# Some Reflections on Research, Instruction, the New Biotechnology and on Pharmacy into the Next Century[1]

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## INTRODUCTION

This paper will consider the interconnected threads of my research interests which led me to focus on a new biotechnology which has evolved over my lifetime. This new biotechnology has had a major influence in the evolution of both my scholarly and teaching interests. I will also briefly analyze the state of its continuing impact on pharmaceutical education and, more particularly, on my perception of its role in the development of pharmacy as a profession. This new biotechnology represents my major focus, as I contribute where I can to the future of pharmaceutical education.

## HYDROGEN-BONDED ION-PAIRS

In the late sixties when the new biotechnology was still very much in the future, we were interested in modeling the energetics of proton-transfer reactions in biopolymers. We suggested that it was possible that the decrease in free energy accompanying the formation of a proton-transfer complex from a hydrogen bonded complex could: (*i*) affect the direction of a conformational transition in a protein;(*ii*), provide a desolvation-dependent driving force in drug-receptor associations; and further(*iii*) represent an elementary feature of enzymatic catalysis(1-3) (Figure 1). Interactions which included proton transfer along a preexisting hydrogen bond had not been previously widely considered in the context of dynamic events in proteins and other biological macromolecules. Still, their importance had been asserted in several biochemical systems of interest[2](4,5).

In simple model systems we were able to measure the formation of hydrogen bonds and their conversion to the corresponding proton-transfer complexes quite easily by spectrophotometric analysis. We evaluated, as best we could, the temperature, solvent polarity and specific solvation dependence of the microscopic equilibrium constants for these processes. Interestingly, depending on the strength of the interacting acid and base, the equilibrium constants needed to be corrected after evaluation of a conductometrically measurable dissociation of the proton-transfer complex. It was no easy task to evaluate the energetic differences between the hydrogen-bonded complex and the corresponding proton-transfer-ion-pair complex, especially to do it in a way relevant to thinking about their contribution to the dynamic behavior of real biological systems. Based on these model studies, we estimated this to be about 1-3 kcal/mole, favoring the proton-transfer complex(2).

The major complication in any *a priori* assessment of the value of individual hydrogen-bonding interactions in a protein was the variable, but generally decreasing presence of ordered and disordered water on proceeding from the exterior to the interior regions of the protein. This assured

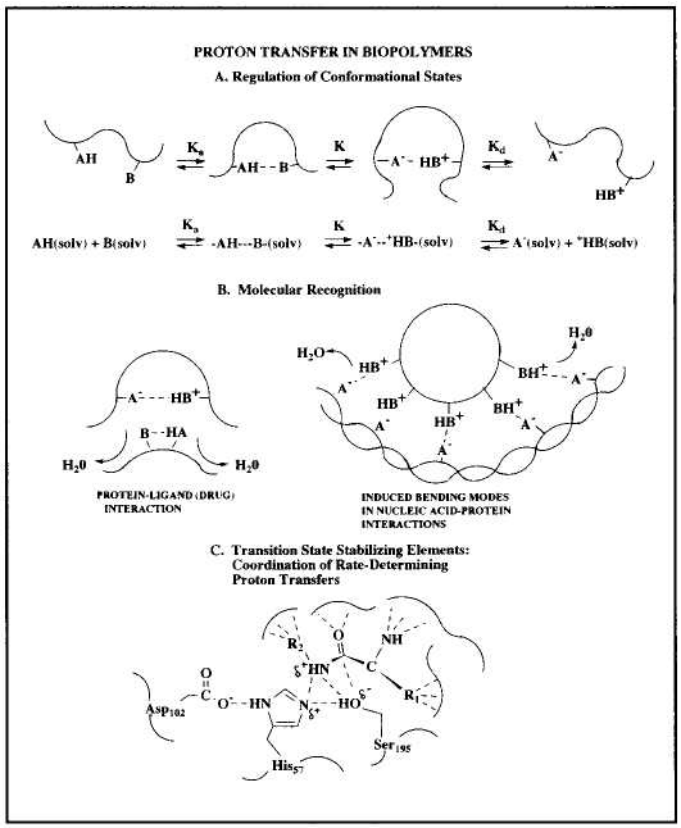


*Fig. 1. Proton Transfers in Biopolymers: A. The role of acid-base interactions in conformational transitions in biopolymers may be considered by the determination of three microscopic equilibrium constants (Ka, K and Kd) in model acid-base systems in mixed aqueous solvent systems with systematic variation in dielectric constants. B. Molecular recognition processes may be driven in protein-ligand (drug) and protein-nucleic acid complexes by sterically induced desolation of the complex, in which hydrogen-bonded and hydrogen-bonded ion-pairs may be seen as a stabilizing influence. C. Coordinated proton transfers energetically facilitate rate limiting proton transfers in enzymatic reactions. A forming transition state for a representative serine protease is shown.*

[1]Paper is based, in part, from the Paul Dawson Biotechnology Award Address, at the AACP Annual Meeting July 11, 1995, Philadelphia PA.

[2]Some early evidence for the important role of ion pairs in the structure of an enzyme comes from the relationship between pH and the structural transition in α-chymotrypsin and the stereochemistry of activation of α-chymotrypsinogen. Both transitions appear to depend on the presence of an enzyme activity conferring Ile16 ($NH3^+$)—Asp 194 ($CO_2^-$) ion pair(4). In the chemical modification of tobacco mosaic virus protein(5) it was shown that the integrity and ability to bear a protonated charge of one or two lysine residues was important to its self-association.

that even surface amino acid residues were asymmetrically solvated, and that the interactions of which a particular amino acid or nucleotide residue might be capable had to be thought about in a way relevant to the specific properties of the microenvironment in which it was found. The term *microenvironment* was rapidly catching on in those days, and it constantly reminded us that there was no isolated, model interaction which could quite mimic it. Increasingly, it seemed we would never be able to assess the microenvironment in detail, and that the best we could do would be to develop a distant, but perhaps still useful, approximation. The idea was to do this using model measurements in mixed solvents where dielectric constants and desolvation properties could be varied systematically. We had not anticipated the appearance of the modern supercomputer with its capacity to consider the structural and dynamic contributions of thousands of water molecules associated in a defined way with the structure of a biomolecule.

As with all research efforts involving model systems we decided what positive contributions we could make and then moved in another direction when it became clear we could not resolve with existing research strategies the increasing divergence between the model system and that which we wished to model. These efforts had been particularly enjoyable to me, in that they had represented a collaboration with Serge Vinogradov and Ron Scott, both protein biochemists who taught me how to think more clearly about the structures of biomolecules. Ultimately, the complexity of solvation in the model systems we were working on, and its fundamental difference from the nature of solvation forces in macromolecules caused us all to move in other directions. Thirty years later solvation and its assessment are still major problems.

## CURARE-MIMICKING NEUROTOXINS AND AcCH—MIMICKING ION-PAIRS

For several years we worked on other problems of modest scope but later returned to thinking about ion pairs in macromolecules in a somewhat unexpected context, namely as critical recognition elements on the surface of one protein, directing its interaction with another(6). Dimetrius Tsernoglou and Greg Petsko had just solved the x-ray structure of a representative curarimimetic neurotoxin and were building a detailed molecular model of it based on their determined coordinates. I remember looking at it for a long time before a hypothesis gradually emerged (after a series of discussions) about how this toxin and others like it were so perfectly recognized by the nicotinic acetylcholine receptor (nAcChR).

It had been known that most of the functionally important amino acid residues were clustered near a β turn at the tip of an extended anti-parallel sheet. This was one of three such features in the molecule, which presented itself at low resolution as a flattened ellipsoid. The structure is shown in increasing resolution in Figure 2, which shows that the protein is held together in a structure reminiscent of a floppy bow tie. Four intrastrand disulfides are interwoven to form the knot, out of which three loops of antiparallel sheet extend. In the final toxin representation in Figure 2 attention is paid to the van der Waals surfaces of the amino acid side chains of residues we thought to be important in the binding: in particular, the four amino acid residues which we thought could create an acetylcholine-mimicking feature in

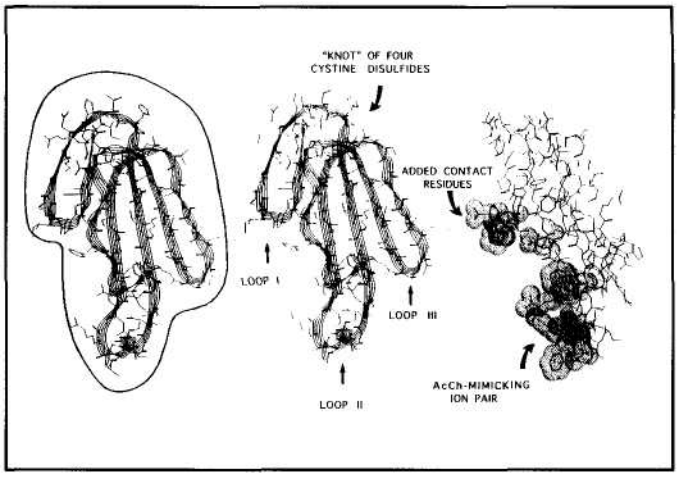


*Fig. 2. Structure of the Curarimimetic Neurotoxins and the AcCh-mimicking Amino Acid Residues derived from Brookhaven Data Base for the crystal structure of the major toxin from the Thailand cobra, Naja naja siamensis, subsequently manipulated into the various forms: the representation on the left shows the general shape (solid outline) of the neurotoxin from the perspective of the nAcChR-interacting face. The overlaid ribbon follows the peptide backbone. Key structural features (the "disulfide knot" and the three principal loops of antiparallel sheet structure) are identified in the central structure, which duplicates the perspective of the drawing on the left. The representation on the far right shows the structure of the same toxin with the same vertical perspective as shown previously, but here without the peptide backbone ribbon and with space filling van der Waals radii indicating the nAcChR interacting residues now turned approximately 90° about the vertical axis with respect to the drawings in the center or left so that the critical interaction between the receptor and the toxin may be viewed in cross-section.*

the form of a hydrophobic-side-chain-solvated Arg-Asp ion pair. While the ion-pair does not form in the crystal structure, and is not expected to form in the absence of a desolvated nonaqueous microenvironment created as the toxin docks into the receptor, we pointed out carefully that the hydrogen-bonded ion-pair was stereochemically allowed without distortion of the peptide backbone made up of the Asp and Arg side chains and the surrounding amino acid residues(6). We also predicted that when the x-ray structures of longer sequence toxins were accomplished that the same stereochemical relationship between the Asp and Arg residues would exist. This was a bit of a leap as three additional amino acid residues and a fifth disulfide bond are incorporated into this region in the "long toxins" relative to the short toxin. When such structures were later solved, our idea turned out to be correct.

The crux of this idea was that steric desolvation associated with the binding of toxin to receptor would, in the course of toxin-receptor association, create an acetylcholine-mimicking ion-pair serving as the principal feature to guide both the accuracy and the intensity of binding. The ion-pair formation would be aided by the indole ring of the Trp above the ion-pair and the phenyl ring in the Phe below it both in terms of the extent of the desolvation of the site as well as in softening the charge on the guanidinium through charge transfer to the aromatic rings. This created a complex which sterochemically resembled, in our minds, the structure of acetylcholine (Figure 3).

The hydrophobic ion-pair idea did not preclude additional secondary contributions to the binding due to hydrophobic, charge-charge and van der Waal's interactions as

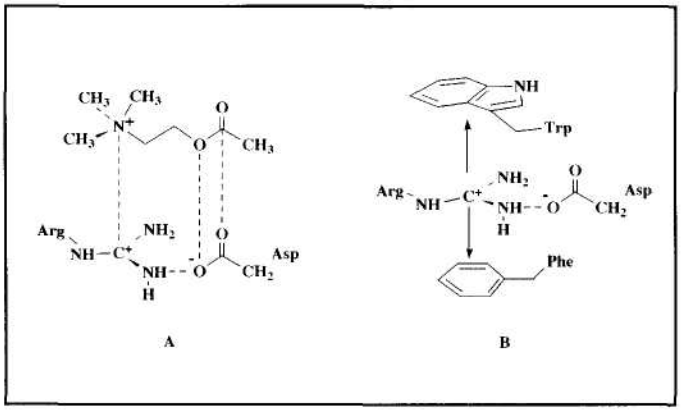


*Fig. 3. (A) illustrates the stereoelectronic similarity of acetylcholine and an Arginine-Asparate hydrogen-bonded ion pair. The positions of the centers of positive charge and the acyloxy oxygens of acetylcholine and the carboxylate of the aspartate are aligned demonstrating the similar distances of the ethanoxy Bridge in acetylcholine and the hydrogen bonded N-H-O salt bridge in the ion pair. (B) shows the approximate alignment of the nearby tryptophan and phenylalanine residues in the toxin allowing for potential charge transfer interactions stabilizing the positions of the aromatic rings in these nearby residues above and below the guanidinium of the Arginine residue.*

nearby structural elements in the toxin matched up with complementary structural elements in the receptor. Indeed, several distant charges on the shorter loops were suggested to create a curare-like matrix of charges on the toxin which might help us understand the much higher affinity of these toxins relative to AcCh(6). Clearly many additional contacts between receptor and toxin must also be important not only as focal points to understand the strength of binding, but also to understand the strong action of the toxin as an antagonist. In that sense, of course, any connection between the molecular surface created by curare and that created by the neurotoxin was important to our argument.

Like all interesting ideas ours created a lot of activity, which I'd like to summarize briefly. We and others focused on the proposed mechanism of action for over ten years. Two distinct approaches were taken. First, were peptides derived from this region of the toxin capable of binding to the nAcChR? The answer to that question was yes. We and others have demonstrated that even linear, unfolded peptides from the putative AcCh-mimicking region bind to the receptor while peptides derived from other regions of the toxin did not. However, the complete reconstruction of binding activity using synthetic peptides has not been possible, nor have we been able to construct peptidomimetic structures based on guanidinium-carboxylate hydrophobically-shielded ion pairs. In part this failed because most of the constrained small molecules considered acted also as zwitterionic detergents, directly denaturing the purified receptor. Perhaps the best current view of required nonbonding contacts between the toxin and the AcChR is assessed in Hawrot's recent NMR analysis(7). This study suggested contacts between the toxin and synthetic peptides based on sequences within the acetylcholine receptor defining the major portion of toxin binding site in the receptor. The receptor-interacting residues in the toxin are illustrated in Figure 2.

The thrust of efforts carried out mostly by others and reviewed in Karlin's Harvey Lecture in 1991 has been to identify the AcCh and antagonist (including toxin) binding

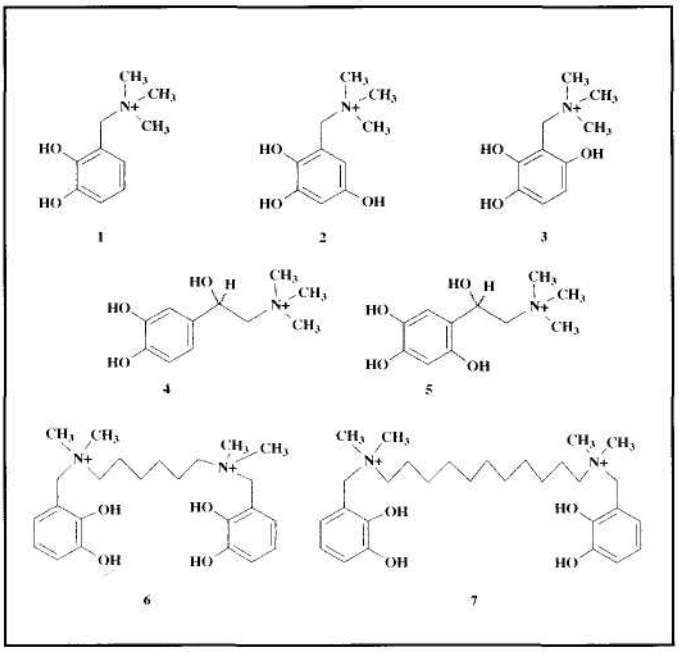


*Fig. 4.: Redox-reactive Affinity Reagents:3-trimethylammoniomethyl catechol (1, TMC), 5-hydroxy TMC (2), 4-hydroxy TMC (3), N,N-dimethylepinephrine (4), N,N-dimethyl-6-hydroxy epinephrine (5), and the bis-3-ammoniomethylcatechol analogues of hexamethonium and decamethonium (6 and 7, respectively).*[3]

sites occurring in two complexation sites located at the interface between the α and γ and the α and δ subunits of the pentameric receptor(8). Each subunit of the receptor is derived from a primitive gene duplication event, thus producing subunits with essentially identical molecular weights. Ligand binding sites which span two subunits may turn out to be an unusual feature of the binding of ligands to even complex receptors. Karlin has suggested that the shorter agonist molecules may actually owe their action as agonists to this feature—as their tight binding to distinct molecular features on the respective subunits of each site may initiate the relative conformational changes which initiate the activation of the receptor. If this be so, then antagonists must essentially fill the site in a perfectly complementary way, preventing the motion characteristic of the agonist in its binding. Some joint efforts between our laboratory and Karlin's helped to define a site for the binding of noncompetitive receptor antagonists(9). This was important as the site for noncompetitive antagonist binding was thought to be near the ion channel. The photoaffinity approach we took using quinacrine azide allowed the time (msec)-resolved photolabeling of the receptor and, as a result, the determination of the probable conformational state of the receptor implicated in the binding of the antagonist. Quinacrine azide was important in photoaffinity studies in a number of other systems as well(10-12).

We also tried to build a site-to-site bridge between the region of the toxin adjacent to the proposed binding site and the interacting site in the receptor. This was accomplished with a mercurialated toxin we defined specifically for this purpose(13,14). Photochemically induced crosslinking procedures also worked. We used catechol oxidation as a theme. This procedure didn't work, but it created more research for

[3]All compounds cited are named trivially as derivatives of 3-trimethylammoniomethyl catechol, hexamethonium and decamethonium. For further chemical details see cited references.

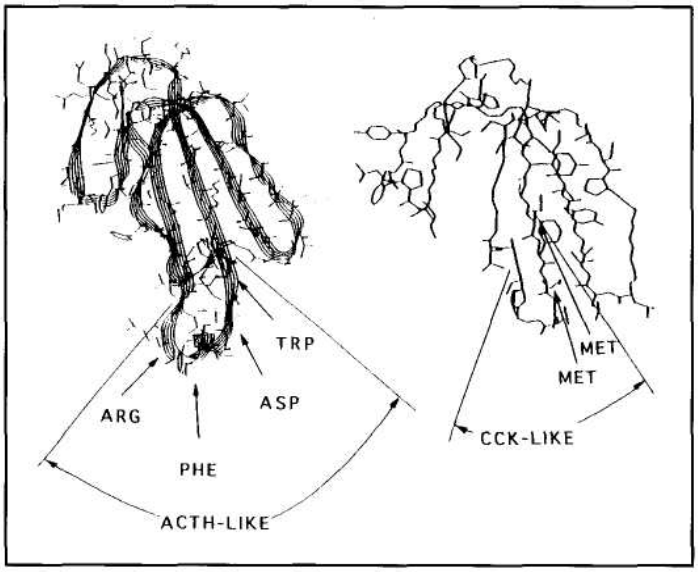


*Fig. 5.: Putative ACTH-like and CCK-like residues identified as in the central Loop II regions of the neurotoxin and the cardiotoxin, respectively. The structural representation of the cardiotoxin is illustrated from approximately the same perspective as the neurotoxin.*

several members of the group in the long term. We made several molecules which have come to be known collectively as catecholines(15-20). These are redox-dependent reactive affinity reagents with some very interesting and unusual properties. The examples shown in Figure 4 possess progressively more specific and increasing inactivating capabilities toward choline-binding macromolecules and may be useful in the study of selective cholinergic inactivation in complex neurobiological systems.

## ARE RECEPTOR BINDING SEQUENCES IN THE NEUROTOXIN UNIQUE?

Thus, it gradually became clear that the central extended looped structure of the toxin was the primary nAcChR-interacting center. How this feature evolved was intriguing. This peptide region represented a sequence of about 6-10 amino acid residues precisely located at the center of the toxin's amino acid sequence. We noted that the corresponding central region of a group of extremely closely related toxins, known as the cardiotoxins, have an entirely different sequence of amino acids within its corresponding central region, whereas the peptide sequences which flank the central regions of the cardiotoxins and the neurotoxins were highly homologous. Indeed, nearly all of the homology between these two classes of evolutionarily related venom proteins was due to homologous sequences in the regions which flank the central sequences. Interestingly, the activity of the cardiotoxins was also associated with its central sequences.

While the central peptide region of the neurotoxins bears a compositional, if not sequence, identity with the N-terminal region of ACTH (a-MSH), the corresponding regions within the cardiotoxins represent CCK-like sequences bearing usually two important methionine residues, one or both of which may be crucial to the physiologic activity of the cardiotoxins (Figure 5). There was no evidence for overlapping physiologic activity associated with these two classes of related toxins. While the neurotoxins expressed a specific, high affinity antagonism toward the

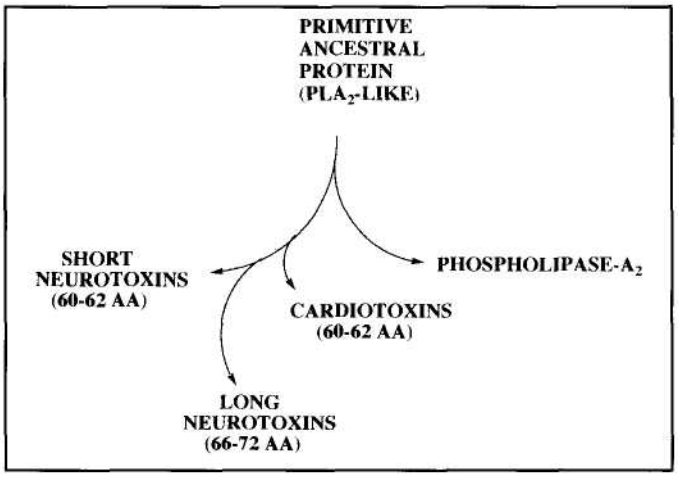


*Fig. 6. Evolutionary relationships among key cobra venom proteins*

nAcChR, the cardiotoxins acted relatively nonspecifically toward a wide variety of cells. The cardiotoxins apparently functioned by dislodging structural $Ca^{2+}$ from membrane surfaces, subsequently destabilizing the membrane structure further through a slower detergent-like interaction. Interestingly, both of these classes of toxins appeared to have evolved from the C-terminal half of a primitive ancestral protein, which was also the precursor of the present day phospholipase $A_2$ enzyme family (Figure 6). All three of these classes of proteins are found in cobra venoms, usually in multivariant forms within each class.

We contemplated how these three classes of proteins might have arisen from a single common ancestor. First, loss of the major N-terminal exons subsequent to a major fragmenting, gene rearrangement event may have given rise to gene fragments producing stable proteins with little biological activity initially but which subsequently evolved into the cardiotoxins and neurotoxins in the case of the gene fragment coding for the C-terminal end of the primitive phospholipase. The two classes of toxins may have then come about through one of two distinct mechanisms: (*i*) they may have evolved completely by chance; that is, through the happenstance of successive mutational events leading eventually to the two toxin subclasses with the two highly divergent central sequences observed, or (*ii*) through the insertion of two distinctly different sequences in different recombinational expansion events in which the new sequences were derived from mobile sequences from more primitive peptide hormone gene elements into the central region of the toxin precursor in an intron-exon shuffling event. Unhappily there was no way to decide easily between these two possibilities. The sequences involved were too short to discriminate between chance evolution versus a possible intron-exon shuffling, one of which brought in new or changed short sequences. To further complicate the matter, it was unclear whether there was a precursor element present in the central region of the C-terminal residue of present day phospholipases.

We might have left it at that had we not taken up a small collaboration with Helene Rauch's laboratory. Her laboratory's primary interest had been in defining the origin and structure of the CNS antigen eliciting experimental allergic encephalomyelitis (EAE), the animal model condition akin to multiple sclerosis in humans. She was also interested in learning how to suppress the model disease, as

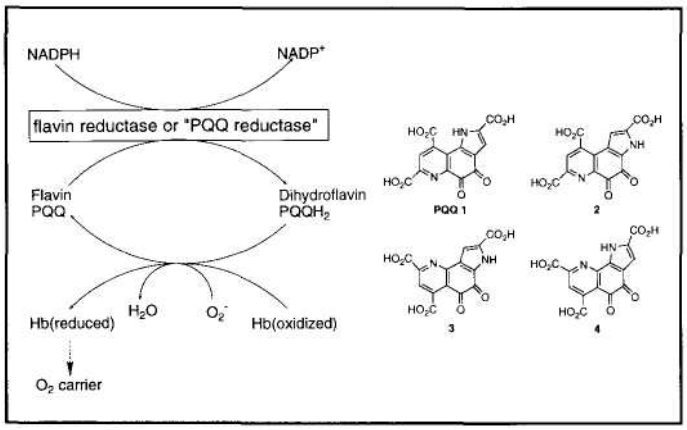


*Fig. 7. The role of riboflavin reductase in the reduction of oxidized hemoglobin may be supplanted by PQQ as shown. Three synthetic isomers of PQQ shown may become versatile structural and mechanistic probes of the PQQ site in riboflavin reductase and for other flavin enzymes capable of utllizining PQQ as well as for PQQ requiring enzymes.*

that would have obvious therapeutic implications. When we considered the differences in the central sequences in the cardiotoxins and the neurotoxins, it became clear that a suitably detoxified neurotoxin might be immunosuppressive whereas the correspondingly detoxified cardiotoxin would not be. We reasoned that the ACTH-like sequences present in the neurotoxins and not in the cardiotoxins may produce immunosuppression. Further, the ACTH-like sequences in the neurotoxins could also resemble sequences within the principle antigen responsible for EAE and thus mimic the immunosuppression (tolerance) produced by the antigen under certain conditions. The antigen, myelin basic protein (MBP), was interestingly known to induce tolerance (or immune unresponsiveness) against challenge with MBP in animals pretreated with the MBP in incomplete Freund's adjuvant.

Thus, we pretreated guinea pigs with reduced and carboxamidomethylated(CAM)-neurotoxin and with CAM-cardiotoxin prior to challenge with MBP. The animals were nearly completely protected by CAM-neurotoxin and completely unprotected by CAM-cardiotoxin. At the time CAM-neurotoxin represented the only naturally occurring protein with protective immunosuppressive properties in EAE other than MBP(21). Subsequently, we demonstrated that the trypsin-derived peptides from CAM-neurotoxin retained essentially all of the immunosuppressive character of the entire peptide; and further, that a substantial proportion of that activity was resident in a ten residue peptide containing the ACTH-like central section of the toxin. Thus, even while we couldn't determine how the ACTH-like central peptide had arisen, it was clear that the neurotoxin had the expected activity whereas the cardiotoxin had the expected inactivity. It will be up to others to decide to what extent the observed immunosuppression is associated with a true ACTH-like effect and to what extent it may represent a tolerance reaction similar to that produced by MBP.

## CURRENT AREAS OF RESEARCH

### Models in Neurodegenerative Disease

We are using the catechol-containing affinity reagents (catecholines) shown in Figure 4 as selective presynaptic cholinotoxic agents. Simultaneously we are using PQQ and the isomeric PQQs we have prepared to determine what features of the catecholine intoxicating reactions can be reversed using PQQs (Figure 7). Protective effects of PQQs are expected based on the documented presence in a variety of tissues of flavin reductases or similar enzymes, which can reduce PQQ and allow the quinol $PQQH_2$ to reduce superoxide. Indeed, the presence of PQQ is intended to reduce the nonspecific toxicity associated with spontaneous oxidation of the catecholines away from the cholinergic sites to which they are affinity-directed. The quinol $PQQH_2$ would also be expected to reduce highly electrophilic quinones generated in the same way. The isomeric PQQs are intended as isosteric probes of the PQQ reducing enzyme systems present in the brain. These isomeric PQQs may be useful as structural and mechanistic probes in enzymes which either use PQQ or riboflavin derivatives as cofactors.

### Interleukin-1 (IL-1) Peptide Antagonists

Recently we began a project which grew out of the realization that we had gone as far as we could go with the neurotoxin-AcChR interaction, and that it would be fun to try to work out some of the details of a protein-protein interaction, where the rewards for new drug design would clearly be greater if we were successful. We've started with the suggestion that the receptor binding region of interleukin 1 (IL-1) was resident in the C-terminal end. This approach was based on: (*i*) the loss of binding activity in C-terminal truncation mutants of IL-1, and (*ii*) a synthetic C-terminal peptide reputed to bind to the IL-1 receptor with high affinity(22). We prepared the C-terminal 33mer and five shorter peptides (11mers or 12mers) representing smaller sections of the 33mer. In some of the systems we've examined so far these peptides have very little activity while, in others, Kd values range as high as $10^{-6}$M. In one system of particular interest we are exploring in collaboration with Marcia McInerney at Toledo, inhibition of IL-1b induced toxicity against pancreatic b-cell islets was observed. Here, IL-1b and synergistic levels of INF-γ suppress the levels of glucose-induced insulin release. This effect can be suppressed with the 33mer. This is an important toxicity effect to suppress as it represents a mitigating problem in the use of various forms of live pancreatic implants, which might otherwise be useful in treating diabetes.

### New Class of HIV-RT Inhibitors

Finally, in regard to current research activities, we have begun work in a completely new area. With the view that there might be more than one functional cleft on the HIV-RT complex, we started exploring simpler chemical forms of known complex natural product inhibitors that seemed to us to be extremely different from other known noncompetitive inhibitors and also completely different from nucleoside inhibitors. We reasoned that development of resistance against the successive or simultaneous application to three mechanistically dissimilar drugs directed against the same key viral enzyme would be slow to develop. But there were also other reasons to pursue these new inhibitors.

We were aided from the beginning by Don Hupe at Parke Davis. Don agreed to let us look at our inhibitors using the cloned wild type enzyme and a cloned double mutant enzyme, which was no longer effective against any of the classes of nonnucleoside inhibitors. With nearly the first compound tested, we observed inhibition at the μM level, and, indeed, observed essentially equivalent levels of inhibition for the wild type and mutant enzymes. This observation

indicated that we had a new inhibitor site. Further, from the kinetics we could see that the inhibitor site behaved uncompetitively but the kinetics remain incompletely defined.

Thus, we had a new class of inhibitors. In addition to measuring their ability to inhibit the polymerase reaction we also decided to simultaneously determine inhibition of the strand transfer process (referring to the separation of the RNA and DNA strands during and following the formation of the RNA-DNA heteroduplex). Ultimately, strand transfer must occur in order to facilitate destruction of the RNA, relocation of the first DNA synthetic strand within the polymerase site, and subsequent synthesis of the DNA complementary DNA strand. Strand transfer and recombination of multiple viruses within the same cell facilitates a major fraction of the multiple-residue-deletion mutations which occur during the course of natural infection. Thus, we reasoned that if we could separate the polymerase and strand transfer inhibitory features of this new class of inhibitors we would have something interesting. In particular, we wanted a strand transfer inhibitor which would not inhibit the polymerase reaction or would at least inhibit it very much less effectively than it inhibits strand transfer.

The concept is very simple. We would like a cell-transportable selective inhibitor of strand transfer. A drug, based on this idea, would interfere only modestly with virus replication to the extent that the strand transfer process is sluggish. It would, however, measurably slow the rate of mutation of the virus and thus afford the immune system of the host an opportunity to neutralize it, under conditions where the virus would be under reduced evolutionary pressure to produce escape mutations.

## BIOTECHNOLOGY IN EDUCATION

### Biotechnology: Where Are We and Where Are We Going?

In regard to education in pharmacy and the related pharmaceutical sciences, we cannot analyze the impact of the new biotechnology alone in the context of the increased numbers of new gene-manipulation based therapeutic agents and diagnostic procedures. The transformative effect of this new technology on pharmacy and the pharmaceutical sciences has been infinitely more pervasive. While the motivation to absorb the new biotechnology into our thinking may have been originally to allow us to talk about a few new biopharmaceuticals and diagnostic procedures based on it, our viewpoint can no longer be so parochial.

Motivation and direction in drug design has always been based on the most current knowledge of the biochemical pathology of disease. Our knowledge of disease is being expanded rapidly by our ability to scan genomic sequences and define distinct marker sites associated with the inheritance of and the predisposition to disease. We can begin to see that this new knowledge will take us, and all other health care practitioners, rapidly in new directions as we approach the start the new millennium.

We now know the position and can analyze the presence of some 4,000-plus marker sites for disease on the human genome. This knowledge will give medicine an entirely new direction in the beginning of the next century and that new direction is already within sight. Standard medical practice will move gradually but persistently with increased emphasis on prevention and prophylaxis in disease. This will also change pharmacy greatly. The outcome will depend largely on what we do now. Pharmacists, if they are sufficiently well trained, will participate more and more in the nuances of therapy but particularly in the therapeutic requirements and decisions associated with long-term preventative care. The dispensing function will virtually disappear in that it will become automatic save certain vital, specialized procedures—and these will probably be "dispensed" mostly by new medical specialists.

**Revolution in Medical Care.** Changes will occur in standard medical care consistent with our ability to manipulate genes and assess the root cause of disease. Cancer incidence will be reduced while heart disease will continue to decline. As people live longer, we will need to retard neurodegenerative disease in order to achieve a net societal benefit.

Cancer will begin to decline as we understand how to reverse or decrease the probability of oncogenic activation. We may learn how to reestablish deleted antioncogenes, or retard their deletion. It is also possible that many cancers will be treated prophylactically with the view of suppressing or controlling them rather than attempting their eradication through the aggressive use of highly cytotoxic drugs. Drugs will be designed routinely to: (*i*) control the expression (transcription) of harmful genes (oncogenes, or integrated DNA derived from DNA viruses or reheated RNA viruses), and (*ii*) stabilize genetically unstable regions of chromosomes which might otherwise allow spontaneous deletion of normal, useful genes, leading to disease. This latter effect may facilitate prophylactic therapy during major organ system development when spontaneous deletions of genes from unstable chromosomal sites may occur at the highest rates. In addition, site-directed inhibition of cancer-specific chromosomal deletions and translocation (*e.g.*, Philadelphia chromosome translocations associated with chronic and later with acute lymphocytic leukemia) may be possible. Also, (*iii*) new drug receptor antagonists will be developed which block receptors we can now clone and study in some detail. Entirely new pharmacologies are likely to develop within this context.

Heart disease will continue to decline as we begin to better understand the obvious predisposing factors and many of the less obvious genetic factors for which marker sites will continue to be found. In cardiovascular disease, therapeutics will be found which are cytoprotective, regenerative, or which target still unresolved control mechanisms in hypertension. We may find ways to revive damaged vasculature. Vegetative regeneration of collaterals may become possible. In regard to cardiac failure, novel antispasmodic, antifibrotic and regenerative agents may evolve. Antifibrotic agents which slow or reverse the development of fibrotic lesions in major organ systems will lessen the pumping demand on the aging heart. Regenerative agents which suppress damage in episodic, preclinical ischemia can reduce long term damage to heart muscle, as well as have useful effects subsequent to acute ischemia in extensive oxygen deprivation.

In 1985, there were but a handful of therapeutics and diagnostics traceable to the use of gene manipulation and related strategies. That number is now increasing geometrically and provides examples of some entirely new kinds of medications, including some extremely clever therapeutic approaches. In addition, the road to regulatory approval is less treacherous as the system gains familiarity with these new medications.

As people begin to live longer we will increasingly be

beset with a host of problems we can well imagine because we are beginning to see them now in microcosm. While there may be some lessening in the incidence of neurodegenerative disease associated with increased attention to lifetime preventative and prophylactic care, new and direct approaches to the retardation of neurodegeneration will be sought. In addition, other organ system degeneration may be slowed or reversed through the development of novel medications. Many of these will be genetically derived or may use organ- or tissue-targeting autologous cells in sophisticated drug delivery systems. Ultimately, death will come, but life will be increasingly productive to late term.

**Curricular Considerations: An Addendum**

What kind of curriculum will such a set of outcomes require, one which I believe we can easily manage and foresee. However, we must first determine what roles pharmacists should assume as we approach the coming revolution in medical care. These are, of course, the roles for which they will need to take responsibility.

The vernacular of the new biotechnology is now well ingrained throughout the basic prepharmacy science curriculum as well as within the basic science curriculum associated with the early phases of professional training. Where we may be lacking and where we may have to play catch-up is in the teaching of novel drug therapies, and in particular new biotechnological strategies for diagnostic and therapeutic regime. Here, we must envision a future which includes out-patient counseling melded with disease prevention and prophylaxis. In foreseeing this future I am hopeful that it is a role pharmacists will define for themselves in a proactive interaction with the entire health care community, and not wait to see what physicians and nurses leave for them to do.

**Computers.** With the advent of bench-top supercomputers it may be possible to integrate the genetic information of large populations in ways that will make it a useful component of health care decision making. This system would be secure, and would provide ready access to both key sequences and marker sites within individual genomes. Such information could be expanded within specific families or groups of families known to be predisposed to specific diseases. The same information would be available to both physicians and pharmacists. While physicians would tend to be interested in this information in regard to treatment alternatives after a disease or condition presents itself, the pharmacist might be well-positioned to deal with prophylaxis and prevention in the context of long-term community care. At this stage, of course, the notion of how overall responsibility for health care would be divided among providers is completely open. However, it is clear that physicians should not attempt to dispense the entire spectrum of services which could be available to individuals in this idealized societal group—and that the role the pharmacist should be focused in the longer term on prophylactic and preventative care in non-life-threatening cases and, in particular, in those cases where nursing care is not required. The vital role of the physician is clearly in dealing with incipient diseases and in establishing conditions for their management, while nurses assist in the usual way. Nurses may, indeed, manage many aspects of the therapy including drug therapy, when the patient requires the care of a nurse in the course of acute disease, during its resolution during recovery, or while in invalid status. The role for the pharmacist then focuses: (*i*) on long-term preventative or prophylactic regime; (*ii*) on consultation with both physician and nurse in the appropriate course of particularly complex therapies or diagnostic procedures; and (*iii*) in the re-establishment of long-term care of the outpatient who no longer needs the critical care of a physician or a nurse.

Some dispensing functions will no doubt continue, particularly in remote areas, but in the main these may be taken over by computers as well as at defined dispensing centers accessed by voice recognition—of both the prescriber and the patient. Physicians, nurses and pharmacists may all prescribe and may all dispense, though both prescribing and dispensing for each will be in a different context and in a quite differently defined set of interrelationships when compared to how each of these groups see each other now. A thorough redefinition of those interrelationships will become necessary as the health care system itself will be redefined.

While much will change, much will remain the same. Boils will need to be lanced. Broken limbs will need to be set. The population will still require ready access to simple pain medications and many other sorts of drugs. Nurses, doctors and pharmacists will still perform many of their currently traditional roles but, not surprisingly, each will acquire new roles as the nature of health care changes.

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