RECOLLECTIONS

From proteases to proteomics

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Abstract

This personal and professional autobiography covers the 50-yr period of 1950–2000 and includes the following topics: History of the University of Washington School of Medicine and its Department of Biochemistry (Mount Rainier and the University of Washington, recruiting faculty, biology, research programs); scientific editing (publication, *Biochemistry*, *Protein Science*, electronic publication); Europe revisited (Heidelberg, approaching retirement, the German Research Center, reunion in Vienna); and 50 yr of research on proteolytic enzymes (trypsin, carboxypeptidases, mast cell proteases, future developments).

History of University of Washington School of Medicine and its Department of Biochemistry

I had never been to the Pacific Northwest before I received an invitation from Dean Edward Turner of the University of Washington School of Medicine to come for an interview. People who knew of my interests in mountains and mountaineering suspected that I was attracted by Mount Rainier, which, however, I did not see until 6 mo after my move to Seattle in September 1950. I was aware that the medical school was new, and the opportunity to build a Department of Biochemistry, more or less from scratch, was challenging.

The trip from Durham, North Carolina, to Seattle was tedious and tiring. I took a commuter flight from Raleigh/Durham to Washington, D.C., and from there to Seattle with four obligatory stops (Chicago; Denver, CO; Salt Lake City, UT; and Portland, OR). I was glad to find some sleep at 1 AM, but the following morning I was awakened at 7:30 by a call from Dean Turner, advising me that my first appointment was to be at 8 AM. I missed that one by a wide margin. The interviews with Dean Turner and with the chairman of the departments of medicine, surgery, anatomy, and microbiology went smoothly. Chair of Medicine Robert H. Williams particularly impressed me, as did Chair of Anatomy Stanley H. Bennett; both of whom turned out to be effective recruiters, open-minded, and friendly. Under Stanley Bennett, the department of anatomy was not conventionally

oriented toward gross anatomy but, rather, toward molecular anatomy down to the levels of electron microscopy and X-ray diffraction analysis. The Department of Medicine was perhaps the best known among the clinical departments of this new medical school. The Health Sciences Division was located in modern, newly built quarters; the spirit of collaboration was excellent; and I gained the general impression of an active and enterprising young team of colleagues who, in the absence of university-operated clinical facilities, were uniquely devoted to biomedical research (Finch 1990).

The Department of Biochemistry had just moved from the Chemistry Department on the upper campus, where it had division status, to the School of Medicine on the lower campus. The faculty included five members, Ed Krebs, Don Hanahan, Carl Kuether, Earl Norris, and Alex Kaplan (clinical biochemistry). There were about a dozen graduate students and two postdoctoral fellows. The task ahead was to enlarge the faculty and space, establish a graduate training program, and promote the department's growth in size, stature, and reputation.

Before visiting Seattle, I had met Ed Krebs on the Boardwalk in Atlantic City, New Jersey, incidental to the annual meeting of FASEB (Federation of American Societies of Experimental Biology), which included the ASBC (American Society of Biological Chemistry). Krebs was not only an effective spokesman for the University of Washington but also became a close personal colleague and friend. Without his insights and continuous advice, I could have hardly carried out my assignment to build up a strong Biochemistry Department.

When I returned to Durham, North Carolina, I received from Dean Turner an offer to become professor and chair-

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Article and publication are at www.proteinscience.org/cgi/doi/10.1110/ps.40101.

man of biochemistry, which I accepted without much hesitation even though my family and I had moved 5 mo earlier into a new house that had been designed according to our needs and specifications. After my arrival in Seattle, Ed Krebs advised me apologetically that he and Don Hanahan had spent the \$50,000 that the dean had offered to equip the department. I assured him that this did not disturb me as I still expected to, and did, receive another \$50,000 for the items that were on my shopping list. In 1953, Dean Turner resigned. As the youngest and most recently appointed member of the Executive Committee of the School of Medicine, I was assigned the honorable task of presenting the dean with a certificate of thanks and appreciation (Fig. 1).

Mount Rainier and the University of Washington

A former dean of the Graduate School whose house overlooked Lake Washington and Mount Rainier opined that if he could not see Mount Rainier in the morning he knew it was raining, and if he could see it, it would rain before the day was over. Mount Rainier (elevation ~14,400 ft) is the most conspicuous landmark of Seattle and the Puget Sound region. With 29 glaciers and 250 miles of trails, this national park attracts tourists, hikers, mountain climbers, and ski enthusiasts. It has also helped the University of Washington attract and retain faculty. As a result, faculty salaries have tended to be somewhat lower than in competing institutions, and faculty turnover has been low. I have never attempted to climb to the summit, but my wife Susi and I have been on the mountain hiking and skiing many times, and rare were the weekends that would find us at home. This was particularly the case after we acquired a cabin on forest service land, located within a few miles from one of the entrances

Fig. 1. As the youngest and most recently appointed member of the executive committee, I was assigned the honorable task of presenting Dean Turner with a certificate of thanks and appreciation (1953).

Fig. 2. Me skiing at age 80, taken at Crystal Mountain, with Mount Rainier in the background.

to Mount Rainier National Park and from the adjacent Crystal Mountain ski area (Fig. 2). We climbed on skis to Camp Muir at the 10,000-ft level dozens of times during the winter and spring and enjoyed the 3000-vertical ft downhill run to the parking area. We were joined on one of these trips by the dean of the School of Medicine, George Aagaard, and on another by Professor Robert Schwyzler of the ETH Zurich while he was a visiting professor of biochemistry in 1964. Our frequent ski trips qualified us to join the “Ancient Skiers” ski club. We are now probably the oldest of the 100 or so members of this elitist organization.

Recruiting faculty

Whenever visitors passed through Seattle I asked them to recommend promising young biochemists who would be interested in and would qualify for a faculty position. One of these visitors was Max Delbrück from the California Institute of Technology (CalTech), who told me of “a crazy Swiss” who insisted on carrying out postdoctoral research at CalTech. George Beadle, the head of biology at CalTech, waved off the Swiss with the comment that he could offer only a small stipend of \$200 per month. The Swiss replied that he was coming anyway; he was assigned, or chose, to work with Henry Borsook on the mechanism of protein biosynthesis. Krebs and I decided to interview this potential candidate and met him in Los Angeles where the American Chemical Society happened to hold its semiannual meeting. We waited patiently at a restaurant until our candidate arrived. He was late because he had not been able to tear himself away from listening to Earl Stadtman’s address as a recipient of the 1953 Paul Lewis Laboratory Award in Enzyme Chemistry of the American Chemical Society. The candidate’s name was Edmond Fischer. We invited Fischer to present himself as a candidate for a staff position, and

soon thereafter he was appointed assistant professor of biochemistry. Little did any of us, Ed Krebs, Ed Fischer, or I, anticipate that this would be the auspicious beginning of a long-term collaboration between Krebs and Fischer that some 40 yr later would earn them the Nobel Prize in Physiology or Medicine.

In the following years, we added Frank Huennekens, Phil Wilcox, and Walter Dandliker, to our staff, followed later by Earl Davie, Paul Bornstein, Milt Gordon, Bill Parson, David Teller, David Morris, Ted Young, John Herriott, Steve Hauschka, John Glomset, Ken Walsh (promoted from Research Associate), Joe Kraut, Brian McCarthy, Bill Rutter, Nina Agabian, and Richard Palmiter. These recruits required a significant expansion of the departmental facilities. This was accomplished first by taking over the nurses' locker facilities on the first floor and converting them into laboratory facilities for my research group. Later, in 1955, we acquired additional space on the fourth floor. We intended to celebrate this expansion by organizing a scientific symposium on enzymes and enzyme mechanisms. That was at a time when the president of the university objected to the planned appearance of Robert Oppenheimer on the campus and, consequently, in protest, our proposed symposium speakers decided unanimously to boycott the symposium that was, therefore, cancelled. Another major space expansion occurred in 1965, when the Department of Biochemistry moved into the newly built Biochemistry-Genetics Wing of the Health Sciences Center. This move was an important milestone.

Biology

President Charles Odegaard and Vice President Fred Thieme appointed a Committee on Biology whose task it was to formulate plans to strengthen the biology program of the University. The committee was first chaired by Stanley Bennett (anatomy), followed by Herschel Roman (genetics) and later by Earl Benditt (pathology) and myself (biochemistry). As a first step, it called for revising and incorporating into the existing and teaching and research programs recent developments in the emerging areas of molecular biology, genetics, biochemistry, and related disciplines. To that end, the committee recommended that the department of genetics, previously affiliated with the College of Arts and Sciences, and that of biochemistry, affiliated with the School of Medicine, be relocated in a new building to be added to the existing health sciences complex. Herschel Roman and I spent many hours planning the basic features of a coordinated program and finally asked the National Institute of General Medical Sciences of the National Institutes of Health for matching building funds. The Institute Director, Frederick L. Stone, was invited to Seattle, and during a lunch engagement in the relatively new Space Needle Restaurant, we succeeded in persuading Dr. Stone of the wor-

thiness of our proposal. We sought and obtained the approval of the Biology Committee; of President Odegaard; of Solomon Katz, the dean of the College of Arts and Sciences; and of George Aagaard, the dean of the School of Medicine to implement the plans in consultation with an architect. The building was designed from inside out, so to speak, taking into consideration the needs of our faculty members (senior and junior) and giving them an opportunity to justify their requirements. After everything was said and done, the size of the proposed building exceeded by a significant margin the available funds. After reducing the size to meet the budget and making provisions for later expansion if and when additional funds might become available, the total construction estimate was \$5 million. Genetics would be housed on the first and second floors; biochemistry on the third, fourth, and fifth floors; and joint facilities including a glass washing facility, a large scale preparation room, and a plant greenhouse for Dr. Gordon's program (the last separately funded by a grant from the National Science Foundation) would be accommodated in the basement. It was probably the only greenhouse located in a basement rather than on the top of a building.

The building was completed in 1965 and dedicated at the fall meeting of the National Academy of Sciences (Fig. 3). The academy was invited by President Odegaard to meet here, even though I was its sole member on the University faculty. The organizing committee sent special invitations to every academy member west of the Mississippi, and 70 members in all attended. They included also Academy President Frederick Seitz and members of the council, seven Nobel Laureates, and the noted nuclear physicists Robert Oppenheimer (now that the university had a new president) and Edward Teller (Fig. 3). The scientific sessions were open to university faculty and students, who filled every meeting room to overflow capacity; the final day featured a symposium on biochemistry chaired by Arthur Kornberg. On the preceding weekend, we organized bus trips to Mount Rainier and to the research laboratories in Friday Harbor, followed by a cocktail party sponsored by the Boeing company. These meetings were the best-attended National Academy meetings outside Washington, D.C.

Research programs

It was clear to us from the very beginning that the field of biochemistry was so large and growing so fast that no single department could represent or embrace all conventional biochemistry areas in their research and teaching programs. We therefore selected one or two major fields to which all members of the department could relate and that could provide an environment that would support different additional research programs. When the department was fully staffed, protein chemistry (Ken Walsh, Phil Wilcox, and myself) and signal transduction and enzymology (Ed Fischer, Ed

Fig. 3. Taken during the meeting of the National Academy of Sciences in Seattle in 1965, marking the opening of the new Biochemistry–Genetics Building. Top row, left to right: Nobel Laureate Linus Pauling, President Charles Odegaard (University of Washington) with President Frederick Seitz (National Academy of Sciences); physicist Professor Robert Oppenheimer. Bottom row: biochemist Britton Chance and Nobel Laureate biochemist Hugo Theorell; physicist Edward Teller, University of Washington; physicist Boris Jacobson and biochemist Hans Neurath; biochemist David Rittenberg with Nobel Laureate Harold Urey.

Krebs, Earl Davie, and Frank Huennekens) were main topics of research. In addition, the general program included biological differentiation and development (Bill Rutter), biochemical evolution (Brian McCarthy), nucleic acids and plant biochemistry (Milton Gordon), biochemistry of lipids (Don Hanahan), and physical biochemistry (Walter Dandliker, Bill Parson, and David Teller). As the department grew and expanded its program, it earned the reputation of being one of the best in the West. Some of the programs will be described elsewhere in this article.

A major effort in assembling this new department was to establish congenial relationships among its members, to develop a spirit of collaboration and interdependence among faculty members so that recognition of one brought credit to all of us. There was no generation gap within the department in those days. As chairman of the department, I always tried to recognize a problem or an opportunity before it became acute, to share my concerns and hopes with the faculty, and to encourage them to express their opinions on important issues. In time, the department acquired the reputation of being among the most congenial ones locally and in the field of biochemistry nationally.

To a significant degree, the strength of a department depends on the quality of its graduate program. There is no segment of a university that practices a closer relationship between teacher and student than the graduate school. Students learn from their mentors' habits of thought and dis-

cipline and the craftsmanship and value judgments in a chosen field. These are handed down from one generation to the next. It is probably no accident that the Nobel Laureate Sir Hans Krebs was a disciple of Otto Warburg, also a Nobel Laureate; who in turn had been a student of the renowned sugar chemist, Emil Fischer; and that this lineage leads to Adolf von Bayer; to Kekule, who discovered the structure of the benzene ring; to Liebig, the founder of organic chemistry; and eventually to Lavoisier, who liberated the physical sciences of his day from medieval notions of Phlogiston. Quality breeds quality.

Scientific editing

Publication

My interest in the process of scientific publications was stimulated by Kurt Jacoby, chief executive officer of Academic Press, who believed that a treatise on protein chemistry seemed very much needed at that time. I finally agreed to assume, together with Kenneth Bailey of the Department of Biochemistry at the University of Cambridge, the editorship of what became a four-volume treatise entitled *The Proteins*. Volume 1A was published in 1953. The preface stated that "the object of this treatise is to present a comprehensive, integrated account of the chemical, physical, and biological properties of the proteins for the benefit of

the advanced students and all those workers trained in different disciplines who are entering the field in fairly large numbers.” The contributing authors were all well-recognized experts and included Pierre Desnuelle, Barbara W. Low, Paul Doty, Peter Geiduschek, and Robert Alberty. The succeeding three volumes were published relatively soon thereafter and included chapters by John Edsall, Irving Klotz, Frank Putnam, Felix Haurowitz, C.H. Li, and Tom Singer. Other contributors were Roy Makham, Thomas McMeekin, J.F. Thompson, N. Michael Green, J.C. Kendrew, Kenneth Bailey, and myself.

The second edition was edited by myself and contained five volumes. The third and last edition, also five volumes, was edited by Robert L. Hill and myself and was published between 1975 and 1982. It reflected the enormous strides molecular protein science had made during that period. Contributing authors included Jerker Porath, Klaus Weber, Ken Van Holde, Irving Klotz, J.T. Finch, Alexander Glazer, Klaus Hofmann, Bruce Merrifield, William Konigsberg, Brian Matthews, Hugh Niall, Russell Doolittle, Emil Smith, William Harrington, Paul Bornstein, Charles Cantor, Nathan Sharon, Yuri A. Ovchinnikov, and Serge Timasheff.

Biochemistry

In 1961 I received a call from David E. Green of the Enzyme Research Institute at the University of Wisconsin on behalf of the editorial committee of the American Chemical Society. They had recommended that I be invited to become founding editor of a new journal in the field of biochemistry. The American Chemical Society was already publishing some 20 chemical journals but none in biochemistry. After some hesitation about adding editorial responsibilities to my chairmanship of the department and my relatively large research program, I decided to accept the position. I was attracted by the opportunity of introducing innovations into scientific publication, an enterprise that had suffered too long from outmoded methods. I also felt that there should be an alternative to the *Journal of Biological Chemistry* that would place greater emphasis on the chemical aspects of biochemistry, particularly in the areas of protein chemistry and enzymology. After extensive consultation with colleagues and friends, the journal was simply named *Biochemistry*. My colleague Milton Gordon agreed to serve as assistant editor. The original editorial advisory board included the following members: Eric Ball, Herbert Carter, Ed Fischer, Heinz Fraenkel-Conrat, Joseph Fruton, Rollin Hotchkiss, Henry Lardy, Alton Meister, Esmond Snell, Herbert Sober, Merton Utter, and Bert Vallee. Warren Wacker served as secretary to the board. We were promised, and indeed enjoyed, full support of the officers and staff of the American Chemical Society, without whom the journal could not have taken off to a running start. The annual meetings of the editors of all the journals published by the society were a source of inspiration and encouragement that

enabled editors to compare experiences and to propose more efficient methods of publication. Volume 1, published in 1962, contained some 1200 pages of text; volume 29, published in 1989, the last year of my editorship, contained almost 12,000 pages. Much of the success of the journal came from the fact that all five associate editors and the editor-in-chief were at the same location and could review all incoming manuscripts day-by-day and could jointly reach editorial decisions. Under the expert guidance of my successor, Gordon Hammes, the journal grew to over 18,000 pages by the end of 1998.

Protein Science

My freedom from editorial responsibilities was not to last very long. In 1990, the membership of the Protein Society, on the recommendation of its council, decided to publish its own journal and asked me to become its founding editor. It did not take much persuasion for me to accept this proposal as it provided an opportunity to combine my lifelong interest in the study of proteins with my interests and experience in scientific publication. Cambridge University Press submitted a bid that the Protein Society found acceptable, and Ralph Bradshaw, in his dual capacity as member of the Council of the Protein Society and treasurer of the American Society of Biological Chemistry and Molecular Biology (ASBMB), negotiated an investment of \$300,000 by the ASBMB to support the journal. All this occurred under the leadership of Finn Wold, president of the Protein Society, whose enthusiasm and dedication were a continuous source of encouragement and support. So were the advice and counsel of David Eisenberg and Ralph Bradshaw. The name of the journal, *Protein Science*, was finally selected from a list submitted by friends and supporters. The initial success was surprisingly encouraging. Despite warnings to the contrary by well-meaning members and colleagues, publication of the journal increased the membership of the Protein Society from ~1800 to ~3000. The intent of the founders was to introduce novel methods of publication, which from the very start included both the kinemage computer graphics program of Jane and David Richardson and, under the experienced and devoted guidance of Stephen White, experimentation in electronic publication. The founding associate editors were Ralph Bradshaw, Tony Hugli, Louise Johnson, Rachel Klevit, and Christopher Walsh. David Eisenberg and Paul Schimmel served as consultants to the editors. Jim Alexander represented the publisher, Cambridge University Press, and guided the publication and interests of the journal with unusual dedication and skill until his untimely death in 1995. With his passing, the journal lost a trusting and dependable friend and manager.

Electronic publication

Some of the basic premises of the current system of scientific publication have been recently called into question

(Marshall 1999). Among the issues raised are the increasing cost of publication and conflict with the commercial interests involved, the fairness of the peer review system, and the need for electronic publication. While this is not the appropriate forum for debating these issues in detail, I would be remiss if I did not express my opinion, based on many years of practical experience as an editor.

As to the question of commercialism and financial gains, no one is in business to lose money and to go bankrupt. This applies also to the publication business. To evaluate the legitimacy of financial gains, one needs to examine and come to an agreement as to what constitutes an adequate charge. Dr. Harold Varmus, one of the spokespersons, suggests that a charge for publication of <1% of the cost of research is excessive (Marshall 1999). This could certainly be debated. However, to minimize and eliminate the influence of commercialism, one should consider first the publication of scientific journals by scientific societies. Some of them subsidize their journals rather than deriving financial profit. Few, if any, are in the publication business for financial gains or are willing to cede control of their journal to a commercial publisher.

While the peer review system is certainly not perfect and is subject to errors of human judgments, no one has yet come up with a better system. It protects science and society against unfounded claims and, in the long run, saves time and effort to prove or disprove such claims. Quick and dirty are both attributes of falsified data or attempts to derive personal gains, and the peer review system is the most effective method to avoid or minimize such falsehoods.

Science, including the biological sciences, is so specialized and diversified that replacement of the current specialty publications by a single all-inclusive publication, electronic or printed, would be totally unmanageable. The current system has the advantage that it directs the authors to the journals in which they are most likely to find articles of interest rather than to search and ignore 90% or more of the publications in more general journals. Libraries face enormous difficulties in keeping up financially with the plethora of new journals because they have failed to eliminate some of the older journals that no longer seem to be relevant to the needs of modern researchers and students.

Finally, there is no assurance that a single, government-sponsored, electronic system of data deposition would be more effective than systems that are based on individual enterprise. So, while the current scientific publication system is far from ideal and needs to be improved and modernized, the alternatives thus far proposed appear to this observer unattractive and in many ways unrealistic.

Europe revisited

Heidelberg

My professional ties to Europe go back to 1970, when I became advisor to the Battelle Memorial Institute, located in

Columbus, Ohio, with satellite operations in Seattle, Frankfurt, Geneva, and Richland, Washington. I was an advisor to the director of the Seattle-based Battelle Research Center, and together with Bill Wiley of the Richland Laboratories, I formally initiated the biology program of Battelle by organizing an international symposium in biology in Seattle. We also traveled to Frankfurt and Geneva to interview potential candidates for employment at Battelle. One of these interviewees was Robert Zwillig, then of the Ruhr University in Bochum, Germany, whom I met in 1970 incidental to the International Congress of Biochemistry, originally scheduled to be held in Italy but then transferred to Interlaken and Montreux, Switzerland. Zwillig was offered a position at Battelle but finally decided to join the University of Heidelberg instead, where he became professor in the Physiology Department of the Zoological Institute. We became friends, and our friendship has continued to this day. A few years later, in 1974, he induced the University of Heidelberg to nominate me for a prestigious senior scientist award of the Alexander von Humboldt Foundation that I won and enabled me to become a visiting professor in the Department of Zoology and an honorary professor, a position that I still hold and cherish today. Susi and I stayed several months in Heidelberg, where I gave lectures on proteins and enzymes. We established long-lasting friendships with the Zwilligs, with Professor and Mrs. Theodor Wieland at the Max-Planck-Institute for Medical Research, and with many other colleagues. Thanks to an invitation by Professor Rainer Jaenicke, an unspent period of my award brought us later for several weeks to the University of Regensburg in Regensburg.

Approaching retirement

My next visit to Heidelberg was prompted by quite different circumstances. In 1979, at age 70, I was to reach mandatory retirement at the University of Washington. In practical terms this meant that I would acquire the flattering title of professor emeritus that is usually conferred on retired professors whose service to the university was especially meritorious. However, I would lose the privilege of applying for my own salary and research grants. I would no longer be required to teach formal lecture courses, but I would lose my regular voting rights on appointments and promotions. In anticipation of this event, the faculty of the Department of Biochemistry unanimously voted to recommend to the university administration that my employment be continued as a research professor. This recommendation was supported by professional colleagues and friends elsewhere, including several Nobel Laureates as well as by Herschel Roman, chairman of the Department of Genetics. Such an appointment would have created a precedent that the university administration was unwilling to establish, fearing that other retirees might make similar claims. This reluctance or unwillingness to differentiate and arbitrate between quality and equality (a topic that I addressed in 1976 in my

University of Washington First Faculty Lecture) prompted the dean of the School of Medicine to disapprove the faculty's recommendation and the president of the university to support the dean. I was, therefore, looking for alternatives to continue my professional career and my research program on proteins.

It so happened that in 1979 I was invited to attend an international conference on proteins in Heidelberg. In the course of this visit, I was asked by a friend who was member of the German Cancer Research Center (DKFZ) whether I would be interested and available to become the next scientific director of that institution, a position that happened to be vacant. Still smarting from the disappointing refusal of the university to continue my employment, I was willing to consider such an offer despite words of caution by some well-meaning friends and colleagues who knew the problems and internal strife within the DKFZ. To explain my qualms and indecision, a brief description of the DKFZ and its history may be in order.

The German Cancer Research Center

DKFZ, as initially constituted, was created by Professor K.H. Bauer to bring under one roof several different basic research programs related to cancer (Wagner and Mauerberger 1989). Each of these programs was independently organized, using the most advanced methods and equipment for their mission. The new center was to be governed by eight institute directors who selected the chairman from among themselves every other year. These institutes were the Institute for Experimental Pathology, the Institute for Biochemistry, the Institute for Virus Research, the Institute for Toxicology and Chemotherapeutics, the Institute for Nuclear Medicine, and the Institute for Informatics and Statistics. To these were subsequently added the Institute for Cell Biology and the Institute for Immunology. The governing bodies were a Committee of Institute Directors (see above), an Administrative Council, and a Kuratorium that included some 18 members appointed by the Ministry for Culture of the State of Baden-Wuerttemberg. Later on, the center moved into new modern research quarters located on the campus of the University of Heidelberg. It also became incorporated into an organization of Large Research Facilities administered by the Federal Ministry for Scientific Research and Technology in Bonn.

Throughout these organizational changes, the center was plagued by internal discord and strife. The daily general administration was vested in two persons, the scientific director and the administrative member. My nomination as scientific director as of May 1980 was controversial because I did not belong to the inner clique and was unwilling to compromise my standards of scientific quality and merit. It was evident from the beginning that any attempts to change the internal structure or the research goals, to introduce

quality review, or to control or to infringe on the self-interests of the institute directors would be vigorously opposed. The annual budget of the Center was 90 million deutsche-marks, but every effort to apportion even a small fraction of the institute budget on the basis of merit was bitterly opposed. My wife's and my name were dragged through the local and federal news media, and all efforts to establish harmonious and constructive internal relations were sabotaged. We went out of our way to have a cocktail party in our rented house to bring together representatives of the DKFZ, the University of Heidelberg, the Max-Planck-Institute for Medical Research, and the European Molecular Biology Laboratory, but our motives were critically examined for hidden agenda or other unwelcome intentions, despite the fact that this was the first time that representatives of these organizations met socially. The opposition against my leadership eventually assumed such proportions that it reached the German parliament.

The director of the Institute for Nuclear Medicine, who was also my predecessor as scientific director, introduced neutron radiation for the treatment of cancer patients even though he knew that there was not sufficient experimental evidence to justify this kind of therapy. This was brought to my attention while he was on a sailing vacation in Florida. On the advice of knowledgeable scientific and clinical colleagues, I ordered that this procedure be discontinued for lack of validating evidence of its effectiveness. This ruling was promptly communicated to my predecessor while he was vacationing, and he voiced his protest in a letter that he mistakenly addressed to me rather than to his deputy director. The gist of the letter was as follows: Although he knew that neutron radiation was worthless therapeutically, he wanted to provoke me to discontinue it. He branded me as incompetent and stupid enough to be caught in that trap and asked his colleague, for whom the letter was intended, to start a newspaper campaign in which the taxpayer should be informed of the loss of an investment of 7 million DM but not to disclose that the procedure had no proven therapeutic value. This incident became the subject of an inquiry by the Committee on Research of the German Parliament in a session to which I, the administrative member of the Directorate, and other members of the scientific staff including the writer of the letter, were invited as guests and witnesses. The letter also reached members of the Social Democratic delegation, who used this opportunity to make it a political issue. In the course of this inquiry, it became clear that the writer of the letter did not know beforehand that so many different circles within the parliamentary committee had been made aware of his intrigues, and following this inquiry he was forced to retire. I decided that enough was enough and submitted my resignation effective December 31, 1981.

In the meantime, the Federal Ministry for Research and Technology appointed an international Blue Ribbon Commission of distinguished scientists to evaluate the scientific

procedures and the research achievements of the DKFZ. The mandate of the commission was to provide advice for improvement of the organization of the DKFZ, to overcome the current internal strife, to analyze the research programs of the various institutes, and to establish a peer review system. The report was submitted to the Kuratorium and to the parliamentary commission.

Following my resignation and final departure from Germany, an interim scientific director was appointed. His tenure was relatively short lived until he was succeeded by the present director, Professor Harald zur Hausen, who instituted fundamental changes in structure, leadership, authority, and programmatic planning. These and other measures enormously improved the atmosphere for research and internal collaboration and made the DKFZ a world-class scientific institution. I feel satisfied that my personal efforts and frustrations have not been in vain.

Reunion in Vienna

In 1972, the government of Austria organized, on the occasion of the National Holiday on October 26, an international congress on "The Future of Science and Technology in Austria." Some 70 scientists were invited, some of whom, like myself, were Americans of Austrian origin, as well as scientists from other foreign countries. Many of the Austrian scientists had fled Austria as it became part of the German Nazi regime. For some this gathering was their first official visit to Austria in 35 yr and, as such, the congress took on some appearance of a meeting of reconciliation. Austrian scientists and engineers also attended the meeting. It was convened by Professor Frederic de Hoffmann (Salk Institute), Professor Victor Weisskopf (Massachusetts Institute of Technology), and distinguished Austrian representatives of science and government. The topic assigned to me was "The Future of Biomedicine," which led me to discuss basic research in molecular biology, biophysics, and biochemistry, as well as drugs, food, and the technological use of enzymes (Neurath 1972).

The scientific program was followed by a social program that included a splendid performance of Mozart's *Magic Flute* in the restored Vienna Opera House and a dinner dance in the imperial castle at Schoenbrunn, the very site at which the Vienna Congress that followed the defeat of Napoleon Bonaparte had been held in the early nineteenth century. That occasion had been the subject of a popular film entitled "The Congress Dances." I also had a dance with Hertha Firnberg, who belonged to the same hiking group as I when both of us had been students at the University of Vienna. Who would have anticipated then that, some 40 yr later, she would become Austrian minister for science? I was also reminded of my student days by my good friend Walter Wodak who had become Austrian ambassador in Moscow and who invited me to visit the chan-

cellery to show me the site where Chancellor Dollfuss had been murdered, signaling the onset of Nazi tyranny in Austria. We former "revolutionaries" were saluted by the honor guard when our automobile passed the entrance gate.

On the last evening, Chancellor Kreisky invited his former old friends to a private party at the "Heurigen," so called because the most recent vintage of wine is traditionally served there. The invited guests included former members of the socialist student organization, like Wodak, Weisskopf, and myself, and others who later held important positions, such as government ministers and a member of the Austrian Supreme Court. It was a strange sensation to be so intensely reminded of bygone days.

50 yr of research on proteolytic enzymes

In many ways, the last 50 yr at the University of Washington were the most exciting and rewarding years of my academic life. It was here that I could build up a long-range research program and derive the benefits of previous experiences in conducting basic research on proteins. It was also the beginning of an era that brought to the study of proteins new experimental approaches for their molecular characterization in terms of isolation, end group and sequence analysis, molecular degradation, and limited proteolysis. These were followed by X-ray crystallography and chemical synthesis and by more precise methods of characterizing the specificity and biological activity of proteins and their derivatives (Neurath 1994). Last but not least, it was the period of the discovery of the double helix and deciphering the genetic code. I was convinced of the merits and necessity of applying any of these methods and advances to our research program on proteolytic enzymes.

My interests in proteases were solicited and stimulated by the simple question What differentiates proteins that catalyze the digestion of other proteins from those that are being digested? In modern scientific parlance, it relates to the specificity and mechanism of action of proteolytic enzymes. I realized that the time was ripe to tackle this fundamental question. Other laboratories, particularly those of Desnuelle in Marseille, France, and of Hartley in Cambridge, England, were following similar tracks. My research group comprised graduate students, postdoctoral fellows, and research technicians, ~25 in total (Fig. 4). They included also Gordon Dixon, William J. Dreyer, John Rupley, Patricia Keller, Frank Tietze, Gerald Reeck, Jean-Pierre Bargetzy, Dorothy Kaufman, and several others whose names are listed in the bibliography. A list of all collaborators ever associated with me is given in Box 1 of this review. The topics of research included certain pancreatic proteolytic enzymes, their structures, precursor activation, mechanisms of action, amino acid sequences, evolution, and phylogenetic variations. One of the focal points was the thesis research by Earl Davie of the chemical changes accompanying the conversion of bo-

Fig. 4. The Neurath research group, taken in 1968. First row (left to right): Charles Nicholas, Ruth Arnon, Ralph Bradshaw, Ken Walsh, Hans Neurath, William P. Winter, Marilyn Sanders, Akintola Aboderin, Ralph Kenner, Neal Robinson, Jack Uren, Roger Wade, Donald MacDonnell. Second row: Richard Granberg, Royce Haynes, Chris Powers, Philip Petra, Karen Lebens, Rosa Mae McDonald, Chengf V. Shih, Carol Roesler, Lynn Matson, Mark Kupperberg, Brita Moody, Gerald Reeck, Lowell Ericsson.

vine trypsinogen to active trypsin (Davie and Neurath, 1955) and its subsequent extension by Davie and coworkers to the blood coagulation cascade (Davie et al. 1991; Titani et al. 1972).

Trypsin

Trypsin has fascinated biochemists, enzymologists, and biologists because it was one of first proteases to be identified and it plays an important digestive and regulatory role in a variety of physiological processes. The enzyme was discovered in bovine intestines by Wilhelm Kuehne, a German physiologist, before the turn of the twentieth century as an inactive precursor (trypsinogen) that is spontaneously converted into the active form either autocatalytically or under the influence of intestinal enterokinase, a unique protease of complex structure. Trypsin was also one of the first proteolytic enzymes to be crystallized by Northrop and Kunitz (Northrop et al. 1948) and shown to follow the thermodynamic criteria of purity. Despite its narrow specificity toward lysyl and arginyl peptide bonds, trypsin is a master enzyme because we have learned from it about zymogen activation, active site mechanism, domain organization, evolution, phylogeny, and irreversible signal transduction, all topics of continuing current interest. Together with chy-

motrypsin, pancreatic elastase, and related enzymes, trypsin belongs to the best-known class of proteases, the serine proteases that have a common charge relay system at their active site. Max Perutz referred to the charge relay system of the serine proteases as one of the best-studied mechanisms in biochemistry. The history of its discovery has recently been recalled by David Blow (Blow 1997, 2000), one of its originators.

Trypsin or trypsin-like enzymes form the enzymatic domain of a variety of enzymes involved in blood coagulation (Titani et al. 1972; Magnusson et al. 1975; Davie et al. 1991) in fibrinolysis, complement, and a great many other proteolytic systems. In all of these, the enzyme domain is associated with specific nonproteolytic domains that act as ligands to target proteins. As reported in the *Handbook of Proteolytic Enzymes* (Barrett et al. 1998), the Swiss Prot data bank contains 667 different trypsins, and the Brookhaven data bank lists 53 X-ray structures of this type of enzyme.

It might be noted in passing that some 50 yr after its discovery, intestinal enterokinase was shown to be a complex multidomain protein even though its biological specificity is uniquely restricted to the activation of trypsinogen. It splits a single lys-ile bond in the N-terminal region of the zymogen. The heavy chain of enterokinase contains do-

Box 1. *Graduate students, associates and visiting scientists associated with Hans Neurath during his active scientific career*

J.P. Greenstein	V. Richmond	M.A. Hermodson
F.W. Putnam	E.S. Awad	G.R. Reeck
E. Elkins Kaufman	J.R. Brown	R.M. McDonald
S. Kaufman	R.N. Greenshields	W.D. Behnke
J.E. Snoke	M. Yamasaki	D.C. Teller
G.W. Schwert	K.A. Walsh	R.W. Tye
M.A. Eisenberg	D.J. Cox	G.M. Hass
G. De Maria	D.A. Deranleau	N.C. Robinson
E. Fredericq	E. Wintersberger	K. Titani
F. Tietze	J.-P. Bargetzi	D.T. Grahm
J.A. Gladner	T. Hofmann	R.W. Kuhn
L.W. Cunningham, Jr.	B.J. Allan	P.H. Morgan
E.W. Davie	E.O.P. Thompson	D. Enfield
F.R. Bettelheim	W.O. McClure	A. Gertler
B.L. Vallee	J.H. Freisheim	B. Pilkaytis
W.J. Dreyer	J.W. Prahl	C. de Haen
Y.D. Halsey	H.N. Beaty	L.E. Anderson
J.A. Rupley	L.L. Houston	M.A. Kerr
R.D. Wade	L.H. Ericsson	R.G. Woodbury
G.H. Dixon	A.G. Lacko	J. Lonsdale Eccles
S. Go	T.M. Rhadakrishnan	M.T. Everett
P.J. Keller	J.G. Beeley	R. Zwillig
E. Cohen	W.P. Winter	T.R. Leary
P.E. Wilcox	P.H. Petra	D.P. Bloxham
J. Kraut	R.A. Kenner	D.C. Parmelee
J.-F. Pechere	S. Russo	H. LeTrong
D.L. Kauffman	R.A. Bradshaw	R. Jaenicke
R.H. Maybury	M. Nomoto	K. Cole
L.B. Smillie	N.G. Srinivasan	S. Conroy
T. Murachi	D.R. Babin	X. Tan
B.S. Hartley	R. Armon	

mains that occur in unrelated proteins. One is related to the LDL receptor; another resembles meprin, which is a member of the recently discovered family of zinc metalloendopeptidases; another occurs in complement C1r; and a fourth domain occurs in macrophage receptor (Kitamoto et al. 1994).

The chemical changes that accompany autocatalytic conversion of trypsinogen to trypsin appeared to us as a most intriguing phenomenon, prompting Earl Davie and me to investigate it at the molecular level. We made use of the novel fluorodinitrobenzene end-group analysis of Sanger in conjunction with purification of the activation peptide on Dowex 50 columns and by paper chromatography in butanol–acetic acid–water mixtures. There was a quantitative correlation between the amount of the N-terminal hexapeptide released and the amount of enzymatic activity appearing during tryptic activation. Although today these experiments could be done faster and with greater accuracy, this investigation was a beginning in elucidating the mechanism of zymogen activation generally and of its physiological significance in particular. In a concurrent investigation with William J. Dreyer (Dreyer and Neurath 1955), the peptide released during the rapid activation of chymotrypsinogen

was isolated and characterized as serylarginine and quantitatively correlated with the conversion of π -chymotrypsin to the δ form. An indication that activation of trypsinogen is also accompanied by structural changes was obtained by measurements of the optical rotation. The ultimate proof of such structural changes came from comparison of the zymogens and the active forms of trypsin and chymotrypsin by Wolfram Bode and Robert Huber, respectively, at the Max Planck Institute for Biochemistry in Martinsried, Germany (Huber and Bode 1978). While our earlier notions of these changes had been necessarily overly simplified, they were compatible with the detailed X-ray structures of the German crystallographers.

These and related phenomena prompted my colleague Kenneth Walsh and me to engage in a long-range research program of the molecular structure of bovine trypsinogen and its conversion to active enzymes. At the same time, P. Desnuelle at the University of Marseille and his coworkers had independently begun a similar project on trypsin and other serine proteases, and we soon found ourselves in a friendly competition with that laboratory. Coincidentally, Brian Hartley, studying the sequence of chymotrypsinogen, came for an extended visit to our laboratory in Seattle to continue his structural analyses. The results of both studies were published within the same year (Hartley 1964; Neurath and Walsh 1964) and clearly gave evidence of the identity of functionally significant amino acid residues in the two proteins and of their common evolution of both structure and function. Earlier notions that the differences in substrate specificity between trypsin and chymotrypsin were solely related to their differences in the S1 site had to be abandoned when it was shown by Perona and Craik (Perona and Craik 1995) that, in addition, two surface loops are changed, indicating that conformational changes at distant secondary sites are also involved. In a review of the role of proteolytic enzymes in biological regulation, published in 1976, Walsh and I summarized the occurrence of zymogen activation in a variety of physiological events of major significance (Neurath and Walsh 1976).

On a side tour of phylogeny, we isolated and characterized trypsinogen of the dogfish and the African lungfish. Other studies prompted us to acquire from the Veterinary School of Washington State University a cow so that we could study, together with Patricia Keller and Elaine Cohen, the protein composition of pancreatic zymogen granules and the rate of incorporation of radioactive amino acids into bovine pancreatic proteases.

Carboxypeptidases

Pancreatic carboxypeptidase A was of interest to us as a by-product of the isolation of bovine pancreatic trypsin and chymotrypsin. The zymogen occurs as a ternary complex composed of procarboxypeptidase A, chymotrypsinogen C,

and proproteinase E (Gomis-Ruth et al. 1995). The isolation of crystalline carboxypeptidase was accomplished by M.L. Anson, who in 1935, produced needle-shaped crystals after tryptic activation of bovine pancreatic juice. Together with Patricia Keller and Elaine Cohen, we first developed a procedure for the chromatographic isolation, characterization, and activation of procarboxypeptidase (Allan et al. 1964). This was followed by a cooperative study with Bert Vallee's laboratory that showed that carboxypeptidase is a zinc metallo-enzyme containing one zinc atom per molecule (Vallee et al. 1960). Little did we anticipate that carboxypeptidase would become one of the most controversial proteases. The identification of the metal was in conflict with earlier studies using less advanced and less sensitive metal analyses that implicated manganese. Also, the presence and state of oxidation of the two half-cystine residues in carboxypeptidase introduced a certain measure of uncertainty in assigning to them functional and structural roles. Later on, together with Ralph Bradshaw and Philip Petra, we found that activation can occur at different adjacent cleavage sites, giving rise to variants of the active enzyme having different N-terminal residues (Petra et al. 1969). Together with Ralph Bradshaw, we ultimately determined the complete amino acid sequence of bovine pancreatic carboxypeptidase (Bradshaw et al. 1969; Neurath et al. 1970). At the same time, Bill Lipscomb and associates determined the X-ray structure of the crystalline enzyme (Lipscomb et al. 1970). The identification of the metal binding ligands and of the mechanism of action of the enzyme became a controversy that at times produced more heat than light and, hence, will not be recapitulated here. Even quite recently, the role of the tyrosine residue in the catalytic mechanism has been called into question (Bukrinsky et al. 1998).

During the last several years, F.X. Aviles and coworkers, together with Robert Huber and Wolfram Bode, have added a new dimension to the study of carboxypeptidase by elucidating the structure and mechanism of activation of bovine pancreatic procarboxypeptidase (Gomis-Ruth et al. 1995). They found that in the heterotrimer, the relatively large activation segment binds the other two subunits and protects the procarboxypeptidase subunit from activation by trypsin. The other two subunits can be activated even in the trimeric state. This is in contrast to the activation of porcine procarboxypeptidase, which only exists as a monomer (Guasch et al. 1992). The large activation segment of the bovine precursor appears to serve also as an intramolecular inhibitor and chaperone, as it does in certain other enzymes, such as the α -lytic protease, a serine protease secreted by certain soil bacteria (Cunningham et al. 1999).

The family of carboxypeptidases proved to be larger and more diverse than originally believed. When we began our investigation, little was known about carboxypeptidase E containing a tyrosine residue in place of Glu 270 of bovine carboxypeptidases A and B or about carboxypeptidase Z, a

serine carboxypeptidase that contains a catalytic triad and an oxyanion hole like the serine proteases but, in contrast to the conventional metalloexopeptidases, catalyzes hydrolytic reactions with large leaving groups (Song and Frickert 1997). In all, 10 metallocarboxypeptidases belong to the gene family of mammalian carboxypeptidases (Novikova et al. 2000). Many of these still await detailed structural and functional analysis.

Mast cell proteases

Together with Richard G. Woodbury, we embarked on a series of investigations of proteases isolated from rat mast cells. They include the isolation and determination of the amino acid sequence of a carboxypeptidase-like protease as well as two chymotrypsin-like enzymes, conventionally referred to as rat mast cell proteases I and II or connective tissue and mucosal mast cells, respectively. They differ from each other in distribution, morphology, histology, and other properties (Woodbury and Neurath 1980). The X-ray structure of rat mast cell protease II was determined by Remington et al. (1988) and shown to resemble that of pancreatic chymotrypsin except for certain specific features that might explain their differences in substrate specificity. A unique mast cell protease for which there is no exact counterpart among the pancreatic proteases is the trypsin-like protease tryptase, isolated from mast cells of human lung tissue. The enzyme is a tetramer that shows 40% sequence identity with pancreatic serine proteases. Unlike pancreatic trypsin, it is not inhibited by most naturally occurring trypsin inhibitors (Sommerhoff et al. 1999). Evidently, a great deal remains to be done to advance our knowledge of the structure and function of mast cell proteases to the level of their pancreatic counterparts.

Future developments

By all accounts, research on proteases and on proteins generally is on the verge of fundamental discoveries of unparalleled proportions. In the protease area, attention is being focused on a relatively new group of enzymes, the caspases, and their physiological role in apoptosis (programmed cell death). The caspases resemble each other in amino acid sequence, activation pathway, and substrate specificity (Thornberry and Lazebnik 1998; Salvesen and Dixit 1999). The virus proteases are another group of enzymes that are receiving renewed attention, particularly the viral encoded proteases that are essential for replication and infectivity (Babe and Craik 1995). Some of them contain a novel catalytic triad of His/His/Ser instead of the conventional Asp/His/Ser of the pancreatic proteases. They also contain a single β barrel fold instead of the two β barrel folds of the pancreatic serine proteases.

Another exciting development relates to the isolation and characterization of multicatalytic proteases, or proteasomes, that digest a variety of cytoplasmic, nuclear, and membrane proteins that have been marked for degradation by polyubiquitin chains. The structure of the 20S proteasome from yeast has been resolved by X-ray crystallography (Groll et al. 1999). Another major advance, not necessarily limited to proteases, is the characterization of the structure and mode of action of chaperones (Horwich et al. 1999) in the folding–unfolding cycle of proteins. They have added a new dimension to the kinetics and specificity of the transition of polypeptide chains during protein synthesis and degradation and thus introduce a new variable into our understanding of this fundamental process. These and related discoveries have taken advantage of new technologies among which mass spectrometry, combinatorial chemistry, the phage display method, and nanochemistry deserve special mention, as they promise to become important tools in current and future waves of discoveries in protein science. By far the most important development relates to proteomics, the realization that a single genome can give rise to many quantitatively different proteomes, such as proteins that are posttranslationally modified and complexed with many other protein and nonprotein molecules (Thornton et al. 1999; Thornton 2001). Whereas for a long time, the ultimate goal was to analyze to the most minute detail the structure and function of single proteins, it now appears that in the postgenomic era this concept will have to be broadened to include the multitude of interactions between proteins and ligands that occur in vivo (Eisenberg et al. 2000). Genomic analysis of many diverse species including human are already yielding a wealth of new information that in the course of time will revolutionize and move the study of proteins once again toward the center stage of biology and medicine. This wave of new knowledge will be based on new and integrated methodologies and concepts that will attract protein scientists, chemists, physicists, geneticists, and other practitioners from every major discipline in the bio-medical science area. If the second millennium was dominated by the advent of molecular biology, the beginning of the current third millennium may well be marked by proteomics and gene technology. These developments may take decades to reach fruition but are already on the scientific horizon.

Note

For the first part of my memoirs, covering the first 15 yr of my professional career, see Neurath (2000).

Acknowledgments

I am indebted to David Eisenberg and Gottfried Schatz for reading an early draft of this review and offering valuable suggestions.

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