
WHO DO WE SERVE?

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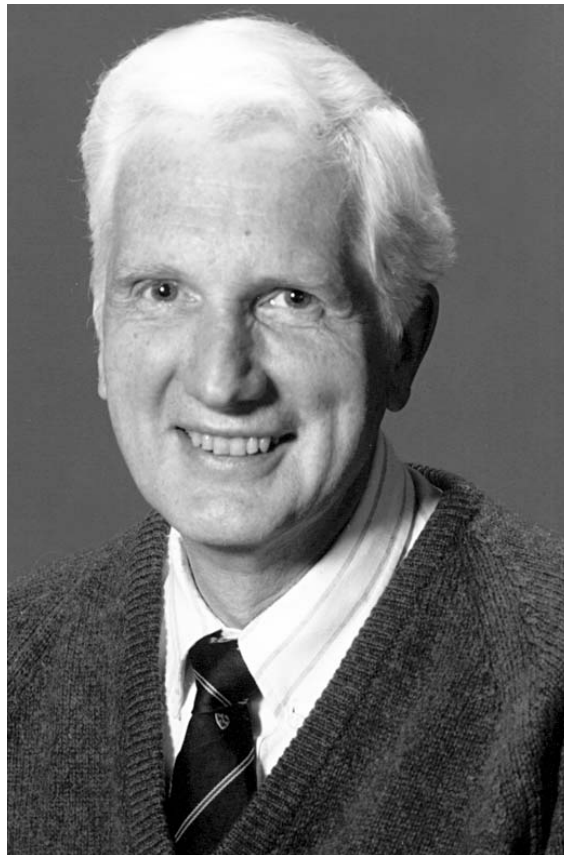
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My career has been an unanticipated odyssey. One might ask: How did you get to here from there? The answer could be: If I had wanted to go to here, perhaps I wouldn't have started from there. How much of the journey has been by choice and how much by chance, you, the reader, will decide.

When I was at University, I majored in Geography and Comparative Literature. I intended to become a graduate student in the Department of Geography at McGill University, at that time the most outstanding department in the world. But I did a strange thing - by choice; I made one application to study medicine. The Admissions Committee of the Medical Faculty at McGill University invited me in, and I accepted. Most of the remaining moves on the journey were a result of chance before choice. With the help of some superb teachers, I discovered that medicine can be both a degree in human biology and an enquiry into its dysfunctions. The opportunity also presented itself to learn about "healing", an important part of medicine.

After graduation (in 1955), I followed the usual path through the internship and residency programs, at which time, I began to appreciate the importance of a particular medical question: Why does this person have this disease now? Later, I found its biological counterpart: each person is unique in biological inheritance and identity, which would translate into a biological view of medicine, and the axiom, "treat not just the disease; treat the patient".

Careers may reflect the role of mentors and I was fortunate in mine. John Beck, Ronald Christie and Alan Ross influenced the route my subsequent journey would take. Alan Ross arranged for my clinical studies to continue at Harvard where I was allowed to indulge in seeking answers to the ques-



tions I mentioned above, and I was involved there in the discoveries of two new diseases in two patients. Both diseases happened to be Mendelian disorders of metabolism, although at the time I did not recognize how well I would be imprinted by those two young patients. John Beck and Ronald Christie helped me to apply for a McLaughlin Traveling Fellowship, to pursue clinical and laboratory experience with Professor Charles Dent at University College Hospital Medical School in London, England. It was there that I discovered what I really wanted to do: although I was 29 years old, with two degrees and 4 years committed to the study of clinical medicine, I wanted to be a clinician-scientist. Harry Harris (Kings College,

London), Charles Dent and Alex Bearn (on sabbatical in London) were now my mentors, in human biochemical genetics; they convinced me to pursue my interests.

Accordingly, I used the chromatographic techniques of the day to investigate one of the patients I had cared for at Harvard. I discovered hyperprolinemia, a heretofore unknown inborn error of metabolism. I also observed that the patient's urine contained three amino acids in excess (proline, hydroxyproline and glycine), whereas the excess in blood was restricted to proline. I experienced one of those sudden insights (I can recall place and time of day) and I surmised that an excess of proline could saturate a shared transporter in the renal tubule and at the same time displace its other substrates, if all three used a shared mediated transport system. (This was quite novel thinking in human biology for the time - the late 1950s). I tested the hypothesis by infusing myself with proline (I was not hyperprolinemic) and the triad of hyperaminoaciduria appeared in my own urine. Two other hardy post-docs in the lab thought this was fun and we repeated the experiment two further times. I was able to present these findings a year later on the plenary session of the American Society for Clinical Investigation and to publish them both in *Nature* and the *New England Journal of Medicine*.

While in London, I met the original patients with "Hartnup disease". My mentors considered the Hartnup phenotype to be an inborn error of amino acid metabolism. I noticed that the findings in the patients were compatible with an inborn error of membrane transport involving a subset of neutral amino acids (again, a novel insight for the times). I was already attuned to think about membrane-located carriers that could recognize group-specific sets of substrates. I thought the Hartnup transporter would be expressed in epithelial cells in both kidney and intestine, probably in the brush-border membrane. I was challenged to produce evidence to support my hypothesis, and I did so. The *New England Journal of Medicine* later published the evidence.

In 1960, I was committed to be Chief Resident in Pediatrics at the Montreal Children's Hospital. The prospect was not delightful. There were better clinicians than myself, and I wanted to continue in research. Alan Ross, my Chairman of Pediatrics, recognized this and supported my wish to develop a lab. Furthermore, he nominated me for a Markle Scholarship and for 5 subsequent years, I was "protected". Thus, I became Canada's first human biochemical geneticist on Faculty. The appointment at McGill allowed me to work with extraordinary colleagues, to create newborn screening programs (for early diagnosis and treatment of genetic diseases) and with those colleagues to create the Quebec Network of Genetic Medicine (Scriver CR et al. *Science* 200 : 946-952, 1978 [PMID 644337]).

By the early 1970s, my colleagues and I were immersed in human biochemical genetics and in the investigation of several new inborn errors of amino acid metabolism. For example, we discovered that there was a subset of vitamin (cofactor)-responsive hereditary disorders of amino acid metabolism, a finding of great significance for the treatment of these particular problems, which we now believe (or know) to involve mutations affecting binding kinetics of the coenzyme or a chaperone-like effect in response to pharmacological doses of the agent. We were also involved in the development of new methods to screen, counsel and treat a variety of Mendelian disorders such as phenylketonuria, Tay-Sachs disease and thalassemia in individuals, families and communities. With hindsight, it was the beginning of "community genetics" (while moving biochemical genetics along), and of an era when something could truly be done to ameliorate "genetic disease". It is not surprising that our involvement in such "translational knowledge" would focus on the treatment of genetic disease, and for over 25 years we have been engaged in a meta-analysis of the modes, progress and efficiency of treatments. Enough to say that biochemists and cell biologists will find challenges enough for many careers here ...!

In other sectors, we continued to investigate mediated membrane transport of amino acids and of phosphate anion. The interest in phosphate trans-

port arose through an investigation of infantile rickets which in Quebec, at the time, appeared as an annual quasi-epidemic related to vitamin D. Inborn errors of metabolism are Mendelian (single-gene) disorders and one tends to think of them rather simplistically; that there is more to consider became a broader theme of our work. For example, homeostasis or the central tendency of metrical trait values (e.g. measured genotypes of amino acid values in plasma) is a classic complex trait. We generated data on the frequency distribution of amino acid values in normal subjects and found both inter- and intra-individual variation; data implying that each person had his or her own measured genotype (i.e. a form of biological individuality). It was my personal introduction to ways of thinking about inherited susceptibility to and risk for common but complex disease. Further discernment came when we measured plasma amino acid values in patients with the Hartnup phenotype. It had become apparent that very few persons with the Hartnup transport phenotype (the aminoaciduria), as detected by newborn screening, ever developed the Hartnup disease phenotype. We found that the individuals who developed the disease phenotype were low outliers for the measured genotype (i.e. the aggregate values for the plasma amino acids involved in the Hartnup transport disorder). While the Hartnup transport disorder is Mendelian, the Hartnup disease phenotype is a complex trait involving the background genotype and putative modifier loci controlling amino acid homeostasis. Furthermore, we found that one could predict a person at risk for the Hartnup disease phenotype and could counsel and respond accordingly.

Our work then became focused on phenylketonuria (PKU), the inborn error of phenylalanine catabolism involving deficient activity of phenylalanine hydroxylase (L-phenylalanine monooxygenase EC 1.14.16.1). Interest in this genetic disease grew significantly when an effective treatment (by low phenylalanine diet) was discovered in the 1950s. PKU became the condition that changed the outlook on medical opportunities for the management of human genetic disease. Our review (1980) for the *New England Journal of Medicine*

focused the mind upon unsolved problems in PKU and when others cloned the PAH gene, a new era of research could begin; we were involved in it as follows:

1. Newborn screening in worldwide populations provided opportunities to sample PAH mutations identified through patients with hyperphenylalaninemia. Evidence of allelic stratification in human populations emerged with the paradigm that “the history of the population is the history of the allele”. We studied this paradigm in detail and became population-geneticists - of a sort.

2. We formed an international consortium and created an online locus-specific mutation database (www.pahdb.mcgill.ca), which became a prototype for LSDBs; it is linked to the newly created Human Variome Project. PAHdb has also taken us into another world and we became involved in bioinformatics and databases.

3. Whereas treatment of PKU was a highly significant development in the field of human medical and biochemical genetics, treatment was neither easy nor pleasant for the patient. We helped to improve the organoleptic properties of the low phenylalanine diet. We also learned that delivery of diet products to patients could be a real problem. In response, we created the National Food Distribution Center as a purchasing and distribution resource for Canada, approved by the Federal and provincial governments. We became food merchants of sorts.

4. Our LSDB showed that 63% of PAH alleles are missense alleles. By means of in vitro expression analysis, we were among the first to show that missense alleles cause misfolding of nascent protein with subsequent aggregation and loss to the proteasome. The paradigm of misfolding proteins due to allelic variation has become a general one in genetics. We had penetrated a little way into the proteomic world.

5. It was known that the phenylalanine hydroxylase reaction required the catalytic cofactor tetrahydrobiopterin (BH4). The scene was set for the discovery of patients reflecting locus heterogeneity rather than allelic heterogeneity. The

locus heterogeneity reflects genes and enzymes involved in synthesis and recycling of BH4. If one could monitor the newborn for evidence of disorders in synthesis or recycling of cofactor, one could identify the affected patient early. This is important because the correct treatment requires replacement of cofactor by pharmacological means; the low phenylalanine diet is not sufficient. Our screening program in Quebec was the first in the world to address this issue systematically.

6. Whereas a few rare patients will have inborn errors of BH4 metabolism, others far more numerous will respond to pharmacological doses of the 6R-BH4 isomer, even though they do not have a primary disorder of cofactor homeostasis. In a collaborative study with colleagues at the Scripps Institute, benefit from cofactor was shown to reflect a chaperone-like effect in some patients; the response is allele-specific. Here was an early demonstration of patient – and allele-specific therapy in a genetic disease. This work creates a new demand for BH4 being met by a corporate response (BioMarin, CA) and a clinical trial. Thus we became involved with the corporate world and the FDA.

7. Lately, we have been investigating enzyme substitution therapy in PKU, using recombinant phenylalanine ammonia lyase (PAL) from yeast. We obtained proof-of-principle (pharmacological and physiological) with PAL substitution in an orthologous mouse model of PKU. This again led to collaboration with the corporate world (BioMarin, CA) – and also introduced us to the animal world of orthologous phenotypes and genes as counterparts of human disease.

The Howard Hughes Medical Institute put me on their SAB for a decade and I was able to play a role in getting the Human Genome Project underway (which may yet evolve into a “Human Phenome Project” (see *J Inherit Metab Dis* 27:305-317, 2004. After the genome - the phenome? [PMID 15190190]). How the field of human biochemical genetics has evolved and what it now offers was the subject of another recent essay (see *J Inherit Metab Dis* 24:93-116, 2001 [PMID 11405353]).

Much time has been spent writing (in long hand) grant applications, progress reports, and articles. It is what we do, is it not! Sometimes the peer review system seems to be the worst possible one; yet it remains the best we have, as I have experienced it.

Education and teaching (academic and public) have always been on the agenda, and quite heavily so. But if the reader has reached this point, you will have noticed recurrent use of the plural pronoun. “We” is the appropriate word because it refers to the extraordinary patients, colleagues, graduate students, and post-doctoral fellows who have populated the place of business these many years; the ones whose names are visible in the papers and remembered in my mind. And then there is the family (the 6 of us plus extensions), at a place I call home, without whom none of this narrative would have happened.

Too much information in this essay! Is there any wisdom? If I could send a message, it would be to highlight the influence and importance of mentors; the joy emanating from creative compatible colleagues; the need to be protected with time to think; the need for good space and stable funding. I thank the McGill institutions, and all the persons and agencies who made those attributes available.

The title at the beginning of this essay is: Who do we serve? To end it, I would add: How do we serve? Why do we serve? The answers fill the days...