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An Interview with a Distinguished Pharmaceutical Scientist

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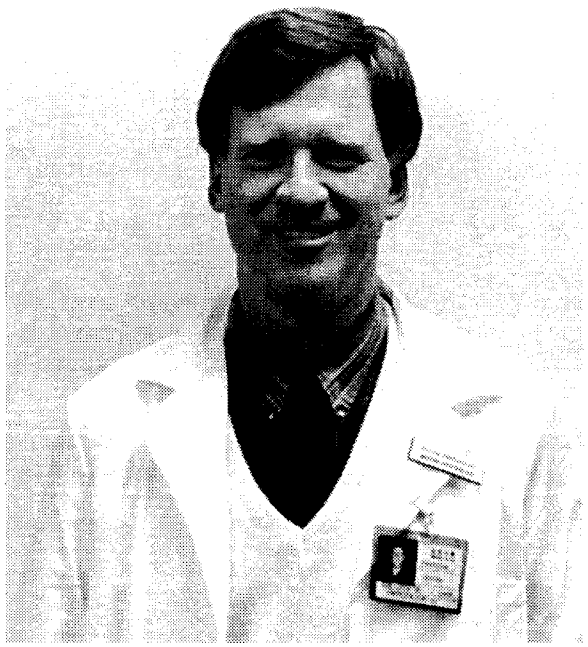
CONGRATULATIONS ON BEING NAMED THE RECIPIENT OF THE AAPS MERITORIOUS MANUSCRIPT AWARD FOR YOUR PAPER ENTITLED, "HUMAN INSULIN RECEPTOR MONOCLONAL ANTIBODY UNDERGOES HIGH AFFINITY BINDING TO HUMAN BRAIN CAPILLARIES *IN VITRO* AND RAPID TRANSCYTOSIS THROUGH THE BLOOD-BRAIN BARRIER *IN VIVO* IN THE PRIMATE," WHICH WAS PUBLISHED IN *PHARMACEUTICAL RESEARCH,* 12:807–816, (1995). WOULD YOU PLEASE TELL US WHAT LED YOU TO THIS WORK?

Response: The origin of this work was in 1980 when I used isolated brain capillaries to test the idea that an insulin receptor was expressed on the brain capillary endothelium, which comprises the blood-brain barrier (BBB) in vivo. We, indeed, found an active BBB insulin receptor (*Diabetes* **30**:757, (1981)), and subsequently showed the BBB insulin receptor is a transcytosing system (*J, Neurochem.* **44**:1771, (1985); *Brain Research* **420**:32, (1987)). The idea of using receptor-mediated transcytosis systems within the BBB to facilitate drug entry into the brain came about with the concept of chimeric peptides. These are formed by conjugating a non-transportable drug to a BBB transport vector such as insulin, transferrin, or cationized albumin. Because the use of insulin as a transport vector, per se, would cause hypoglycemia, we originally used cationized albumin as a transport vector. However, we subsequently used monoclonal antibodies (MAb) to the rat transferrin receptor, and then, as described in our 1995 *Pharmaceutical Research* paper, we used a MAb to the human insulin receptor (HIR) as a BBB transport vector. The anti-human insulin receptor MAb is 9-fold more active than any of the anti-transferrin receptor MAbs as a BBB drug transport vector.

HAVE YOU FOLLOWED UP ON THIS EXCITING TECHNOLOGY? IF SO, WHAT ARE THE RECENT FINDINGS?

Response: Yes, we reported in the October, 1997 issue of *Journal of Clinical Investigation,* that a peptide radiopharmaceutical could be effectively delivered through the primate BBB in vivo by conjugating the peptide pharmaceutical to the anti-human insulin receptor MAb. This peptide radiopharmaceutical, $A\beta^{1-40}$, is a potential brain amyloid imaging agent, and is a potential diagnostic agent for Alzheimer's disease. In the absence of a BBB drug delivery system (DDS), there is no measurable brain uptake of this labeled peptide, because the molecule is not transported through the BBB. However, a robust "2-deoxyglucose"-like scan of the living primate brain is obtained when the peptide radiopharmaceutical is conjugated to the anti-HIRMAb BBB drug delivery system (*J. Clin. Invest.,* **100**:1804 (1997)). In the future, we hope to humanize receptor-specific MAbs to allow for BBB transport of drugs in humans.



HOW DOES YOUR TECHNOLOGY BASED ON RECEPTOR-MEDIATED TRANSCYTOSIS COMPARE WITH OTHER TECHNOLOGIES FOR ENHANCING BLOOD-BRAIN BARRIER TRANSPORT IN TERMS OF CLINICAL PROMISE?

Response: Other brain drug delivery strategies include craniotomy-based brain drug delivery, liposomes, and drug lipidization. Craniotomy-based drug delivery strategies such as intracerebro-ventricular infusion or direct intracerebral implants allows for very limited distribution of drug within the brain because these controlled release strategies rely on diffusion for drug penetration into the brain. Diffusion is a poor drug delivery vehicle to the brain. Craniotomy, per se, is also not a particularly satisfying approach to solving the brain drug delivery problem. Liposomes, even 40–80 nm small unilamellar vesicles, are too large to cross the BBB and are not effective brain drug delivery vehicles in the absence of specific targeting mechanisms. Drug lipidization has the disadvantage that the plasma area under the concentration curve (AUC) decreases in proportion to the

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increase in blood-brain barrier permeability-surface area (PS) product following conjugation of the drug to a lipid-soluble substance. However, liposomes can be targeted to the brain by tethering a receptor-specific MAb to the tip of a polyethyleneglycol strand attached to the surface of the liposome (*Proc. Natl. Acad. Sci.* **93**:14164, (1996)). Chimeric peptides use endogenous receptor-mediated transport systems, and there are also endogenous carrier-mediated transport systems that could also be used to transfer across the BBB small molecule drugs that have a molecular structure mimicking an endogenous nutrient.

YOU ARE A LEADING FIGURE IN DRUG TRANSPORT ACROSS THE BLOOD-BRAIN BARRIER. WHAT LED YOU TO THIS CHALLENGING AREA OF INVESTIGATION? WHAT ARE YOUR KEY FINDINGS? WHERE WOULD THESE FINDINGS LEAD TO?

Response: I was introduced to the blood-brain barrier because I needed a summer job and I happened to apply to the laboratory of Professor William H. Oldendorf at the UCLA School of Medicine. Professor Oldendorf hired me for the summer of 1970, and during that time, I learned his brain uptake index (BUI) technique, which provided a new approach to the rapid measurement of BBB permeability. I subsequently used the BUI technique to carry out my own studies both in medical school and in my internal medicine residency at the Boston University Medical Center. In 1978, I was appointed Assistant Professor at the UCLA School of Medicine, and I used exclusively the BUI technique for the first 12–18 months of my laboratory investigation at that time. The key finding that has been made in the subsequent years regarding the study of BBB transport is that endogenous transport systems within the BBB can be used to safely deliver drugs to the brain that are normally not transported through the BBB. If the CNS drug delivery problem is solved, this could open up the "bottle-neck" formed by the BBB problem in the overall CNS drug development mission.

TO WHAT EXTENT WAS YOUR CHOSEN FIELD SHAPED BY YOUR CLINICAL TRAINING?

Response: In 1976–78, I was trained as an endocrinologist, and my first NIH grant was entitled "Endocrinology of the Blood-Brain Barrier." This initial grant ultimately led to the present day work on the receptor-mediated transcytosis of chimeric peptides through the BBB.

WHAT ARE THE OTHER ASPECTS OF BLOOD-BRAIN BARRIER THAT YOU ARE INVESTIGATING? WHAT IS THE SYNERGY BETWEEN THEM AND YOUR DRUG TRANSPORT WORK?

Response: I am also investigating the molecular biology of the BBB GLUT1 glucose transporter and focusing on specific post-transcriptional mechanisms that regulate the BBB glucose transporter gene expression. I have used the tools of molecular biology to execute these studies, and I have found it very convenient to use these same tools for the brain drug delivery work. These techniques allow for cloning of receptor-specific monoclonal antibody genes as well as the construction of MAb/avidin fusion genes and expression of MAb/avidin fusion proteins.

WHAT IS YOUR VIEW ON THE ROLE OF GENE THERAPY IN NEUROLOGICAL DISEASES?

Response: I believe it is premature to consider the role of gene therapy in neurological diseases. The isolation and cloning of a gene is analogous to CNS drug discovery. If the drug (or the gene) is not transportable through the BBB, then it is not a feasible therapy, until suitable CNS drug delivery strategies are devised. The application of gene therapy or antisense therapy to the brain is analogous to the application of neurotrophin-based neuropharmaceuticals. In the case of neurotrophins, considerable CNS drug discovery occurred in the complete absence of a parallel program in CNS drug delivery. Since neurotrophins do not undergo transport through the BBB, it is not surprising that extensive phase III clinical trials using neurotrophins for CNS disorders have been uniform failures. The particular aspects of CNS drug delivery that must be focused on with respect to gene medicines is the development of (a) effective linker strategies that conjugate the plasmid-based gene medicine to a BBB transport vector, and (b) the ability to build into the brain gene targeting mechanism an intracellular endosomal release mechanism. Drug delivered to cells via receptor-mediated mechanisms will be entrapped in the intracellular endosomal system, and drugs such as antisense agents or gene medicines that must be delivered to the cellular cytosol are ineffective pharmaceuticals in the absence of effective and safe endosomal release mechanisms.

WHAT IS YOUR VIEW ON THE CURRENT STATE OF BLOOD-BRAIN BARRIER DRUG TRANSPORT RESEARCH?

Response: Blood-brain barrier drug transport research is still an "outpost" on the scientific frontier that, despite the importance of this field to the overall CNS mission, is populated by a surprising small number of people worldwide. Accordingly, more than 99% of the worldwide CNS drug development effort is devoted to CNS drug discovery, and less than 1% of the worldwide CNS drug development effort is devoted to CNS drug delivery. I am not sure why there is such an imbalance between CNS drug discovery and CNS drug delivery, but I believe this is attributed to the fact that there are so few BBB scientists that are currently being trained worldwide. There is simply an insufficient pool of scientists trained in blood-brain barrier research to populate even a small fraction of the number of CNS drug delivery scientists needed at both academic institutions and industry. I believe one of the reasons for this underdevelopment of fundamental research in the BBB field is because neither the neurosciences nor the physiological sciences integrated BBB research within the body politic of those entities. Moreover, I believe that BBB scientists in the future will be largely trained within the pharmaceutical sciences.

WHAT IS YOUR VIEW ON CULTURED BOVINE BRAIN ENDOTHELIAL CELL LAYERS AS A RESEARCH AND DRUG DEVELOPMENT TOOL? HOW HAS THIS TOOL BEEN MISUSED?

Response: When brain endothelial cells are grown in tissue culture, the tissue-specific gene expression within the brain capillary endothelium that accounts for the remarkable transport properties of the BBB in vivo, are severely down-regulated. Even when brain endothelial cells are grown in co-culture with

astrocytes, the endothelium is still de-differentiated with respect to brain capillary endothelium in vivo. For example, the electrical resistance across the brain capillary endothelium in vivo is on the order of 8,000 $\Omega \cdot cm^2$, and the highest electrical resistance found in tissue culture is only 5–10% of the in vivo value. The "in vitro BBB" is much leakier than the BBB in vivo. However, the more serious problem with the in vitro model is that many of the receptor-mediated transcytosis and carrier-mediated transport systems that operate at the BBB in vivo are severely down-regulated in tissue culture. If the tissue culture model was used to screen L-DOPA as a potential neuropharmaceutical that is capable of transport through the BBB in vivo on the neutral amino acid carrier system, then this pharmaceutical would be completely missed as a potential neuropharmaceutical. This is because the neutral amino acid carrier in the in vitro BBB models is markedly down-regulated. The transport of L-DOPA across the monolayer in tissue culture occurs primarily by free diffusion based on the lipid solubility of the molecule (*J. Pharmacol. Exp. Ther.* **253**:884, (1990)).

WHAT ARE THE FUTURE CHALLENGES IN DRUG DELIVERY TO THE CENTRAL NERVOUS SYSTEM?

Response: The challenge to brain drug delivery in the future is to deliver drugs to the brain without craniotomy. The best approach for this is to use endogenous transport systems. This is a good example of how tightly connected are the pure and applied sciences. It is my view that it is artificial to think of a separation between the pure and applied sciences. Pure science is viewed as studying the fundamental transport biology of the brain capillary endothelium and applied science is viewed as developing practical BBB drug delivery strategies. However, the latter is achieved by studying the cell biology of BBB transcytosis and other transport mechanisms. Therefore, there really is no distinction between pure and applied sciences in this context.

DO YOU CONSIDER YOURSELF A PHARMACEUTICAL SCIENTIST? WHY SO?

Response: Yes. Pharmaceutical science is the formulation and delivery of drugs, and delivery of drugs to the CNS is the ultimate challenge to the pharmaceutical sciences.

HOW WOULD YOU DEFINE PHARMACEUTICAL SCIENCES?

Response: The pharmaceutical sciences have traditionally focused on drug formulation and controlled release, and these areas have fundamentally emanated from the material sciences. However, in the future, I believe the pharmaceutical sciences will focus on drug targeting using endogenous transport systems and this form of future pharmaceutical sciences will find its roots in transport biology, which is fundamentally a different scientific discipline than the material sciences. I also believe that in the future there will be considerable fusion between the material sciences and the transport biology sciences. For example, targeting liposomes or nanoparticles across cellular barriers by receptor-mediated transport systems is an example of how both the material sciences and transport biology sciences are used to develop a drug targeting strategy.

WHAT DO YOU THINK HOLDS THE KEY TO YOUR SUCCESS AS A PHARMACEUTICAL SCIENTIST?

Response: I have been able to formulate drugs in a way that allows the drug to utilize natural, endogenous transport systems within the BBB to cause CNS delivery of a pharmaceutical, that, in the absence of the BBB drug targeting system, would not be capable of transport through the BBB in pharmacologically significant amounts. I have also placed high value on optimizing the pharmacokinetics of the drug.

WHAT ARE THE 2–3 ACHIEVEMENTS THAT YOU ARE MOST PROUD OF? WHY?

Response: I contributed to hormone transport theory with respect to plasma protein binding effects in vivo (reviewed in the 1998 *Handbook of Physiology*), to the development of the chimeric peptide approach for brain drug delivery (reviewed in the 1991 book, *Peptide Drug Delivery to the Brain*), and to the molecular biology of the BBB GLUT1 glucose transporter (reviewed in the 1993 book, *Blood-Brain Barrier: Cellular and Molecular Biology*).

WHAT WAS THE TURNING POINT IN YOUR DISTINGUISHED CAREER?

Response: I exclusively used the BUI technique for the first 12–18 months of my laboratory career as Assistant Professor of Medicine at UCLA in the late 1970's. However, I realized that an integrated approach to BBB research would require a cellular and molecular analysis, and this was not possible with sole reliance on conventional whole organ physiologic techniques. Therefore, in 1980, I decided to establish methods for isolating brain capillaries from initially animal brain and then human autopsy brain. The use of isolated brain capillaries subsequently led to a series of biochemical and molecular biological studies of the brain capillary transport processes. I chose not to investigate multiple biological problems with a specific technique, but rather to examine specific biological problems with multiple in vivo and in vitro techniques.

CAN YOU NAME THE TWO OR THREE INDIVIDUALS WHO HAVE MADE A DIFFERENCE IN YOUR CAREER? HOW SO?

Response: The individual who made the most difference in my career was Professor William H. Oldendorf, who introduced me to the blood-brain barrier field in the summer of 1970. For the next 22 years, until his death in 1992, I frequently had the privilege to discuss various problems with him and was always amazed by his unique intellect.

PHARMACEUTICAL SCIENTISTS ARE FACED WITH THE DILEMMA OF HAVING TO PUBLISH IN BIOMEDICAL OR BASIC SCIENCE JOURNALS AND HAVING TO PRESENT IN THEIR SPECIALTY MEETINGS IN ADDITION TO THE PHARMACEUTICAL SCIENCES VENUES. DOES IT MEAN THE CUTTING EDGE SCIENCE WILL NOT LIKELY BE FEATURED IN THE PHARMACEUTICAL SCIENCES FORUM?

Response: As the pharmaceutical sciences evolve in the future emphasizing drug targeting based on the biology of endogenous transport processes, the lines between the basic and pharmaceu-

tical sciences will be blurred and any distinction between the two will be less important. This is because the innovation of new drug targeting strategies will require basic investigations of the cellular and molecular biology of membrane transport.

WHAT WOULD BE YOUR ADVICE TO OUR JUNIOR PHARMACEUTICAL SCIENTISTS WHO ARE ABOUT TO EMBARK ON THEIR CAREERS?

Response: I would recommend that young pharmaceutical scientists focus on learning transport biology so they can build careers in the cellular/molecular aspects of drug targeting through endogenous transport mechanisms.

WHAT WOULD BE YOUR ADVICE TO OUR SENIOR PHARMACEUTICAL SCIENTISTS IN THEIR RELATIONSHIP TO THEIR JUNIOR COLLEAGUES?

Response: I would recommend that senior pharmaceutical scientists encourage their junior colleagues to be their competitors of the future.

HOW HAS YOUR PHILOSOPHY OF EDUCATING GRADUATE STUDENTS BEEN CHANGED OVER THE YEARS?

Response: Because I have been in a Department of Medicine for the last 20 years, I have actually not had the privilege of having any graduate students. I have, however, trained many post-doctoral fellows, and I have noted that during recent years, the complexity of projects has increased substantially, and as this complexity increases, post-doctoral colleagues require more supervision. This, of course, is hard to do when a laboratory supervisor has a very busy schedule.

HOW HAS YOUR PHILOSOPHY OF MENTORING JUNIOR COLLEAGUES CHANGED OVER THE YEARS?

Response: I have become pessimistic that junior colleagues will ultimately chose an academic career and direct their own laboratory. The dual forces of (a) growth of the biotechnology industry and further growth of the large pharmaceutical firms, and (b) the unpredictability of NIH funding, provides powerful incentives for young colleagues to select careers in industry rather than academia. On the other hand, the quality of science done in industry in the future will continue to increase.

WHAT ARE THE FUTURE CHALLENGES TO THE PHARMACEUTICAL AND BIOMEDICAL SCIENCES?

Response: I believe the future challenge of the pharmaceutical and biomedical sciences is to recognize that the line between pure and applied science is not only thin but anachronistic. NIH was founded on 1940's concepts that the government should fund pure science. Drug delivery and drug targeting is generally viewed as an applied science. Thus, academic research has heavily favored those sciences that are viewed as "pure" and de-emphasized those sciences that are viewed as "applied." I believe it is this tradition that underlies the present day imbalance between CNS drug discovery, which emanates from the molecular neurosciences, and CNS drug delivery, which is viewed as applied science. In fact, the development of effective CNS drug targeting strategies requires fundamental cell biological investigations of capillary endothelial transcytosis systems. Moreover, the use carrier-mediated transport systems to facilitate drug delivery requires fundamental knowledge on the molecular biology of BBB nutrient transport systems. As a result, a distinction between pure and applied science has never made much sense to me.

WHAT IS THE PLACE FOR ENTREPRENEURSHIP IN ACADEMIA?

Response: I would prefer to use the term technology transfer, and I believe that there is not only a place for this in academia, but that it is essential for all academic scientists to participate in technology transfer. The taxpayer funds academic science on the belief that this science will ultimately lead to new cures for human diseases. Therefore, it is not possible to fulfill this expectation without efficiently transferring technology from the academic laboratory to industry. Effective technology transfer from academic laboratories is the single most important factor for future sustained funding of academic science.

Thank you very much.