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The invitation of the Editorial Committee to write a prefatory chapter deeply affirms my long commitment to biochemistry. I feel honored to be the first woman, I trust the first in a long succession, to be so chosen. My preoccupations with the intermediary metabolism of amino acids and proteins began in 1937 when research laboratories in this country were occupied with the chemistry of amino acids and with nutritional studies. Major contributions were being made on the functional role of vitamins in nutrition and in elucidation of the chemical structure of vitamins. In the field of sterols, activity was reflected in major contributions to the nature of vitamin D and to the structure and action of the estrogenic hormones. Not until I became thoroughly immersed in research on amino acid metabolism did I catch up with the biochemical developments in European and English laboratories emerging from the elucidation of the function of vitamins as cofactors in enzymatic catalysis, thus stimulating and making possible the pursuit of in vitro approaches to oxidative metabolism that was soon to follow.

The earliest in vitro approach to the general problem of nitrogen metabolism was accomplished by Hans Krebs in 1932 in studies on urea formation with surviving liver slices which led to his far-reaching concept of a metabolic cycle pulled by arginase, whereby urea is formed from NH_3 and CO_2 in a stepwise manner through the agency of carrier compounds participating in the turnovers of the cycle. This was soon followed by Kreb's work on the oxidative deamination of L-amino acids and D-amino acids. I have always looked on these studies as closely related approaches to the two most basic questions in nitrogen metabolism. Decades elapsed before all the implications of Kreb's observations could be fully realized, or for that matter before the metabolic significance of the work of Euler and Adler on glutamic dehydrogenase in 1938, or that of Braunstein and Kritzmann on transaminase in 1939, could be fully understood.

Then there were, starting in 1937, the stimulating studies on the intermediary metabolism of amino acids and proteins by Schoenheimer and his collaborators with ^{15}N-labeled compounds. Following after, came the increasingly abundant and magnificent elucidations of biosynthetic pathways in amino acid metabolism that were accomplished, in the biochemical studies of the next three decades (1), through enzymology and mastery of reaction mechanisms and that are actively continuing at the present time. Now we are provided with detailed chemical knowledge of intracellular events and some approximation of metabolic complexity within the cell and within the intracellular compartments. Biochemistry is approaching closer to its goal.

Not all of it burst forth at once. For me, as for any biochemist given so long a span to view, the intellectual excitements sprung not alone from linking metabolic events to biological processes; as much derived from appreciation of conceptual and experimental changes marking the uneven progress of a problem from its earliest recognition, through successive stages, before attaining resolution, or going on to a second and a third resolution. The experimentalist carries about a growing inventory of mental imprints to which frequent extensions are made. These are continuous records of the irregular progressions and ideological contingencies that mark the pace of progress. In this remarkably productive span of forty years, the imprints linking recognition to solution have frequently become buried in a host of new questions.

EARLY YEARS

It is far easier to explain how I became interested in nitrogen metabolism than to explain how I became a biochemist, other than to say I grew into biochemistry. It is not a simple matter to break self-imposed habits of

personal restraint or to overcome a natural reticence, yet we have come to acknowledge that early influences are strongest in shaping our lives and the directions that scientists follow are of interest to other scientists. As I sift out personal family influences and the emotional, human, aspects of family circumstances, so closely do they accord with the patterns of motivation we now accept that I begin to wonder whether my memory has not selected too well among my childhood recollections.

To start further back, my parents, to avoid persecution as Jews, emigrated to New York from a poor Russian village well before the turn of the century, arriving separately, the home builder first, mother and child following later. By the time I was born, in 1903, my father had managed to establish a manufacturing business here, and except for two interruptions, I was raised and educated, and have worked, in New York City.

I grew up as an only daughter among four sons; my twin brother and I were the youngest by a gap of seven years. We were four or five years old when, during a brief period of prosperity, we moved to Mt. Vernon, the New York suburb, where our home was located in an open, countrylike area. My brother and I, sharing our friends, roamed about adventurously, unlike city children, and later attended an excellent and pleasant grade school nearby.

My mother, gentle and self-effacing, was very much taken up with seeing to family needs. In almost all matters she gave us her unquestioning support. I was always closely attached to her. My father was a person of many interests, independent opinions, and strong convictions; of course he greatly influenced me. He expressed his affection by teaching me to read before formal schooling began and, of an occasional evening when we were a bit older, he gave us arithmetic questions, chosen out of a Russian problem book, to be solved by reasoning.

Except for a few years of early Hebrew biblical studies, my father was an entirely self-educated man and an omnivorous reader and collector of Hebraica, and of the late 19th century classics in literature, philosophy, and history. Shelves full of books in several languages were among my earliest childhood memories. Choosing among the novelists, I spent many childhood hours reading Tolstoy, Dostoevski, Chekhov, Ibsen, George Eliot, Zangwill, Mark Twain, and others drawn from the public library.

Childhood memories include recollections of a close family friend of whom I was very fond. He was the head of the Section of Semitic Languages at the New York Public Library, and an incorrigible bibliophile by profession. He would arrive for a Sunday visit with the *New York Times* and a small pair of scissors tucked into his vest pocket to clip the book reviews he systematically perused; I then carefully examined and arranged them.

Our home library also contained simplified expositions on the great technical inventions: electricity, the telegraph and telephone, the incandescent bulb, and the internal combustion engine. These too were part of my childhood reading. Unlike most intellectual men of European birth, my father was mechanically inclined and managed, each time we moved, to find room for a home workshop where he made working models of ingenious devices of his own invention. As a fascinated child I often watched him of an evening. I grew up knowing how to use tools, although I was more occupied with the usual feminine skills.

About the time grade school was completed our family circumstances changed, and we returned to the city and to our former simpler way of life. The high school I attended in New York was a new and excellent one. There my choice of courses gave preference to sciences and mathematics. The physics course especially stands out. It was taught by a young man who had recently received his doctorate and who took great pains to set up and present very lucid, absorbing demonstrations. As the four years came to an end, I was without guidance in my desire to pursue a scientific education, except for feedback from older friends. In that way my wish to attend a university open to women centered on Cornell, with its excellent chemistry department and scholarship possibilities. Parental objections to my plans were later withdrawn upon the arrival of a letter announcing the award of a scholarship. I was the only one of their five children to choose an academic education.

At Cornell I selected a chemistry major with all the attendant requisites and puzzled my way through the course work. I would have liked to exchange impressions or to clarify some point with my fellow students but felt discouraged, being innately very shy. In most of my courses, with scarcely an exception, they were all men. It seemed to me they were bound by tradition to ignore coeds, but perhaps they too felt shy. Actually our interests differed since most of the chemistry undergraduates expected to find jobs in industry and many of the courses were therefore oriented in that direction. What I minded was the duality of my college days, for the friends I found were students of the liberal arts and I could not talk to them about my course work. The character of that great department has since undergone many changes. This was before the theoretical developments of atomic structure were to make chemistry more comprehensible to students. Later on, in smaller classes, I discovered that instructors welcome questions. As an undergraduate my exposure to organic chemistry was brief and left me without memorable impressions. During my last semester I managed to carry out a very minor research problem under Wilder D. Bancroft, whose lectures on colloid chemistry had provoked my interest, and I caught glimmerings of another world when I sat in on a few of the lectures given by James Sumner in the School of Agriculture.

Toward the end of college, in that time for decisions, neither medical school nor an industrial position seemed feasible or suitable. With my genuine interest in chemistry, and despite the long hours spent in afternoon lab sessions, I felt impatiently eager to see what the work in almost any laboratory would be like before making further decisions. My learning had been chiefly out of books and I did not see how I could extend it or make use of it. On returning to New York in 1924, my first job gave me experience in analytical chemistry but was uninteresting. My second brought me to a clinical laboratory where I became engaged in pediatric research for several years, and where I learned of the importance of electrolyte balance in body fluids. Through their bearing on pediatric problems, I read papers of Gamble, Ross, and Tisdall, of Stadie, of Hastings and Sendroy, and others. I had gained access to a library of biochemical and medical journals to feed a growing interest in what was then called physiological chemistry.

During this time I began to attend evening graduate courses in chemistry at Columbia University given by Becker, Hammet, Taylor, and Nelson and became familiar with the sort of research problems, including enzyme studies, that the graduate students were working on. A year or two later I was drawn back by Selig Hecht's lectures, vividly describing his biophysical studies on rhodopsin. These roamings sharpened my focus. It would be essential, if I were ever to move into the new areas for which biochemistry held out the promise, that I go on for a doctorate. My decision was framed in this way six or seven years after completing college.

Thus in the early 1930s, in the depth of the depression, I left my job when I was accepted as a graduate student by Hans T. Clarke into the Department of Biochemistry at the Columbia College of Physicians and Surgeons (P & S). I had entered at a good time, near the beginning of an era distinguished by remarkable advancements in which organic chemistry played a prominent role. I was to see that my good fortune in being the student of a most gifted organic chemist privileged me to come under the influence of his scientific aims and high standards. I gained immeasurably as I witnessed his freedom from prejudice in the selection of graduate students and gifted biochemists obliged to leave Europe in the 1930s for political reasons.

Since I entered graduate training in a medical school's biochemistry department, one of the traditional routes in this country, it is more than nostalgia that makes me think it of interest to describe the spirit of the department I found myself in. Clarke's method of choosing graduate students was a mystery on which we often speculated. As he has since described it in his prefatory chapter of 1958, he relied primarily on his own remarkable judgment, which was based entirely on a personal interview. The candidate, I will add, was informed of his decision before leaving his office. Though nowhere was there ever more than a whisper of administration, we quickly learned of the course work expected of us. In Clarke's

wisdom, physiology was included, for which I have always been grateful. Except for occasional discussions with him, his students were left alone to sink or swim, especially when there was little progress to report. The problems differed widely; the thesis work had to make a contribution acceptable to the *Journal of Biological Chemistry* or the *Journal of the American Chemical Society.* Clarke's discernment and influence netted a high yield for biochemistry. In the few pages of restrained pride he allowed to it in his prefatory chapter, his references to the accomplishments of his students were as unduly modest as he was himself.

All graduate students attended the course given to medical students; this was mitigated by having us all together in one large lab. Informality was encouraged and indeed we learned from each other. At one point, since neither student seminars nor graduate courses were held in the department, we organized one of our own, and invited the staff to lecture. We felt a part of the biochemical community when Clarke brought his distinguished visitors into the graduate lab to meet and chat with us; the visitors would often be Dakin or Vickery or du Vigneaud, whose papers we were familiar with.

So that I might complete my course work, Clarke, quite characteristically, cleared the way for a part-time job in the department. Thus I became involved in clinical studies on the follicular hormone and on a uterine contracting substance. As it turned out later, the report on that work constituted the first recorded description of the uterine contracting properties of a prostaglandin. It was characteristic of Clarke's interest in new biochemical developments that he allotted bench space to a practicing gynecologist interested in applying new hormonal developments to clinical problems.

To prepare us for a biochemical future, familiarity with the micro techniques of Pregl was acquired through practice analysis of new compounds, usually our own. As Clarke's student, a bit of his great skill as a glass blower rubbed off on me. I felt my way through two unfruitful areas before settling into my thesis work, a problem in amino acid chemistry on the interaction of formaldehyde with cysteine. In the course of establishing the structure of the stable compound thus formed, thiazolidine carboxylic acid, I learned a great deal about the chemistry and properties of amino acids. Some years later, after restriction on penicillin research was lifted, I learned how the properties characteristic of that thiazolidine contributed in some degree to the elucidation of the structure of penicillin. In my long retrospective view I can now add that it was not until 1965 that it became possible through the studies of Strominger to propose the complex mechanism of the antibacterial action of penicillin; those studies continue on. Research in biochemistry was extended into new areas by the gifted biochemists who came to P & S during the 1930s with Clarke's direct or indirect aid; the

contributions of R. Schoenheimer, K. Meyer, Z. Dische, E. Chargaff, and later H. Waelsch, are fruits of Clarke's generous humanity, his remarkable discernment and his stress on the importance of the chemically oriented viewpoint in the development of biochemistry. Our graduate lab confabs were even more enlivened by their occasional visits.

Late in 1936 when the time came to proceed to postdoctoral research in the academic world outside P & S, some time was lost in finding a suitable opening. Other students finishing up at this time were men, and they had been well placed. Several teaching positions were suggested in women's colleges, but in those years they lacked graduate teaching programs and research facilities. In time a research opening arose some distance from home which I accepted with reluctance because of my father's ill health. His loss a few months later and the need to assume responsibility for my mother's care were painful, traumatic experiences; they brought me back to New York.

ISOTOPE STUDIES ON THE METABOLISM OF AMINO ACIDS AND PROTEINS

At this time I had the professional good fortune to be invited back to P & S to work with Rudolf Schoenheimer. He had, two years earlier, launched a program with David Rittenberg on fatty acid metabolism, with deuterium labeling, which I had witnessed with great interest as a graduate student. Now he was preparing, with Rittenberg, to begin a study of amino acid and protein metabolism with ^{15}N labeling. Harold Urey gave his generous support to this highly original approach, for soon after his isotope discoveries he became very active in promoting the application of isotopes to biological problems. The keen anticipation that I felt in taking part in this work was accompanied by considerable trepidation at the prospect of handling the first precious ^{15}N-enriched samples entrusted to me. Rittenberg had just completed the construction of a mass spectrometer and no further delays were anticipated.

We began with the idea that the Folin hypothesis, which held that the main bodily constituents (fats, carbohydrates, and proteins) were metabolically inert except for a small amount of wear and tear, could be tested through the application of isotopic labeling. After methods were worked out for the synthesis of several amino acids containing ^{15}N in the amino group, the labeled amino acid was administered to an animal, and the metabolic fate was determined after isolation of the administered compound from acid hydrolysates of the proteins of body tissues. The results were entirely unexpected and we were in a stew of excitment. The labeled amino groups were found in many amino acids of body proteins, in addition to the one adminis-

tered. In consequence, only a portion of the ^{15}N in the given amino acid was found in excreted urea. Earlier attempts to determine the metabolic fate of amino acids were carried out with derivatives entirely foreign to the body. The first isotope results confirmed the idea that a natural amino acid could be tagged with ^{15}N without changing its physical or chemical properties. This gave biochemistry an entirely new tool with which to follow the fate of a suspected metabolite in the intact animal and to detect its participation in a multiplicity of hitherto unrecognized reactions. A number of different amino acids containing ^{15}N were given: the isotope was always incorporated into the amino groups of most protein amino acids, whether the isotopic compound administered was an amino acid or ammonium citrate.

As a participant in these studies with intact animals, I enjoyed the remarkable experience to be able to apply, with the aid of an isotope, the criteria of proof inherent in the approach of the organic chemist to the experimental testing of old and new concepts of metabolism. The black box was beginning to open. One could not have had a more illuminating introduction to the study of nitrogen metabolism.

In the course of the remaining few years of Schoenheimer's life, the results of many studies of this kind culminated in the formulation by Schoenheimer of the concept of *The Dynamic State of Body Constituents* in three Dunham Lectures given at Harvard (2). The evidence gathered in the span of a few years pointed to a rapid replacement, through synthesis and breakdown, of all bodily constituents as a continuous process, whether they be fatty acids or fats, or amino acids or proteins. It is scarcely possible to overestimate the importance of the concept of protein turnover to biochemical thought and of the allied concept of the metabolic pool. Protein turnover has since been much studied and expanded in function; because it is a process capable of multiple functions, a few questions remain to puzzle over. I learned invaluable lessons, for Schoenheimer possessed extraordinary biochemical insight and was gifted in devising highly imaginative yet beautifully simple experiments, designed to elicit a definitive answer to the question asked. It is for this reason, I believe, that the new concepts of nitrogen metabolism gained through isotope application exerted so great an influence on the development of biochemistry in the direction of intermediary metabolism. Few biochemists are now familiar with the original isotope findings, nor can they recall their very great impact (2, 3, 4).

In the course of those studies the continuity of biochemistry extended backward through organic chemistry, as well as forward, for in conjunction with isotopes and a mass spectrometer, I was adapting and making use of synthetic procedures of which several had been described decades earlier, in the 1890s. The numerous isolations of amino acids from protein hydrolysates involved laborious, pragmatic procedures. The number of specific amino acid salt-forming precipitants then available was limited, and ion

exchange chromatography had not yet been conceived. It was not difficult to plan, in half an hour, an experiment that took many months to complete. I was particularly taken up with a series of experiments designed to investigate amino group transfer with the essential amino acid L-leucine, into which I had introduced two isotopes, ^{2}H for the carbon chain and ^{15}N for the amino group. In this study we hoped also to add to the growing evidence pointing to a continuous rapid process of amino acid replacement in body proteins.

A number of body tissues were so investigated; some, like liver, showed a much faster rate of turnover than did muscle, which was among the slowest. Was there a distinction, were only certain proteins of specific function subject to turnover, while structural proteins remained inert, or were all bodily proteins involved in the turnover process? In addressing the question of specificity, proteins exhibiting some degree of functional specificity and having properties permitting selective isolation, such as the several plasma proteins and circulating antibody protein, were chosen for study. Only the first experiment of this series, conducted in collaboration with Michael Heidelberger, was in progress at the time Schoenheimer was writing his Dunham Lectures. The others had not been started when we received the shocking news of his death. He was striking and dynamic, as stimulating in daily collaboration as were his concepts for the future development of biochemistry.

The ensuing year was occupied with the completion of the protein turnover experiments and the writing of manuscripts relating to that work, and with the fate of D-lysine and D-glutamic acid. This was an ongoing interest, for which isotope labeling was admirably suited, in the metabolic fate of the unnatural isomers of amino acids. Our studies had shown earlier that D-leucine and D-tyrosine could undergo inversion to the natural form through reamination. In contrast D-lysine, unable to undergo reversible deamination, was largely excreted unchanged. In contrast to the highly reactive L-form, the fate of D-glutamic acid proved to be even more surprising; it resisted oxidative degradation and was almost entirely excreted in the form of the D-isomer of pyrrolidone carboxylic acid. Meister's later finding that ring closure is catalyzed by an enzyme showing specificity for the D- isomer has prompted speculation as to the metabolic function of the enzyme. Looking backward at enormous progress on bacterial cell wall structure, to assign a function related to the removal of cell wall degradation products seems the most satisfactory.

Several of our conclusions on amino acid metabolism were related to contemporary work by others in many ways: the growth studies of Rose, and of Berg, and the in vitro experiments of Braunstein and Kritzmann. Krebs' concept of the urea cycle was confirmed in a thought-provoking way:

> The arginine of the proteins has thus a double function. In addition to possessing significance as an important protein constituent, it participates in the urea cycle.
>
> In all the experiments in which isotopic ammonia and amino acids were fed, the isotope concentration of the amidine nitrogen of the protein arginine was always less than that in the corresponding urinary urea. This observation can be explained by assuming that an appreciable fraction of the newly formed arginine is directly broken down to urea, while the remainder is reincorporated into proteins.

I have quoted from the third of the Dunham Lectures (2) for the way in which this carefully worded interpretation keeps scrupulously within the limits allowed by the experimental design, and then reaches over to two of the newly found continuous processes: protein turnover and amino acid reutilization.

The period in Schoenheimer's laboratory left me with an abiding view of the broad aspects of nitrogen metabolism as a background and a guide.

STUDIES ON AMINO ACID OXIDASES

David Green returned to this country after several years in Gowland Hopkins' department in Cambridge when the United States entered the war. He settled in the Department of Medicine at P & S, one of several clinical departments that supported and housed research appointments in biochemistry. The war years had interrupted the flow of foreign journals and I had gone to Green's book *Mechanisms of Biological Oxidations* to bridge this gap, covering as it did the remarkable and highly significant advances in the flavoprotein and pyridine nucleotide enzymes coming out of enzymological research in European and English laboratories.

Green had again become interested in amino acid oxidation following on an earlier interest in D-amino acid oxidase and when, in 1942, he asked me to join him in studies on the oxidation of L-amino acids, I looked on this as an opportunity to continue my interest in nitrogen metabolism through enzymatic approaches. As a result of Krebs' original observations, and the work of others, it was known by then that D-amino acid oxidase was a flavoprotein. Krebs had also observed the oxidative deamination of L-amino acids in liver slices, but in his experiments this activity could not be separated from cellular structure.

In my change to new problems, the use of the ultraviolet spectrophotometer and the Warburg manometer were joined with organic chemistry.

During our association several flavoprotein amino acid oxidases came to light. Our most noteworthy accomplishment, the outcome of considerable study, was the isolation in 1945 of an L-amino acid oxidase from rat kidney

with broad amino acid specificity; some 12 or 13 amino acids were active as substrates. The prosthetic group proved to be riboflavin phosphate, two of which are bound per molecular weight of 120,000. Except for the old yellow enzyme of Warburg and Christian, this was the only other flavoprotein with riboflavin phosphate as prosthetic group. Our purified enzyme proved to have a very low catalytic turnover number.

More than a decade later, in 1957, work by Meister and collaborators, in which reversibility was achieved anaerobically with the very active L-amino acid oxidase from snake venom, made highly significant additions to the elucidation of the stepwise mechanism of dehydrogenation by flavin adenine dinucleotide (1).

With much reluctance, especially since I had enjoyed the work with a flavoprotein amino acid oxidase, I came to forego the idea that our enzyme, also present in the liver, might play a significant role in amino acid deamination. It was left then to explain the oxidative deamination of L-amino acids, observed by Krebs, as proceeding through transamination coupled to the dehydrogenation of glutamate, the suggestion of Braunstein and Byschkov in 1939. That this is a reversible pathway should, however, remain associated with their concept. During this period I had learned a great deal about enzymology and had become very eager to pursue new facets of nitrogen metabolism.

Green's enthusiasms were responsible, in 1943, for the start of a discussion group in New York, called the Enzyme Club, which brought together investigators of common interests and which still continues to meet. In 1946 toward the end of our collaboration, I was occupied in establishing the structure of a peptide from yeast containing *p*-aminobenzoic acid attached, through the carboxyl group, to a polyglutamic acid peptide chain of 10 or 11 residues. This turned out to be analogous to an essential component of the folic acid molecule, the structure of which was published simultaneously. Green, renewing an old interest in oxidative metabolism, was planning to start an Enzyme Institute at the University of Wisconsin.

EARLY STUDIES ON ARGININE AND UREA SYNTHESIS

Thus far my associations had been with investigators who were obtaining outside support from private foundations. Though I was much in favor of spending some postdoctoral years in association with gifted investigators, and I had been fortunate in this way, my hopes for an opportunity to explore new areas of nitrogen metabolism ran together with the awareness that appointments in medical schools were extremely rare for women. In periods of transition, young investigators should be allotted some time to putter

around, to get on and off bad tracks and on to good ones, in an interlude free of outside obligations. In the 1940s, a faculty appointment represented one of the few ways of providing such opportunities; research needs were far less costly than they are today.

At another crucial turning point, I was again fortunate to be asked to join the Department of Pharmacology at New York University College of Medicine by Severo Ochoa when he became chairman of that department in 1946. He succeeded James B. Shannon who had just been invited to NIH, where his ideas shaped the future of basic biomedical research. Much of the new work conducted by Shannon and his collaborators on antimalarial agents, during the war, had been developed in this department. We inherited two modern rebuilt laboratories, well designed for biochemical work, rebuilt others, and installed a cold room. For a newcomer the first task was to prepare for the pharmacology course. I began by devoting part of the summer to reading the lucid, monumental pharmacology text by Goodman and Gilman, perhaps the first comprehensive text to bring the old and the new pharmacology together.

Otto Loewi had joined the department a few years before, a refugee from the University of Gratz. We became great friends. In 1946 he was in his early seventies and actively investigating the effects of calcium ions on the contractions of the frog heart. For him, pharmacology, physiology, and biochemistry merged into each other. This was characteristic of his way of thinking, and of his rich scientific past. He was friendly and curious about the new research going on in the department, especially in my lab. Many years before, in 1902, he had carried out a decisive experiment on the origin of body proteins, since overlooked, in which he fed a protein autolysate to an animal and found that it successfully maintained the animal in nitrogen equilibrium. He rightly concluded that the free amino acids in the autolysate were being used for protein synthesis. I had the pleasure of hearing that account from him as well as dramatic and amusing accounts of his discovery of acetylcholine and of several other discoveries. In 1953, while he was drafting a retrospective chapter for *Annual Review of Physiology* (Volume 16), we enjoyed many discussions on the importance of stressing basic concepts, speculation, imagination, and intuition in scientific research, lest they disappear.

The war was well over in 1946, research and journal communications were restored, and the research atmosphere was optimistic. The stimulation of advances in cofactor structures on mechanisms of enzymatic catalysis, and the freshly realized advantages of working with broken cell suspensions, gave biochemists an opening into oxidative metabolism and coupled phosphorylation. Ochoa was an innovator in this field. The turnover of visitors, postdoctoral fellows of promise, and graduate students

reflected a general interest among young biochemists in the potentialities of investigating all areas of intermediary metabolism through the study of isolated systems. In that period, well before the great expansion of biochemistry, our lunch-time discussions were taken up with exchanges on some puzzling observations and on journal articles as new areas of intermediary metabolism began to unfold. Efraim Racker, then in the department of microbiology on the floor below, frequently joined us with a fresh supplement of interests.

While the teaching trimester was in progress, I puzzled over amino acid metabolism and urea formation. In our studies with ^{15}N-labeled amino acids, we had found invariably that the isotope was present in highest abundance in the amino acid administered. Glutamic and aspartic acids showed the next highest uptake of ^{15}N; the concentration found was almost as high as in the excreted urea. Transaminases were coming increasingly under study. The highly active preparations obtained by Green, Leloir, and Nocito in 1945, and by O'Kane and Gunsalus in 1947, were impressive, and reinforced my idea that the two dicarboxylic amino acids were as closely linked to urea formation as to the general turnover of amino groups among other amino acids. Meanwhile my experiments on urea synthesis, which began with respiring rat liver homogenates in Warburg manometers with citrulline and glutamic, or aspartic acids, and some cofactors, were soon changed to anaerobic studies with the idea that the process I was interested in did not, on chemical grounds, have to involve dehydrogenation; and then they were again changed, to take the direction I wished to follow in the enzymatic pursuit of urea formation. As new developments in coupled phosphorylation were rapidly emerging, I began to see that the less well understood, probably analogous, oxidative processes going on in respiring liver homogenates were interfering complications. To avoid them, I resorted to acetone treatment to inactivate oxidative metabolism. Refrigerated centrifuges and freezers were not yet available, nor were commerical sources of DPN, ATP, oxaloacetic acid, α-ketoglutaric acid, or any of the phosphorylated intermediates of glycolysis. All of these I had to prepare in the laboratory by isolation or synthesis. L-Citrulline had then to be synthesized also; only the racemic form was commercially available.

About a year later, I was able to send in a brief report on the conversion of citrulline to arginine and urea, proposing a new mechanism for the acquistition of nitrogen through a reaction in which citrulline condenses with aspartate with the utilization of ATP to form an intermediate which is then cleaved to form arginine and fumaric acid. The intermediate was the new amino acid, argininosuccinic acid, provisionally assigned the structure of a disubstituted guanidine. According to this mechanism one half the nitrogen of urea orginates in asparate, rather than NH_3. The ATP required

to form the new C–N bond explained the dependence on structure and respiration in Krebs' experiments.

The Williams-Waterman Fund of the Research Foundation was then supporting individual research projects. On the recommendation of Ochoa, the Foundation gave me support for one year in the amount of $3000, all but $500 of which was budgeted for technical help. I can attest to my inexperience in such matters. Although I was much in need of help in the lab and financial support, the application I submitted consisted of one brief paragraph. Thereafter the Growth Committee of the American Cancer Society came into existence, setting wise precedents in its policy of support for basic research and in reviewing procedures for grant applications. For the next seven or eight years I was their grateful recipient. Their support enabled me, at a fairly early stage of the work, to retain technical help and obtain equipment.

Since earlier reports from the laboratories of H. Borsook and P. P. Cohen had assigned the role of nitrogen donor to glutamate in the conversion of citrulline to arginine and urea in respiring slices or homogenates, it seemed necessary to present the results of experiments designed to explain and reconcile the contradictory results stemming from unrecognized metabolic interrelationships. The generation of ATP and many of the substrates required for the synthesis of citrulline, arginine, and urea were being provided by the oxidations of the citric acid cycle and by associated nitrogen transfers when glutamate was provided as the nitrogen donor rather than aspartate.

It became clear to me that the urea cycle, the citric acid cycle, and coupled phosphorylation are metabolically linked through transamination, and function as a metabolic bicycle in the intact cell. In my visualization, the highly active transaminase was assigned the essential metabolic function of a go-between to catalyze the transfer of amino nitrogen, through glutamic and asparitc acids into urea, thus insuring a continuous supply of keto acids as nitrogen carriers. Although these time-consuming experiments may have slowed my progress in other directions, the need for clarification in the interpretation of experiments of this kind gave me the opportunity to formulate these interrelationships in conceptual terms when the details of ATP generation coupled to mitochondrial oxidations were in the early stages of investigation in liver tissue.

I have gone into some general aspects and would like to add occasional detail, with the readers' indulgence, anticipating that the new and urgent problems in nitrogen metabolism awaiting experimental investigation become more visible when we see where we have arrived and by what routes.

As a result of Krebs' experiments and earlier chemical work, it was appreciated that the synthesis of urea was an energy-consuming process.

That some part of the total energy requirement must be supplied by the phosphate bond energy of ATP could now be seen in the biosynthesis of argininosuccinate in the liver cytoplasm. The findings were helpful in drawing attention to masking of a similar requirement in the conversion of ornithine to citrulline by enzymes localized within the mitochondria of liver cells. A few years later, the requirement for ATP in the formation of Compound X, an intermediate in citrulline formation, was shown by Grisolia and Cohen, and then the identification of Compound X as carbamyl phosphate, through chemical synthesis, by Jones, Spector, and Lipmann completed the demonstration. Thus it became evident that energy utilization for urea synthesis is confined to the formation of the newly formed C–N bonds in the carbamyl and guanidino groupings of the two new intermediates.

The separation of citrulline from arginine biosynthesis by intracellular compartmentation requires the passage across the mitochondrial membrane of substrates and products common to cytoplasm and mitochondrion. A number of investigators have been prompted in recent years to take up the study of specific mitochondrial transport mechanisms to facilitate access and egress of aspartate, ornithine, and metabolically related compounds.

The new C–N bond formed to make argininosuccinate represents the primary synthesis of the guanidino grouping. Much of my subsequent efforts were devoted to the clarification of the mechanism of utilization of phosphate bond energy in the formation of this type of C–N bond. With some progress in the direction of enzyme separation, argininosuccinate—synthesized by the first enzyme and cleaved by the second—could be prepared in small amounts. We advanced in slow, alternating stages on the mechanism of ATP-utilizing condensation of citrulline with aspartate, and we advanced on the mechanism of cleavage, when fumarate was found to be the second product of the reaction. To escape this frustrating cycle, purification of the cleavage enzyme was given urgent priority. Through reaction reversal this enzyme would hopefully provide supplies of the intermediate adequate for use as a substrate and for characterization and structural studies.

Guided by prediction, argininosuccinate was isolated from incubation runs as the alcohol-insoluble barium salt. In my efforts to prepare the amino acid in the free isoelectric state, pH 3.2, the crystals I obtained proved to be an anhydride of the intermediate. When this stage was reached all the pieces of a puzzle fell into place. In the structure of this amino acid lay the proof I had been seeking for the new mechanism of nitrogen transfer, and I felt then the sense of elation and discovery that the scientist experiences.

To form the guanidino group of arginine, the transfer of nitrogen from aspartate occurs so as to circumvent the participation of free NH_3. A new

universal metabolic pattern becomes evident in the utilization of aspartic acid and the release of fumaric acid, two metabolites common to major pathways. The pattern can again be seen in repeated application to the biosynthesis of C–N bonds in other metabolites. The conversion of inosinic acid to adenylic acid proceeds by two analogous reactions, and Buchanan and collaborators showed that nitrogen atom 1 of the purine ring is derived from aspartate by analogous steps. For many species uric acid rather than urea represents the form in which nitrogen is excreted. It is pertinent, for reasons of chemical and metabolic significance, that nitrogen in positions 3 and 9 of the purine ring, previously thought to come from NH_3, actually derive from glutamine. One of the two pyrimidine ring nitrogens originates in aspartate.

Sometime earlier, I had provided the necessary evidence that the activity was more than commensurate with in vivo rate requirements for urea formation. Nevertheless the unusual properties of argininosuccinate gave rise to doubts. The compound had escaped detection in plasma and liver tissue, and being highly polar could not permeate cellular membranes. Attempts to elicit a precursor-product relationship, either in supporting the growth of a bacterial or eucaryotic mutant in the arginine pathway or as a precursor of urea in liver slices or liver perfusion, have been invariably negative. The lack of evidence of this nature, in contrast to urea-stimulating effects shown by NH_3 and glutamine, led to speculation that this compound may not lie directly in the pathway, despite ample genetic and enzymatic confirmation for the pathway gained from studies on arginine synthesis in procaryotic and eucaryotic microorganisms and in higher plant species.

It was not until 1958 that news of the first occurrence of argininosuccinate in man came to me in a letter from R. G. Westall. He was the gifted and indispensable chemist in the Department of Pediatrics at the University of London. Dent and his colleagues had described a mentally abnormal child exhibiting very marked derangements of nitrogen metabolism, which were probably of genetic origin. This patient excreted 3 or more grams a day of an amino acid which Westall identified as argininosuccinate. Yet, for some, doubts remained as to the role of this amino acid since the patient continued to excrete urea. Influenced by the "all or none" hypothesis, the reasoning went that a metabolic derangement associated with a genetic error of an enzyme of the urea cycle should be a lethal mutation. I will refer to these abnormalities again, later on.

In 1953 our advances with the cleavage enzyme and the chemical characterization and proof of structure of argininosuccinate, including the evidence that the product of the synthetic step was identical with the substrate for the cleavage step, were described in two lengthy papers. At this point in our progress my sights were set a long way ahead and there was much to be done. Even the subdued modulations of retrospection do not allow me

to say that the course was always serene or that it went without turbulence. Experimentation is for me a very human, personal, and intense activity. In experimental design, and in responding to the urge to strengthen proof by reducing the number of experimental variables, there are creative and aesthetic as well as intellectual aims and pleasures. All of this is what keeps investigators in their laboratories. Because of the elusive nature of the reactions I was involved with, it seemed all the more important, for cellular and biological integrations, to continue with my in-depth studies. The turn of events enabled me to do this.

ARGININE AND UREA SYNTHESIS AND RELATED PROBLEMS

When the New York University School of Medicine moved to a new building in 1954 and Ochoa accepted the Chair of Biochemistry, I was left in a dilemma which was happily solved by the return of Efraim Racker to New York. After some years at Yale, he was about to reorganize and head the Department of Biochemistry at the Public Health Research Institute in New York, which I was very pleased to join. My move to the Institute would, I hoped, be my last. The department had ample space for a good start and was to undergo renovations to provide more extensive facilities for the preparative needs of an incoming group of biochemists with enzymological leanings.

I had been gathering material together for a review in *Advances in Enzymology* at the invitation of F. F. Nord who started the series, and planned to cover the recent progress in citrulline and arginine metabolism in relation to urea formation. Investigations in this area were accumulating. Some phases were in a puzzling stage, adding difficulty to the undertaking, but it was an appropriate time to see how it all held together; it compelled me to clarify my own point of view. The review was completed in the new quarters while we were settling in (5).

I looked forward to the prospects ahead. Some main features in the problems of isolation and structure of the intermediate were behind us and basic aspects of the enzymes we were concerned with such as stoichiometry and reversibility had been established. This and earlier work had been carried out with two gifted assistants, Barbara Petrack and Olga Rochovansky, who moved to the Institute to carry out their thesis problems with me. One stayed on to continue as a collaborator. Our work could not have developed as it did without them. It was a source of pleasure, later on, to see them well along in their own careers in biochemistry.

The need for preparing enzymes from slaughterhouse sources on a scale sufficiently large to sustain progress was always a matter of great concern and labor. Our new facilities were well planned for this purpose. Almost

everyone in the department, especially in those years, had to start from scratch, however much the problems and enzyme sources varied. Racker had brought a lively group together. By mutual consent our seminars, always a good yardstick, were frequent and prolonged.

As the work moved forward, additional space was made available to me and I was encouraged to turn to NIH to seek funding to cover a second associate. In my many years at the Institute, the total number of associates can be counted on the fingers of one hand. Later on, when the nature of the problem required it, I sought collaboration outside.

Research support from NIH over a period of many years, and the feeling of being appropriately situated, have permitted me to continue a relatively unhampered pursuit of problems in nitrogen metabolism with long-range goals in mind. Since our group has been small, the pattern has tended toward longer papers and less frequent publication. Possibly this reason, or the absence of wide interest in my field, or my sex, accounts for the small number of postdoctoral applications I have received. I will add that my career as a biochemist has been a more difficult one because of my sex.

My proclivities seem best suited to full-time research. Although I am an uncomfortable lecturer, I regret not having taught biochemistry for I place a high value on teaching, for student and lecturer alike. I have missed the wider cultural and social activities of a university community. Life in New York has in part made up for that lack with its endless sources of nourishment and pleasure to feed aesthetic needs.

When several problems were going well and our almost continuous large-scale enzyme preparations could be temporarily suspended, I would give myself the luxury of trial and exploration into new phases or new problems. I sometimes resorted to extreme measures to prolong those precious hours. Nonetheless, the outside activities and exchanges of professional life, editorial reviewing and other activities biochemists feel obliged to share in, were placed in the essential category. The hours lost were more than offset by the intellectual stimulations. With increased facilities I could extend our studies into the metabolism of other guanidino compounds, then quite obscure. Out of that interest came our enzymatic studies in 1956 on the mechanism of guanidino acetic acid formation. New insight into donor-acceptor relationships in the transamidination process was gained with the application of selective isotope labeling. I became interested in 1957 in the mechanism of action of the bacterial enzyme catalyzing the desimidation of arginine to citrulline previously found by others. This proved to be a purely hydrolytic reaction, unrelated in mechanism to the biosynthetic conversion of citrulline to arginine. The purified enzyme continued to interest us as a tool for the preparation of L-citrulline selectively labeled with ^{18}O in the ureido grouping. The enzyme is present in *Mycoplasma* and were

it not for the warning of Schimke, the organism might continue to be a hidden scavenger of arginine in tissue cell cultures.

With Hadassah Tamir in 1963, an interest in the enzymatic basis for the old nutritional finding that arginine is an essential amino acid for avian species revealed enzymatic limitations. In becoming purinotelic, avian species have lost the ability to convert ornithine to citrulline, while the ability to convert citrulline to arginine has been retained since the enzymes for this step are present in the kidney, but are there without function.

Owing to the labile properties of anhydride I of argininosuccinate, in contrast to those of anhydride II, I became unsatisfied with my previous assignment of structure and undertook, in 1966, a reexamination of both anhydrides with preparations selectively labeled in different moieties of each compound. This led to a revised structure for anhydride I in which the proton at the δ-nitrogen of the ornithine moiety is involved in the anhydride linkage, thus pointing to an unexpected reactivity of that grouping more in keeping with the chemical properties. I placed much importance on the reactivity of guanidino compounds in connection with their role as phosphogens, and feeling that the chemical evidence for anhydride I was not altogether definitive, I undertook in 1969, in collaboration with Arthur Kowalsky who was then at the Johnson Foundation, examination of the structures of the two anhydrides and related guanidino compounds through studies of the proton magnetic resonance spectra. This work confirmed anhydride linkage involving the δ-nitrogen and proposed structural revisions for creatinine and several other guanidine anhydrides.

Meanwhile in collaboration with Olga Rochovansky, our energies and long-range plans continued to be focused on argininosuccinate synthetase. Difficulties arising from product inhibitions, including the particularly strong inhibition by PP_i, had been circumvented earlier. Searches for a much more rapid assay and improved purification procedures became relatively simple to overcome, compared to the snags and obstacles we encountered before reaching elucidation of the reaction mechanism. Evidence gained from the results of experiments with ^{18}O-citrulline, and the fact that cleavage takes place at the α,β-pyrophosphate linkage of ATP, suggested that phosphate bond energy is utilized to activate citrulline; condensation should proceed through transadenylation in a stepwise manner. Among other snags, the fact that with citrulline and ATP, PP_i remained tightly bound to the enzyme posed new difficulties in the way of proof. Persistently renewing our pursuit a few years later, direct proof was obtained through evidence of a citrulline-dependent cleavage of ATP, through pulse-labeling experiments, and by inducing PP_i release from the enzyme in the presence of an analogue of aspartate having enzyme-binding capacity but lacking the capacity to condense with citrulline.

When Augusta Schuegraff joined me for a period, we became immersed in a study of the energetics of the synthetase reaction. With the collaboration of Robert C. Warner of New York University School of Medicine, we could show by taking the Mg-complexed species of ATP and PP_i into consideration that $\Delta G^{\circ\prime}$ for the hydrolytic cleavage of ATP at the α,β-pyrophosphate bond is more negative by several kcal than cleavage to ADP and P_i. Thus a mechanism involving transadenylation from ATP is energetically more favorable and obtains a second boost from PP_i hydrolysis.

In collaboration with Henry Hoberman of Albert Einstein College of Medicine, the stereospecificity of the β-elimination reaction catalyzed by the cleavage enzyme was established in relation to reactions catalyzed by fumarase and aspartase.

In association with Evelyn Havir, work was renewed on the cleavage enzyme with an improved assay and purification procedure. Homogeneous crystalline enzyme was obtained, leading us into a number of subsequent studies with Richard Bray, Carol Lusty, and Olga Rochovansky on subunit dissociation, oligomeric structure, structure-function relationships, and regulation. Our progress in this direction was often stimulated by elegant contemporaneous contributions to the understanding of protein structure in relation to enzyme catalysis. Somewhat later our knowledge of the synthetase, the less stable enzyme of the two, was also advanced in this direction. With both enzymes, at the right stage and opportunity, mechanisms for catalytic regulation at the molecular level were profitably explored through kinetic analysis.

During this period, knowledge of the behavior and properties of the enzymes of citrulline biosynthesis from ornithine had been moved to advanced stages by studies in the laboratories of P. P. Cohen, S. Grisolia, M. E. Jones, M. Marshall, and others. D. M. Greenberg and others had brought our knowledge of arginase to an advanced stage.

NEW QUESTIONS AND NEW PROBLEMS IN NITROGEN METABOLISM

In 1973 I undertook a review for *Advances in Enzymology* at the invitation of A. Meister. The time for bringing together all of the work on the five urea-cycle enzymes and related biological advancements was almost past due, so extensive had it grown (6). Placing the enzymes in sequence at this point, our knowledge gives us the explanation for the running of the cycle and shows us each built-in operational advantage: advantageous compartmentation and energy drive, advantageous reaction equilibrium, assurance of system operation through the relative proportions found in vivo among the five participating enzymes. The so-called coupling enzymes, ornithine

transcarbamylase and arginase, are normally present in large excess over each synthetase, thus maintaining, through enzymatic design, a remarkable efficiency of operation reflected in the low steady-state concentrations of the amino acid intermediates of the cycle. Now we can ask how it is regulated in response to flux, and how the supply of substrates derived from oxidative metabolism is regulated, and innumerable other questions. The synchronization of urea production with gluconeogenesis has been repeatedly observed. How does the operation of the urea cycle adjust to this flux and what share is borne by metabolic systems in muscle? Assuming appreciable sequestering of NH_3 in the form of glutamine, does the regulation of glutamine synthetase, glutamine transaminase, and the urea cycle interact with gluconeogenesis?

Regulation of the activity of the urea cycle enzymes can occur through increased enzyme synthesis in response to increases in dietary protein load. The results of R. T. Schimke show adaptive, coordinated increases in net amounts of each of the urea cycle enzymes gained over a period of several days. P. P. Cohen and his colleagues found that the increases in each of the urea cycle enzymes were coordinated during the development of ureotelism in tadpole metamorphosis. Cohen and also N. C. R. Räihä demonstrated progressive coordinated increases in the livers of rats during prenatal development.

Familiarity with the intermediates and enzymes participating in urea synthesis has contributed to the recognition of human metabolic abnormalities of genetic origin associated with a deficiency of enzymatic activity in the liver. Inborn errors affecting each of the urea cycle enzymes have been described. Among the earliest to be recognized were partial losses in activity of argininosuccinase and argininosuccinate synthetase since these are associated, respectively, with elevated plasma levels of argininosuccinate or citrulline, accumulating behind the partial block, and with their excretion in the urine. With early diagnosis the number of reported cases of nonlethal and lethal mutational variants for each enzyme deficiency has increased rapidly. In addition to characteristic biochemical changes, afflicted infants and children with partial deficiencies of any one of the enzymes exhibit certain features in common: mental deficiency, neurological changes, abnormal growth and development to some degree, protein intolerance, and hyperammonemia, particularly in response to a protein load.

The normal level of NH_3 in the plasma is extremely low, about 0.03 mM; neurological manifestations begin to appear when the concentration is increased three- or fourfold. It is now generally accepted that the convulsions, coma, and other neurological changes are the result of elevated ammonia levels in the blood and brain. The appearance of hyperammonemia as a common finding, regardless of the enzyme affected, is difficult to under-

stand. What flexibility in kinetic adjustment is available when one rate-limiting step or component is substituted for another? The nature of the genetic abnormalities of urea cycle enzymes and the metabolic derangements constituting their phenotypic biochemical expression has generated new questions and new urgencies.

How shall the young biochemist or clinician wishing to address his or her questions to the problems raised by abnormal nitrogen metabolism become informed, how to become informed about many other metabolic abnormalities of genetic origin also amenable to alleviation through early diagnosis, yet lying in metabolic pathways elaborated in past decades? As biochemistry has moved in new directions, becoming a fragmented discipline through sheer increase in bulk, departmental stress and textbook treatment of intermediary metabolism have been contracted or transformed into metabolic maps, or the space has almost entirely been given over to major and more recent biochemical developments of new biological significance. Can we not develop viable alternatives to counteract permissive obsolescence of our knowledge?

Urea formation has long been regarded as a mechanism for NH_3 detoxification by clinicians, biochemists, and comparative biologists alike. With all our present advances, we do not know what portion of the general pool of amino nitrogen passes through the stage of free NH_3, en route to arginine and urea formation or incorporation into protein. In addition to aspartate and glutamate, enzymatic transaminations include almost all amino acids. In vivo studies directed at this problem may be thwarted by isotope equilibration through reversible reactions. The numerous roles of glutamine in NH_3 utilization, and in general transamination, elaborated by Meister, constitute further important mechanisms that function through bypass or uptake. Possibly regulation of these systems is affected in abnormalities of the urea cycle enzymes.

It is not an accident that most of Meister's studies on glutamine synthetase were carried out with the enzyme from brain. The activity in brain is high, yet we do not know why an elevation in the normally low plasma level of NH_3 produces metabolic changes in the brain and causes brain damage. The problem has been a focus of frequent speculation over the years. A fresh experimental approach to the study of brain metabolism was contributed by Heinrich Waelsch. He was an old friend and our talk often reverted to discussions on the role of glutamic acid and glutamine in the brain, for glutamic acid metabolism had engaged his interest for some time. In 1962, only a few years before his death, two of his papers appeared that have since become models for the study of brain metabolism (7, 8). Following on the intercarotid administration to cats of $NaH^{14}CO_3$ or $^{15}NH_3$, or both,, the distribution of isotopic labeling was determined in aspartate, glutamate, and

glutamine. This approach permitted the conclusion that the metabolism of the dicarboxylic amino acids occurs in cellular compartments in cerebral tissue, and that the brain contains the enzymatic activities of the citric acid cycle and major metabolic pathways found in other organs. To mention another important finding, the initial fate of the infused NH_3 was traced in the normal animal to a considerable increase in brain glutamine without a corresponding decline in glutamic acid.

Waelsch and others have found protein turnover to be an active process in brain tissue; considerations of structure and function have permitted no more than general interpretations. It is not known why mental deficiency accompanies many genetic disorders of metabolism. This too has been a subject of frequent speculation. For urea cycle disorders, protein synthesis from an abnormal amino acid pool would seem a plausible explanation. But how about other disorders: does almost any derangement of brain metabolism affect the composition of the amino acid pool? The question is raised without overlooking the functions and the limitations of the blood-brain barrier. Considerable uncertainty continues to surround the underlying process of brain development. Is brain development complete at the time of birth, or does the process continue into the early postnatal period?

Appreciating the latitude allowed me, I confess to playing iconoclastic games, speculating on the possibility that glutamine, rather than NH_3, has the role of substrate in carbamyl phosphate formation in ureotelic species as in lower forms, recalling in my backward look the elusive trail of very fast reactions; speculating on the possibility that all the complexities of intermediary nitrogen metabolism, the processes for reutilization of amino and amide nitrogen, for amino acid reutilization in protein turnover, represent elaborate measures for the conservation of nitrogen, inherited from our distant past and overlain with homeostatic mechanisms. Protein is the food essential to man. Agronomists tell us it is the food most difficult to grow and in the shortest supply in the Third World. Some nutritionists ask whether limited protein intake in infancy and childhood affects mental development and others ask how little is the least amount of protein to suffice. The biochemistry of nitrogen metabolism is faced with innumerable imperatives.

My active life continues with its customary experimental urgencies and daily sense of anticipation. As long as I am able to work in this way, my place is quite properly in the laboratory. In Woods Hole, where I spend summer holidays in busman's fashion, the continuation of the emeritus tradition in universities over the nation is much in evidence. It is very refreshing to see that there is room for all ages. On the New York scene, I have the example of a number of old friends, biochemists of my own vintage, to look to. Throughout much of our scientific careers, the pursuit

of basic research in the biomedical sciences has been explicitly acknowledged as an invaluable national asset to be kept alive and well nourished for immediate and future gains. Now that fiscal problems begin to affect these values, it is to the scientists we must look to restore recognition of the crucial role of basic research in scientific progress.

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