1990 RICHARDSON LECTURE

Challenges for Neurology in the Nineties: Will We Survive?

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It is a great honor for me to present the Richardson Lecture this year. Although I was not personally acquainted with Dr. Richardson, his contributions to Canadian neurology are legendary, not the least of which is immortalized in the disease he and his colleagues first described in 1964: Steele-Richardson-Olszewski syndrome.

I have entitled my talk "Challenges for Neurology in the 90s: Will We Survive?", to highlight some of the issues that I believe are important for us to consider as we embark upon our academic enterprise during the last decade of the 20th century. There are a number of opportunities as well as, potentially, major difficulties that we face, and I wish to focus attention on some of them.

Recent Advances in Molecular Genetics of Neurological Diseases

I should like to begin by making a few comments about the remarkable progress that has been made in the field that I have spent much time thinking about in recent years. This is the impact of molecular genetics on discovering the underlying cause of neurologic diseases, particularly those that are genetic. The solution to these problems lies in the understanding of their pathogenesis by analysis of the mutant genes that cause them. During the first part of this report, therefore, I will review current advances in a few of these arenas with a focus on the contributions that have been made in neurology departments. I will describe Huntington's disease, Alzheimer's disease and tumors of the nervous system that appear to be caused by deficiencies in a new group of factors, referred to as *recessive oncogenes* or *growth suppressor factors*.

Advances in the application of molecular genetic techniques to neurological diseases have occurred at a remarkable pace since 1980. Definitive strategies are now available to answer questions that once seemed impossible. The use of DNA probes that exhibit restriction fragment length polymorphisms (RFLPs) together with linkage analysis has resulted in the chromosomal localization of the mutant gene in over 25 diseases.[1,2] The actual gene defect has been identified in Duchenne dystrophy and in retinoblastoma.[2] These advances now offer opportunities to define the precise molecular basis of inherited diseases and to develop rational treatments.[3]

Huntington's Disease (HD)

Molecular Genetics

The application of RFLPs and linkage in families with HD resulted in location of the mutant gene to the short arm of chromosome 4.[4,5] Progress in isolating the gene for HD has been, however, unexpectedly difficult. The original probe G8 (D4S10) linked to HD is located approximately 5×10^6 bp from the telomere on the short arm of chromosome 4.[6] The HD gene is located between D4S10 and the telomere and is separated from the DNA marker by about 4×10^6 bp. The identification of additional DNA markers telomeric to D4S10[6-9] has permitted extensive genetic mapping of the region. Most pedigree analyses looking for recombinations between markers and the HD gene indicate that the HD gene lies within 500,000 to 1 million bp of the telomere. The proposed physical order of probes on chromosome 4 is D4S10-D4S95-D4S90-HD-telomere. However, two recent linkage studies have challenged this conclusion, suggesting that the gene is more proximal (between D4S10 and D4S90 for example).[10-12]

To date, all HD families studied are linked to chromosome 4.[13] The mutation rate in HD is very low and it has generally been considered that all cases of HD arise from the same mutation. The recent data showing that some cases of HD are caused by a defect more proximal on 4p raise the possibility of non-allelic heterogeneity; i.e., that more than one mutation in the outer two million bp of chromosome 4p may occur.

Identifying the actual mutation in HD remains a formidable task. Genetic studies in homozygotes confirm that the HD mutation is dominant; therefore, a single chromosome mutation is sufficient to cause the full phenotype. Lacking any cytogenetic clues (translocation, deletion, etc.), it may be necessary to sequence the entire region in both normal and abnormal chromosomes (normal and affected) and it is not entirely clear what to look for. At the outset, genes identified by structure (promoter

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regions, start sites, TATA boxes, etc.) will need to be examined one-by-one — both for tissue localization and putative functions — and for evidence of mutations. Fortunately, the daunting task lying ahead is being shared in a collaborative initiative involving a number of laboratories.

Alzheimer's Disease (AD)

Molecular genetic studies have advanced on several fronts in the past two years.[2,14] Of particular importance have been studies to 1) localize the gene in familial AD (FAD), and 2) molecular/-cellular biological studies of the amyloid precursor protein (APP).

Familial AD (FAD)

The original pedigree analysis of four families with early onset FAD indicated linkage to chromosome 21q.[15] Subsequent studies permit the following conclusions:

1) It is likely that a subset of patients with FAD (usually with early age of onset) are due to an abnormality on chromosome 21.[16,17] 2) However, it is also likely that some pedigrees of FAD, such as the Volga-German family reported by Schellenberg et al,[18] are not due to a defect on the region of chromosome 21 implicated in the early onset cases. Recent studies that failed to confirm linkage with probes on the chromosome 21q in older onset AD patients have now suggested linkage to chromosome 19.[19,20] Progress in locating the gene responsible for FAD is likely to progress even more slowly than in HD.

Amyloid Precursor Protein

The extraordinary developments in our understanding of the biology of the amyloid precursor protein (APP) has provided a series of hypotheses concerning its role in the pathogenesis of AD. These may be summarized as follows.

1) The ß (A_4) protein is a 4.2 kDa peptide that is deposited in both neuritic plaques and the walls of small cerebral vessels in AD. Although the normal cellular locus of APP is primarily neuronal, extracellular deposition of APP in AD occurs both in brain and in other tissues.[21,22]

2) There is no evidence for over-expression of the APP gene transcripts in the brain in either sporadic AD or in FAD, although over-expression does occur in some cases of Down syndrome.[2,14]

3) There are at least three alternative mRNA transcripts produced by alternative splicing of RNA from a single gene in brain.[2] There is no evidence for more than one gene for APP, which is located telomeric to the FAD-linked markers on chromosome 21 and separated from them by 8 to 15 x 10^6 bp. The full-length complementary DNA (cDNA) for the B-amyloid protein encodes a protein of amino 695 acids (APP_{695}). The alternative larger forms have 751 (APP_{751}) and 770 (APP_{770}) amino acids, respectively.[2,14]

4) These larger forms of APP are of particular interest because they contain domains recognized by structure to be serine proteinase inhibitors of the Kunitz type. [21,22] Recent comparisons of the APP structure with other known proteins show that the secreted form of APP contains an amino-terminal portion identical to protease nexin -2 (PN-2). PN-2 is known to inhibit chymotrypsin and trypsin and to also inhibit proteases associated with the growth factors, epidermal growth factors (EGF) and nerve growth factor (NGF).[21,22]

5) Despite tantalizing hypotheses concerning the role of Kunitz-type protease inhibitors in the pathogenesis of APP deposition in brain or other tissues, it remains only that — a hypothesis. There is no direct convincing evidence for abnormal metabolism of APP in brain to establish whether its deposition is a primary or secondary factor in the pathogenesis of AD.

6) The source of APP in brain and other tissues remains problematic. APP cannot be detected readily in blood. The possibility that it arises from non-neural tissues and reaches the brain via altered blood vessels (altered blood-brain barrier) has not been proven nor excluded.[22]

7) An alternative mRNA for APP is described in which the 208 amino acids in the carboxyl-terminal region are deleted and replaced by 20 amino acids with homology to the Alu repeat family. It is speculated that this represents a fourth form of APP (it lacks the transmembrane domain of APP).[22]

8) The biological effects of the B-amyloid peptide, or secreted form of APP, have been studied with conflicting results. Both neurite promoting[23] and neurotoxic effects have been shown to occur.[24] Further studies of these important biologic effects will be required to determine whether these responses are physiologic or pathologic, and whether APP deposition in brain contributes to neuronal destruction in AD.

In recent months several additional observations have been reported concerning the cell biology of APP.[25-27] The first aspect is the structural homology of APP to other proteins and the emerging hypotheses that abnormal cell cycling of the protein may lead to abnormal tissue deposition. It is speculated, for example, that PNII is released by proteolytic cleavage near the cell membrane and that the released moiety can inactivate serine proteases in the extracellular space and then by binding to the cellular membrane be internalized and recycled.[22]

One of the major disappointments in the aftermath of the discovery of the structure of APP and the location of its gene on chromosome 21, was the failure of linkage to FAD, and the realization that mutations of the APP gene are not found in AD. The deposition of amyloid-staining material in hereditary amyloid polyneuropathy is known to occur because of a mutation in the amino acid sequence of transthyretin.[2]

Two recent papers show unequivocally that mutations in amyloid can cause CNS disease.[26,27] The association of cerebral amyloid angiopathy with intracerebral hemorrhages has been advanced by the study of four Dutch families with autosomal dominant inherited cerebral angiopathy. These cases show deposition of amyloid in vessels and in senile plaque-like structures (however, they do not have neurofibrillary tangles, an important distinction from AD). Van Broeckhoven and collaborators[26] showed close linkage to the APP gene in these families with a LOD score of 7.59 at theta 0.0. The hypothesis that the ß-amyloid gene might be the cause of the disease is strongly supported by Levy et al[27] who show that there is a mutation in the APP gene in these families. The site of the mutation and the type suggest that it may be the cause of the disorder. Using oligonucleotide probes based on the known intron sequences that flank the two exons encoding APP, together with the polymerase chain reaction, they amplified the sequences that contain the two exons. A single mutation in the genomic DNA from two female brains from different families revealed a single point mutation in both at nucleotide 1852. The mutation substitutes cytosine for guanine. Each patient had one normal allele. The mutation was

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not present in an 83-year-old female with AD or in four normal controls. The mutation abolished a restriction site identified with MBOII. Loss of this site could be detected in PCR-amplified genomic DNA. Analysis of two patients showed that the gene was polymorphic. The problem then was to determine whether this is the cause of the disease. Oligonucleotide probes containing the mutation hybridized at high stringency in other family members but not in controls, indicating that the mutation follows the disease occurrence in all patients studied thus far, without exception. The G to C transversion in the third of the triplet codon results in substitution of glutamine for glutamic acid at position 22 of the APP. How this structural modification leads to polymerization and deposition of amyloid is unknown.

As a result of these studies and those of other investigators, it is evident that there are now three autosomal dominant conditions that cause cerebral disease characterized by amyloid deposition in the CNS.

1) Amyloid angiopathy with a mutation in beta amyloid (Dutch disease).[26,27] 2) Gerstmann-Sträussler syndrome with a point mutation in the prion protein.[28] 3) Icelandic hereditary cerebral hemorrhage with amyloidosis where the brain tissue is not affected (only blood vessels). In this disease the amyloid fibrils are derived from Cystatin C, a lysosomal inhibitor of cysteine proteinases.[29] It has been shown that the point mutation results in the substitution of glutamine instead of leucine. In both the Dutch and Icelandic diseases the amyloid derives from mutations in protease inhibitors that may be present in the circulation. The significance of the glutamine substitution and its role in the deposition of amyloid fibrils is intriguing but unknown. 4) Finally there is familial Alzheimer's disease — where the cause of the defect remains unknown, but in which to date no mutations of APP have been found.

CNS Tumors and Suppressor Oncogenes

Understanding cellular processes that determine normal cell differentiation and the termination of cell division are important for discerning mechanisms of tumor formation. Tumor development "may be viewed as the gradual emancipation of a clone of somatic cells from the complex controls that regulate its growth."[30] An oncogene is a gene that causes abnormal cellular division leading to tumor formation. Growth suppressor or tumor suppressor genes, also sometimes called anti-oncogenes, belong to another class of growth regulatory gene whose normal function is the suppression of cellular division and growth. There is now evidence that loss or mutational inactivation of "recessive cancer genes" plays a role in the formation of retinoblastoma, meningioma, acoustic neuroma and hemangioblastoma (von Hippel-Lindau disease).[2]

Retinoblastoma

Retinoblastoma usually presents clinically as a unilateral, sporadic malignancy of childhood.[31,32] About 15% of cases occur bilaterally and are inherited as an autosomal dominant trait. The locus for the tumor suppressor gene in retinoblastoma is chromosome 13q14; deletions or mutations of DNA in this region on both chromosomes cause tumor development. "Inherited tumors occur when retinoblasts with one defective gene copy in this region due to a germinal mutation undergo a second somatic mutation ('second hit') of the chromosome carrying the normal allele, resulting in the loss of the remaining normal copy of the gene. Loss of both copies of the growth suppressor gene is believed to result in tumor formation."[14]

The anti-oncogene in retinoblastoma has been identified.[33,34] A 4.7 kb RNA transcript that codes for a protein (Rb) of 816 amino acids and molecular weight of about 105K daltons has been identified. The Rb protein locates to the nucleus and is believed to be a DNA-binding protein. Recent work suggests that transforming DNA viruses act to relieve the suppression caused by growth suppressor genes by combining with the RB protein.[14]

Acoustic Neurofibromas

Tumor tissue obtained from patients with bilateral acoustic neuromas demonstrated loss of a region of chromosome 22.[35,36] A panel of DNA probes from chromosome 22 showed linkage in two large families with NFII. It is speculated that this form of the disease is analogous to retinoblastoma due to loss of a cell growth-controlling gene on chromosome 22. Similar speculations are made about DNA deletions on chromosome 22 in meningioma.[37]

von Hippel-Lindau Disease

von Hippel-Lindau disease is an autosomal dominant disorder with inherited susceptibility to several tumors (hemangioblastoma, pheochromocytoma, and renal cell carcinoma). Deletions on chromosome 3p occur in renal cell carcinomas. Seizinger and colleagues[38] examined markers in this region in nine families with von Hippel-Lindau disease and showed that the disease is linked to chromosome 3p25. They speculated that absence of suppressor genes may be the cause of both the inherited and sporadic forms of the disease.

What Promise Does the Future Hold?

The O/R Paradox

These examples illustrate the extraordinary power and potential of using molecular biologic tools to approach directly some of the most serious and hitherto unsolvable diseases that affect the nervous system. In fact, the problem facing us during the next decade is not one of ideas and approaches, but rather one of limitations of resources and funding to carry on the work we should be doing. I call this the O/R paradox. And this does not refer to the operating room! The paradox to which I refer is the opportunity/resource paradox. This is a national problem, indeed an international problem. It is a problem in our neurological departments. One can illustrate it by considering the 4000 genetic diseases known to affect human beings. Of these, perhaps half are known to affect the central nervous system. The techniques are available to solve all of them if the resources to carry forward the necessary investigations were available. But, for the first time in the history of the biologic sciences, we face the very serious issue that we have insufficient resources, both in terms of person power and grant support, to carry the work forward at the pace we wish to do so. Indeed, one can envision emerging in the years ahead serious discussions about the rationing of research that will carry us to the brink of making tough decisions about which diseases or initiatives deserve attention.

Who is to decide which diseases deserve most attention? Increasingly the pressure will be felt politically and we will be called upon to make choices in terms of allocations of resources

to change the directions of our research. I can state an example from my own experience. When I went to the Massachusetts General Hospital in 1978 I had no intention of changing the course of my own research interest from neuroendocrinology. However, the opportunities that arose from a congressional mandate to fund research in Huntington's disease led to our application for a Center Without Walls. The application was successful and resulted in a major portion of my own interest turning toward this one particular disease. I estimate that over the course of the past ten years between twenty and thirty million dollars have been expended on research into this one difficult and relatively rare problem. Yet, we still do not have the gene isolated and there will be a further substantial amount of money expended before it is discovered. So opportunities set the course of direction of research and the political pressure and the political mechanisms that are so active in our time frequently determine decisions about what we choose to do.

This issue comes to attention in other ways as well. Having recently attended the sixth International AIDS Conference in San Francisco I can attest to the extraordinary power of the body public to protest the slow advance of science and to illustrate the pressures applied by a segment concerned about the disease that affects them, i.e., AIDS, and the painful impact it has on other scientists and on governmental leaders who are criticized for doing too little too late.

The other example that comes to mind is the current controversy surrounding the human genome project, which is scheduled to be funded in the U.S. at approximately fifty to one hundred million dollars annually for the next five years. This area of science has been criticized by other scientists as draining resources away from small grant projects in order to achieve a big science initiative. In both of these examples, the issue revolves around resources available and not about the opportunities present in carrying the work forward. The two big complaints that we read about regularly in the newspapers now is the controversy between big science and little science advocates and the controversy between spending money for specific disease-related issues as opposed to curiosity driven fundamental research initiatives.

The O/R paradox is a failure of society — a failure of our leaders in high places to seize the opportunity afforded by science.

The E/D Risk

The second large issue that is not unrelated to the first is what I will refer to as the E/D risk. Again, this medical metaphor is not an analogy to emergency departments, but rather to expectation/disillusionment risk (or expectation/disappointment risk). Because science has created opportunities and has become much more visible publicly, the expectation that science can solve problems is held by a substantial component of our society. This view is by no means held by all, since the rise of anti-intellectualism and opposition to the scientific method has also appeared in a major way in our times. This skepticism is also shared by some within the medical community. Dr. Freymann, a Family Medicine faculty member of the University of Connecticut wrote recently, "Americans are moving rapidly toward a new paradigm of health care. Until recently most Americans were convinced the key to health was conquest of disease through science."[39] According to others, the public is abandoning the view that mankind can master nature through science. Three years ago Alan Bloom's book *The Closing of the American Mind* emerged as a best seller.[40] Bloom likened science to "the absurdity of a grown man who spends his time thinking about gnats' anuses". "We have become too persuaded about the utility of science," Bloom states, "to perceive how shocking and petty the scientists' interest appear . . . If science is just for curiosity's sake, which is what theoretical men believe, it is nonsense, and immoral nonsense, from the viewpoint of practical men." These are not the ravings of a Haight-Ashbury junkie – Bloom is a distinguished professor of philosophy at the University of Chicago. These are worrisome signals.[41]

In general, however, there remains a strong sense among our patients and the public that science, if directed, can solve diseases in the same way that enabled us to put a man on the moon. I sense, in my contact with families affected by Alzheimer's disease, that they fully expect that we will find a solution from one of our various approaches to the problem of the decline of intellectual functions associated with aging. And yet, realistically, it seems doubtful that during the next decade we will find any definitive answer to the mechanisms that cause this disease, and unlikely that we will find a treatment that will enhance or prolong intellectual function. This produces a serious potential problem, if we do not communicate adequately. The expectations of our accomplishments may lead to a sense of sellout or disillusionment at our failure. Again, the experience with the AIDS epidemic has taught us a great deal about the effectiveness of a vocal group of affected patients in bringing attention to the inadequacies that they perceive in a system of research and the development of effective drugs. This topic, which consumes many of our health care pages in the daily newspapers in the United States, demonstrates poignantly that we need to adopt new methods of communication to assure that we are heard and that the expectations perceived are realistic. The E/D risk could come about from a failure of communication. We need to be careful what we say can be done.

So far I have considered global and generic issues that are really inherent in all of our academic activities within our medical schools and research institutes. I wish to turn now to some of the risks that I perceive for neurology itself.

The P/M Dilemma

This issue speaks more directly to the future of academic neurology. The P/M dilemma is one of promises/missed opportunities which may result from a failure of vision on our part. It relates both to the opportunities afforded by science and to the ways in which these can be brought to bear in an effective way upon our everyday research practices in neurology. It is clear that the momentum of science is such that developments and advances occur on a weekly or monthly basis. I would illustrate this by referring to the polymerase chain reaction called PCR, which was awarded a prize by *Science* last year as the "molecule of the year".[42] This distinguished award (given for the first time) addressed the extraordinary power of this new scientific method to multiply small pieces of DNA and to generate quantities sufficient for detailed base pair sequence analysis. First viewed as an arcane technique that would only be understood by a few specialized laboratories, this technique has quickly become so generally important in molecular diagnosis, and in every form of molecular biological research, that it has simply revolutionized the capacity of our scientists to analyze DNA.

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This kind of advance is unlikely to occur in clinical departments without close and meaningful scientific affiliation with basic scientists, either by bringing them into our own departments or by establishing effective collaborations with them. It was my own belief that big science and little science and important disease related neuroscience research carried out in clinical departments can only be accomplished by the recruitment of Ph.D.'s to our departments, by giving them full academic status, and by giving them the opportunity for tenure advancement in the same fashion that we accomplish this for our clinical appointments. Sometimes this same objective can be accomplished by developing effective relationships with basic science departments. But I strongly believe that we cannot advance the scientific mission of our departments without such affiliations. Mike Bishop of the University of California, San Francisco, who was awarded the Nobel Prize in 1989 for his work with Harold Varmus on oncogenes said recently, "Individuals trained originally as physicians are prominent among the elite of our biomedical scientists: I cite Harold Varmus as an example. And Ph.D.'s have done great things in the study of human disease: consider the path-breaking work of Lew Kunkel on muscular dystrophy (I chose Lew as my example because he and I are alumni of the same small liberal arts college in Pennsylvania). Nevertheless, the two camps bring distinct perspectives to the fray. So we should seek a better meeting of the minds. We should find ways to initiate Ph.D.'s into the mysteries of the human organism. Some few now manage the initiation on their own, but many more are eligible. And we must change the way we prepare physicians to do research. Most contemporary graduates of medical school and specialty training are not qualified to conduct creditable research at the cutting edge of our discipline."[41] These words of advice do not mean that physicians/scientists will not exist in the future — but we should not underestimate the length of the training period — 3-5 years post residency (plus the cost). And the need to protect time afterward.

The affiliation with basic neuroscientists has *another important implication* for neurology. I want to emphasize that many of the really important discoveries will come from unexpected sources. The nematode *C. Elegans* may well tell us important things about genes that cause CNS degenerative disease.[43] *Drosophila* was the species used to identify the genes that encode for potassium channels in excitable membranes.[44]

Economic Disincentives

Practical problems are most likely to hinder the progress of neurology in the 90s. There is a decline in the perceived importance of the cognitive specialties in medical practice and with it increased economic burdens to survive! The economic pressures are resulting in our best students entering radiology, ophthalmology, and some to neurosurgery. Modern imaging techniques divert patients from the neurologists. As one of our prominent neurosurgeons was heard to say recently, albeit in jest, "a good CT or MRI scanner is worth a roomful of neurologists."

Our colleagues in internal medicine and general surgery are referring patients past us directly to neurosurgeons. We may need to develop closer working relationships, including financial arrangements, with our neurosurgical colleagues. The economic pressures being felt currently cannot be overemphasized as a drain from the academic enterprise — preventing us from protecting our researchers.

Ethical Issues

The last challenges that I will consider are those having to do with the broad ethical issues that face us today. We and others recently developed techniques to provide presymptomatic testing for patients with Huntington's disease.[45,46] Patients offered assistance of this kind require careful assessment and attention. We found out quickly that this problem was not a simple one. Those affected by the disease, i.e., those at risk for the gene defect, soon imposed their concerns and wishes upon us in terms of how to proceed with the testing. What might have initially been perceived as interference led to a very effective evolution of principles of symptomatic testing that I believe have in some senses set the stage for the application of these techniques to other diseases in the future.

Another issue with which I have dealt extensively and about which there seems at the moment to be little likelihood of change, at least in the United States, is the issue of *fetal tissue transplantation* or the use of fetal tissue for alleviation of neurologic disorders. I had the opportunity to serve on a panel formed by Jim Wyngaarden, then director of the NIH, to deal with the issue of whether scientific advances available by the use of fetal tissue were important for the scientific community. Although the scientists on the panel were unanimous in their belief that this research was both legal and necessary, the opposition to it under the concerns expressed by those opposed to abortion that the use of fetal tissue would increase the incidence of abortion led to the current permanent moratorium on such research. So in this case, scientists are unable to proceed with scientific questions because of political and ethical concerns.

I should like to end my comments by reflecting in a more optimistic way upon the extraordinary moment that we see in front of us. To quote Satchel Paige, "Don't look back, something may be gaining on you." I think it is important to plan rationally and positively about the extraordinary moment that we find ourselves in with respect to the next decade. There are opportunities that would not have been dreamed of even five years ago. The tools to accomplish things in science and research that affect patients who have neurologic diseases are now available in unbelievable ways. The opportunities to pursue science are there. We clearly will need to develop strategies that are effective, that are responsible to the tax payers, and that are responsible to our patients.

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