On the boundary between development and neoplasia

An interview with Professor G. Barry Pierce

JUAN ARECHAGA*

Department of Cell Biology, University of the Basque Country, Leioa, Spain

Only four decades ago, the conceptual relations between cancer, cell differentiation and embryonic development were not at all clear to scientists, nor were there many willing to investigate such relations experimentally. With very few exceptions, pathologists largely ignored what was going on in embryology, while embryologists, except for the the field of teratology, were not at all interested in pathology, much less in neoplasia. The fusion of the methods and approaches of embryology and pathology was the work of a few pioneers, one of the most remarkable of whom is undoubtedly Professor G. Barry Pierce. It is therefore with great admiration and gratitude for his contribution to developmental biology that this volume is dedicated to him.

Gordon Barry Pierce was born on July 21, 1925 in the town of Westlock (Alberta), Canada. After serving in the Canadian Army during World War II, he enrolled in the University of Alberta, earning a BSc (Biology) in 1948, MSc (Anatomy) in 1950 and MD in 1952. Following his years of internship and residency in pathology at the University of Alberta Hospital, Pierce transferred to the University of Pittsburgh in 1955 to train in experimental pathology under Frank Dixon. In 1961, following three years as an Assistant Professor of Pathology at Pittsburgh, he moved to the University of Michigan, where he took up an appointment as Associate Professor of Pathology. In 1964, he was named a lifetime Professor of the American Cancer Society. This distinction was followed in 1968 by an invitation from the University of Colorado in Denver to accept a Professorship and chair the Department of Pathology. The University named him Centennial Distinguished Research Professor of Pathology in 1982 and, in 1988, awarded him its highest honor as University Distinguished Professor.

Throughout his professional career, Professor Pierce has won a host of academic prizes and awards, including the Pope Memorial Gold Medal in Medicine in 1952, the American Urological Association's Guiteras Award in 1962, and two *Honoris Causa* doctorates, one awarded in 1982 in Medicine by the University of Granada in Spain, the other, in Science, by Scotland's University of Glasgow in 1984. Other distinctions include the Rous-Whipple Award from the American Association of Pathologists, granted in 1983, the Gold Headed Cane in 1991 and membership on many prestigious scientific committees and boards, including the NIH, ASEP, FASEB, ACS, AAP, the Jackson Laboratory, the Council for Tobacco Research and La Jolla Cancer Research Foundation. Professor Pierce has also done a great deal of editorial work on developmental biology journals, such as *Developmental Biology*, *Differentiation* and *The International Journal of Developmental Biology*, and has served on the editorial boards of various experimental pathology publications, including *Laboratory Investigation*, *Cancer Research*, *Leukemia Research* and *Anticancer Research*. Barry Pierce's terms as president of the International Society of Differentiation, the American Association of Pathologists and the Federation of American Societies of Experimental Biology are particularly worthy of note.

However, it is the quality of his experiments and, above all, the validity and originality of his scientific proposals that give us a better idea of the true dimensions of his professional career. Mario Bunge, the philosopher of science, has written that the merit of a given research project should be judged by the size of the problem taken on. On this reckoning, Barry Pierce has enjoyed a particularly successful career. A brief survey of his professional writings will show us how he has gone about solving the problems he has set himself over the years.

After completing his Master's thesis (Pierce, 1950), he became

***Address for reprints**: Department of Cell Biology, School of Medicine, University of the Basque Country, E-48940 Leioa, Spain. FAX: 34-4-464.89.66.

0214-6282/93/$03.00

Printed in Spain

interested in the study of gonadal tumors. The period (1955-1961) spent working at the University of Pittsburgh with Frank Dixon was most productive, and a brief summary of his findings during these years will help to understand his subsequent career:

- Embryonal carcinoma cells are the multipotential stem cells of teratocarcinomas (Pierce *et al.*, 1960a), a hypothesis elegantly confirmed some years later by Lew Kleinsmith by *in vivo* cloning experiments (Kleinsmith and Pierce, 1964).
- *In vivo*, embryonal carcinoma cells differentiate to form histologically benign tissues very similar to normal ones (Pierce and Dixon 1959a), a process which can be modulated *in vitro* (Pierce and Verney, 1961). The most striking confirmation of these proposals was obtained in the mid-seventies in a series of key experiments in which mouse chimeras were produced from embryonic carcinoma cells (Brinster, 1974; Mintz and Illmensee, 1975; Papaioannou *et al.*, 1975).
- Teratocarcinoma can be used as a model system in developmental biology (Pierce, 1961).

Among his other interesting contributions from this period were the experimental conversion of teratocarcinoma into ascites variants with mass production of the characteristic "embryo bodies" (Pierce and Dixon, 1959b), studies on tumor endocrine function (Pierce *et al.*, 1959; Verney *et al.*, 1959) and initial approaches to an eventual therapy (Midgley *et al.*, 1959; Pierce *et al.*, 1960b; Midgley and Pierce, 1961; Pierce *et al.*, 1962a).

A second highly productive stage in Professor Pierce's career began with his transfer to the Department of Pathology at Michigan and continued on through his early years at Colorado. What opened the way was the histogenic comparison he made between the "hyaline substance" synthesized by murine transplantable yolk sac carcinoma and Reichert's membrane of the early mouse embryo (Pierce *et al.*, 1962b). This work was followed by a series of ultrastructural and immunocytochemical studies, the results of which are briefly summarized below:

Experimental demonstration of the epithelial origin of certain basement membranes, when the prevailing view at the time was that these were produced exclusively by the condensation of extracellular matrix ("ground substance") of connective origin around the cells. The first step was to establish a murine yolk sac carcinoma cell line capable of synthesizing basement membranes *in vitro* and in the absence of connective tissue (Pierce *et al.*, 1962b). At the same time, it was shown that conversion to an ascites variant gave rise to tumor cell coated spheroid aggregates containing a fairly clear, homogeneous nucleus of a hyaline substance similar to the basement membrane. This led to its mass isolation and purification in order to produce polyclonal antibodies, which were then put to use in indirect immunofluorescence techniques — then known as Coons' technique — using histological sections, at a time when such methods were hardly common practice (Midgley and Pierce, 1963; Pierce *et al.*, 1963, 1964a). The subsequent absorption of the antibodies by basement membranes of mesenchymal origin demonstrated that membranes of epithelial origin possessed specific antigens, making it possible to carry out a differential study of the basement membranes in various tissues (Pierce and Nakane, 1967a).

Establishment of the chronology of basement membrane development in the mouse embryo: the discovery that Reichert's membrane and neoplastic basement membranes of epithelial origin both reacted to the same antibodies encouraged him to go further into the study of the origin and distribution of basement membranes during embryogenesis (Pierce, 1966).

- Subsequent research into the chemical composition of basement membranes (Mukerjee *et al.*, 1965; Lee *et al.*, 1969) and into the cytological bases for their synthesis in pathological circumstances (Pierce and Nakane, 1969; Johnson and Pierce, 1970; Martínez-Hernández *et al.*, 1974, 1976; Pierce *et al.*, 1982a). According to Pierce's description, basement membrane was collagen proteins with a mucoprotein antigenic determinant, later shown to be laminin. It was synthesized in the rough endoplasmic reticulum and exteriorized directly from that organelle.
- Due to the need for greater specificity and resolution in the techniques used in many of the above-mentioned studies, Barry Pierce began searching for more sensitive, highly specific immunocytochemical methods applicable especially to electron microscopy (Sri Ram *et al.*, 1963; Pierce *et al.*, 1964b). Particularly significant was his idea of conjugating enzymes to antibodies to increase their cytochemical effect (Nakane and Pierce, 1967a, 1967b), now a routine technique in numerous laboratories.
- The skill and originality acquired in the use of immunocytochemical methods also gave rise to the discovery that syncytiotrophoblast produces chorionic gonadotrophin and is derived by differentiation from cytotrophoblast (Midgley and Pierce, 1962; Midgley *et al.*, 1963; Pierce and Midgley, 1963; Pierce *et al.*, 1964c).

Although his major scientific contributions during this period mainly involved the study of basement membranes, syncytiotrophoblast and the development of new cytochemical methods, Barry Pierce also carried out a series of important histopathological and ultrastructural studies that were crucial for clarifying the histogenesis of certain tumors, their relation to processes of cell differentiation (Gray and Pierce, 1964; Pierce and Beals, 1964; Beals *et al.*, 1965; Pierce and Nakane, 1967b; Pierce *et al.*, 1967; Pierce and Abell, 1970; Pierce *et al.*, 1970; Wyllie *et al.*, 1973; Lehman *et al.*, 1974; Pierce and Fennel, 1976; Nogales *et al.*, 1977; Pierce *et al.*, 1977) and the possible spontaneous regression of some tumors using non-toxic treatments (El-Bolkainy *et al.*, 1967). As a result of this work and of the projects mentioned earlier, there began to form in his mind an idea concerning the interpretation of the neoplastic process: *the "stem cell" theory of cancer.*

Pierce's crucial insight into carcinogenesis is that it is due to a flaw in the normal process of tissue renewal. In this view, neoplastic tissue is a *"caricature"* of normal tissue in the sense that there is a gross overproduction of undifferentiated stem cells for each cell that differentiates. These ideas were developed and published in a series of review articles and lectures (Pierce, 1967; Pierce, 1970; Pierce and Johnson, 1971; Pierce and Wallace, 1971; Pierce, 1972; Pierce, 1974a,b,c; Pierce *et al.*, 1974; Pierce, 1975a,b; Pierce, 1976; Pierce, 1977a,b; Pierce and Cox, 1978) and, especially, in his book *Cancer. A Problem in Developmental Biology* (Pierce *et al.*, 1978).

The most decisive empirical support for Pierce's ideas on differentiation in cancer came in the mid-seventies when the laboratories of Ralph Brinster, Beatriz Mintz and Richard Gardner independently produced animal chimeras with murine teratocarcinoma cells, as mentioned earlier. Pierce learned the

Barry Pierce (third from right, rear) after joining the Department of Experimental Pathology at the Univeresity of Pittsburgh (1956). Prof. Pierce is standing just to the left of Frank Dixon (bow-tie).

embryonic micromanipulation techniques from Clement L. Markert, then at Yale, and began to delve into the biological mechanisms whereby neoplastic cells are regulated during development of the embryo.

First, it was necessary to develop test procedures for quantifying the capacity of embryonic microenvironments to regulate growth and tumor malignancy both *in vivo* (Pierce *et al.*, 1979) and *in vitro* (Wells, 1982). Once this was possible, Pierce was able to show the specific way in which embryonal carcinoma is regulated (Pierce *et al.*, 1982b) and to demonstrate that at least two factors are involved: selective cell contacts and the fluid that fills the blastocyst cavity (Pierce *et al.*, 1984).

While this work was being done, Leo Sachs's group in Israel demonstrated that the injection of leukemia cells into the placenta of a 10-day mouse fetus led to hematopoietic maturation and the appearance of normal leukocytes carrying leukemia cell markers in the circulating blood of the mature animal (Gootwine *et al.*, 1982). So, in some way the leukemia cells had been induced to differentiate by an embryonic microenvironment distinct from that of teratocarcinomas. Pierce's group, convinced of the epigenetic nature of neoplastic differentiation and encouraged by the results of these experiments, then began to study embryonal regulation of other tumors during organogenesis, focusing especially on neuroblastoma (Podesta *et al.*, 1984; Wells and Miotto, 1986) and melanoma cells (Gerschenson *et al.*, 1986). As a result of this work, Pierce developed the idea that tumor cells could be regulated in the appropriate "embryonic fields" — a temporal and anatomical site in the embryo which abrogates the malignancy of cancers derived from that field's normal lineages. Postulating that *if one embryonic field can regulate its closely related carcinoma, there may be an embryonic field capable of regulating each type of cancer,* he was able to point towards the very real possibility of treating neoplasia biologically (Pierce *et al.*, 1982c; Pierce, 1983; Pierce *et al.*, 1983; Pierce, 1985; Pierce *et al.*, 1986; Pierce and Speers, 1988). At the same time, studies were made concerning the topographical fate of various teratocarcinoma cell lines introduced into embryos during the first stages of development (Pierce *et al.*, 1987), and their possible correlation with the processes affecting cell differentiation of the embryonal inner cell mass (Pierce *et al.*, 1988).

The most recent period in the biography of Barry Pierce is marked by the study of the processes of cell death during early embryonic development with a view to gaining a better understanding of

regulatory processes in neoplasia. Initially, these studies arose from the above-mentioned observation that different teratocarcinoma cell lines suffered different fates in the embryo, especially with regard to their chimera-forming capacity. As a result of numerous experiments, Pierce reached two basic conclusions: 1) Like the inner cell mass — of which teratocarcinoma is the pathological equivalent (Pierce, 1967; Evans and Kaufmann, 1981) — embryonal cancer cells have the capacity to evolve into either trophectoderm or primitive ectoderm. 2) There must be some toxic factor in the fluid of the blastocoel to account for the programmed cell death (apoptosis) of pretrophectodermal cells in the ICM and of teratocarcinoma cells with pretrophectodermal potential.

Given the apparent difficulties of experimenting with the fluid content of the blastocoel, Pierce and his group began to use in their studies giant blastocysts made by the aggregation of several morulae. Empty zona pellucidae containing the test cells were injected into them to demonstrate the effect of the fluid on the cells. Large amounts of pseudoblastocoel fluid from the cystic "embryoid bodies" of a particular type of ascitic transplantable teratocarcinoma were used to try and identify the responsible molecules (Pierce *et al.*, 1989; Parchment *et al.*, 1990a). Thanks to this technique, they were able to conclude that the apoptosis of cells with trophectodermal potential is caused by the catabolism of polyamines mediated by enzymes such as amine oxidases (Gramzinski *et al.*, 1990), and pointed to the probable role of hydrogen peroxide and a developmentally regulated glutathione-dependent protection mechanism (Pierce *et al.*, 1990, 1991). A similar activity was also demonstrated in systems other than the fluid of the blastocyst (Parchment and Pierce, 1989; Parchment *et al.*, 1990c), providing the hypothesis with more general confirmation from outside the confines of early embryonic development. As a result of all these studies, major progress was made in our understanding of the process of tumor growth and towards the possibility of developing non-cytotoxic, biological therapies for cancer (Parchment *et al.*, 1990b; Pierce, 1991).

This has been a brief description of the professional career of Barry Pierce, a scientist whose work has been of such importance that we often tend to overlook other equally remarkable facets of the man. Professor Pierce is, for example, a highly talented and entertaining lecturer, a skilled photographer and also a craftsman whose extraordinary manual dexterity serves him well not only in the lab but also in his carpentry shop. But perhaps the qualities that define him best and have made him so beloved a personality are the kindness and generosity that pervade all his dealings with others.

The following interview was held in Professor Barry Pierce's office at the University of Colorado Medical School on August 21, 1991, and affords, through its first-person account, a much clearer image of the man and his scientific stature than any biographical sketch possibly can.

Pathologists are not often trained in embryology, just as embryologists do not usually receive training in pathology. However, your case was an exception. So, let me start this interview by asking you how you came to be trained in both fields. Who were your teachers and what do you remember of those first years of your career?

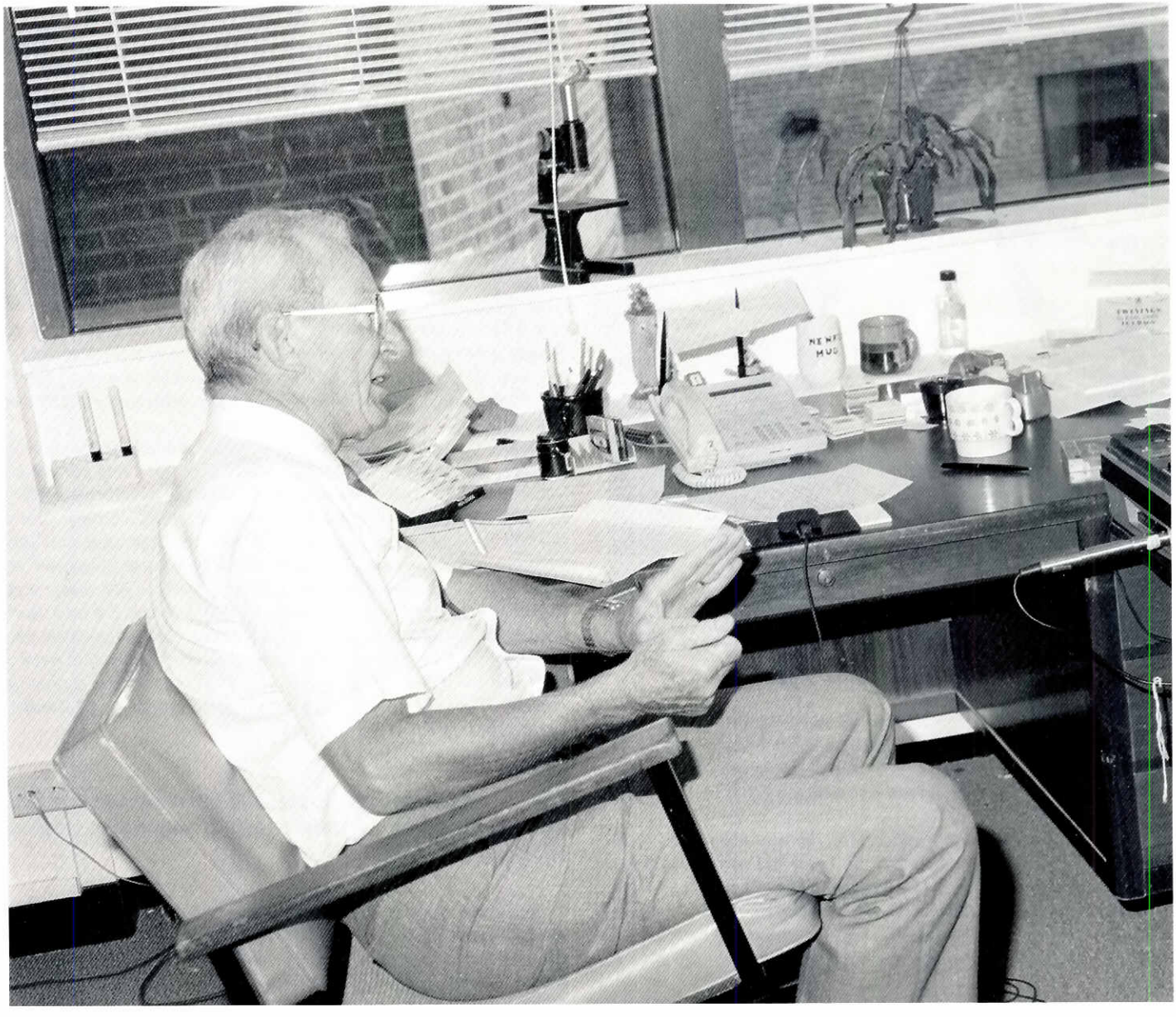
I was never formally trained in embryology and became a scientist via the backdoor. I was raised on a farm north of Edmonton, Alberta, and attended a one-room school. From this I learned that if you wanted to know something, you learned it. My father was a cardiac invalid and the most dramatic thoughts that I have concerning my early days are of when Dad was ill and one of the men would go to the telegraph which was 7 miles away, call the doctor who was 27 miles away, and he would come in his snowmobile across the great fields with a huge plume of snow soaring up behind the propeller. That was my motivation for going to medical school — to be a physician. Later, in medical school, R.F. Shaner and H.E. Rawlinson introduced me to medical science, and I was smitten by it. I took a year out of medical school to work with Professor Rawlinson on a problem related to breast cancer. It was one of the most wonderful years of my life. I had the opportunity to work on a problem of my choice with a dynamic man and at the same time be close to Professor Shaner, who was probably the greatest intellect that I have ever known. Through his influence I decided upon a career in pathology. So, these two started me in science.

The thing that captivated me was the intellectual freedom that the experimenter had. As a physician, you are rightfully required, by oath, to look after anyone who needs your help, but as a scientist you can choose problems commensurate with your interest and your abilities. This is what I mean by intellectual freedom and it is a heady kind of freedom.

After medical school, I did a required rotating internship and then two years of pathology residency, which constituted most of my formal training as a pathologist. During this period I looked after a 3-year-old boy who had a testicular cancer. He died, which was terrible, but what disturbed me was our ignorance of testicular cancer. It bothered me that we did not even know the diagnosis of the tumor that killed him. So, I decided then that I was going to be a scientist and work on testicular cancer.

I sought out Frank J. Dixon, who had written the fascicle on testicular cancer for the Armed Forces Institute of Pathology series and was Professor of Pathology at the University of Pittsburgh. By the time I reached Pittsburgh, he was interested in immunology, and less interested in testicular tumors, but he had the veteran's hospital made into a treatment center for testicular cancer so I was assured plenty of human tumors. He decided that a good approach would be to heterotransplant human testicular tumors into cortisone-treated hamsters. In a short time I had lines of heterotransplanted choriocarcinoma, both from the testes and from ovaries, and embryonal carcinoma of the testis, but teratocarcinomas failed to grow in hamsters. If tumors had embryonal carcinoma plus features of differentiation, they just would not transplant. This was a great disappointment. One of the embryonal carcinomas, with no overt features of choriocarcinoma, synthesized human chorionic gonadotropin, as did the choriocarcinomas. This then led to the idea that embryonal carcinoma was the precursor of choriocarcinoma in the testis, which then led to the elegant work of Rees Midgley, who worked out the morphogenesis of trophoblast in the monkey placenta and showed that the cytotrophoblast was the undifferentiated proliferative cell type of the placenta and that the syncytium was the differentiated functional form of this tissue. This was contrary to accepted dogma, and was not received favorably by reproductive biologists. Eventually, Rees showed that the HCG localized in cytotrophoblast by others was a diffusion artifact. That settled that!

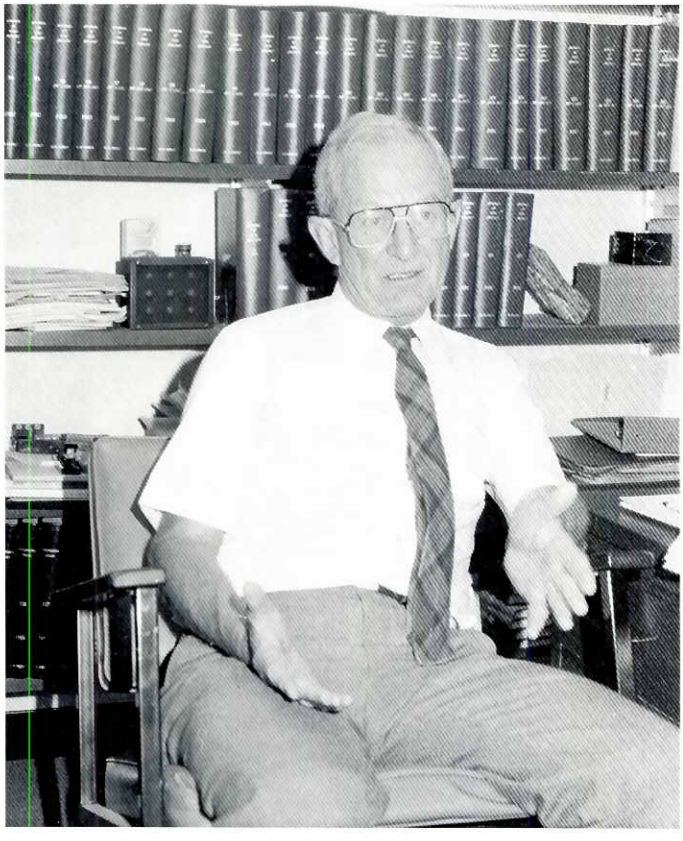
It was necessary to use transplantable mouse tumors to study teratocarcinoma. Dr. Dixon had postulated that embryonal carcinoma was probably a multipotential precursor cell of these tumors, but



this idea was not widely accepted, particularly in Great Britain where embryonal carcinoma was not even recognized as an entity. We showed that embryonal carcinoma of the mouse was multipotential and that the embryonal carcinoma cells corresponded to cells of the preimplantation mouse embryo. This is what made me realize that I needed some understanding of embryology.

Accordingly I attended the summer course in marine embryology at Woods Hole in the late 1950s, which put me in contact with Ed Zwilling, a most creative experimentalist. Ed was a superb fellow, a good teacher who was generous with his time. We became close friends. I also learned a great deal from Mac Eds, a superb scholar, who later became Dean of Medicine at Brown University. John Saunders and Nelson Spratt were also helpful and enthusiastic.

I also learned a lot of embryology from Roy Stevens. I started working with the Fekete ovarian teratocarcinoma in 1956, and then discovered Roy Stevens' strain 129 testicular tumors. Roy was a Holtfreter-trained embryologist who went to the Jackson Lab in the early 50s, and in 1954 published a paper on spontaneous strain 129 teratomas with C.C. Little. He even described embryoid bodies in them. I learned how to mass produce the embryoid bodies by converting the solid tumor to an ascites variant, and immediately told Roy. He was very interested in these bodies and when he published a paper some months later, he was very gracious to me and we became very close friends. He was interested in the genetics



of testicular tumors and made important discoveries in that field. He was also interested in the experimental production of the tumors using embryo transplants. This became a very important tool in testicular tumor research as did our discovery of mass producing embryoid bodies.

It was a very interesting situation. He was interested in the genetics and the development of the tumors. I was interested in their neoplastic aspects, the relationship of embryonal carcinoma to their differentiated derivatives and the possibility of developing differentiation therapy. I was particularly interested in why, when the histiotypic differentiation occurred, abrogation of malignancy resulted.

So, you can now see that I really was not formally trained in embryology and have great gaps in this discipline, but I had wonderful friends who helped me.

Very soon, in the late fifties, you demonstrated that embryonal carcinoma cells could differentiate into normal cells in a manner similar to the way in which embryonic cells transform into adult cells. At that time, this was a revolutionary idea that went against the established dogma of "once a cancer cell, always a cancer cell". How were these novelties received by the scientific community?

I will tell you an anecdote that will give you part of the answer. When Frank Dixon and I demonstrated that embryonal carcinoma cells of the mouse were multipotential and evolved benign progeny (it was left to Brinster 15 years later to show they were normal), I sent the paper to the journal *Cancer,* which is clinically oriented, in keeping with my interests in pathology. The paper was under review for six months. I knew it was safe because I had received a card saying that it had been received. Finally, one of the associate editors telephoned saying that there was nothing wrong with the data. I was quite relieved, but then he said they could not publish it. Upon inquiring as to why not, he said "everybody knows that cancer cells cannot differentiate". I said to him, "well if the data are sound, then cancer cells *can* differentiate".

There was a very long pause. I thought the nice man had had a stroke or something like that, but finally he said, "we will publish your paper if you change the title". Now the title was a jawbreaker. It was *Teratocarcinogenesis by differentiation of multipotential cells*. Again, he asked, "will you change the title?" I said, "I will if it is the only way I can get the paper published". I changed it to *Teratocarcinogenesis by metamorphosis of multipotential cells.* I am sure that many people believe that this was one of my gaps in the understanding of embryology, but I assure you it was not. It was just pressure.

I learned from this experience that the position of editor deserves respect, but the person in the office may not. Well-performed research with the n+1 control is readily accepted by creative people; the others can be a nuisance, but they do not count for much. There are two kinds of people in the scientific world. There are the "yes-butters", who represent the vast majority. They are the people who, when told a neat idea that came into your mind as you were riding your bicycle to work, will say "yes, but", and then tear the idea down usually with technical trivia. There is a small, elite group of people, however, that say "now, if that were true, then you would expect such and such, and if that is also true you could further expect so and so". If the idea is poor, it generates no progression of thought. Yes-butters have limited horizons.

How did you overcome the objection that teratocarcinoma cells might be an exception to the established rules of oncology?

We were continually bombarded with the objection that teratocarcinomas were not typical of tumors in general, so we confirmed all of the things that we found in testicular tumors, in breast cancer, the squamous cell carcinomas of the skin, and adenocarcinomas of the colon. All of these tumors differ only in the potential for differentiation of their stem cells: embryonal carcinoma forms the three germ layers, breast cancer stem cells form only glandular epithelium, etc.

The studies on squamous cell carcinoma of the skin were done by Carol Wallace while still a premedical student. She demonstrated by a combination of embryologic dissection and labeling of cells, followed by light and electron micrography, that the stem cells of the squamous cell carcinoma of the skin could differentiate into well-differentiated squamous cells, which were incapable of proliferating and forming a tumor. We then studied the melanotic and amelanotic cells of melanomas to understand their relationship, and this led to some interesting observations concerning progression. You see my interests have always been in neoplasia, but I looked at the problem in a way a bit different from my contemporaries.

What about the concept of "dedifferentiation" as a way of explaining the undifferentiated appearance of some malignant tumors?

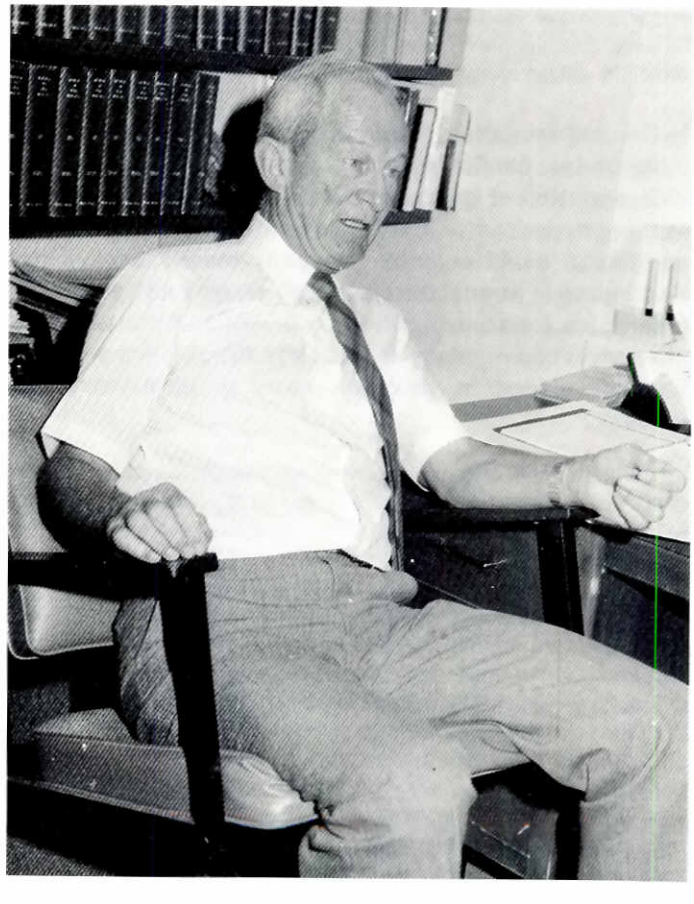
I believe there is no basis in fact for the idea of dedifferentiation as a mechanism in carcinogenesis. Our oncologic ancestors required an explanation for the appearance of undifferentiated malignant tissue in well-differentiated organs. This was long before anything was known about tissue renewal, which embodies the idea that mature cells of an organ were continually generated from undifferentiated cells in a regulated manner. Studies of teratocarcinoma by virtue of its potential showed that there was a flow of differentiation as we see it in early development. Squamous cell carcinoma showed that there was a flow as we see it in tissue renewal. Roy Stevens, in his transplantation experiments, demonstrated clearly that the cell of origin of teratocarcinoma was the primordial germ cell. This is the stem cell of the species, and from this cell type all of the differentiated tissues develop by proliferation and differentiation. Roy and I showed that the normal primordial germ cell is no more and no less differentiated than the embryonal carcinoma cells to which it gives origin. There is an overproduction of undifferentiated malignant cells in the tumor. This is not the result of dedifferentiation; rather it is merely the overproduction of undifferentiated cells that have a limited potential for differentiation. This is the caricature. What goes on in tumors is the antithesis of dedifferentiation. But, that does not mean to say that dedifferentiation does not occur in biology. If you remove the lens from the eye of an amphibian, the uveal tract cells will depigment and reform a new lens and a new iris. That is true dedifferentiation because the cells lose their differentiated features and gain the potential to redifferentiate into another tissue. No malignant tumor has ever been shown to be able to lose differentiation and gain potential.

In the early seventies, you arrived at the idea that normal tissue stem cells were the origin of the malignant tumors, establishing your theory of "cancer cells as a caricature of the normal process of tissue renewal". Could you please summarize for our readers the main points of this concept?

First, you give me too much credit. By then, Jacob Furth had already cloned leukemia cells and S. Makino had studied stem cell lines in transplantable tumors. Where did these stem cells come from? The data suggest that carcinogenesis involves a normal stem cell that is undifferentiated and gives rise to undifferentiated malignant stem cells. Whereas normal stem cells proliferate in a regulated manner to produce the correct number of differentiated cells for replacing senescent ones, the malignant stem cell proliferates and forms a mass of undifferentiated progeny, only a few of which differentiate. The term caricature means a gross misrepresentation, and in this case, proliferation and differentiation are grossly misrepresented. The undifferentiated mass is the result.

There were many theories about the original cell giving rise to cancer when I was entering research. One, sustained by Cohnheim, said cancer arose in embryonic cell nests. This hypothesis was quickly discarded for lack of support, but I think he was correct. Normal stem cells develop at the time of morphogenesis and are determined for a particular differentiation. These undifferentiated cells respond to the embryonic environment by proliferating, and to that of the adult by proliferating and differentiating. Clearly the adult stem cells are the same as the embryonic ones and when they become malignant and if they express regulatory factors (i.e. ACTH in lung cancer) then ACTH will have been produced by embryonic stem cells. What is important is the idea that malignant stem cells are a caricature of the normal stem cells at the time of their induction in the embryo. In this sense malignant stem cells are excellent models of normal embryonic counterparts.

Now the concept also allows for therapy. A tumor may be cured by removing or killing all of the malignant cells. You can also cure a tumor by making all of the malignant stem cells differentiate. This has been done using retinoic acid with teratocarcinoma cells. Retinoic acid induces endodermal differentiation and other cell types from embryonal carcinoma. It is also possible, on the basis of the Brinster experiment, to re-regulate malignant stem cells in their appropriate embryonic environment. Thus, when we understand the process of embryonic induction and the role of growth factors, both positive and suppressive, that interact and regulate proliferation and differentiation of normal stem cells in organogenesis, we should be able to re-regulate malignant stem cells to behave as normal ones. Armin Braun was the first person to show the presence of tumor growth factors. He did this with plant teratomas in 1956, but not many people have given him much credit for that brilliant work. I believe that the ultimate cure for cancer will be through the re-regulation of malignant stem cells to benign stem cells, using the principles of embryonic induction and the growth factor action that occurs at the time of organogenesis.



If your theory is correct, every cancer cell might have a normal stem cell progenitor which could be the target of the carcinogenic agents. However, it is well known that not all tissues have a definable stem cell compartment. How can you explain the origin of the malignant counterpart of this normal tissue?

Normal stem cells have been demonstrated in a wide variety of epithelial tissues, although they have not been demonstrated in all of them. Stewart Sell, among others, has now shown that there are stem cells in the liver, so as information accumulates more and more stem cell systems will be discovered. The situation with regard to connective tissues is less clear. We studied chondrosarcomas and normal cartilage and found that the cells of each contain well-developed profiles of endoplasmic reticulum and other evidence of differentiation. Chondrosarcoma cells that proliferated rapidly appeared well differentiated but were not synthesizing as much chondromucoprotein. Those in the central masses of the tumors where much chondromucoprotein had accumulated were not synthesizing DNA. Whether or not the accumulation of matrix regulates cell division in this type of system or whether there are subtle things we cannot see remains to be determined.

Carcinogenesis is known to involve cells capable of cell division. Thus whether or not mesenchymal tissues really contain stem cells or not does not matter, because the proliferating cells could respond to carcinogenic insults and develop into a tumor, the appearance of which would in large part correspond to the appearance of the targeted cells. These tumors then would caricaturize the proliferation and differentiation of the normal cells. I do not think it matters to the validity of the concept if mesenchymal and other tissues contain stem cells in the classic manner or not. The principle will be pretty much the same.

In the mid-seventies, Ralph Brinster, and very soon other laboratories, confirmed your previous result on the *in vivo* differentiation of teratocarcinoma cells. After a series of striking experiments it was shown that murine embryonal carcinoma could colonize the early mouse embryo and participate in normal development. These findings opened new vistas for studying the way in which the mammalian blastocyst can regulate cancer cells. What types of assays were developed in your laboratory to understand this process?

Before we talk about the techniques, I would like to say a word about Ralph Brinster. He was the first to produce chimeric mice using embryonal carcinoma cells. His work was quickly confirmed in a very, very elegant manner, but there should be no confusion as to who made the first chimeric mice with embryonal carcinoma cells.

Discovery of the manner by which the blastocyst regulated embryonal carcinoma cells could lead to the new forms of therapy that I mentioned previously. Bob Wells developed a tissue culture colony assay which was extremely reproducible and clearly showed the effects of the blastocyst upon embryonal carcinoma cells. We showed that two factors were responsible for regulation of the embryonal carcinoma cells. The first one was contact of the cells with trophectoderm. Interestingly, only the inner surface was regulatory, and it was only regulatory when the embryonal carcinoma cells were also bathed by blastocoel fluid.

We analyzed blastocoel fluid to see what the factor was. We learned from Roger Pedersen and Akiko Spindle a technique for making giant blastocysts. We emptied zona pelucidae and used the empty egg shells as carriers in which to put embryonal carcinoma cells for testing. Then the zona pellucidae carriers with their cells were put into giant blastocysts to measure the effect of the fluid on the cells. Well, we did not find what the differentiating factor was, but we did learn some interesting things about programmed cell death.

In addition to what has been said about the regulation of embryonal carcinoma cells by the blastocyst, you were able to show that other cancer cells could be regulated sometime later in development, during early organogenesis. Could you explain your hypothesis and main experimental results concerning the regulation of malignant cells by "embryonic fields"?

We began to study regulation of cancer cells by other embryonic fields because of the logistical problems in working with blastocysts. There were so few cells and so little blastocoel fluid, we thought that other cell types might be easier to study in their appropriate embryonic situations. Accordingly, Dr. Podesta studied the regulation of neuroblastoma cells in the neural crest migratory route and showed that they were regulated. Kathy Graves studied melanoma cells in the embryonic limb bud and found that they were regulated. Leo Sachs and his associates in Israel put leukemia cells in the 10-day-old mouse placenta and found that some of these cells colonized the marrow and produced functional, circulating, leukemia-derived leukocytes. In other words, these animals were chimeric, but only in the leukopoietic tissues. It turned out that we learned some important things from these studies. First of all, there are embryonic fields capable of regulating at least four kinds of malignant cells. Secondly, none of these systems were really more amenable to study than the blastocyst and embryonal carcinoma cells. And thirdly, Ralph Parchment was able to show that melanoma cells placed in the limb were not differentiated as we had anticipated but instead were killed. So, all of the regulation that occurs in the embryo may not necessarily be by differentiation. Cell death is an important part of embryonic development and possibly these destructive mechanisms can be specific for malignant cells and can be utilized clinically.

This is a such a fascinating question that I'd like to discuss it a bit further. In recent years you have launched a series of interesting experiments concerning the mechanism of cell death during embryogenesis and its relationship with the process of regulation of cancer cell differentiation and growth. Could you tell us about this in more detail?

I mentioned that cell death is an integral part of normal development. It even occurs as early as the blastocyst stage. This was really first studied elegantly by El Shirshaby and Hinchliffe in the mid 70s. It turns out that the inner cell mass of the early blastocyst has the potential to make trophectoderm *in vitro*, but after programmed cell death has occurred during the transition from early to late blastocyst, the inner cell mass of the resulting late blastocyst lacks this potential. We wondered if the purpose of this programmed cell death was to rid the inner cell mass of redundant pretrophectodermal

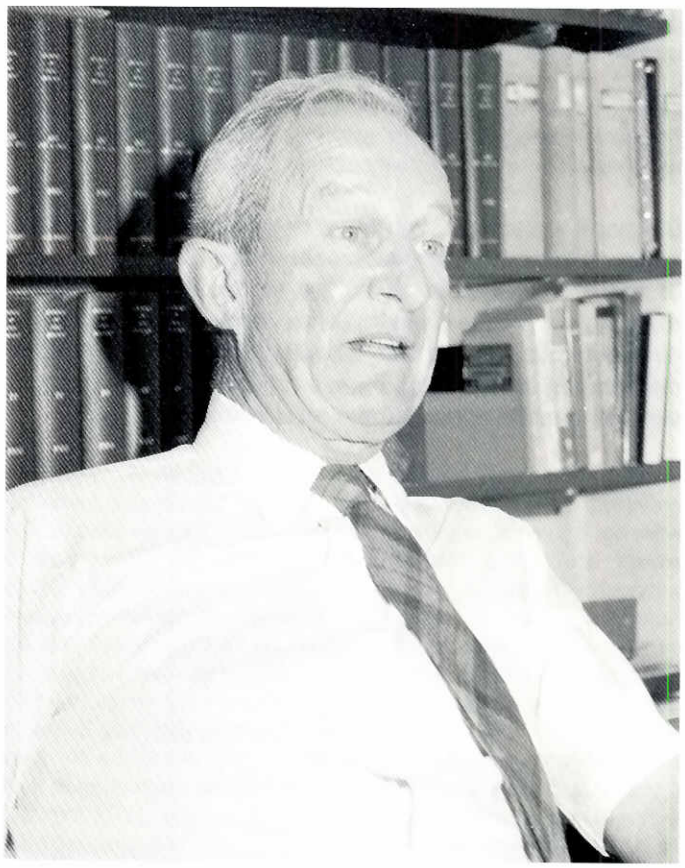
cells. We studied this and found that embryonal carcinoma cells with pretrophectodermal potential died in blastocoel fluid, but embryonal carcinoma cells with embryonic potential were not killed. Bob Gramzinski and Ralph Parchment showed that the mechanism of death of these pretrophectodermal cancer cells was hydrogen peroxide generated by the oxidation of polyamines, by amine oxidases. This led to the idea that programmed cell death in the blastocyst might be mediated by hydrogen peroxide, but this has not been proved. Everything is compatible with the hypothesis, however.

There is an important point to be made here, and that is that the blastocoel fluid that was used in the preliminary experiment in this this study was not derived from normal blastocysts, because they only contain approximately a nanoliter of fluid each. The material analyzed was obtained from the cystic embryoid bodies of a line of embryonal carcinoma called C44 that corresponded to late blastocysts. This points out yet another use of the idea that tumors are caricatures of the process of tissue renewal. C44 tumor cells were a caricature of the late inner cell mass that produced something that is important in development, in this case, the agents that probably mediated programmed cell death.

Until now, we have seen in this interview how embryology helped you to understand an important pathological phenomenon like cancer. Let me try to look in your biography to see the other side of the problem: how the study of pathological material can help to understand embryonic processes better. I think that this was the situation with your research on the origin of the basement membranes in the embryo, wasn't it?

We became interested in basement membrane through ascites conversion of testicular teratomas in 1958. They underwent progression with repeated passage of the tumors in the ascites. A highly malignant, rapidly growing cell type that synthesized a peculiar hyaline-kind of material resulted. It was not amyloid and that left basement membrane as an alternative. In the early mouse embryo, there is a thick basement membrane that lies between trophoblast and parietal yolk sac. It is called Reichert's membrane. The tumor that we were working with proved to be a parietal yolk sac carcinoma by electron microscopy and the neoplastic extracellular matrix and Reichert's membrane had similar antigens. It was clear that the tumor was making basement membrane. The prevailing concept concerning the origin of basement membranes at that time was that they were formed by condensation of ground substance. Our work showed that they were synthesized by epithelial cells. We then did some chemistry in conjunction with Sri Ram that showed that it was a glycoprotein and x-ray diffraction studies showed that the molecule contained collagen. Timpl, of course, later showed that the glycoprotein was laminin and others showed that the collagen was collagen type IV, but I was not really interested in basement membrane as much as I was interested in differentiation in cancer.

There was considerable resistance to the idea that a tumor could synthesize molecules of normal cells and that tumor basement membrane could be used as a counterpart of normal basement membrane. This surprised me because at that time the immunologists were using the myeloma proteins as models of immunoproteins and their work was accepted. No-one doubted that



HCG made by choriocarcinoma was HCG. Now nobody argues at all if a tumor makes a growth factor or hormone.

Important technical tools were also developed during your studies on the basal lamina of normal and tumor cells. What can you tell us about the immunoperoxidase technique?

I remember the development of this technique with a great deal of pleasure. Rees Midgley, Sri Ram and I had just written a critical review of ferritin-labeled antibody technique. We had been trying to utilize it in the localization of basement membrane antigens in parietal yolk sac cells. The rough endoplasmic reticulum was filled with what appeared to be basement membrane antigens and we performed biosynthetic studies and labeling studies using ferritin-labeled antibodies. The labeled antibody was just too large to penetrate intact cells and we were extremely frustrated. One day, coming home from lunch, I asked Sri Ram — a superb protein chemist who conjugated fluorescent molecules to antibodies using bifunctional reagents — why we couldn't couple an enzyme to antibody and then localize the conjugate histochemically using the enzymatic activity of the enzyme. Rees Midgley thought this was a marvellous idea and that summer, a young medical student, Donald Rawlinson, who was the son of my mentor back at the University of Alberta, began to work with us on this problem. We were able to make workable conjugates of phosphatase to antibody. The follow-

ing year, Paul Nakane joined our group. In no time at all, he showed that peroxidase was a better enzyme than phosphatase for this technique. Then he developed methods of minimal fixation of cells to preserve architecture, yet allow penetration of the conjugates into the cells. He developed all kinds of new embedding techniques. The development of the peroxidase-labeled antibody technique really represents the creativity of Paul Nakane.

On many occasions, you have been invited by scientists engaged in basic biological research to explain the benefits of using cancer cells as probes of development. In this regard, your proposal to use teratocarcinoma as a model in developmental biology has yielded splendid results in modern mammalian embryology. Please tell us about other cancer cells as models for developmental biology studies.

As I mentioned before, the interpretation of our work was criticized because some people believed the teratocarcinomas were not typical of cancers in general. Bill Cox and I decided that we would study the relationship between the stem cell of adenocarcinomas of the colon of mice and the development of normal stem cells in the mouse. These tumors contained mucous cells, columnar cells, undifferentiated cancer cells and occasional endocrine cells. By cloning the undifferentiated cells *in vivo* we were able to obtain tumors with undifferentiated cells, mucous cells, columnar cells and endocrine cells, indicating that they had common malignant and normal stem cells. In examining the literature it turned out that so-called APUD cells had been postulated to have a common histogenesis. On the basis of studies using formalin-activated fluorescence, the migration of neural crest cells had been studied in the gut. It turned out that these cells did go to the gut, but probably into the neural plexes that developed at the same time that the endodermal cells were differentiating into mucous, endocrine and columnar cells. We were not alone in believing that the endocrine cells were of endodermal origin. Andrews in South Africa had done some brilliant studies on this topic, and ours were really confirmatory of those studies. I am sure that there are many, many other examples. I have already mentioned the myeloma proteins as models of immunoglobulins. Any tumor can serve as a convenient model of the normal process. One must always remember that negative results do not mean anything, and any positive results must always be confirmed in the normal tissue. There is much to be learned by using tumors as models of tissue renewal.

These kinds of studies also have important consequences in orienting modern cancer therapeutics. I would like to ask you now about the principles for a differentiation therapy of tumors.

The people that use cytotoxic therapy for cancer have learned that many of the agents enhance differentiation in the tumors. This has now led to a search for chemicals that specifically affect or enhance the differentiation of malignant cells. The idea would be that all of the malignant stem cells would be converted into benign, if not normal, cells. The final option would be to take advantage of embryonic regulation of cancer. In the case of embryonal carcinoma, when the malignant cell is placed in the embryonic environment, it is regulated. Thus, the autocrine and paracrine secretions in the normal embryonic environment that develops the normal stem cell can regulate the malignant stem cell. Understanding these factors should lead to a non-cytotoxic treatment for cancer that would make malignant stem cells become benign stem cells.

As I mentioned, melanoma cells injected into the embryo were killed. The mechanism of programmed cell death might be developed as a specific kind of cytotoxic chemotherapy. The problem with cytotoxic chemotherapy is its lack of specificity and destruction of many normal cell types. If we could enhance programmed cell death in a tumor, then the tumor cells specifically should be killed. There is good reason to believe that this might work, because what has been interpreted in tumors as necrosis resulting from overgrowth of blood supply is not necrosis at all. It is massive apoptosis or programmed cell death. Now, if that programmed cell death could be enhanced, there could be tremendous reduction in tumor mass.

Finally, having addressed your scientific background in pathology and embryology, may I ask you for a personal definition of cancer from the developmental point of view?

I do not think I am able to do this. I like the idea that tumors are caricatures of the process of tissue renewal because it focuses on growth and differentiation, which are clearly major problems that have to be solved in the understanding of cancer. That is not really a definition because it does not take into account any of the biological activities of tumors that need to be explained, such as invasion or metastasis. These are integral parts of the cancer phenotype. I'm afraid I cannot put all these together in a developmental concept, just off the top of my head.

Your answer is appreciated anyway, especially since such a risky question deserves an equally cautious answer.

Acknowledgments

I would like to thank the Chairman of the Department of Pathology of the University of Colorado Health Sciences Center at Denver, Prof. Lázaro Gerschenson, for his help and consideration in arranging the interview. I am also indebted to Laurie Bogue and Clairene Mraz, who arranged the material for the interview and transcribed it from the tape recorder, to Robert McCullough, photographer of the U.C.H.S.C. Department of Pathology and to Prof. Frank Dixon for the group photograph. Also, the economic support provided through grants from the Autonomous Basque Government and the University of the Basque Country (Spain) is very much appreciated.

References

BEALS, T.F., PIERCE, G.B. and SCHROEDER, C.F. (1965). The ultrastructure of human testicular tumors. I. Interstitial cell tumors. *J. Urol. 93:* 63-73.

BRINSTER, R.L. (1974). The effect of cells transferred into the mouse blastocyst on subsequent development. *J. Exp. Med. 140:* 1049-1056.

EL-BOLKAINY, M.N., PIERCE, G.B. and FRENCH, A.J. (1967). Regression of an adrenal cortical carcinoma by estradiol treatment. *Cancer Res. 27:* 1846-1854.

EVANS, M.J. and KAUFMANN, M.H. (1981). Establishment in culture of pluripotential cells from mouse embryos. *Nature 292:* 154-156.

GERSCHENSON, M., GRAVES, K., CARSON, S.D., WELLS, R.S. and PIERCE, G.B. (1986). Regulation of melanoma by the embryonic skin. *Proc. Natl. Acad. Sci. USA 83:* 7307-7310.

GOOTWINE, E., WEBB, C.G. and SACHS, L. (1982). Participation of myeloid leukaemia cells injected into embryos in hematopoietic differentiation in adult mice. *Nature 299:* 63-65.

GRAMZINSKI, R.A., PARCHMENT, R.E. and PIERCE, G.B. (1990). Evidence linking programmed cell death in the blastocyst to polyamine oxidation. *Differentiation 43:* 59-65.

GRAY, J.M. and PIERCE, G.B. (1964). Relationship between growth rate and differentiation of melanoma *in vivo*. *J. Natl. Cancer Inst. 32:* 1201-1211.

JOHNSON, L.D. and PIERCE, G.B. (1970). Changes in antigenicity of basement membrane during wound healing. *Dev. Biol. 23:* 534-549.

KLEINSMITH, L.J. and PIERCE, G.B. (1964). Multipotentiality of single embryonal carcinoma cells. *Cancer Res. 24:* 1544-1551.

LEE, P.A., BLASEY, K., GOLDSTEIN, I.J. and PIERCE, G.B. (1969). Basement membrane: carbohydrates and X-ray differentiation. *Exp. Mol. Pathol. 10:* 323-330.

LEHMAN, J.M., SPEERS, W.C., SWARTZENDRUBER, D.E. and PIERCE, G.B. (1974). Neoplastic differentiation: characteristics of cell lines derived from a murine teratocarcinoma. *J. Cell. Physiol. 84:* 13-27.

MARTÍNEZ-HERNÁNDEZ, A., FINK, L.M. and PIERCE, G.B. (1976). Removal of basement membrane in the involuting breast. *Lab. Invest. 34:* 455-462.

MARTÍNEZ-HERNÁNDEZ, A., NAKANE, P.K. and PIERCE, G.B. (1974). Intracellular localization of basement membrane antigen in parietal yolk sac cells. *Am. J. Pathol. 76:* 549-556.

MIDGLEY, A.R. and PIERCE, G.B. (1961). Effect of colchicine and X-radiation on the differentiation of human embryonal carcinoma. *Cancer Res. 21:* 545-549.

MIDGLEY, A.R. and PIERCE, G.B. (1962). Immunohistochemical localization of human chorionic gonadotrophin. *J. Exp. Med. 115:* 289-294.

MIDGLEY, A.R. and PIERCE, G.B. (1963). Immunohistochemical analysis of basement membranes of the mouse. *Am. J. Pathol. 43:* 929-943.

MIDGLEY, A.R., PIERCE, G.B., DENEAU, G.A. and GOSLING, J.R.G. (1963). Morphogenesis of syncytiotrophoblast *in vivo:* an autoradiographic demonstration. *Science 141:* 349-350.

MIDGLEY, A.R., PIERCE, G.B. and DIXON, F.J. (1959). Nature of colchicine resistance in golden hamster. *Science 130:* 40-41.

MINTZ, B. and ILLMENSEE, K. (1975). Normal genetically mosaic mice produced from malignant teratocarcinoma cells. *Proc. Natl. Acad. Sci. USA 72:* 3585-3589.

MUKERJEE, H., SRI RAM, J. and PIERCE, G.B. (1965). Basement membranes. V. Chemical composition of neoplastic basement membrane mucoprotein. *Am. J. Pathol. 46:* 49-57.

NAKANE, P. and PIERCE, G.B. (1967a). Enzyme-labeled antibodies: preparation and application for the localization of antigens. *J. Histochem. Cytochem. 14:* 929-931.

NAKANE, P. and PIERCE, G.B. (1967b). Enzyme-labeled antibodies for the light and electron microscopic localization of tissue antigens. *J. Cell Biol. 33:* 307-318.

NOGALES-FERNÁNDEZ, F., SILVERBERG, S.G., BLOUSTEIN, P.A., MARTÍNEZ-HERNÁNDEZ, A. and PIERCE, G.B. (1977). Yolk sac carcinoma (endodermal sinus tumor). Ultrastructure and histogenesis of gonadal and extragonadal tumors in comparison with normal human yolk sac. *Cancer 39:* 1462-1474.

PAPAIOANNOU, V.E., McBURNEY, M.W., GARDNER, R.L. and EVANS, R.L. (1975). Fate of teratocarcinoma cells injected into early mouse embryos. *Nature 258:* 70-73.

PARCHMENT, R.E. and PIERCE, G.B. (1989). Polyamine oxidation, programmed cell death, and regulation of melanoma in the murine embryonic limb. *Cancer Res. 49:* 6680-6686.

PARCHMENT, R.E., GRAMZINSKI, R.A. and PIERCE, G.B. (1990a). Neoplastic embryoid bodies of embryonal carcinoma C44 as a source of blastocoele-like fluid. *Differentiation 43:* 51-58.

PARCHMENT, R.E., GRAMZINSKI, R.A. and PIERCE, G.B. (1990b). Embryonic mechanisms for abrogating the malignancy of cancer cells. In *Effects of Therapy on Biology and Kinetics of the Residual Tumor, Part A: Pre-clinical Aspects* (Eds. B. Fisher, M. Lippman, J. Ragaz and L. Simpson-Herren). Wiley-Liss Inc., New York, pp. 331-344.

PARCHMENT, R.E., LEWELLYN, A., SWARTZENDRUBER, D. and PIERCE, G.B. (1990c). Serum amine oxidase activity contributes to crisis in mouse embryo cell lines. *Proc. Natl. Acad. Sci. USA 87:* 4340-4344.

PIERCE, G.B. (1950). *The Iron Content of Mammary Epithelium.* Master's Thesis. University of Alberta.

PIERCE, G.B. (1961). Teratocarcinomas, a problem in developmental biology. *Canadian Cancer Conference* Vol. 4. Academic Press Inc., New York, pp. 119-137.

PIERCE, G.B. (1966). The development of basement membranes of the mouse embryo. *Dev. Biol. 13:* 231-249.

PIERCE, G.B. (1967). Teratocarcinoma: model for a developmental concept of cancer. *Curr. Top. Dev. Biol. 2:* 223-246.

PIERCE, G.B. (1970). Differentiation of normal and malignant cells. *Fed. Proc. 29:* 1248-1254.

PIERCE, G.B. (1972). Differentiation and cancer. In *Cell Differentiation* (Eds. R. Harris, P. Allin and D. Viza). Munksgaard, Denmark, pp. 109-114.

PIERCE, G.B. (1974a). The benign cells of malignant tumors. In *Developmental Aspects of Carcinogenesis and Immunity.* 32nd Symposium of the Society for Developmental Biology. Academic Press, San Francisco, pp. 3-22.

PIERCE, G.B. (1974b). Cellular heterogeneity of cancers. In *Chemical Carcinogenesis: Part B* (Eds. P.O.P. Ts'o and J.A. Di Paolo). Marcel Dekker Inc., New York, pp. 463-472.

PIERCE, G.B. (1974c). Neoplasms, differentiations and mutations. *Am. J. Pathol. 77:* 103-118.

PIERCE, G.B. (1975a). Nonproliferating cells of tumors. In *Comparative Leukemia Research* (Eds. Y. Ito and R.M. Dutcher). University of Tokyo Press, Tokyo/Karger, Basel, pp. 271-272.

PIERCE, G.B. (1975b). Teratocarcinoma: introduction and perspectives. In *Teratomas and Differentiation* (Eds. M. Sherman and D. Solter). Academic Press Inc., New York, pp. 3-12.

PIERCE, G.B. (1976). Origin of neoplastic stem cells. In *Progress in Differentiation Research* (Eds. N. Müller-Bérat *et al.*). North-Holland Pub. Co., Amsterdam, pp. 269-273.

PIERCE, G.B. (1977a). Relationship between differentiation and carcinogenesis. *J. Toxicol. Environ. Health 2:* 1335-1342.

PIERCE, G.B. (1977b). Neoplastic stem cells. In *Advances in Pathobiology. Cancer Biology IV* (Eds. C. Borek, C.M. Fenoglio and D.W. King). Stratton Intercontinental Medical Book Corporation, New York, pp. 141-152.

PIERCE, G.B. (1983). The cancer cell and its control by the embryo. *Am. J. Pathol. 113:* 117-124.

PIERCE, G.B. (1985). Carcinoma is to embryology as mutation is to genetics. *Am. Zool. 25:* 707-712.

PIERCE, G.B. (1991). Regulation of embryonal carcinoma by blastocyst, tumor suppressor genes, and metastasis. In New Approaches to Cancer Therapy. Meeting Report of the First International Symposium of Hiroshima Cancer Seminar, Hiroshima, Nov. 1990 (Ed. E. Tahara). *J. Cancer Res. Clin. Oncol. 117:* 269-272.

PIERCE, G.B. and ABELL, M.R. (1970). Embryonal carcinoma of the testis. In *Pathology Annual* (Ed. S.C. Sommers). Appleton-Century-Crofts, New York/Meredith Corp., pp. 27-60.

PIERCE, G.B. and BEALS, T.F. (1964). The ultrastructure of primordial germinal cells of the fetal testes and of embryonal carcinoma cells of mice. *Cancer Res. 24:* 1553-1567.

PIERCE, G.B. and COX, W.F. (1978). Neoplasms as caricatures of tissue renewal. In *Cell Differentiation and Neoplasia* (Ed. G.F. Saunders). Raven Press, New York, pp. 57-66.

PIERCE, G.B. and DIXON, F.J. (1959a). Testicular teratomas. I. Demonstration of teratogenesis by metamorphosis of multipotential cells. *Cancer 12:* 573-583.

PIERCE, G.B. and DIXON, F.J. (1959b). Testicular teratomas. II. Teratocarcinoma as an ascitic tumor. *Cancer 12:* 584-589.

PIERCE, G.B. and FENNELL, R.H. (1976). Latent carcinoma and carcinoma *in situ*. *Conference on Spontaneous Regression of Cancer.* National Cancer Institute Monograph 44. US Department of Health, Education and Welfare. Public Health Service. National Cancer Institute, Bethesda, MD, pp. 99-101.

PIERCE, G.B. and JOHNSON, L.D. (1971). Differentiation and cancer. *In Vitro 7:* 140-145.

PIERCE, G.B. and MIDGLEY, A.R. (1963). The origin and function of human syncytiotrophoblastic giant cells. *Am. J. Pathol. 43:* 153-173.

PIERCE, G.B. and NAKANE, P. (1967a). Antigens of epithelial basement membranes of mouse, rat and man: a study utilizing enzyme labeled antibody. *Lab. Invest. 17:* 499-514.

PIERCE, G.B. and NAKANE, P. (1967b). Nuclear sex of testicular teratomas of infants. *Nature 214:* 820-821.

PIERCE, G.B. and NAKANE, P. (1969). Basement membranes. Synthesis and deposition in response to cellular injury. *Lab. Invest. 21:* 27-41.

PIERCE, G.B. and SPEERS, W.C. (1988). Tumors as caricatures of the process of tissue renewal: prospects for therapy by directing differentiation. *Cancer Res. 48:* 1996-2004.

PIERCE, G.B. and VERNEY, E.L. (1961). An *in vitro* and *in vivo* study of differentiation in teratocarcinomas. *Cancer 14:* 1017-1029.

PIERCE, G.B. and WALLACE, C. (1971). Differentiation of malignant to benign cells. *Cancer Res. 31:* 127-134.

PIERCE, G.B., AGUILAR, D., HOOD, G. and WELLS, R.S. (1984). Trophectoderms in control of murine embryonal carcinoma. *Cancer Res. 44:* 3987-3996.

PIERCE, G.B., ARÉCHAGA, J. and WELLS, R.S. (1986). Embryonic control of cancer. In *Cellular Endocrinology: Hormonal Control of Embryonic and Cellular Differentiation* (Eds. G. Serrero and J. Hayashi). Alan R. Liss Inc., New York, pp. 67-75.

PIERCE, G.B., ARÉCHAGA, J., JONES, A., LEWELLYN, A. and WELLS, R.S. (1987). The fate of embryonal-carcinoma cells in mouse blastocyst. *Differentiation 33:* 247-253.

PIERCE, G.B., ARÉCHAGA, J., MURO, C. and WELLS, R.S. (1988). Differentiation of ICM cells into trophectoderm. *Am. J. Pathol. 132:* 356-364.

PIERCE, G.B., BEALS, T.F., SRI RAM, J. and MIDGLEY, A.R. (1964a). Basement membranes. IV. Epithelial origin and immunologic cross reactions. *Am. J. Pathol. 45:* 929-960.

PIERCE, G.B., BULLOCK, W.K. and HUNTINGTON, R.W. (1970). Yolk sac tumors of testis. *Cancer 25:* 644-658.

PIERCE, G.B., DIXON, F.J. and VERNEY, E.L. (1959). Endocrine function of a heterotransplantable human embryonal carcinoma. *A.M.A. Arch. Pathol. 67:* 204-210.

PIERCE, G.B., DIXON, F.J. and VERNEY, E.L. (1960a). Teratocarcinogenic and tissue-forming potentials of the cell types comprising neoplastic embryoid bodies. *Lab. Invest. 9:* 583-602.

PIERCE, G.B., DIXON, F.J. and VERNEY, E.L. (1960b). A critical evaluation of furacin as an antitesticular tumor agent. *J. Urol. 84:* 569-574.

PIERCE, G.B., GRAMZINSKI, R.A. and PARCHMENT, R.E. (1990). Amine oxidases, programmed cell death and tissue renewal. *Philos. Trans. R. Soc. Lond. B 327:* 67-74.

PIERCE, G.B., JONES, A., ORFANAKIS, N.G., NAKANE, P. and LUSTIG, L. (1982a). Biosynthesis of basement membrane by parietal yolk sac cells. *Differentiation 23:* 60-72.

PIERCE, G.B., LEWELLYN, A. and PARCHMENT, R.E. (1989). Mechanism of programmed cell death in the blastocyst. *Proc. Natl. Acad. Sci. USA 86:* 3654-3658.

PIERCE, G.B., LEWIS, S.H., MILLER, G.J., MORITZ, E. and MILLER, P. (1979). Tumorigenicity of embryonal carcinoma as an assay to study control of malignancy by the murine blastocyst. *Proc. Natl. Acad. Sci. USA 76:* 6649-6651.

PIERCE, G.B., MIDGLEY, A.R. and BEALS, T.F. (1964b). An ultrastructural study of differentiation and maturation of trophoblast of the monkey. *Lab. Invest. 5:* 451-464.

PIERCE, G.B., MIDGLEY, A.R. and SRI RAM, J. (1963). The histogenesis of basement membranes. *J. Exp. Med. 117:* 339-348.

PIERCE, G.B., MIDGLEY, A.R. and VERNEY, E.L. (1962a). Therapy of heterotransplanted choriocarcinoma. *Cancer Res. 22:* 563-567.

PIERCE, G.B., MIDGLEY, A.R., SRI RAM, J. and FELDMAN, J.D. (1962). Parietal yolk sac carcinoma: clue to the histogenesis of Reichert's membrane of the mouse embryo. *Am. J. Pathol. 41:* 549-566.

PIERCE, G.B., NAKANE, P. and MAZURKIEWICZ, J.E. (1974). Natural history of malignant stem cells. In *Proceedings of the 4th International Symposium of the Princess Takamatsu Cancer Research Fund* (Eds. W. Nakamura, T. Ono, T. Sugimura and H. Sugano). Univ. of Tokyo Press, Tokyo, pp. 453-469.

PIERCE, G.B., NAKANE, P., MARTÍNEZ-HERNÁNDEZ, A. and WARD, J.M. (1977). Ultrastructural comparison of differentiation of stem cells of murine adenocarcinomas of colon and breast with their normal counterparts. *J. Natl. Cancer Inst. 58:* 1329-1345.

PIERCE, G.B., PANTAZIS, C.G., CALDWELL, J.E. and WELLS, R.S. (1982b). Specificity of the control of tumor formation by the blastocyst. *Cancer Res. 42:* 1082-1087.

PIERCE, G.B., PANTAZIS, C.G., CALDWELL, J.E. and WELLS, R.S. (1982c). Embryologic control of malignancy. In *Mechanisms of Chemical Carcinogenesis* Vol. 2 (Eds. C. Harris and P. Cerutti). Alan R. Liss Inc., pp. 531-536.

PIERCE, G.B., PARCHMENT, R.E. and LEWELLYN, A. (1991). Hydrogen peroxide as a mediator of programmed cell death in the blastocyst. *Differentiation 46:* 181-186.

PIERCE, G.B., PODESTA, A. and WELLS, R.S. (1983). Malignancy and differentiation: the role of the blastocyst in control of colony formation. Teratocarcinoma stem cell. *Cold Spring Harb. Conf. Cell Prolif. 10:* 15-22.

PIERCE, G.B., SHIKES, R. and FINK, L.M. (1978). *Cancer: A Problem of Developmental Biology.* Prentice Hall, Englewood Cliffs, New Jersey, pp. 242.

PIERCE, G.B., SRI RAM, J. and MIDGLEY, A.R. (1964). The use of labeled antibodies in ultrastructural studies. *Int. Rev. Exp. Pathol. 3:* 1-34.

PIERCE, G.B., STEVENS, L.C. and NAKANE, P. (1967). Ultrastructural analysis of the early development of teratocarcinomas. *J. Natl. Cancer Inst. 39:* 755-773.

PODESTA, A.H., MULLINS, J., PIERCE, G.B. and WELLS, R.S. (1984). The neurula stage mouse embryos in control of neuroblastoma. *Proc. Natl. Acad. Sci. USA 81:* 7608-7611.

SRI RAM, J., TAWDE, S.S., PIERCE, G.B. and MIDGLEY, A.R. (1963). Preparation of antibody-ferritin conjugates for immuno-electron microscopy. *J. Cell Biol. 17:* 673-675.

VERNEY, E., PIERCE, G.B. and DIXON, F.J. (1959). The biology of testicular cancer. III. Heterotransplanted choriocarcinomas. *Cancer Res. 19:* 633-637.

WELLS, R.S. (1982). An *in vitro* assay for growth regulation of embryonal carcinoma by the blastocyst. *Cancer Res. 42:* 2736-2741.

WELLS, R.S. and MIOTTO, K. (1986). Widespread inhibition of neuroblastoma cells in the 13-to-17-day-old mouse embryo. *Cancer Res. 46:* 1659-1662.

WYLIE, C.V., NAKANE, P. and PIERCE, G.B. (1973). Degrees of differentiation in nonproliferating cells of mammary carcinoma. *Differentiation 1:* 11-20.