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YAFFE AWARD LECTURE

Ideas, Collaboration, and Replication

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Those of us who have made a commitment to academic medicine have a real interest in contributing to learning. It may be research, or it may be teaching—perhaps a combination. I wish to tell you how such a sequence of ideas, collaboration, and replication has been important to me in the early days of my academic history, but most especially since coming to Penn State College of Medicine and the Penn State Children's Hospital at Hershey. It is a great honor to receive the Yaffe award, named for Dr. Sumner Yaffe (Figure 1) who is personally responsible for the very significant advances made in pediatric therapeutics over the past 35 years. More importantly, he has been a significant mentor for many of us for over six decades.

My first exposure to pharmacology was as a second year medical student at Harvard Medical School. The pharmacology course was a rigorous one taught by very dedicated teachers and researchers. The Chair was Otto Krayer, already a legend not only for his work in pharmacology but also for his extraordinary ethical stance during the dark days of Nazi rule in Germany.[1] After my marriage at the conclusion of the second year, my wife Anne worked as a secretary in the Pharmacology Department office, and through her I heard many stories of the activities of the members of this department. I was able to work with Dr. John Blinks on the force velocity relationship of cardiac muscle and the influence on various drugs on this relationship.[2] It is a great coincidence that Dr. Sumner Yaffe's first paper was also on cardiac muscle, and when published, introduced by Dr. Otto Krayer.[3]

ABBREVIATIONS AAP, American Academy of Pediatrics; PKU, phenylketonuria; TSA, Tourette Syndrome Association

In the summer of 1971, Dr. Nicholas Nelson (Figure 2A) telephoned me at my position at the George Washington University School of Medicine in Washington D.C. He said that he wanted me to come to the then new College of Medicine of the Pennsylvania State University at Hershey. I said I would like to visit the new medical school plus see the town itself, for in spite of being a native of Pennsylvania and passing Hershey many times on the turnpike, I had never been to the town. He said, "No, you don't understand, I want you to come and work

Figure 1. Sumner J. Yaffe, MD, pictured with his daughter. Christine Yaffe, MD is pharmacologist whose research focuses in the area of the central nervous system.

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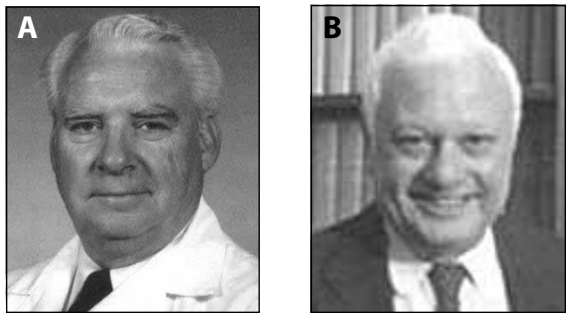

Figure 2. (A) Dr. Nicholas Nelson, founding chair, Department of Pediatrics, Penn State University. (B) Dr. Elliot Vesell, Chairman of Pharmacology, Penn State College of Medicine and the Penn State Children's Hospital.

here in Hershey." I said, "I can't do that; last month I purchased and moved into our first home." In a most sympathetic and supportive fashion, he said: "Sell it."

I had another reason for being interested in Hershey. The newly appointed Chairman of Pharmacology was Dr. Elliot Vesell (Figure 2B) a friend of many years who had been the very first person I had met when I arrived at Harvard Medical School in 1958. I decided to come to Hershey for three reasons, hence the title of this talk. I felt Hershey was an exciting atmosphere where the exploration for ideas was encouraged and supported. I had never been in a medical school, having been born and raised, academically, in a Children's Hospital. I thought it would be exciting to be in a medical center with everything under one roof (it really was in those days!). I anticipated opportunities for collaboration. Dr. Vesell generously offered me a joint appointment in his Department of Pharmacology. I certainly did not anticipate that I would be able to collaborate with such a large number of colleagues in seven other departments. And finally, I looked forward to working closely with students and residents, something which did not readily occur at my previous location.

I believe that the desire for replication is very strong in physicians, whether in full-time practice or whether in a teaching environment. The interest we have in working with students and residents identifies this desire at replicating our kind. Indeed, the word "doctor" does come from the Latin word "to teach". An important ingredient in replication as well as research is continuity. Our ideas build on others' ideas, and progress usually travels paths that others have begun. I do have a concern that young people today beginning careers of teaching, care, and research and move around too rapidly to acquire the contacts, experiences, and inspiration necessary to pursue ideas. Success comes only to the prepared mind.

Two ideas that I have pursued have come rather suddenly in the course of laboratory work in the general area of pharmacology. After my internship year in pediatrics, I was very fortunate to be able to spend two years in the Laboratory of Biochemical Pharmacology in the National Institute of Arthritis and Metabolic Disease at the NIH. My senior mentor was Dr. Herbert Tabor, and I was assigned to work in the laboratory with Dr. Robert Schimke. We did have a laboratory technician, but both Dr. Schimke and I did most of the actual bench work. We were interested in the regulation of enzyme (protein) levels in tissue. For the enzymes we selected, administration of substrate (amino acid), through either diet or injection, stabilized the enzyme involved in that particular amino acid's metabolism; it blocked enzyme degradation. Administration of a corticosteroid (hydrocortisone) increased the enzyme level many fold, depending on the enzyme. Isotope label studies determined that the corticosteroid increased the rate of synthesis of the enzyme. The rate of synthesis is a zero order kinetic function; the rate of degradation, first order (Figure 3). Administration of both the substrate and the corticosteroid results in a synergestic curve (top curve in Figure 3). Looking at the curves and writing the equations for the rate constants enable us to show that there is indeed a dynamic equilibrium in these particular body constituents.[4] The rate of synthesis equals the rate of degradation. The particular contribution of this research was to show that the response of an enzyme to either increased synthesis or increased degradation depends of the half-life of the enzyme.[5] All enzymes will show that same degree of response, but the time course will be different.

I am aware that this important area of research, the regulation of enzyme levels in tissue, is still not yet totally clear. For example, although the amount of certain enzymes may increase many fold, total liver protein may only increase 30%-40%, regardless of the stimulus.

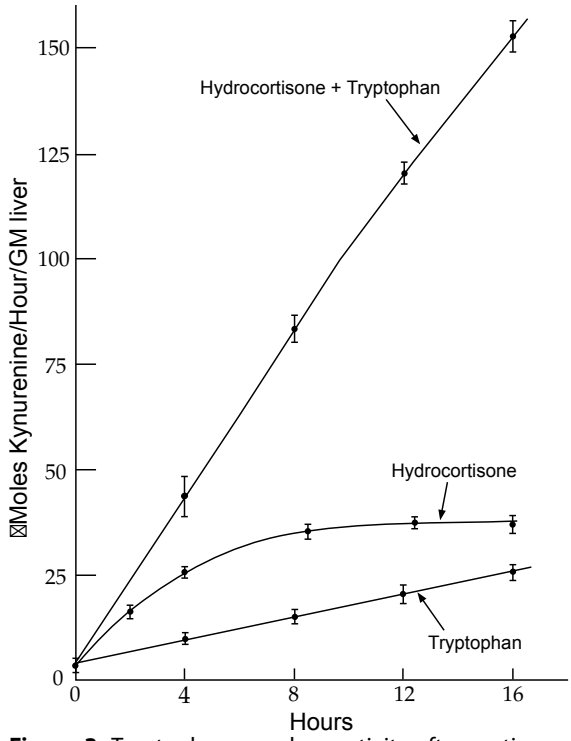

Figure 3. Tryptophan pyrrolase activity after cortisone, tryptophan or both.
Reference 4. Reproduced with permission of the American Society for Biochemistry and Molecular Biology.

We are not knowledgeable about other molecular controls of protein synthesis, but still must translate them to the intact organ and intact organism. Enzyme regulation has an important influence in the management of normal nutrition, drug response, and response of the patient to disease states. Pediatrics is a special case. For many substances, the rate of synthesis is not obviously equal to the rate of degradation for the growing infant and child. What controls this? What controls the increase in body mass as well as the precise and elegant differentiation that the various tissues undergo? I am very optimistic that molecular biological knowledge and techniques will elucidate this most important secret of human biology.

The second idea I would like to discuss is one that I continue to pursue: the excretion of drugs and chemicals in human milk. This idea occurred very suddenly whilst I was looking over a paper written by Elliot Vesell concerning the genetic control of the hepatic metabolism of the compound antipyrine.[6] This drug, with a single exception (it is an ingredient in the analesgic Auralgen, used for external ear canal pain), is no longer used in the United States as an analgesic. I was struck by two observations about the behavior of this drug: it is distributed to total body water, and the distribution is rapid.

Because of a long-standing interest in breast feeding, I was aware of how primitive is our knowledge of the excretion of drugs into human milk. What about antipryine as a model compound? Does it cross into milk, and does it teach us anything about the appearance of a compound into human milk? Dr. Vesell shared my enthusiasm to look into these questions, and we gave our first patient antipyrine by mouth. The drug was administered only to lactating women who were weaning their infants, and thus no infant was exposed to the drug through the mother's milk. I have mentioned how important it is to be aware of colleagues' interests and experience. Dr. Vesell has conducted over 100 studies with antipyrine, involving administration of this compound to several thousand individuals. This drug appears promptly in milk and has a first order decay identical with that of the drug in plasma.[7] It fact, the levels are virtually identical. It is very important to stress how promptly this drug appears in milk; within 10 minutes of oral administration. This was a new and quite unexpected finding.

Previous reports in the literature concerning drugs in milk usually looked at a single measurement of the drug at various times from maternal ingestion. Our work was one of the first to follow the time course of the appearance and disappearance of a drug after the initial dose in the same patient. The study was the first to show such correlation between the plasma and milk levels, and finally, it was the first study to permit the estimation of the exposure of the nursing infant to maternal drug. Lactation also seems to significantly affect drug metabolism. During lactation, the antipryine half-lives were doubled compared with half-lives measured months after lactation had been concluded. Subsequent studies from our laboratory have shown similar patterns for the excretion of acetaminophen, caffeine, and the most interesting of all, chocolate, or rather an active component, theobromine. Our work with azulfidine and isoniazid indicates that a metabolite may be transferred rather than the parent compound.[8,9] In the case of isoniazid

(INH), its metabolite, acetylisoniazid, may be not only the active compound, but it may also be the toxic one as well. We worry about the small infant being exposed to even the small amounts found in our study.

This work on drug excretion into human milk has resulted in our Medical Center being recognized as a national resource for the information on the excretion of drugs and chemicals into human milk. The Committee on Drugs of the American Academy of Pediatrics (AAP) completely revised the Statement on this subject in November 1989 with revisions in 1994 and 2001.[10] A revision is currently in the planning stages. The AAP has also published a chapter on maternal medication use in the book *Breastfeeding for Physicians*.[11]

There remain many unanswered questions in this area. How precisely are drugs transferred into milk? What is the role of environmental chemicals in human milk—the concentration of most of the environmental chemicals measured in human milk are of the order of nanograms or picograms per milliliter. Could this be hazardous to the nursing infant? The most common questions currently asked are concerning the use of central nervous system drugs by the nursing mother: antidepressants, antipsychotics, and tranquilizers. For almost all of these compounds, the total amount possibly transferred to the infant over 24 hours is quite small. For most of these compounds, very small amounts can be found in human milk and, in some cases, in the plasma of the nursing infant's urine. Is this amount hazardous? Can an effect be measured by current neuropsychological testing? Will knowledge of the molecular biology of the central nervous system help us? This is not a trivial problem.

Each year in the United States alone, about 1.5 million newborn babies receive most or all of their nourishment from human milk at a time of important development of the central nervous system. There is a national emphasis to increase the rate of breastfeeding even higher than the current 70% of infants discharged from nurseries. We are learning much about the imprinting processes that occur early in infancy on many organs. In this research, I have collaborated with many people, chiefly the nursing mothers who so enthusiastically took part in these studies, and I wish to thank all of them for their help and support. We must be committed to making breast feeding as safe as possible.

In 1972, as part of my general pediatric practice, I saw a patient with public outbursts of obscene language. He also had motor tics. I had remembered a patient during my residency with the diagnosis of Tourette Syndrome. I promptly made the diagnosis and started him on haloperidol. Knowledge of pediatric dosing of psychotropic drugs was very limited 35 years ago; the dose I used gave him severe extrapyramidal symptoms requiring intravenous diphendyramine the very next day. With proper adjustment of dosing his symptoms dramatically disappeared, and he was able to function in a normal classroom; the coprolalia disappeared.

I became very interested in Tourette Syndrome and other tic disorders and over the ensuing years have seen approximately 700 patients. It is an example of obtaining satisfaction in tackling a difficult area with interest, training, and experience. I enjoy my involvement as a member of the Medical Advisory Board of the Tourette Syndrome Association (TSA). As a general pediatrician, I am involved in educational efforts by TSA designed to encourage general pediatricians and family physicians to assume the care of a majority of children with tic disorders.

The next idea I wish to mention actually started with a mother who lived in a small community near Hershey. She asked her pediatrician, "Why do I have to take Heidi to Philadelphia to receive her check-ups for PKU?" As a result of her question and resultant inquiries, the Hershey PKU center was established in August 1983 with one patient. At the end of the first year, we had 9 patients. Currently, we have over 150 patients on a phenylalanine-restricted diet. Women with PKU are a special concern if they become pregnant.

We participated in the National Institutes of Health National Collaborative Maternal PKU Study which followed the reproductive outcomes of women with PKU. At the Hershey PKU clinic, there have been over 40 infants born to mothers with homozygous PKU. If the maternal serum phenylalanine levels can be maintained between 2 and 6 mg/dL, these babies are, so far, indistinguishable from normal

babies. It takes a great deal of collaboration to care for all of these patients and the complexities of their diets, with the monitoring of their plasma phenylalanine levels. Nutritional and secretarial help are indispensable for providing the best clinical care for these patients. The PKU Clinic is another example of the ability of people at Hershey with such a supportive atmosphere, to start from scratch and acquire significant expertise in the field of PKU nutrition. I know that the growth of such programs not only provide clinical care to patients, but also serve to teach and provide research opportunities. Through my clinical work in PKU, I have been able to collaborate with Harvey Levy of Boston and Flemming Guttler of Copenhagen,Denmark, on several research projects.[12,13]

Those of us who have chosen to remain as members of teaching faculties have done so primarily because we hope that by remaining in this academic atmosphere we may learn something and pass it on. Collaboration makes this so much easier and has the advantage of opening new avenues of investigation; and around the cycle goes.

The occurrence of ideas is unpredictable, especially among those of us who also have clinical duties. Some ideas simply do not work; others are incorrect. I have been fortunate to work with individuals in very diverse areas; we have had enjoyable collaboration, and I believe we have done a part in discovering something new.

Dr. Samson Jacob and I have shared for a very long time a joint interest in the immunology of connective tissue disease. We have demonstrated that patients with "autoimmune" diseases such as lupus have antibodies against their own nucleic acid enzymes: RNA polymerase, protein kinase NII, and poly(A) polymerase.[14] In the case of the first enzyme, RNA polymerase, this was the first demonstration of antibody production against a nucleic acid component with functional properties. Further work is necessary to determine if this may ultimately be a site for therapy.

Drs. Arthur Hull Hayes and Jack Luderer, both from the Department of Medicine, collaborated on antihypertensive therapy in a child with systemic vasculitis.[15] The patient received intravenous sodium nitroprusside for the control of severe hypertension for 28 days. This experience introduced me to the current dilemma that most drugs are not approved for use in children. The current *Physicians Desk Reference* lists pediatric indications (labeling) for only about 30% of the drugs, and most of them are antibiotics. Pediatric colleagues have cooperated on initiatives to change this. Prominent in these efforts have been the Committee on Drugs of the American Academy of Pediatrics, pharmaceutical industry researchers, the Food and Drug Administration, and the National Institute of Child Development of the National Institutes of Health. Dr. Yaffe's great contribution is the establishment of the NIH-sponsored Pediatric Pharmacology Research Units (PPRU), for which I have had the honor to serve as Chair of the Network Steering Committee (Table).[16]

The project with Dr. Kay LaNoue (Cell and Molecular Physiology) was an amazing example of laboratory and personal cooperation. We had a patient whom we strongly suspected, on the basis of enzyme assay of his peripheral white cells, to be lacking normal activity, or any activity, of the important enzyme pyruvate dehydrogenase. After the patient died, the family gave us permission to measure the enzyme in the different organs. At 11 a.m., Dr. LaNoue devoted herself and her entire laboratory staff (n = 7) to processing the tissues for measurement of oxidative metabolism. Since this was a collaborative project with Dr. Douglas Kerr's laboratory at Rainbow Babies and Children's Hospital in Cleveland, we picked up his technician at the Harrisburg airport at 1:30 p.m., and had her back in Cleveland, with the processed tissues safely preserved in liquid nitrogen, by supper time. That research produced the first example of a patient with a systemic deficiency of the first component of this, the most complicated of mammalian enzymes.[17]

The final subject of my talk is replication. Part of the great privilege of being at Penn State College of Medicine is participating in the education and growth of our medical students. Each year, we have a student research symposium. All medical students must complete a research project prior to graduation. The quality of these presentations is very high; indeed over 60% are eventually published. This is a tribute to the faculty of the College of Medicine and to

Table. List of Pediatric Pharmacology Research Units, National Institute of Child Health, National Institutes of Health (1994-2009)

Site	Principal Investigator
1994-1999 (N = 6)	
Arkansas Children's	Thomas Wells
Columbus Children's	Philip Walson
Louisiana State University, Shreveport	John Wilson
Rainbow Babies and Children, Cleveland	Jeffrey Blumer
University of Tennessee, Memphis	Russell Chesney
Wayne State (moved to Kansas City)	Ralph Kauffman
1999-2004 (N = 13)	
Children's Hospital of Philadelphia	Beverly Lange
Children's Mercy, Kansas City MO	Ralph Kauffman
Cincinnati Children's	Floyd Sallee
Columbus Children's	John van den Anker
Louisiana State University, Shreveport	John Wilson
National Jewish Hospital, Denver	Stanley Szefler
Rainbow Babies and Children, Cleveland	Jeffrey Blumer
Texas Children's Hospital, Houston	David Poplack
University of California, San Diego	James Connor
University of Tennessee, Memphis	Russell Chesney
Wayne State, Detroit	Jack Aranda
Yale University	William Tamborlane
2004-2009 (N = 13)	
Children's Hospital of Philadelphia	Peter Adamson
Children's Mercy, Kansas City MO	Gregory Kearns
Children's National, Washington DC	John van den Anker
Cincinnati Children's	Alexander Vinks
Kosair Children's, Louisville KT	Janice Sullivan
North Carolina/Duke	Danny Benjamin Ross McKinney
Rainbow Babies and Children, Cleveland	Jeffrey Blumer
Texas Children's Hospital, Houston	David Poplack
University of California, San Diego	Edmund Capparelli
University of Texas, Southwestern, Dallas	George McCracken
University of Utah	Robert Ward
Wayne State, Detroit	Jack Aranda
Yale University	William Tamborlane

the interest and enthusiasm of our students. I can best speak of the students who have chosen pediatrics.

In the rather short history of Penn State (37 graduating classes),we have graduated 387 (12%) students from the College of Medicine who have or are completing pediatric residencies. A large number of these remain in academic posts, continuing the tradition of replication of teachers, and in several cases, achieving national recognition which we all are proud to share. We are equally proud of our graduates who have gone into practice—in their way they are also contributing to replication. They are educating parents, children, and medical students by serving as preceptors to third- and fourth-year students for clinical experiences. I had the privilege to have responsibility for medical student affairs for 15 years. Easily, the most exciting aspect was watching the preparations of the fourth-year students for their residencies and following their subsequent careers. Collaboration was also a very important part of that administrative responsibility. I am very grateful to the many faculty members of my medical school who supported me and, more importantly, the students. This support by the faculty for our students continues and shows

the dedication of this faculty to education and thus replication.

The training (replication) of pediatric pharmacologists is an area that is of concern to all of us. There remains too little academic and financial support. My own medical school stopped a pharmacology course for medical students years ago; there is an attempt to integrate pharmacology with case-based learning and also the systems approach with mixed results. Expertise needed to develop into a clinical pharmacologist is changing. There is appropriate emphasis on the role of molecular biology best illustrated by both pharmacogenetics and the search for biomarkers that will facilitate studying pharmacodynamics. The discipline is important, as the clinical pharmacologist may be the best person to translate "translational" science. Physicians and pharmacists understand human biology; they must be trained to understand and translate molecular pharmacology.

Of all my ideas, my best idea occurred 48 years ago when I was invited on a blind date. I was so taken by this young lady that I proposed to her and fortunately was accepted. We have had a wonderful life together with many adventures and in our own collaboration have been blessed with replication. All of the replicates are here today: our daughter Jean, our sons Douglas, Alexander, and Gordon. Also my two wonderful daughters-in-law: Julie Cramer and Diane Berlin. My daughter Jean has also ideas of her own and has also replicated giving us our granddaughters Emily and Rebecca. To all my colleagues and friends, but especially to my family, I am so very grateful.

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