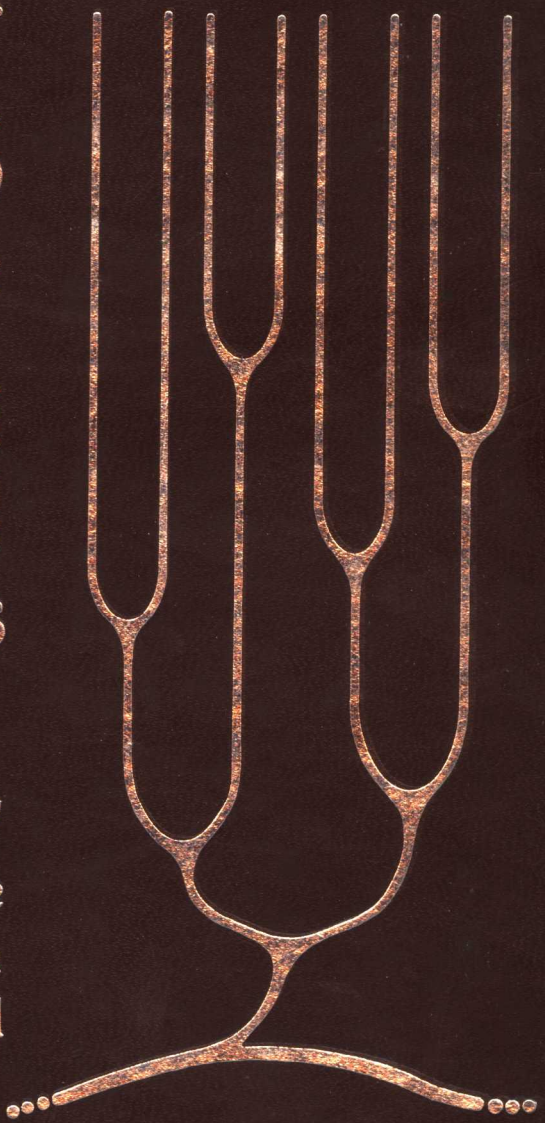
Stem Cells

Of Renewing Cell Populations



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Dr. Charles P. Leblond

C. P. Leblond

The dynamic processes taking place within organs, tissues, and cells have always held a special fascination for Dr. C.P. Leblond, in full accord with his own personal dynamism. The vigor of his approach to biology has been and remains a constant inspiration for his many colleagues and students.

An enthusiastic teacher of histology at McGill University since 1941, Dr. Leblond's research productivity is impressive. So far, he has contributed close to three hundred articles to the scientific literature. Those who have worked close to him know the real significance of this figure, since Dr. Leblond's rigorous scientific thinking and perfectionism usually blossom most fully at the time of writing, sometimes to the despair of his students or collaborators!

The technique of radioautography developed by Dr. Leblond in collaboration with Dr. L. Belanger in the mid-1940's was perfectly adapted to view biological phenomena dynamically as a function of time. This technique, now utilized in both light and electron microscopy, provides a most elegant tool to analyze the kinetic processes taking place within either cell populations or the cells themselves. Using radioautography Dr. Leblond and his associates have extensively investigated a wide variety of problems in histology and cell biology, some of which are represented in the present symposium. The diversity of Dr. Leblond's interests may be illustrated by the following examples of areas in which he has made major contributions: the mode of renewal of the intestinal epithelium (studies that started as early as 1947) and of several other cell populations (skin, thymus, esophagus, stomach, testis, etc.), the formation of the thyroid hormone using radioactive iodine as a tracer; the formation and calcification of bone and tooth; the synthesis of proteins in various cells using ^{35}S-labeled methionine as a tracer; the role of the Golgi apparatus in the synthesis of glycoproteins using labeled amino acids as precursors, etc.

It is evident that Dr. Leblond has a particular flair for discerning the significant facts that lead to discoveries. This remarkable quality, combined with his overwhelming enthusiasm in pursuing methodically a research project, has made him eminently capable of training numerous students. Indeed, he is "un maître" in the classical sense of the word and in the best scientific tradition. This facet of Dr. Leblond's personality is happily coupled with warmth and good humor. After long hours of hard work, there is always a place for bursts of joyful relaxation.

Throughout his prolific career Dr. Leblond's scientific endeavors have been acknowledged by a long list of honors, including the Flavelle Medal of the

Royal Society of Canada (1961), the Gairdner Foundation Award (1965), the American College of Physicians Award (1966). A Fellow of the Royal Society of Canada since 1951 and the Royal Society of London since 1965, he also received an Honorary Doctor of Science Degree from Acadia University, Wolfville, Nova Scotia in 1972.

The pioneering studies of Dr. Leblond on the kinetics of cell populations led him to the identification and analysis of the behavior of the "stem cells," which are at the origin of each cell line. Because of his continuous interest in this area of investigation, and also of the importance of this topic in biology, it was felt that an international symposium on stem cells was the best possible way to acknowledge Dr. Leblond's scientific endeavor and honor him as a leading scientist in biology.

Preface

This publication constitutes the proceedings of a symposium entitled, "Stem Cells in Various Tissues" which was organized as a tribute to Charles P. Leblond on the occasion of his sixty-fifth birthday.

A major scientific goal in planning the symposium was that of gathering together investigators working in a wide variety of fields who would not otherwise have the opportunity to interact with one another. For the first time, workers from many disciplines, using several distinct techniques, were able to exchange information on stem cells in a variety of organ systems studied under normal steady state conditions, as well as during growth, aging, regeneration, and neoplasia.

The chairman of the meeting was L.F. Lamerton, widely acknowledged as the *eminence grise* of the field. His opening address and closing summary will indicate to readers why he was considered to be the obvious person to voice both the first and the last word. We are grateful to him for undertaking this arduous task with such skill and grace.

The conference was made possible by generous financial support from the Medical Research Council of Canada, the National Cancer Institute of Canada, le Conseil de la Recherche en Santé du Québec, the Faculty of Medicine and the Faculty of Graduate Studies and Research, McGill University. The organizing committee, consisting of J.E. Till, Y. Clermont, and ourselves, is grateful to those who came so willingly to take part in the program, to those who came to listen and to discuss, and to all those who helped behind the scenes to make the conference a successful and happy scientific occasion. Finally, we thank Academic Press for their assistance in publishing the proceedings.

A.B. Cairnie
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Chairman's Opening Address at the Symposium on Stem Cells: A Tribute to C. P. Leblond

L. F. LAMERTON

Institute of Cancer Research, Sutton, Surrey, England

This meeting is to honour Professor Charles Leblond. I had wondered why I was given the great privilege of being Chairman and have concluded it was because I am old, old enough to have been in the field during the major biological revolution in which Charles Leblond has played such an important part.

I am, in fact, not quite old enough to have experienced the full impact of the first biological applications of artificial isotopes in the 30's and the early 40's with which the name of George Hevesy will always be associated, and the recognition of the 'dynamic state of body constituents', the title of Schoenheimer's lectures of 1941. The concept of the constituents of the body being in a state of flux, of course, is not a new one. Heraclitus, in the 5th century B.C., believed it. However, the concept was largely forgotten until markers of atoms became available in the form of isotopes, mainly radioactive, though one must not forget that the classic work of Schoenheimer and Rittenberg in 1936 on fatty acid turnover was done with deuterium, which was a stable isotope.

But this was a biochemist's world, and biochemists were too preoccupied with the complexities of their own subject to allow them to pay much attention to the biological problems arising from the heterogeneity of the tissues with which they were dealing, and this led to many difficulties and uncertainties in the interpretation of experimental data.

It was Charles Leblond who recognized the need to study turnover at the cellular level; not only recognized the need but developed the techniques, and over many years has not ceased to produce new and original findings and classic work in the field.

The technique of high resolution radioautography first described by Belanger and Leblond in 1942 has been, and is, of profound importance in biological work because, as Leblond himself has expressed it, it gives histology a fourth dimension - time. The ability to study turnover at the cellular level has opened up a Pandora's box (or as some who are for a quiet life might say, a nest of vipers!) and the fields of cell metabolism and cell population kinetics studied by radioautographic methods now engage a great army of workers.

I am sure Professor Leblond will understand me when I say we should all be grateful he is not a mathematician. This doesn't mean that he has not recognized the place of mathematics in his work and has not collaborated most effectively with very expert mathematical colleagues; but if he had been more mathematically inclined himself, he might have been seduced away from the microscope to indulge in the luxury of devising hypothetical control systems. He did not succumb, and I think he would maintain that before one begins to work out control mechanisms, it is essential to have a thorough understanding of the biology of the system. This remains for us, particularly at the present time, a very important lesson, with computers lurking around every corner demanding employment! I see Professor Leblond's hand in the program of this meeting in that most of the sessions start with a paper on the question of the identification of the appropriate stem cell population.

One of the great debts we owe to Professor Leblond and his colleagues is the classification of the tissues of the body based on their proliferative characteristics. Their application of the principles of the steady state to the renewing tissues of the body has been very fruitful and, increasingly, has concentrated attention on the stem cell populations - comprised of those cells that have the dual property of self-maintenance and of providing cells for the maturation pathway.

The classical theory of control of stem cell population, as presented by Osgood, was based on the assumption of asymmetric division of stem cells and, in a sense, was more a mathematical device than a biological theory. A most important contribution to the subject was made by Professor Leblond when, with Marques-Pereira, he demonstrated that this theory of stem cell control, at least in a simple form, was certainly not applicable to all tissues and possibly to none. This study also raised, in a clear fashion, the fundamental issue of how far the characteristics of stem cells are a consequence of their microenvironment. Is there, as the work suggested, a spatially defined zone in which cells can

continue to divide indefinitely without moving on to the maturation pathway? This is a theory with a number of attractions - it shows how a limit could be set to the size of the stem cell population, and could explain the relatively slow turnover of stem cells in most unstimulated tissues as the result of some density-dependent inhibition of division. The problem remains of the particular characteristics of the environment that allow the cells to be shielded from maturation influences; and, in the case of the skin, the problem is made more complex by present work - which we shall hear about - suggesting that the basal layer is by no means entirely composed of stem cells. And then there is the nature of the feed-back message changing the rate of output of cells from the stem cell compartment. Would this primarily be a proliferative stimulus, causing cells to enter the maturation compartment, perhaps as a result of population pressure, or a maturation stimulus leading secondarily to increased proliferation, in other words, *vis a tergo* or *vis a fronte*, or both?

The question of the extent of the influence of the environment in determining stem cell behaviour is, of course, bound up with the ability of cells to proliferate indefinitely and produce cells for the maturation pathway when transplanted into other environments. This requires experiments involving clonogenic assay as, in fact, do many other types of study one would like to do with stem cells. Histological and cell turnover studies can give important clues about stem cell populations, but since the stem cell is defined prospectively, in terms of its future behaviour, the information is bound to be very limited unless we also have functional assays at our disposal.

It is interesting, but perhaps not surprising, that the break-through in providing clonogenic techniques for cells of normal tissues also came from Canada, from the Ontario Cancer Institute. Among their many achievements these workers have demonstrated a remarkable talent for recognizing the significance of some often rather odd biological phenomenon as the basis for quantitative studies of fundamental processes. For us the important example of this was the spleen colony technique of Till and McCulloch for hemopoietic stem cells, and this ushered in the second phase of stem cell investigation which, of course, has now been extended to a number of other tissues, as we shall hear over the next three days.

By means of clonogenic techniques a great deal of information is being gathered about the proliferative behaviour, response to various agents and other characteristics of the

repopulating cells, but I believe the real need now is for the integration of the two approaches, histological and clonogenic. One problem here is the relationship between the effective stem cell population *in vivo* and the cell populations measured by various types of clonogenic assay, which has a bearing on the question as to whether the stem cells in various tissues represent a population quite distinct from the maturing cells or whether under stress, for instance under the conditions of clonogenic assay, some cells may revert to stem cell behaviour. However, I suspect that the major problem in the integration of histological and clonogenic approaches is the unequivocal identification of the position and extent of the clonogenic cell population within the tissue architecture. For the gut and the skin, we may be somewhere near the solution. For the bone marrow we need much more information on microarchitecture, and this itself could help in the solution of many outstanding problems.

A great deal of data is going to be presented over the course of the next three days and it is important that the wood should not be lost amongst the trees or, perhaps I should say, the stem amongst the flowers. I think that when there is any doubt, speakers should define what they mean by 'stem cells', and particularly if they use such odd terms as 'committed stem cell'. Let us not forget that the initial stem cell of the body, the fertilized ovum from which all the other stem cell populations are derived, is itself committed to producing *Homo sapiens* and, in fact, still further committed to producing either male or female. Let us relate, whenever we can, our observations to the basic problems, on the one hand, of the nature and mode of operation of the messages to the stem cell compartment that control the rate of output of cells at maturation and, on the other, to the factors that give a cell 'stem' properties. Somehow I feel we shall have enough to talk about.

Now this meeting, which will explore so many facets of dynamic and functional histology, must be very gratifying to Professor Leblond. The voice crying in the wilderness has now become a roar, even if it still has to penetrate some of the fastnesses of classical histology and pathology. Professor Leblond must feel justly proud that the subject which he has been so instrumental in initiating and developing has become one of the major growing points of modern biology.

Session I

Stem Cells in the Intestine