RECOLLECTIONS

Reaction mechanisms, catalysis, and movement

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My approach to science developed from a conservative education and an inquiring father. My father loved to discuss almost any question, and did so with great enthusiasm. He began to discuss the meaning and philosophy of life with me when I was 6, and continued to do so for the rest of his life. I often found this difficult to handle. I struggled to find simple conclusions from these discussions that I could understand, with limited success.

Some 10 years later, my cousin told me how much she enjoyed engaging my father in a discussion. He would bring forth a strong opinion on some question, which might be philosophical, and she would take the opposite position. She would then

gradually shift her position and he would follow an opposing position, until he was arguing with great enthusiasm in favor of her original position. This led me to search for an approach to the world that I could understand, which I found in biochemistry and, later, in chemistry.

Education – Elementary

I attended the Calvert School in Baltimore for 6 years. This school had a set of fundamentals that every student was expected to learn. When one had mastered the fundamentals, a red seal was pasted after one's name in a square on a board that listed all the students in the class. Exceptionally good performance merited a gold star on top of the red seal. At the time, I thought that this was a stuffy, old-fashioned approach. But after seeing the results of more progressive, disorganized education in more modern schools, I have concluded that it was very valuable. It emphasized the importance of clear-cut, concrete

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William P. Jencks graduated from Harvard Medical School in 1951. After an internship in the Medical Service at Peter Bent Brigham Hospital (1951–1952), Dr. Jencks spent 2 years in postdoctoral work with Fritz Lipmann at the Massachusetts General Hospital (1952–1953 and 1955–1956). This period was interrupted by 2 years in the Army at the Army Medical Service Graduate School of the Walter Reed Army Medical Center. Dr. Jencks was Chief of the Pharmacology Department during the second year. Following this, he spent a year in R.B. Woodward's laboratory at Harvard.

In 1957, Dr. Jencks joined the Graduate Department of Biochemistry at Brandeis University, where he has remained since. His research has centered around 3 areas: (1) the mechanism of catalysis of acyl and carbonyl group reactions in water; (2) the mechanism of acyl transfer and hydrolysis reactions catalyzed by enzymes; and (3) the mechanism of the interaction of small and large molecules with each other in aqueous solution. Recently, Dr. Jencks has been particularly concerned with the way in which reaction mechanisms are determined by the lifetime of reaction intermediates in general acid–base catalysis, carbanion, carbocation, and ligand exchange reactions, and the use of structure–reactivity interactions to characterize transition states.

Dr. Jencks is a member of the American Society of Biological Chemists, Alpha Omega Alpha, and served as Councilor of the American Chemical Society (1989–1992). He is a fellow of the American Association for the Advancement of Science and former Councilor of the American Academy of Arts and Sciences (1971–1975). In 1971, Dr. Jencks was elected to the National Academy of Sciences. He was elected a foreign member of the Royal Society in 1992. He received the American Chemical Society Award in Biological Chemistry sponsored by the Eli Lilly Company in 1962 and he was the recipient of the American Society of Biological Chemists Award sponsored by Merck, Sharp, and Dohme Research Laboratories Division of Merck and Company, Inc. Dr. Jencks is the author of a text, *Catalysis in Chemistry and Enzymology* (1969), and a review, "Binding energy, specificity and enzyme catalysis—The Circe effect" (1975), that updates much of this text; co-author of a recent biochemistry text (Abeles et al., 1992); and has published more than 360 articles in scientific journals.

Fig. 1. William P. Jencks.

accomplishments—one finishes one thing and then goes on to the next. This approach has been invaluable in science. I have tackled many questions that I did not understand initially, one step at a time. After a (variable) time, the answers usually became obvious and clear. If they did not, in a reasonable time, the project was dropped. Stopping work on a project is at least as important as starting it.

After finishing the 6th grade in Baltimore, I went to the public school for (part of) a year in Taos, New Mexico. I walked into the wrong room and registered for the 9th grade instead of the 7th grade. This was an easy year. The next year I returned to Baltimore and entered the 10th grade in St. Paul's school. This was a hard year.

High school

St. Paul's school was remarkable. It was very small, but it won the state lacrosse championship almost every year. This was a consequence of *school spirit*. Every student in the school participated in every athletic event, as a cheering spectator if not a player. This determination brought results. The players put everything they had into every game and St. Paul's did very well in basketball, as well as lacrosse. It did less well in football because it did not have a stock of heavy players.

I was no athlete—I was small and somehow managed to avoid almost all athletics. I felt that I was an outsider who had little to contribute to the school and its spirit. So I compensated with other activities, including managing the library and yearbook, and editing the school newspaper. I was flabbergasted when I graduated and was given the award for school spirit. I learned from this that accomplishment depends more on determination, and some sense of direction, than it does on intrinsic ability. This applies to science—I am far from brilliant, but I am determined, and I have slowly worked my way toward an increased understanding of several areas of biochemistry and chemistry. I was surprised to find recently that my coworkers and I have published some 360 papers in my 36 years at Brandeis.

More education

I went to Harvard with an interest in chemistry, took first-year chemistry, and changed my major to English. The chemistry course was comprehensive, but the significance and interest of the facts that were poured into our brains were not at all apparent. This has led me to the conclusion that it is more important to teach questions than answers—at least, the questions should be presented before the answers. It is not stimulating for a student to memorize answers to questions that he or she has not asked.

I finally went to Harvard Medical School because I couldn't think of what else to do. I learned little of interest in the pre-clinical years of medical school. A prodigious number of facts were poured into us by a prodigious number of teachers, each of whom tried to tell us everything they knew in one or a few lectures. We examined an even larger number of slides, some ancient, some less so. Few *questions*, or interesting answers to questions, were raised or discussed.

However, I had the good fortune to spend 2 summers at Woods Hole, where I took the physiology course and had the opportunity to carry out some research with George Wald on the blue pigment of the lobster shell (Wald et al., 1948). The

prosthetic group of this pigment, astaxanthin, is a red carotenoid, but when it is combined with the protein, it becomes blue. It is possible to reversibly denature the protein with heat—it turns red, then returns to blue on cooling. The mechanism of this remarkable behavior is still not established, but it may arise from distortion of the double bonds in the bound carotenoid chain that reduces the degree of alternation of single- and double-bond character in the chain and decreases the excitation energy. There is no covalent bonding of the carotenoid with the protein. This work stimulated an interest in biochemistry.

Internship

After completing medical school, I interned at the Peter Bent Brigham Hospital for a year. This was surely the hardest, but possibly the most useful year of my life. Interns have heavy responsibilities even at the Brigham, which is a research-oriented hospital. An intern always has more that should be done than possibly can be done, so he or she learns to identify and deal with the important problems first and get to less important problems later, or not at all. This provided invaluable experience in how to get things done. A number of M.D.s have worked in my laboratory subsequently. They generally know little when they arrive, but they know how to get things done and, within a few weeks, they are progressing faster and more effectively than more knowledgeable postdoctoral fellows with Ph.D.s.

Postdoctoral years and lipoproteins

I decided at this point that medicine was too complicated; it is too difficult to reach a simple conclusion from study of an organism that is as complex as a human being. Therefore, I became a postdoctoral fellow for 2 years with Fritz Lipmann at his research laboratory in the Massachusetts General Hospital and examined the properties of the fatty acid activating enzyme. This enzyme utilizes ATP and catalyzes transfer of the *leaving group*, a carboxylate oxygen anion, by forming a mixed anhydride of the fatty acid and AMP. The anhydride is then attacked by the sulfur atom of Coenzyme A to give the fatty acyl-CoA product (Jencks, 1953; Jencks & Lipmann, 1957). This was an unusual class of reaction at the time, but a large number of activation reactions that involve the abstraction of an oxygen atom from an acyl group or phosphate through an anhydride intermediate have been identified subsequently.

The postdoctoral fellowship was interrupted by the Korean War. I was drafted for 2 years as a physician and, after some grueling basic training at Fort Sam Houston, was assigned to Emmet Durrum's laboratory in the Army Medical Service Graduate School at Walter Reed Hospital. Here we developed a method for protein separation and analysis by paper electrophoresis and carried out a clinical study on serum proteins and lipoproteins (Jencks & Durrum, 1955; Jencks et al., 1955, 1956a, 1956b; Langan et al., 1955). This work showed, in 1956, that the relative concentrations of α and β , or heavy and light, lipoproteins provided a much better indication of atherosclerosis than did the serum cholesterol level (Jencks et al., 1956a).

After the second year in Lipmann's laboratory, I realized that I would have to learn some chemistry if I wanted to understand how enzymes catalyze reactions. Lipmann sent me to R.B. Woodward's laboratory to learn some chemistry. Woodward

was busy when I arrived, but his secretary said to wait in the Chemistry library and she would call me as soon as he was free.

Two weeks later, she called me from the library. Woodward talked with me for an hour and showed me a space in which I could work. There were 16 postdoctoral fellows in Woodward's laboratories, working in groups of 4 on 4 different projects. Woodward spent many hours with *one* of these groups when a project was going forward, but seldom talked with the other groups or with his graduate students, most of whom worked in the basement. I didn't know much about synthesis, so I did some experiments on my own. I managed to ask Woodward a question when I saw him in the hall at midwinter (after I assured him that it was a short question), and spent a second hour with him when I left at the end of the year. However, I learned a lot by attending Paul Bartlett's course in physical-organic chemistry.

General acid–base catalysis

During this year I examined reactions of acetylimidazole and found that imidazole catalyzed the hydrolysis of acetylimidazole. This puzzled me because the reaction of imidazole with acetylimidazole would only give acetylimidazole. After some reading, I realized that I had rediscovered general base catalysis, in which a base accelerates the reaction by abstracting a proton from a water molecule as the water is attacking the acyl compound (Equation 1, Fig. 2). The catalysis is slower in deuterium oxide than in water, with a solvent deuterium isotope effect of $k_{\text{H}_2\text{O}}/k_{\text{D}_2\text{O}} = 3.6$. This isotope effect shows that the catalysis involves abstraction of a proton from water and that the proton transfer is concerted with attack of water on the acyl group in the rate-limiting step. This work was completed and published 2 years later, after I joined the new Department of Biochemistry at Brandeis University (Jencks & Carriuolo, 1959a, 1959b).

Many reactions are catalyzed by acid or base, but enzymes cannot increase the concentration of H^+ or OH^- ions to bring about catalysis. Betts and Hammett observed that the rate of ammonolysis of methyl phenylacetate in methanol is 3/2 order in $[\text{NH}_3]$ (Betts & Hammett, 1937). General base catalysis and general acid catalysis provide attractive mechanisms for catalysis by enzymes, which can utilize the imidazole group of histi-

dine or other basic groups in the active site to remove a proton from an attacking nucleophilic reagent and utilize general acids to add a proton to a substrate in the transition state. Enzymes cannot change the pH of the solution in which they reside, but they surely can, and do, donate and accept protons by utilizing basic and acidic groups at the active site for general base and general acid catalysis.

We examined this catalysis in some detail for the aminolysis of phenyl acetate and subsequently for a number of other reactions. Catalysis can be brought about by proton transfer to or from the substrate by acids or bases in the solution. General base catalysis is utilized by serine proteases to abstract a proton from the hydroxyl group of serine as it attacks the acyl group of a substrate molecule that is bound in the active site. In 1961, we showed that the hydrolysis of *N,O*-diacetylserinamide, a model for the acyl-enzyme intermediate of serine proteases, is catalyzed by imidazole buffers. The catalysis is 2-fold slower in deuterium oxide. This shows that the reaction is true general base catalysis of proton removal from the water molecule as it attacks the serine ester, in a concerted reaction (Anderson et al., 1961).

Similar catalysis and isotope effects were observed with a series of esters (Jencks & Carriuolo, 1961) and general base catalysis was observed for reactions of the acyl group of acetyl phosphate monoanion with a series of primary and secondary amines. However, pyridines and other tertiary amines attack the phosphate group of acetyl phosphate to give a phosphorylated pyridine, which can be trapped by fluoride ion to give fluorophosphate (Di Sabato & Jencks, 1961a).

Reaction mechanisms—Appearance and reality

At this time we made a serious error, by confusing *appearance* and *reality*. We examined 9 criteria to characterize the transition state for the hydrolysis of acetyl phosphate, all of which showed that the hydrolysis occurred through a dissociative transition state with a large amount of bond breaking and little or no bond formation to water. We concluded that the hydrolysis proceeded through a monomeric metaphosphate monoanion, which then reacted rapidly with water. The values of $\Delta S^\ddagger$ for hydrolysis of the mono- and dianions of acetyl phosphate are

Fig. 2. Equations 1, 2, and 3.

close to zero, compared with $\Delta S^\ddagger = -29$ e.u. for the diester acetyl phenyl phosphate; there is no significant solvent deuterium isotope effect; hydrolysis is much faster than the hydrolysis of acetyl phenyl phosphate; the ρ value of 1.2 for hydrolysis of the dianions of substituted benzoyl phosphates showed that the transition state is strongly dissociative, the hydrolysis occurs with P–O cleavage, the addition of acetonitrile has little effect on the rate of hydrolysis with acetyl phosphate but decreases the rate with acetyl phenyl phosphate, $\Delta V^\ddagger$ is close to zero for acetyl phosphate but is $-19 \text{ cm}^3 \text{ mol}^{-1}$ for acetyl phenyl phosphate, and the solvolysis of acetyl phosphate dianions in 7 M sodium perchlorate gives pyrophosphate as a product. All of these results are consistent with a reaction that proceeds with a large amount of bond breaking in a strongly dissociative transition state to form a metaphosphate intermediate (Di Sabato & Jencks, 1961a, 1961b).

But they don't prove it. These results show that the transition state for the hydrolysis of acetyl phosphate is strongly dissociative, as would be expected for the formation of metaphosphate; there is little or no nucleophilic attack by water in the transition state. However, none of these results *proves* the existence of a monomeric metaphosphate intermediate; they show that the transition state resembles metaphosphate, but they do not demonstrate that a monomeric metaphosphate intermediate with a significant lifetime is formed in the reaction. In fact, the methanolysis of phenyl [^{16}O , ^{17}O , ^{18}O] phosphate monoanion and 2,4-dinitrophenyl phosphate dianion in aqueous methanol proceeds with complete inversion at phosphorus. This requires that these reactions must occur by bimolecular displacement or, possibly, by a preassociation mechanism in which a short-lived monomeric metaphosphate intermediate reacts with an adjacent solvent nucleophile faster than it can rotate to give racemization (Buchwald et al., 1984). However, the solvolysis of phenyl (R)-[^{16}O , ^{17}O , ^{18}O] phosphate in acetonitrile containing 1 M *tert*-butyl alcohol gave predominant racemization (Friedman & Knowles, 1985). This is consistent with the formation of a metaphosphate intermediate but does not exclude transient phosphorylation of acetonitrile that gives rise to partial racemization in the *tert*-butyl phosphate product through a sequence of steps that proceed with inversion.

We learned from this that the *appearance* of a transition state, as measured by structure–reactivity correlations, solvent effects, isotope effects, and other criteria, usually does not tell us what is the *mechanism* of a reaction, although it may give hints. The mechanism must be established by determining what are the number and the sequence of individual steps in the reaction, i.e., the number, nature, and sequence of Gibbs energy maxima that occur along the reaction path.

General acid–base catalysis and bell-shaped pH-rate profiles

It has been known for many years that the reactions of aldehydes and ketones with hydroxylamine and semicarbazide exhibit striking maxima in their pH-rate profiles. These maxima had been attributed to the opposing effects of general acid catalysis of the addition of the amine to the carbonyl group and a decrease in the concentration of the nitrogen base at low pH, as it is protonated (Barrett & Lapworth, 1908; Conant & Bartlett, 1932; Hammett, 1940).

However, I found that the addition of semicarbazide or hydroxylamine to aldehydes and ketones at neutral pH results in a rapid decrease in the ultraviolet absorption of the carbonyl group, before the semicarbazone is formed. This shows that the nucleophilic attack of semicarbazide is fast and that the rate-limiting step for semicarbazone or oxime formation is the loss of water from the carbinolamine addition compound. This dehydration step is subject to general acid catalysis by buffer acids, which donate a proton to the leaving hydroxyl group (Equation 2, Fig. 2) (Jencks, 1959). Thus, the bell-shaped pH-rate profile arises because there is a change in the rate-limiting step, from rate-limiting attack of semicarbazide at low pH, where the concentration of free semicarbazide is very low, to rate-limiting acid-catalyzed dehydration of the carbinolamine addition compound at high pH, where the concentration of acid is low and the acid-catalyzed dehydration step is slow.

Catalysis by imidazole and kinetic ambiguity

We were interested in the catalytic properties of imidazole because at that time the imidazole group of histidine was thought to be a nucleophilic catalyst in the catalysis of acyl transfer reactions by enzymes. Stadtman and others had shown that imidazole could catalyze the transfer of acyl groups from acyl adenylates to thiols, phosphate, and other nucleophiles (Stadtman, 1954). We confirmed these results and showed that the rate of reaction of acetylimidazole with carboxylic acids is proportional to the concentration of the free acid, RCOOH, rather than the more nucleophilic carboxylate ion. This puzzled us at first, until we realized that the reaction occurs through the kinetically equivalent attack of the acetate anion, which is a good nucleophile, on protonated acetylimidazole, which is a highly reactive acylating agent (Equation 3, Fig. 2).

This showed us that the rate law that is obtained from kinetic experiments only tells us what is the stoichiometric composition of the rate-limiting transition state of the reaction; it does not tell us where the different atoms are located in the transition state. This kind of kinetic ambiguity is not uncommon for reactions in which protons are involved; it must be resolved in order to establish the reaction mechanism. There are often several very different mechanisms that can be written for a given reaction and rate law, depending on where the proton is in the transition state (Jencks & Carriuolo, 1959a, 1959b).

“High energy” acyl and phosphate compounds

There was some question at this time as to whether acyl esters and thiol esters were both “energy-rich” compounds, so we determined the difference in ΔG° for reactions of these compounds by measuring the equilibrium constant for the transfer of an acetyl group between the oxygen and sulfur atoms of mercaptoethanol. The equilibrium constant was found to be 56, favoring the oxygen ester, which corresponds to a more favorable hydrolysis of the thiol ester by $2,600 \text{ cal mol}^{-1}$ (Jencks et al., 1960). This is a significant, but modest, difference. We finally realized that most of the driving force for the hydrolysis of esters that makes both of these compounds “energy rich” comes from the dissociation of a proton from the carboxylic acid product, which is favorable by some $-3,300 \text{ cal mol}^{-1}$ at pH 7.4.

The catalysis can involve hydrogen bonding to an acidic or basic group in the transition state, trapping an unstable inter-

mediate by adding or abstracting a proton, or proton transfer that is concerted with the formation or cleavage of bonds to heavy atoms. All of these mechanisms have been identified and characterized (Jencks & Carriuolo, 1959b, 1960; Jencks, 1972).

Another mechanism that is critical to catalysis is *destabilization*, or increasing the Gibbs energy of the bound substrate in the enzyme-substrate complex. It is true, by definition, that an enzyme must stabilize the transition state of a reaction in order to bring about catalysis, but it is not always realized that it is equally important that it *destabilize* the bound substrate. If the enzyme were to stabilize the transition state and the bound substrate equally, the barrier for reaching the transition state, $\Delta G^\ddagger$, would be unchanged and there would be no catalysis. This destabilization can be brought about by distortion, desolvation, and other mechanisms.

A particularly important mechanism for increasing the Gibbs energy of the substrate, so that it can reach the transition state easily, is through loss of entropy of the bound substrate in the E-S complex. This fixation of the substrate of an enzyme in *exactly* the correct position relative to catalytic groups, and often relative to another substrate in the active site, makes a much greater contribution to catalysis than has often been assumed. Instead of a factor of 55 for 1 M solutions, which would be obtained by replacing a water molecule by a reacting molecule, this factor can be as much as 10^8 M. This large number arises from the stiffness of a covalent bond. In order to form a typical covalent bond between 2 molecules some 30 entropy units must be lost. This corresponds to a factor of approximately 10^8 for 1 M solutions. The factor is proportionally larger for the more dilute solutions of reactants that exist under physiological conditions (Page & Jencks, 1971). Thus, induced intramolecular and loss of entropy make a major contribution to catalysis.

It has, of course, been well known from the beginning of studies of catalysis by enzymes that bringing substrates together, with each other and with catalytic groups at the active site, is important for catalysis. This has given rise to an extraordinary number of different words from different laboratories to describe the exact fixation of the substrate relative to catalytic groups and to other substrates that may be in the active site. The best known of these terms are perhaps "orbital steering" and "stereopopulation control." Science is stimulated by controversy and it has been useful to have these terms, in order to stimulate controversy and interest in these problems. However, the proliferation of new words in order to describe a decrease in entropy represents an increase in entropy, and an increase in entropy represents disorder and confusion. Therefore, it is fortunate that the rate of discovery of new terms is finally tapering off. This should make discussions of enzyme catalysis more easily understandable, although perhaps less exciting.

Movement in biological systems

Ever since I learned that biochemists were studying muscle contraction and active transport, I have been fascinated by the question of how movement is brought about in biological systems, such as muscle contraction and the active transport of ions and other molecules. Some 12 years ago I decided to try to understand such processes and perhaps contribute to a further understanding of how such reactions occur. These reactions are called *coupled vectorial processes* because they bring about movement when, and only when, a source of chemical energy is provided

and used to drive the vectorial process of movement. The most common source of energy is, of course, ATP. In the reverse direction, the synthesis of ATP from ADP and phosphate in oxidative phosphorylation is a coupled vectorial process that is driven by the transport of protons across a membrane.

I started by talking to friends who were working on coupled vectorial processes. I asked them what they were trying to find out, in order to understand how ATP brought about movement. They were always happy to talk about the result of their last experiment, but none of these experiments seemed to address the question directly: What is the process that is responsible for the *coupling* of ATP hydrolysis, a chemical reaction, to the movement of 2Ca^{2+} ions into sarcoplasmic reticulum vesicles, a vectorial reaction, by the calcium pump, or the coupling of ATP synthesis to the movement of hydrogen ions across the mitochondrial membrane, in such a way that ATP hydrolysis or synthesis occurs when, and only when, the vectorial process also occurs?

Cecile Pickart examined the mechanism of the calcium pump of sarcoplasmic reticulum by studying the individual steps of the reaction in single turnover experiments, using a rapid quench apparatus. She was able to characterize the individual steps of the reaction and we concluded that the essential steps of the process could be described by the simple 4-state scheme shown in Figure 3. We later found that the identical scheme had been proposed by Makinose in 1974.

We identified all of the steps of this scheme and characterized them kinetically. The reaction cycle does what it is supposed to do: it transports 2Ca^{2+} ions, at micromolar concentrations in the cytoplasm, into the lumen, at millimolar concentrations, and cleaves 1 molecule of ATP to ADP and P_i . Furthermore, the reaction is completely reversible. But where is the *coupling* between ATP hydrolysis and Ca^{2+} transport? In what step does the coupling occur?

We concluded that there is no *step* in which the coupling occurs. The coupling is defined by *specificities for catalysis* of chemical and vectorial reactions. What is remarkable about these reactions is that the specificities of the enzyme for catalysis *change* in different states of the system (Jencks, 1980; Pickart & Jencks, 1984).

Fig. 3. A 4-state scheme for the individual steps of the calcium pump in the endoplasmic reticulum.

The chemical specificity of the enzyme is for catalysis of enzyme phosphorylation by ATP and dephosphorylation of the enzyme by ADP, when 2 cytoplasmic Ca^{2+} ions are bound to it. However, the specificity *changes* when the Ca^{2+} ions dissociate or are transported into the vesicle: the free enzyme reacts only with inorganic phosphate to form the phosphoenzyme, and the phosphoenzyme reacts only with water in the reverse direction.

The vectorial specificity is for binding and dissociation of cytoplasmic calcium by the free enzyme, but it changes to dissociation and binding of luminal calcium by the phosphoenzyme.

Thus, the chemical reaction of phosphorylation and dephosphorylation controls the specificity of the vectorial reaction of calcium binding and dissociation, and the vectorial reaction of calcium binding and dissociation controls the specificity of the chemical reaction, the reversible phosphorylation by ATP with $\text{E} \cdot \text{Ca}_2$ or by inorganic phosphate with E.

If all of these specificity rules are obeyed, the reaction is completely coupled: ATP will be hydrolyzed when, and only when, 2 calcium ions are transported into the vesicle. If any *one* of the rules is disobeyed, the reaction will be completely uncoupled or will not occur at all.

Very similar coupling rules for the sodium-potassium ATPase and for the gastric proton ATPase define the mechanism for coupling the chemical reaction of ATP hydrolysis to the vectorial reactions of Na^+ and K^+ transport across the cell membrane and of H^+ transport into the stomach. The synthesis of ATP that is driven by proton transport in oxidative phosphorylation is brought about by a very different mechanism, which can be described by a different set of coupling rules. These rules do not, of course, represent new discoveries; they are only meant to provide a simple way to describe what is responsible for the coupling of a vectorial reaction to a chemical reaction, with a high degree of specificity and efficiency (Jencks, 1989).

These recollections recall a few of the questions and answers that we have encountered over more than 3 decades. But my most important recollection is that I have been extraordinarily fortunate to have collaborated with such a large number of talented and committed students, postdoctoral fellows, and visiting scientists, who have been responsible for almost all of the work that has come out of this laboratory.

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