library. I was impressed with the high quality of the students and staff, and it was good to serve again as a teacher of those principles that govern man viewed as a heterogeneous system. I felt very much at home.

But the most memorable part of my trip to Iran was the visit we made to Persepolis, the ancient capital of Persia, near Shiraz. Much of this impressive city, magnificent in its architecture, magnitude, and setting, still stands in spite of the ravages of Alexander and time. As I looked at the tombs of Darius and his successors, fragments of Xenophon's Anabasis which I had read in Greek in high school came to mind. My memory is that the armies marched parasang upon parasang, to and fro, "Kai Kata Gen, Kai Kata Thalattan" (both by land and by sea). I seemed to see Miss Marthens and Miss McClellan waiting there for me to give them an account of my Anabasis. All I could say was: "I have come 'Kai Kata Gen, Kai Kata Thalattan.'"

If these meandering reminiscenses seem dated and only a collection of unrelated biochemical experiences, it should be remembered that they are history and are indications of how far we have come in biochemical knowledge in this century. Nor are they unrelated experiences as viewed by my per-

spective. Each begat the other, and though at times they seemed to be separate and distinct, eventually they proved to be related. At the least, they gave me new insight into the composition and biochemical behavior of the organism and its parts.

In retrospect, my life seems to have been a succession of happy accidents studded with friendships. Trained originally as a physical chemist, then as a physiologist, I am indeed a biochemist in name only. There are those who might say that I've earned my living all my life by measuring pH—and they wouldn't be far from right.

I only hope that those of you who are young and chance to read these words, encounter such friends and have as much fun in labs as I. Cooperation, communication, and competition have put man on the moon. There is no reason today why you shouldn't "hitch your wagon to a star."

LITERATURE CITED

1. Hastings, A. B., *Am. J. Physiol.*, **49,** 134 (1919)
2. Hastings, A. B., Mahowald, T. A., Fanestil, D. D., Longmore, W. J., *Fed. Proc.*, **21,** 82 (1962)
3. Hastings, A. B., *Public Health Bull.*, **117** (1921)
4. Hastings, A. B., *J. Biol. Chem.*, **46,** 463 (1921)
5. Van Slyke, D. D., Hastings, A. B., Heidelberger, M., Neill, J. M., *J. Biol. Chem.*, **54,** 481 (1922)
6. Van Slyke, D. D., Hastings, A. B., Neill, J. M., *J. Biol. Chem.*, **54,** 507 (1922)
7. Hastings, A. B., Van Slyke, D. D., Neill, J. M., Heidelberger, M., Harington, C. R., *J. Biol. Chem.*, **60,** 89 (1924)
8. Henderson, L. J., *J. Biol. Chem.*, **41,** 401 (1920)
9. Van Slyke, D. D., Wu, H., McLean, F. C., *J. Biol. Chem.*, **56,** 765 (1923)
10. Van Slyke, D. D., Hastings, A. B., Murray, C. D., Sendroy, J., *J. Biol. Chem.*, **65,** 701 (1925)
11. Hastings, A. B., Sendroy, J., *J. Biol. Chem.*, **61,** 695 (1924)
12. Van Slyke, D. D., Cullen, G. E., *J. Biol. Chem.*, **30,** 289 (1917)
13. Van Slyke, D. D., *J. Biol. Chem.* **48,** 153 (1921)
14. Shock, N. W., Hastings, A. B., *J. Biol. Chem.*, **104,** 565; 585 (1934)
15. Shock, N. W., Hastings, A. B., *J. Biol. Chem.*, **112,** 239 (1935)
16. Hastings, A. B., Dowdle, E. B., *Trans. Assoc. Am. Physicians*, **73,** 240 (1960)
17. Fanestil, D. D., Hastings, A. B., Mahowald, T. A., *J. Biol. Chem.*, **238,** 836 (1963)
18. Longmore, W. J., Hastings, A. B., Harrison, E. S., *Proc. Natl. Acad. Sci.*, **52,** 1040 (1964)
19. Hastings, A. B., Longmore, W. J., *Advan. Enz. Regulation*, **3,** 147 (1965)
20. Hastings, A. B., Eichelberger, L., *J. Biol. Chem.*, **117,** 73 (1937)
21. Eichelberger, L., Hastings, A. B., *J. Biol. Chem.*, **118,** 197 (1937)
22. Eichelberger, L., Hastings, A. B., *J. Biol. Chem.*, **118,** 205 (1937)
23. Hastings, A. B., *Harvey Lectures*, **36,** 91 (1940)
24. Hastings, A. B., Buchanan, J. M., *Proc. Natl. Acad. Sci.*, **28,** 478 (1942)
25. Conant, J. B., Cramer, R. D., Hastings, A. B., Klemperer, F. W., Solomon, A. K., Vennesland, B., *J. Biol. Chem.*, **137,** 557 (1941)
26. Vennesland, B., Solomon, A. K., Buchanan, J. B., Cramer, R. D., Hastings, A. B., *J. Biol. Chem.*, **142,** 371 (1942)
27. Vennesland, B., Solomon, A. K., Buchanan, J. M., Hastings, A. B., *J. Biol. Chem.*, **142,** 379 (1942)
28. Renold, A. E., Ashmore, J., Hastings, A. B., *Vitamins Hormones*, **14,** 139 (1956)
29. Ashmore, J., Karnovsky, M. L., Hastings, A. B., *Radiation Biology and Medicine*, **29,** 738 (Addison-Wesley, Reading, Mass., 1958)
30. Ashmore, J., Cahill, G. F., Hastings,

A. B., *Recent Progr. Hormone Res.*, **16,** 547 (1960)
31. Longmore, W. J., Hastings, A. B., Harrison, T. S., Liem, H. H., *Am. J. Physiol.*, **212,** 221 (1967)
32. Hastings, A. B., *Perspectives Biol. Med.*, **8,** 84 (1964)
33. MacCallum, W. G., Voegtlin, C., *J. Exptl. Med.*, **11,** 118 (1909)
34. Howland, J., Marriott, W. McK., *Quart. J. Med.*, **11,** 289 (1917)
35. Hastings, A. B., Murray, H. A., *J. Biol. Chem.*, **46,** 233 (1921)
36. Hastings, A. B., Murray, C. D., Sendroy, J., *J. Biol. Chem.*, **71,** 723 (1927)
37. Sendroy, J., Hastings, A. B., *J. Biol. Chem.*, **71,** 783; 797 (1927)
38. Holt, L. E., Jr., La Mer, V. K., Chown, H. B., *J. Biol. Chem.*, **64,** 509, 567 (1925)
39. Hastings, A. B., Sendroy, J., *J. Biol. Chem.*, **65,** 445 (1925)
40. Roseberry, H. H., Hastings, A. B., Morse, J. K., *J. Biol. Chem.*, **90,** 395 (1931)
41. de Jong, W. F., *Rec. Trav. Chim.*, **45,** 445 (1926)
42. Straub, W., *Verhandl. Ges. deut. Naturforsch. Aerzt.*, **84,** 192 (1912)
43. McLean, F. C., Hastings, A. B., *J. Biol. Chem.*, **107,** 337 (1934)
44. McLean, F. C., Hastings, A. B., *J. Biol. Chem.*, **108,** 285 (1935)
45. Glimcher, M. J., Les Tissus Calcifiés, *V European Symp.*, **3** (1968)
46. Hastings, A. B., Shimkin, M. B., *Science*, **103,** 605, 637 (1946)