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Robbins Conjecture An Autobiographical Investigation

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ABSTRACT: Chromatin structure is now believed to be dynamic and intimately related with cellular processes such as transcription. Over the past few years, high-resolution structures for the histones have become available. These structures and their implications for nucleosome organization are reviewed here.

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1 Prologue

The following is the log of a day in the life of a typical 35-year-old faculty member at one of today's research universities:

0730—breakfast meeting of the Committee on Revision of the Graduate Preliminary Examination; 0900—give today's lecture in the beginning course for premeds; 0950—put off student questions after the lecture because….; 1000—meeting of the Building Committee to discuss the color for repainting the 4th floor hall; 1100—meet with today's departmental seminar speaker to tell her all the wonderful things that "you" are doing in your laboratory; 1130—explain to your starting graduate student that you would like to discuss his thesis proposal, but that today is the deadline for a letter of recommendation for one of your other students who is applying for a postdoctoral fellowship; 1200—emergency Departmental lunch meeting to decide how to respond to a claim by unannounced EPA inspectors that a waste bottle for organic solvents in your hood was not stoppered; 1330—missed an appointment with a potential postdoc previously arranged for 1300, use the unexpected 30 minutes to scan and reply to your E mail; 1400—meeting of the University Junior Appointments Committee in the Humanities (you are the token scientist); 1530—call from the Provost asking you to serve on an Ad Hoc Committee to examine the claims of fudged data by a whistle blower; 1545—start the rewrite of a grant request that had already received rave reviews but got a priority of 1.28, which was .02 below the payline for this round; 1630—attend Department seminar; 1745—call your spouse and explain that Bill W. had called in sick and you had been asked to stand in at the dinner for the seminar speaker—yes you remember that it is "concert night" but can't someone else go in your place—after all you just have to tend out.

This may be a little exaggerated, but not by much. Is this really what research is all about, what you were trained for and looked forward to doing with your life? Where is the fun and excitement of discovery? Or, God forbid, is this the new definition of "fun"?

2 How it was

2.1 Setting the Stage, Precollege Days

With the essential help of his mother, Marianna Middlebrook Richards, the author arrived on scene in 1925. At that time his father, George H. Richards, had graduated from Yale in 1903 and was now well underway with a law firm set up by himself and a friend in 1911, located in New York. (Although the family's winter address was New York City, both sides were descended from early pilgrims who moved to the colonies of Fairfield and New London in the early 1600s. For the next three centuries, many members of the Richards/Middlebrook clans spent all or most of their time in what later became the State of Connecticut.) Older sister, Marianna, was 14, and second sister, Sarah, was 11; brother, William, had died in 1923 at age 3 from a strep infection.

High school years were spent at Phillips Exeter Academy. The excellent science department even permitted certain students the unsupervised run of the laboratories outside of class hours. This attitude played a strong role for my roommate John King (destined to be a physicist) and me in cementing our commitment to scientific careers. (I believe that no such access would be permitted today in any school owing to fear of legal actions.) We learned many useful trades at that time with Strong's text on *Procedures in Experimental Physics* as a major resource: glassblowing to the level of a two-stage mercury diffusion pump, a differential thermometer to clock the hours of sunlight, an attempt to measure the universal gravitational constant. For the latter we made a torsion pendulum with a 20-min period in a vacuum chamber, and we used 100-pound cannonballs borrowed from the village green as moveable weights. This particular project was unsuccessful but very educational! I ground and polished a 6″ parabolic mirror for a reflecting telescope, made the system for the Foucault test, and learned how to put on a silver coating with the Brashear process. King was a real expert in the electronics of that period, and both he and I knew most of the RCA Tube Manual by heart. This was all great fun. (Some years later one of my great disappointments was the overnight disappearance of the need for any knowledge of the Tube Manual, shortly after the appearance of the transistor in 1948!)

2.2 MIT, Harvard, and Postdoctoral Years

For those with a scientific bent, the Massachussetts Institute of Technology (MIT) was the obvious choice for college, especially in the middle of World War II. John King and I both started there in 1943. My stay was interrupted by an uneventful two-year stint in the Army, after which I finished Course V, the undergraduate major in chemistry. King graduated from Course VIII and eventually joined the physics faculty at MIT. As an elective I happened to take Bert Warren's one-year physics course in X-ray diffraction, which covered almost all aspects of diffraction except the determination of the structure of organic compounds. Nonetheless it turned out to be very useful, and indeed it determined the direction of my future research.

At this point my sister intervened again and sent me to see John Edsall and Larry Oncley in EJ Cohn's department in the Harvard Medical School. Admission to the graduate program was arranged. Since my sister knew my interests, I assumed she had made the right decision; it never occurred to me to check around anywhere else, and I don't remember in those days that big efforts were made by universities to solicit applications and then wine and dine those who did apply. If you wanted a graduate program, a number of places would let you apply without any fuss. In Cohn's department, on the order of 100 professional scientists were worrying about proteins; five of these were graduate students. I am sure to this day that this is about the right ratio. The five of us were effectively invisible.

Stig Claesson from Upsala suggested that we try a density gradient column based on xylene-bromobenzene mixtures, following the procedure developed for other purposes by Linderstrøm-Lang in Copenhagen. I did. It worked and was quite quick and easy, and the results agreed with the microbalance values. (This turns out to be a useful procedure for measuring the density of any solid sample because a sharp line indicates purity, and mixtures and impurities are easily seen and not averaged (16)). Since the beta lactoglobulin crystals contained only water and protein, the composition was established by dry weight analyses. Thesis complete; PhD obtained; great fun all the way through.

After receiving a PhD, I spent an additional year working directly with Edwin Cohn. I did some team teaching with Frank Gurd in the first year of the then-new, all-encompassing Medical Science program for first-year medical students. To indicate other applications for his protein separation procedures Cohn arranged that I share the stage in the auditorium of the Boston Museum of Science with a farmer and a cow. The latter was attached to a milking machine, which was attached to a milk separator, which fed the whey into a precipitation chamber, which transferred the suspended precipitate to a centrifuge, which delivered the dissolved precipitate to the automatic machinery developed for protein fractionation of blood plasma. I was supposed to explain to the lay audience what was going on! Both were "interesting experiences"!

It was time to leave Boston. Chris Anfinsen suggested Linderstrøm-Lang and the Carlsberg Laboratory in Copenhagen as the place for aspiring protein chemists. Having met Lang during one of his many visits to the United States, I could sense what a marvelous man he was and I agreed with Chris's choice. In the fall of 1954, my wife Heidi, Hans Clarke's second daughter, and I with our two small daughters went to Copenhagen. A description of that wonderful visit is covered in some detail elsewhere (27). By the end of the year at Carlsberg I had found that limited proteolysis of Ribonuclease-A with subtilisin Carlsberg gave a high yield of an altered enzyme (Ribonuclease-S), which was still an active nuclease, and that these two forms of the enzyme could be easily separated by chromatography (21). I did not recognize at that time that the postdoctoral years are usually the best part of one's scientific career: no exams to worry about, no assigned duties; play in the lab and generally lay out your own project.

2.3 Early Years at Yale

During the year at Carlsberg I received the offer of a faculty position in the Biochemistry Department at Yale from Joe Fruton, then chairman of Biochemistry. Not only was I pleased to have a job, but there was also pressure, implied but never expressed, from the family to accept. My father, uncle, and earlier ancestors had all attended Yale. I was the black sheep for having gone to MIT, but perhaps I could make amends within the family by joining the University as an employee. As it turned out I have spent all of my scientific career at that institution, far more in total years at Yale than the student years of all of my ancestors combined. However, I came to realize that you can only be considered a "Yale man" if you attend as an undergraduate. By the same token, I could never claim to be a "Harvard man" because I was there only as a graduate student. I am not aware that MIT fractionates its students in the same way.

I showed up in New Haven in 1955. The first person I met in the corridor of the Biochemistry Department was Maxine Singer, who introduced herself as a graduate student with Fruton. I had no clue, of course, as to the heights to which she would rise during her own career. It was an exciting department with lots of young people on the staff all roughly my own age. My teaching assignment was the biophysical section of the biochemistry course. This turned out to be easy because John Edsall had recently published a long chapter on all the physicochemical methods, in the first edition of *The Proteins*. I knew the students were unlikely to read it, even when it was given as a reference; thus I was able to seem remarkably well informed as a lecturer!

The Ribonuclease-S project, which Lang had kindly allowed me to take away and continue, clearly required amino acid analysis to establish the site of the proteolytic cleavage. After running through the Stein and Moore analytical procedure by hand a few times (ninhydrin assays on 300–400 tubes), laziness demanded mechanization. A system was set up that exactly duplicated the manual process. The Dowex-50 resin column dripped into a collecting funnel that fed a glass tube bent into a repeating figure eight (homemade, of course) that sat in a boiling water bath. After a predetermined number of drops, ninhydrin reagent was added automatically to the funnel. A relay then pulled on a lever that tipped the tube.

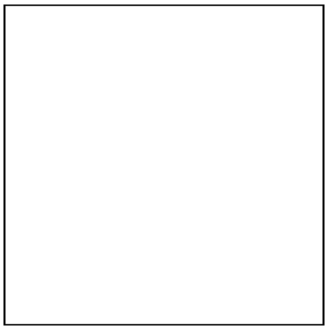

Figure 1: The histone dimer. The homodimer of HMfB (107) is used as an example. This dimer has a perfect twofold axis of symmetry, which is only approximate in the heterodimers. The strand from the N-terminal HSH motif of one member pairs with the C-terminal strand of its partner to form a β-bridge (4), and the ends of the N-terminal helices are also close together to form another paired element (4).

Table 1: Some consequences of hierarchy in RNA folding and of the large difference between the energy content of the secondary and tertiary structures

Kinetic traps
Early stages to the molten globule state
Ensemble of loose states with most or all 2-D elementary motifs
Rather insensitive to single mutations and to magnesium concentration
Sensitive to kinetic traps
Large negative ΔGs of formation (RNA helices)
Late stages toward native 3-D fold
All-or-none and highly cooperative processes involving specific tertiary interactions between preformed substructures and domains
Very sensitive to single mutations and to magnesium concentration
Small negative ΔGs of formation

The contents of each segment then passed on to the loop in the next turn of the figure eight. After about 15 min total in the bath, the final tip dumped the now-developed sample into a cuvette, which was read by a colorimeter and recorded on a strip chart. This Rube Goldberg device provided Werner Klee, my first graduate student, with data for a nice thesis on the conversion of all the amino groups in the enzyme to guanidinium groups using the O-methyl isourea reaction (12). After Werner finished, the device was replaced, with some sorrow, with a copy of the much better continuous flow model developed by Stein and Moore, the precursor of the later commercial Spinco machine that became the workhorse for the biochemical community.

3 THE RESEARCH COMPONENT

At this time in a career, the standard academic procedure takes hold; responsibilities pile up. Research is soon done only by graduate students and postdoctoral fellows. My group has been run in the “disorganized mode.” Each individual has usually ended up doing what they wanted, but with no particular relation or coordination between the different projects. For grant purposes, of course, we have written up the applications as though there were one or more clearly achievable goals and a logical set of experiments by which to obtain the necessary data. The title of the grant was very general and has not changed in 35 years: "Study of Proteins in Solution, Interfaces and Solids." We could hide under that and do almost anything without violating the regulations! The following is a nonchronological sampling of some of the work.

3.1 The Ribonuclease-S System—Chemistry and X-Ray Scattering

Paul Vithayathil was my first postdoctoral associate. He came to work with Joe Fruton, but Joe persuaded him to work with me, an act for which I became eternally grateful. Paul skillfully carried out a large variety of chemical modification experiments on S-peptide, the small 20-residue piece of RNase-S, to try to identify the groups important for catalytic activity and to find out what was responsible for the very tight binding to the other component, S-Protein. One could only alter the peptide chemically or enzymatically at that time. Merrifield synthesis and the genetic approaches were still years away. When Paul returned to a position in India, he had exhausted about all the work that could be done with chemical techniques, and we had a catalogue of functional group importance in S-peptide (29). His 12 was an essential component of the active site. All other formal charges had only minor effects. Met 13 was important for the binding; the other nonpolar residues could not be changed.

During this time it became increasingly clear that a detailed 3D structure was essential to go much further in interpretation. Kendrew had shown in 1959 that protein structures were obtainable with myoglobin; the isomorphous replacement procedure for phase determination worked. David Harker (of Harker Section fame) and his associates had been working for some years on ribonuclease-A. I was getting impatient for the structure and decided to go after RNase-S, which Paul had already crystallized. Because it was a "different" protein, I could squeak by without having it considered direct competition with Harker, which would have been unacceptable in those days. Each crystallogaphic lab had a "right" to its own protein.

3.2 Properties of Proteins in the Crystalline State

In the early 1960s, while waiting to get the major structure project properly launched, we played around with some other questions. Some skeptic at a seminar on protein crystallography would almost always ask: "Why should there be any relation between the structure of a protein in a crystal and in its biologically relevant form in solution?" Except for faith, no direct evidence was available to squelch such skepticism. Marilyn Doscher and I decided to put it to rest. We knew that protein crystals had a lot of internal solvent with properties similar to water. We would just diffuse in a small substrate and see if it came out as product. She did this with all the proper controls, using nucleoside cyclic phosphates as the substrates. The crystals clearly were active under conditions of no demonstrable activity in the mother liquor (6).

Also at this time we developed a flow-cell to hold the crystal in the X-ray beam so that liquids of varying composition could be made to flow over it (36). The diffraction pattern was seen to change when the substrate was in the solution and to return to the starting pattern when the substrate was washed out. Without the structures that came later, one could not know from the X-ray data whether the intensity changes were due to the bound substrate or the bound product, but the biochemical evidence showed that the conversion had occurred.

3.3 The Optical Comparator (Fred's Folly)

Just after the solution of the RNase-S structure, I was granted a sabbatical leave. This translated into a marvelous year in David Phillips's lab in Oxford. The construction of physical models of the myoglobin and lysozyme structures had been terrible chores, involving months of going back and forth between a 3D stacked electron density map and measurements on the growing model. There must be a better way. Calling up the memories of the telescope mirror and the microbalance, it suddenly seemed that a large-scale beam splitter would be just the thing. Big half-silvered mirrors (one way glass) are easily obtained from the mirror-making companies. So I built the "Folly" just outside of David's office, and the RNase-S model, seen through the mirror as though immersed in the density map, was assembled in a relatively short time (23). A number of these "instruments" were built by the now increasingly successful protein structure labs around the world. This was a bit like a very small-scale version of the clipper ship era. In spite of the enormous literature describing the ships, that famous period lasted only about 10 years, after which the vessels powered by steam engines took over. In the same fashion the Follys disappeared overnight when the computer graphics revolution got to the stage of ease of use and commercial availability. Shortly after the "death" of the Folly, I found, thanks to my nephew Howard Wellman, a theater major at Cornell, that I had an unrecognized and unacknowleged debt to the world of magic. The half-silvered mirror played an important role in vaudeville shows in the late 1800s, for example converting a member of the audience, brought on stage, into a skeleton and then revivifying him. Those of you who are interested in arcane bits of historical trivia may find amusement in the appendix to a short review article on map fitting (26).

3.4 Membrane Protein Geometry

In the early 1970s, Jim Staros came to my lab as a graduate student with a remarkable amount of synthetic organic and photochemical experience from his undergraduate days at Dartmouth. Prompted by Jeremy Knowles' recent introduction of aryl nitrenes as photoaffinity labels, Jim synthesized NAP-taurine, a nitrophenyl azide with a strong anionic group that would not pass through a lipid bilayer. He used this reagent to study membrane protein geometry (32). This work was the start of a whole series of reagents developed over the next few years by various individuals who passed through the lab.

The closest we came to high resolution data on a membrane "protein" was Bob Fox's structure for alamethicin, a pore-forming peptide crystallized from a water-free organic solvent (7). He proposed a plausible detailed model for the voltage-gated pore as a high oligomer of the monomeric structure found in the crystal.

3.5 Protein Structure Analysis

Just before the start of our efforts on membranes, BK Lee came to the lab with a great interest in computation. In the course of producing a transparent, stacked-sheet, packing model of RNase-S, where the sections cut through the vanderWaal's envelope around each atom, he realized an alternative use for the same program. By simply increasing the radius of each atom by a fixed amount, he could calculate a surface area defined by a probe sphere of any specified size. Thus was born an algorithm for the numerical approximation of surface accessible area that can easily be applied to any molecular model whose atomic coordinates are known. His 1971 paper (14) on this subject is one of the few whose citations increase continually with time even today, 25 years after its publication.

Tim Richmond (31) and Mike Connolly (4) both spent some time in the lab some years later, and after leaving, both independently solved the nontrivial geometrical problem of providing an analytical solution for the description of the intersection of three spheres of different sizes (Richmond–31; Connolly–4). Even though an exact analytical solution is now in hand for calculating either the solvent-accessible or molecular surface area, the original Lee approach is still widely used. Practically all X-ray structure papers today refer to some surface area characteristics.

Volume was next. In the early 1970s I was called for jury duty in New Haven. This citizenly chore turned out to be a blessing in disguise. In each court case either the prosecution or the defense did not want an academic on the jury. Thus for two or three months I spent each day in the jury room with no phone or other disturbance. This time permitted the rapid coding of the Voronoi procedure for calculating atomic volumes, which I had been put onto by John Finney from his work with JD Bernal. Each lunch recess I ran up the street to the computer center and put the morning's effort on punch cards and started the debugging. It all went well and produced quantitative estimates of the packing quality in proteins (24). The results were that the average packing density was in the same range as that found for molecular crystals of small molecules; thus the packing was about as tight as could be expected. However, the packing density throughout the whole molecule was nonuniform, and there were packing defects and cavities (25). All of this suggested that the unique structures of native proteins might be explained by simple geometric arguments based on the hard sphere approximation and that function might be related to packing density. While the proposal retains an element of truth even today, the actual picture turns out to be much more subtle.

Roughly a decade later, Jay Ponder came to the lab and dramatically extended this work with PROPAK, his program for calculating the "tertiary template," or the inverse folding problem that predicts the sequences compatible with a fixed main chain conformation (18). The number of such sequences is exquisitely sensitive to the packing density chosen as acceptable. Interestingly, the real proteins do not appear to have the highest possible packing density. The number of allowed sequences, while generally quite large, is still only a tiny fraction of the combinatorial total, i.e. one should expect homologous proteins with different sequences but the same common structure as found experimentally. The predictions are not perfect; for a given interior packing unit (5 or 6 residues) a number of rotamer combinations are possible for a given sequence. This is not seen in the actual X-ray structures where the core residues invariably have one unique conformation. The program, however, has been very useful and provocative.

3.6 A Pseudopod into NMR

As a graduate student in the period before the multidimensional pulse sequences had been developed, David LeMaster asked to work in the lab on an NMR project. He had the idea of an orchestrated approach through labeling with stable isotopes to produce samples where the interpretation of the spectra could be dramatically simplified. The proposal was for detailed spectral editing far beyond what any protein NMR lab had done at that time. I asked him why he wanted to come to my lab since I knew next to nothing beyond the *Scientific American* level about NMR. He said he knew that but discounted it because he already knew exactly what he wanted to do and only needed space and money to carry on. This was a refreshingly different approach for an entering graduate student, so I said OK.

Much of the work was in the preparation of the necessary labeled amino acids. Completely on his own, David got to know more about intermediary metabolism in *E. coli* than anyone in the department; he deleted appropriate enzymes in his production strains so that the planned label positions would not get scrambled and combined this work with detailed studies of procedures for the preparation of highly pure amino acids in gram quantities using displacement chromatography. The atoms in the amino acids were carefully arranged to avoid adjacent ^{13}C neighbors and thus to eliminate the coupling, which would only confuse the spectrum. He selected *E. coli* thioredoxin as the protein of choice: The genetics were in hand; the protein was very stable, and it was small enough for NMR procedures of that time. Also the expression strains had to be made auxotrophic for all the relevant amino acids. All of this was in his original proposal and was actually accomplished (15). This work has not been properly recognized because the two-dimensional procedures that appeared well after he was in the middle of his work swept aside any other approaches. It is likely that interest will return as the movement to carry out NMR studies on ever larger proteins continues. One aspect of his work, random fractional deuteration to minimize spin diffusion, is well established today.

3.7 Pressure as a Variable

Walter Kauzmann has pointed out that knowledge of the PV term in the expression for total energy would have to be known in as much detail as the TS term before we could claim understanding of any process or phenomenon such as the hydrophobic effect. Because temperature is much simpler to control than pressure, only a tiny number of labs are carrying out such studies.

For most inorganic and organic crystals of known structure, only changes in unit cell dimensions with pressure are needed to establish the compressibility of the material. As discussed earlier for density, such measurements on a protein crystal are not enough because the composition of a crystal may change with pressure. Complete and separate structure determinations at two or more pressures are required along with the crystal densities at each pressure.

Over the years the lab has been chipping away at some problems in protein structure and the origin of function. Many colleagues in addition to those mentioned above have been involved. Some 15 to 20 different proteins or protein systems have been studied. This work was carried out during a great period in the history of protein chemistry.

4 THE NONRESEARCH COMPONENT

We all complain about not having enough time to talk with our students and perhaps to carry out some research ourselves. The reasons for difficulty in saying "no" to requests for our time are complex, but a common factor is the response to opportunities for recognition by our peers.

4.1 The Jane Coffin Childs Fund

One such opportunity was the offer I received for the Directorship of the Jane Coffin Childs Fund for Medical Research. I could not resist giving it a try and served as Director from 1976 to 1991, when I turned over the helm to Joan Steitz. It was not a major time commitment because all of the actual work was carried out almost singlehandedly by Elizabeth Ford, the Executive Director. The principal role of the Director was to keep the Board of Scientific Advisors properly "stocked." This turned out to be quite easy. Little arm twisting was required. In recent years the JCCF has spent all of the available funds on Postdoctoral Fellowships, considered the most highly leveraged and effective use of its resources at this time. As of 1996, the Fund's 59th year, well over 800 fellows have been supported. This group along with those of a few other similar private foundations have turned out to be major players in biomedical research not only in the United States but around the world. My association with the Childs Fund was both pleasurable and worthwhile.

4.2 Scientific Societies

Other opportunities for recognition were provided by the scientific societies who are always looking for officers to run the group for a year or two. To ensure democracy, there is always an election ordinarily with two candidates for president. These candidates are usually ambivalent. One hopes the election will be close so that whoever "loses" will not be too upset. However, it is not clear who wants to "win." By "winning" you inherit a chore, while by losing a close election, you have evidence of peer regard without having your research time further eroded by actually having to do something!

4.3 Departmental Chairmanships

At the request of Yale's President Brewster, in 1963 I moved from the Medical School Biochemistry Department to the Biophysics Department on the north campus, taking over as chairman of the group, then renamed Molecular Biology and Biophysics to recognize the new era just beginning. The original Biophysics Department, focused on radiation biology, had been set up just after WWII by Ernie Pollard.

The University was trying to attract Clem Markert to be chairman of the Department of Biology. He said yes he would come if he could have the new MB&B department folded in as well. We took umbrage at that and said "No." Negotiations continued. The upshot was that MB&B was allowed to stay as a separate department as long as it changed its name. In 1964 it became the Department of Molecular Biophysics. "Molecular Biology" was too politically sensitive so, cutting off our noses, neither department was allowed that phrase in its title.

Yale decided to amalgamate the Departments of Biochemistry in the Medical School and Molecular Biophysics in Arts and Sciences into a single unit. I was asked to chair the new unit on my return from leave and was unable to force myself to say "no." After hours of discussion the joint faculties settled for the name Molecular Biophysics and Biochemistry, MB&B, the same initials we had had five years earlier before the Markert episode!

Although the warnings were in the wind, this was still the Golden Age, and we were supplied with a number of positions to fill. We managed to get as new junior faculty Don Engelman, Tom and Joan Steitz, Peter Moore, Dave Ward, and John Cronan. We also had some empty slots we did not really need, and so we gave some to the new Cell Biology group during the negotiations that extracted George Palade from Rockefeller University. Later on we gave up some more to help set up Human Genetics. All of this was interesting, but was not science, and could not compare with research excitement. Negotiating with the top brass is almost always an ulcer producing exercise anywhere. Today the problems of a chairman are horrendous and make anything that I ran into in the early 1970s pale to insignificance.

5 "THEM AND US"

5.1 Indirect Costs and Circular A-21

In my view, the big change within the universities began in 1965. Prior to that date both administrations and faculties were engaged in a joint effort with the same goals, and they were, to a large extent, mutually supportive. In 1963 the universities, acting collectively, decided to go for full cost recovery in the conduct of scientific and engineering research. Before that date, grants from the government were considered grants-in-aid, i.e. they assisted, but did not replace, whatever the university otherwise had arranged for the support of research. The faculty began to realize only slowly what had happened; in fact, overnight, research changed from being viewed as pure scholarship to being a "profit center." The governing document was, and is, the infamous Circular A-21, which describes how one computes indirect costs. Earlier, the granting agencies had tacked on about 25% to the direct costs of a grant in order to help with the administrative, library, utility expenses, etc. Under A-21 a very detailed and complicated set of formulas was and is used to compute the reimbursable indirect costs. Needless to say the numbers were much larger than 25% and represented a very large new income stream for the universities. Since this was not "new money" but simply a redistribution of the available research funds between indirect and direct costs, the latter being what the faculty can spend to carry out their research, a wedge had been driven between the administration and the faculty. Subsequently the two groups have become more like adversaries than colleagues.

Indirect costs are real expenses; the only question is who should be responsible for paying them. Today the transition is complete and apparently irreversible. Each academic scientist and engineer runs a cottage industry. He or she is required to raise the funds to support the research and to pay "rent" for the space. At one stage an attempt was made to require faculty to keep time sheets as the basis for "accurate" cost accounting of their varied activities. This caused an explosive reaction in the sciences, but we received no support from the law faculty who just laughed because those who practice law do this routinely. Now knowing how difficult such an exercise can be, some of us have become much more skeptical of our personal legal bills.

5.2 EPA and OSHA

Government regulations have increased dramatically, especially during the last decade. This increase appears to be correlated both with increases in the national R&D budget, now large enough to attract intense congressional attention, and with the American craving for absolute safety from birth to death. OSHA and EPA are special targets of faculty wrath. Applying regulations developed for safety in large production and manufacturing facilities to research laboratories is just idiotic and indicates a lack of understanding of how the research enterprise actually works. There is a pervasive dissatisfaction with the timidness of university administrations collectively in acquiescing to such rules rather than trying to fight them. MIT received a lot of plaudits when it stood up and fought the government in court about a problem in administration, the application of antitrust laws to graduate student stipends, and MIT won! The other universities were sitting on the sidelines watching, very much like the penguins in Antarctica who, when one of their number dives in, all peer over the edge of the iceflow to see if it is eaten by a leopard seal, before they go swimming themselves. However, none of the universities have sought legislative relief from the oppressive laboratory regulations.

5.3 Scientific Misconduct

While a source of occasional amusement in the past, scientific misconduct is a non-funny hot topic today. It is not clear why this is so. The daily papers are filled with reports of egregious misconduct of accountants, lawyers, bankers, stock brokers, defense contractors, nondefense contractors, politicians, of course, and even judges. Why should the scientific community be required by regulation to attend courses on how to behave ethically? Apparently scientists are expected to live up to higher standards than are other groups. I do not see why misconduct should be defined by discipline. Fraud or sexual harassment are not associated with any particular field of activity. Plagiarism occurs in all areas where the written word is important. Data fudging is just as important in the financial world as it is in science. To top it off, none of these other fields have the strong, nonanthropogenic corrective pressure that is provided in the study of natural phenomena. I have been unable to think of a form of misconduct that is specifically and solely associated with science.

As opposed to misconduct, "non-ethical behavior" is in the eye of the beholder. Different individuals will interpret the same event differently. We see this in all kinds of people, even in our friends on occasion, and they presumably in us, but this choice of personal style is not "misconduct" that requires legal action. If more individuals recognized the mileage that one gets by leaning over backwards to acknowledge the work of one's colleagues, a good segment of the turmoil over scientific misconduct would disappear overnight. Much of the rest could be handled by listening without jumping to immediate conclusions. A major culprit seems to be ego.

My own experience with a claim of misconduct was in the Gallo-Popovic affair concerning the first isolation of the AIDS virus. Whatever the truth of the matter may have been, the issues were considered to have been closed by a signed agreement between the French and the American governments on the priority claims of the Montagnier and Gallo labs for isolation of what is now called HIV. The accusations were reignited by a very long and detailed article published in November 1989 in the Chicago Tribune by the investigative reporter John Crewsdon.

Along with seven others, I was appointed a member of a panel of advisors to the Director of NIH. We did not investigate the charges; another group did that. We were to advise on the process and to comment on the final report of the investigation. This group was set up because of the international prominence of Dr. Gallo and the intimate involvement of the press from the very beginning. As far as I know, no such group was set up in any of the other cases. I now believe that it was not a good idea. I can see, admittedly only in retrospect, that the process was almost certain to produce, as it did, frustration for all parties to the dispute, especially for the Panel itself limited to its advisory role.

Montagnier's group had sent a sample of the French virus to NIH, and the Gallo lab had cultured this material successfully, separate from their own isolates. Later it was shown that the HIV particles isolated from one of the Gallo Lab's own cultures, the one used to develop the antibody test for HIV, was identical to the French virus. The basic question in the investigation turned out to be whether the French virus appeared in the Gallo culture through an accidental contamination or through a purposeful addition. Since the French virus was in the NIH lab, and since cross-contamination of viruses is not an uncommon problem, either explanation was a possibility. During this period, in the early 1990s, the Panel and the close-reading public were provided with an example of the stark differences between the legal and the scientific communities on the meaning of words. Eventually the case against Popovic was thrown out by the HHS Departmental Appeals Board, and the case against Gallo was dropped. A convincing answer to the major question has never appeared in public.

Investigation of conflicting claims in cases of alleged misconduct is a specialty in its own right. Some people are very good at it, but they are very unlikely to be your standard faculty member. Investigation is not a trial. It is a search for the truth and the necessary facts to establish it. The investigator must be scientifically literate, must know when independent advice in special areas is required and how to get it, and, most especially, must have experience in recognizing lies, dissembling, and deliberate misrepresentation. This is not daily fare for most faculty. Due process is not involved at this point. The investigator should describe the findings in a report, may suggest whether further action is indicated or not, but should not decide guilt or innocence, or punishment. The next step would be to set up a group, mostly scientists, to hold a hearing based on the report of the investigator. Due process begins at this point: the calling of witnesses by both sides, cross-examinations, etc. Such a procedure parallels the court system, but differs in that prompt action is a major goal and there is a predetermined timetable. Later appeals procedures can be used when required.

A single individual or group should not be the investigator, the judge, and the jury all in one. I do not believe that the current procedures being adopted by the HHS Office of Research Integrity and by universities are appropriate solutions to these problems. Even the HHS Departmental Appeals Board has a spotty record. Its function, composition, and procedures need to be carefully reconsidered. The recent addition of at least one scientist to an Appeals Panel is a step in the right direction. A reasoned discussion of many of these problems entitled "The

Laboratory Is Not a Courtroom" has been provided by Charles Maechling, Jr (17).

"WE HAVE MET THE ENEMY AND HE IS US." (Walt Kelly in the comic strip POGO)

5.4 The Scientific Literature

Regarding the content of papers in one small corner of the scientific literature, half a dozen of us in the structural biology community got together in the late 1980s and produced a public letter with over 150 signatures requesting that journals demand public deposition of model coordinates for any paper on a protein structure accepted for publication. Normal expectation for any scientific paper is that the data[1] will be available to the reader for evaluation of the claims made by the authors. This request was not met with uniform acceptance by either the crystallographic community or the journals (1). However, the pressure has been maintained by the action of more and more editorial boards, recently even *Nature*, and especially by NIH in their requirements for grant applications. Thus an original serious concern is rapidly becoming a nonproblem. Also through grant rules NIH is gently pushing toward a solution to a more general problem, the exponential literature expansion.

As one who watches the electronic revolution with amazement, I feel that the communication problem is not being effectively addressed. The current enthusiasm oversells the Internet. The unexpected or the unsought-for may be lost. The speed with which one can now make searches in the literature is indeed dramatic; the cross-referencing is very convenient, and the pictures lovely. However, reliability depends on the completeness of the available data bases and on the users' ability to select the appropriate key words. Today any science reported before the earliest date in a data base simply does not exist, but is often the origin of the original idea or concept, and may be as or more important than the latest modification. I doubt that browsing is any more successful with the screen than with a book in the library. The attractiveness of the system seems to be particularly associated with speed. While the pages can be made to fly by, the reading ability of most individuals remains what it always was. One cannot read text on a screen any faster than in a book. Nor can you absorb or think about it any faster.

The real problem centers on the volume of literature that is published. New journals appear at the same rate now as single papers used to not so many years ago. With or without electronic assistance there is no way for anyone to absorb all the information any faster than before. You cannot be an expert in everything. The result is that the segment of science that you can know in any detail is getting to be a smaller and smaller fraction of the total. These segments as always have a tendency to develop their own language, thus complicating casual communication.

All of this has produced the "wandering minstrel syndrome," a return to the Middle Ages. Along with, or in spite of, the Internet, the seminar circuit is humming. At any institution you can usually arrange to spend each entire day

[1]Several individuals have pointed out that the molecular model does not represent "data," but rather an interpretation of the data. Although the model is very useful and is what most individuals using the Data Bank are looking for, the actual data, the set of corrected intensities for each reflection, should also be deposited. Refinement will become increasingly common as refinement techniques are improved. Such data has always been accepted by the Protein Data Bank, and its deposition should become routine. This can be of use to the originating laboratory where complete loss of older data is not an unusual problem.

doing nothing but attending seminars, the easiest way to keep up to date without bothering to read screen or book.

We have a major problem in organizing and digesting information, and the current efforts are only chipping away at the difficulty. In your own field a paper will usually contain only one idea or datum that you need to know or remember. The rest of the paper is just "noise" from which you must extract this relevant bit. The present format of title/abstract/body is an attempt to help but has to be pushed much further to make even a dent in the problem. Here the electronic options could become very important, but an approach completely different from hard copy publication is required. The viscosity provided by copyright, finances, security and acknowledgment of credit will inevitably slow things down considerably.

A simultaneous approach could be simply an absolute decrease in the amount of literature, a reduction in the number of "minimal publishable units." This cannot be legislated, but it could be effected by a reduction in publication pressure, much of which comes from attitudes and procedures on appointments and promotions. The single most important change would be in bibliographies submitted to appointment and promotion committees. The uniform adoption by all institutions of an absolute restriction to a maximum of, say, 12 publications independent of the rank or position under consideration would help a lot over time. This is the procedure for nominations to the National Academy of Sciences and has already been adopted by some universities. If a case cannot be made for significant scientific contributions from such a list, then there are no such contributions. The pressure would then shift to producing a few first class papers. A stream of minimal ones, regardless of their number, would serve no purpose in terms of peer recognition.

6 EPILOGUE

The title of this article was provided as a question. The answer seems to be that the "fun" is still potentially here. This may be the most exciting period in history for many if not all of the sciences. The problem is that any individual, following the brief apprenticeship phase, has less and less time to engage in research and to enjoy it. An option must be provided for the young to protect their time. The reduction of committees and the simultaneous defense of a democratic system is an enormous challenge, but it should be addressed. Let's all hope that the PROLOGUE is indeed overstated and that the fun component can be reinstated.

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