

## G. Barry Pierce – mentor

DOUGLAS E. SWARTZENDRUBER\*

*Department of Biology, University of Colorado at Colorado Springs, Colorado Springs, USA*

It is a privilege to participate in this *Festschrift* honoring the career of Dr. G. Barry Pierce. Throughout the past 25 years, there are innumerable examples of the impact that Dr. Pierce has had on my career. However, I would like to use two descriptors that characterize many of our professional and personal interactions: serendipity and mentoring. Serendipity means «finding valuable things not sought for», and a mentor is «a close, trusted, and experienced counselor or guide.»

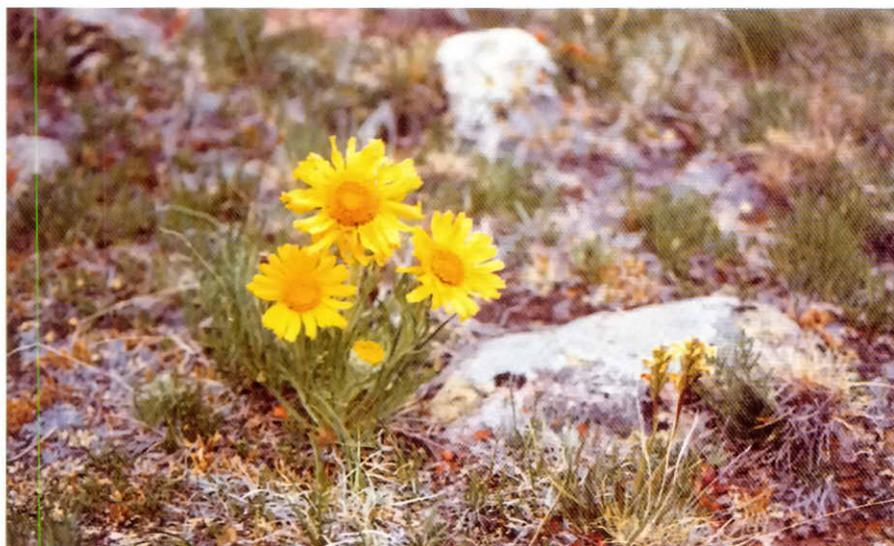
First — serendipity. In the Spring of 1968, I graduated from Goshen College (IN) with a Bachelor's degree in natural sciences. A college classmate, Marlin Nofziger, had graduated at semester and had found a research technician position in a pathology laboratory at the University of Michigan. Marlin told me that there would be other positions available because the laboratory was moving to the University of Colorado. I travelled to Ann Arbor for an interview and, in the Fall of 1968, moved to Denver to join the research laboratories of Drs. Pierce and Nakane. It was through this set of fortuitous circumstances that I came to know Dr. Pierce, first as an employer, then as the Chairman of the Department in which I pursued my graduate studies, and finally as a mentor and member of my Dissertation Committee.

Second — mentoring. During my years at the University of Colorado Health Sciences Center, Dr. Pierce provided encouragement, guidance and scientific insight, and was always quick with a thoughtful question or a challenging comment. His weekly research meetings were an important learning forum for research assistants, graduate students, residents and faculty. Results were discussed, new experiments were designed, and strategies were planned for answering specific questions. And most importantly, Dr. Pierce would discuss how the research pieces fit into the bigger topic of the biology of cancer.

I began my graduate research project in the laboratory of Dr. John M. Lehman in 1972. Dr. Lehman and Dr. Pierce had begun a collaborative project to combine Dr. Lehman's SV40 experimental system with the mouse teratocarcinoma system of Dr. Pierce. The goal was fairly straightforward: infection of teratocarcinoma stem cells with SV40 would provide well-characterized genes and gene products that could be readily followed as the malignant stem cells gave rise to well-differentiated, and often benign, progeny. The first task was to establish the teratocarcinoma *in vitro*, and to characterize growth and developmental potentials. Dr. Lehman, Dr. Wendell C. Speers (a pathology resident working in the lab) and I successfully established several cell lines and demonstrated that their *in vitro* growth and differentiation closely mimicked *in vivo* development (Lehman, *et al.*, 1974). We determined that frequent subculture would greatly enrich for stem cells whereas long-term nutrient feeding without subculture would promote differentiation to a wide variety of cell types.

Once teratocarcinoma cultures were established, my task was to infect the stem cells with SV40 and then assess the regulation of expression of SV40 genes, such as T antigen, as the stem cells differentiated. Even though I was convinced that all of the experimental procedures had been carried out correctly, numerous attempts to infect the stem cells failed. Since SV40 is nonpermissive in mouse cells, infection with a permissive virus (polyoma) was also attempted, without success. However, I noted that if any differentiated cells developed in the culture, they were susceptible to infection with both SV40 and polyoma. When these results were discussed at the research meetings, serendipity and mentoring again were both evident. Something very unique was occurring, in that the stem cells were innately resistant to infection, but upon differentiation, the progeny became susceptible in typical mouse-

\*Address for reprints: Department of Biology, University of Colorado, Colorado Springs, CO 80933-7150, USA. FAX: 719-593.1239.



Alpine sun flower. Trail Ridge, 1970. Courtesy of Dr. Pierce.

system fashion. Thus, the emphasis of my thesis research shifted to focus on the resistance of the stem cells to infection with small, DNA viruses (Lehman *et al.*, 1975; Swartzendruber and Lehman, 1975; Swartzendruber *et al.*, 1977). Interestingly, the teratocarcinoma system again mimicked normal developmental biology in that stem cells of the very early mouse embryo also resist such viral infections. I was fortunate to encounter such a unique set of experimental findings. These initial findings have been greatly expanded and investigated in depth by students and fellows in Dr. Lehman's laboratory, as well as in other laboratories.

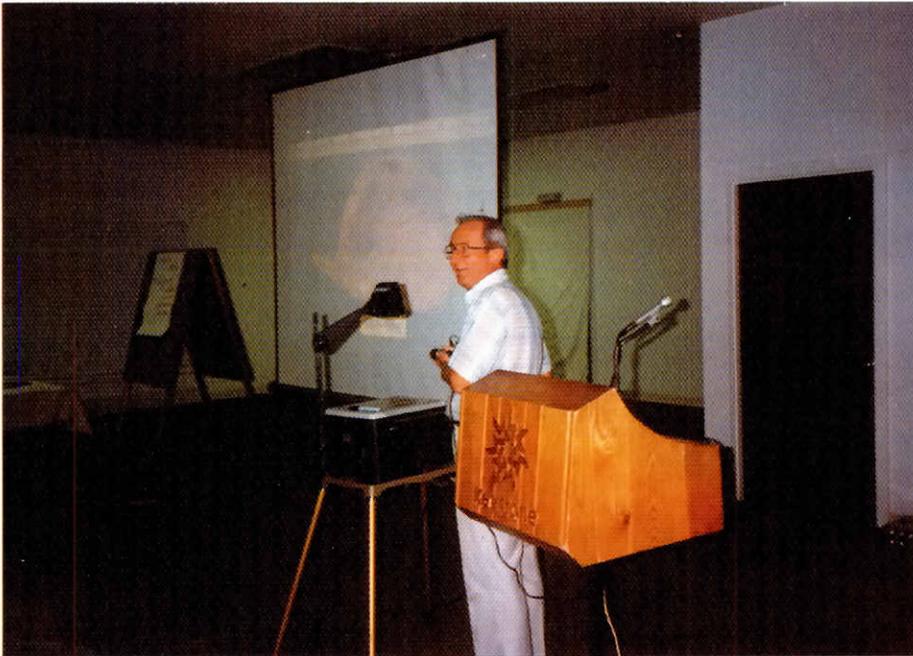
In 1974, I moved to the Los Alamos National Laboratory to begin a postdoctoral fellowship. As I began to develop my own experimental investigations of the biology of tumors, and even today as I do the same, there are several key guiding insights that Dr. Pierce made very clear: i) a tumor is a *tissue*; ii) all tissues including tumors are innately heterogeneous, from molecular genetic properties to phenotypic characteristics to differentiation potential to growth kinetics; and iii) tumors are often caricatures of tissue renewal. At Los Alamos, it became clear to me that flow cytometry was a powerful tool for quantitative analysis of tumor heterogeneity in the teratocarcinoma system as well as in other experimental and clinical neoplasias. High-speed single cell analysis provides the means to analyze single cells isolated from tumor tissue, to quantitatively assess a wide variety of genotypic and phenotypic characteristics of individual cells, and to determine population distributions of such characteristics. As a fellow and a staff member at Los Alamos, I utilized single- and multiparameter flow cytometry to quantitate *in vitro* differentiation of teratocarcinoma cells (Swartzendruber, 1976; Swartzendruber *et al.*, 1976, 1979; Hoffman and Swartzendruber, 1979). I also developed several flow cytometric techniques to assess tumor cell heterogeneity, including discrimination of cycling cells from noncycling cells (Swartzendruber, 1977a,b) and kinetic assessment of enzyme activities in individual cells (Martin and Swartzendruber, 1980). These techniques were used to assess both spontaneous and induced differentiation of teratocarcinoma stem cells. (Swartzendruber *et al.*, 1980a,b). Thus,

flow cytometric techniques provided quantitative information concerning attempts to direct the differentiation of malignant stem cells.

Many of the types of studies carried out in my laboratory in New Mexico were extended to human neoplasia while I was in the Department of Developmental Therapeutics at the M.D. Anderson Hospital in Houston. In collaboration with Dr. Bart Barlogie and his co-workers, quantitative flow cytometric analyses of cell cycle kinetics and other genotypic and phenotypic characteristics of human malignancies were carried out in an attempt to develop rational approaches to chemotherapy (summarized in Barlogie *et al.*, 1983).

As my interest in human cancer increased, the principles of tumor biology put forth by Dr. Pierce continued to be central to my studies; that is, neoplasia is a problem in developmental biology, cancers arise via abnormal stem cell development, and understanding the innate heterogeneity of tumors is key to developing effective treatments. During the past several years, much of my research in tumor biology has been towards understanding the growth kinetics of human breast cancer. Breast cancer tissue, like any normal renewing tissue, is characterized by heterogeneity. The cells within the tissue often display a variety of morphologies, differentiated functions, proliferative capabilities, genotypes and metastatic potentials. This innate heterogeneity has confounded the understanding of the biology of breast cancer and has obfuscated the search for effective treatments. Specifically, adjuvant chemotherapy and hormonal therapy extend disease-free survival, but are not curative for the majority of patients.

In collaboration with Dr. Michael Retsky and co-workers, a computer model has been developed that simulates the biological characteristics of breast cancer, including growth kinetics. Although most standard chemotherapy is based on constant, exponential (or Gompertzian), regular (homogeneous) growth kinetics, our studies show that like many characteristics of cancer *tissue*, growth kinetics are also heterogeneous (Retsky *et al.*, 1987, 1989). Computer modelling has reemphasized the need for an appreciation of



**G. Barry Pierce doing the two things he enjoys most: lecturing and taking pictures.**  
*Courtesy of Dr. Stewart Sell.*

the complex nature of cancerous tissue, challenged the old paradigm of breast cancer growth and treatment, and provided the basis for a new paradigm (Retsky *et al.*, 1990, 1993).

Throughout the years since I graduated from the Pathology Department at Denver, Barry Pierce's work has influenced my own. His mentoring extended beyond the research laboratory to the classroom, to informal discussions with peers and students, and to explanations of cancer to nonscientists. I am fortunate to have Barry as a mentor, colleague and friend.

#### *Acknowledgments*

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