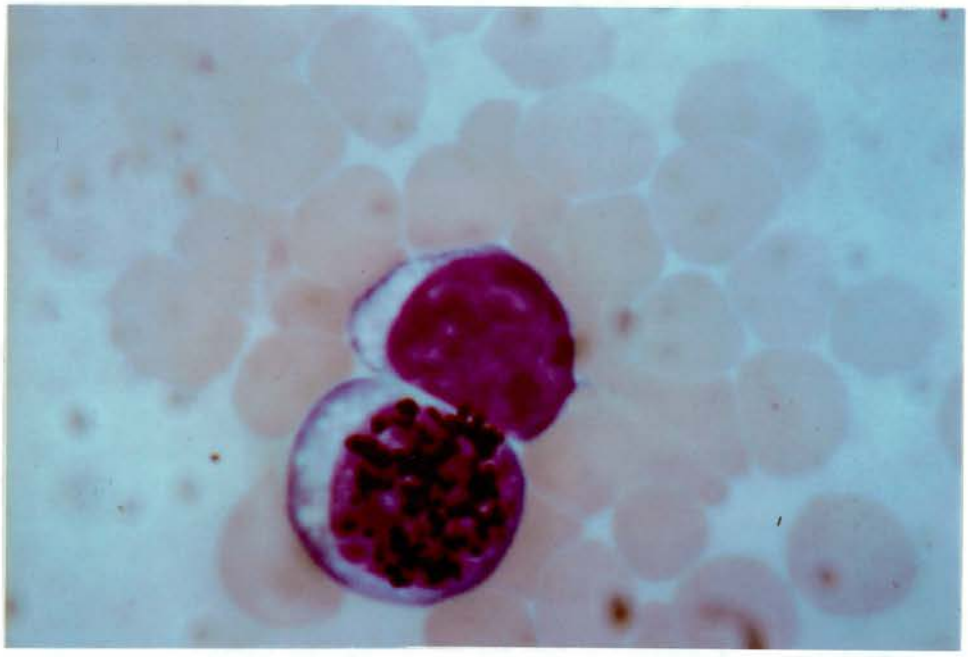
MORPHOLOGICAL PATTERNS OF PERTURBATION AND RECOVERY IN POPULATIONS OF BLOOD CELLS AND THEIR PRECURSORS

An annotated selection of publications submitted for the
Degree of Master of Science
by Cäcilia A. Villiers-Briscoe

Department of Biology and Preclinical Medicine
University of St. Andrews, October 1991

FRONTISPIECE

The development of the compound microscope coincided with the synthesis of dyes which were successfully used to differentiate the several varieties of blood cells one from another. Subsequently, techniques have become available which have enabled specific molecules to be localized with precision and permitted cellular activities to be demonstrated, thus autoradiography using tritiated thymidine has enabled DNA synthesis to be monitored and resulted in the rapid accumulation of detailed information about the kinetics of cellular proliferation.



In the photomicrograph the cell labelled with tritiated thymidine which was synthesizing DNA at the time of fixation is readily distinguished from the adjacent unlabelled cell.

CONTENTS

page

DECLARATION.....5

ACKNOWLEDGEMENTS.....7

ABSTRACT..... .8

LIST OF PUBLICATIONS.....10

A. THE DISCOVERY AND CHARACTERISATION OF BLOOD CELLS AND THEIR PRECURSORS.....14

I Historical Introduction.....15
II The Development of the Compound Microscope.....17
III The Application of Dyes.....20

B. THE PERTURBATION OF HAEMATOPOIETIC TISSUE.....23

Introduction.....24
a) By Endotoxin.....27
Publication: 4
b) By Cytotoxic Drugs.....41
Publications: 5,8,1,7
c) In Mutant Mice.....68
Publications: 21,22

C. HAEMATOPOIETIC STEM CELLS.....71

I The Stem Cell Compartment of Haematopoietic Tissue.....72
II The *in vivo* Isolation of Haematopoietic Cell Clones.....74
III The Regulation of Isolated Haematopoietic Cell Clones.....76
IV Haematopoiesis *in vitro*.....77
V The Haematopoietic Microenvironment...78
a) Haematopoietic Stem Cells.....81
Publications: 2,3,6,9,10,12,11
b) Regulation of Haematopoietic Stem Cells.....124
Publications: 13,19
c) Microenvironment.....135
Publications: 16,17

D. THE RECOGNITION AND CLASSIFICATION OF THE LEUKAEMIAS.....138

I Historical Introduction.....139
II The Induction of Leukaemia by Ionizing Radiation.....144
III Observations on Induced Myeloid Leukaemias.....149
1.) Introduction.....149
2.) Materials and Methods.....151
3.) Results.....155
4.) Tables.....159
5.) Discussion.....170

Leukaemia.....177
Publications: 14,15,20,18,23

REFERENCES.....195

ILLUSTRATIONS

page

Frontispiece.....1
Cell in DNA Synthesis labelled with Tritiated Thymidine

Salvador Dali.....148
Galacidalacidesoxyribonucleidacid

Normal Murine Bone Marrow.....174

Leukaemic Murine Bone Marrow.....174

Normal Murine Spleen.....175

Leukaemic Murine Spleen.....175

Cells synthezising DNA in
Leukaemic Murine Bone Marrow.....176

Cells synthesizing DNA in
Leukaemic Murine Spleen.....176

DECLARATION FOR THE DEGREEE OF M.SC.

I, Cäcilia Villiers-Briscoe, hereby certify that this thesis has been composed by myself, that it is a record of my own work, and that it has not been accepted in partial or complete fulfilment of any other degree or professional qualification.

31. Oct. 1991

Signed Date

All of the differential counts referred to in this submission were performed by me and all of the photomicrographs were taken by me.

In submitting this thesis to the University of St. Andrews I understand that I am giving permission for it to be made available for use in accordance with the regulations of the University Library for the time being in force, subject to any copyright vested in the work not being affected thereby.
I also understand that the title and abstract will be published, and that a copy of the work may be made and supplied to any *bona fide* library or research worker.

Willst du dich am Ganzen erquicken,
So musst du das Ganze im Kleinsten erblicken.

Johann Wolfgang von Goethe

If in the whole you would delight
In the smallest the whole must come to sight.

Translation by John Williams, Department of German

ACKNOWLEDGEMENTS

I am indebted to Professor D. Brynmor Thomas for providing the initial spark to this thesis, for inspiration throughout and - above all - for leading me away from microbiology into the depths of haematology some twenty years ago, thus instilling into me a lifelong, unfading interest in and enthusiasm for blood cells.

Many thanks are owed to Dr. A.C. Riches for his time, patience, advice and humour. His vast knowledge of the subject and his generosity in sharing it helped to make this thesis complete.

I also thank Simon Robinson for not abandoning attempts to make me computer-literate, Eleni Kavnoudias and Dimitrios Bairaktaris for taking me gently to the higher echelons of word processing, Jean Melville and Annika Norton for moral support often in form of laughs and coffee, and finally, I wish to express my gratitude to my children Julia, Caroline and Henry for their understanding and to my husband, Ray, for being so patient, supportive and encouraging and for 'sorting out' my German word order.

ABSTRACT

The development of microscopy, coinciding with the synthesis of appropriate dyes, allowed blood cells to be differentiated one from another. The accessibility of blood cells and their extravascular precursors allowed full advantage to be taken of these techniques. As a result, information about the patterns of production and accumulation of blood cells was readily exploited in the diagnosis, management and analysis of haematological disorders.

An attempt is made to elucidate the behaviour of haematopoietic cell populations, which are normally in a state of dynamic equilibrium. To obtain an insight into this equilibrium, the system was exposed to measured perturbations by endotoxin, cytotoxic drugs and X-irradiation. The analysis is based mainly on microscopical observations of cell morphology, but also includes observations on the behaviour of transplanted haematopoietic cells and the results of assays conducted to monitor changes in the stem cell compartment.

In vivo and *in vitro* observations on radiation-induced murine myeloid leukaemias reveal similarities with the human form of the disease. Fifteen leukaemias were studied in detail with particular reference to their cellular morphology and the proliferative status of cells derived from different haematopoietic tissues.

The present classified selection of publications illustrates the use and continuing importance of morphological techniques in investigations designed to provide information about the perturbation of

haematopoiesis, the properties of the earliest precursors of the blood cells and the manifestations of abnormal production or accumulation in the leukaemias.

LIST OF PUBLICATIONS

1) Briscoe CV (1970)
The proliferation and differentiation of transplanted bone marrow cells derived from mice treated with mustine hydrochloride.
Journal of Anatomy **107:** p395
With JG Sharp and D.Brynmor Thomas

2) Briscoe CV (1971)
An approach to the morphological characterisation of the haematopoietic stem cell.
Journal of Anatomy **108:** p597
With JG Sharp and D Brynmor Thomas

3) Briscoe CV (1973)
Stem cells indicated by transplantation assay. (SCIBTA).
British Journal of Haematology **24:** p662
With JG Sharp and D.Brynmor Thomas

4) Briscoe CV (1973)
The effects of intravenous injections of endotoxin on the distribution of cells between murine blood and bone marrow.
Journal of Anatomy **114:** p407
With D Brynmor Thomas

5) Briscoe CV (1973)
Effects of mustine hydrochloride on murine haematopoietic and lymphoid tissues.
Chemotherapy **18**: p304
With JG Sharp and D Brynmor Thomas

6) Briscoe CV (1973)
The quantitative association of proliferative capacity with cellular morphology in transplanted murine bone marrow.
Experimental Hematology **1:** p55
With V Littlewood, JG Sharp, and D Brynmor Thomas

7) Briscoe CV (1974)
Proliferation and differentiation of transplanted bone marrow from mice treated with nitrogen mustard.
Experimental Hematology **2:** p1
With JG Sharp and D Brynmor Thomas

8) Briscoe CV (1975)
Prolonged effects of nitrogen mustard on mouse haematopietic and lymphoid tissues.
Scandinavian Journal of Haematology **14:** p313
With V.Littlewood, JG Sharp, and D Brynmor Thomas

9) Briscoe CV (1976)
The morphological equivalent of the haematological stem cell compartment.
I. Transplantation studies
Journal of Clinical Haematology and Oncology **6**: p23
With V Littlewood, AC.Riches, JG Sharp and D Brynmor Thomas

10) Briscoe CV (1976)
The morphological equivalent of the haematopoietic stem cell compartment.
II. Kinetic studies.
Journal of Clinical Haematology and Oncology **6:** p35
With V.Littlewood, AC Riches, JG Sharp and D.Brynmor Thomas

11) Briscoe CV (1976)
The kinetic properties of the haematopoietic stem cell compartment in the mouse.
Journal of Anatomy **122:** p717
With V.Littlewood, AC Riches, JG Sharp and D.Brynmor Thomas

12) Briscoe CV (1977)
The morphological characterization of the haematopoietic stem cell (CFU-S).
Journal of Anatomy **124:** p21
With JG Sharp and D Brynmor Thomas

13) Briscoe CV (1983)
Separation of murine fetal liver cells by Percoll to study factors regulating haematopoietic stem cell proliferation.
Journal of Anatomy **136:** p625
With KA Darwood, AC Riches and D Brynmor Thomas

14) Briscoe CV (1986)
Investigation of the induction protocol for radiation-induced myeloid leukaemia.
Leukemia Research **10:** p872
With I Doherty, M Hepburn and AC Riches

15) Briscoe CV (1987)
Transplantation and morphological studies of primary and passaged murine radiation-induced myeloid leukaemias.
Leukemia Research **11:** No 11 p1001
With I Doherty, M Hepburn and AC Riches

16) Briscoe CV (1987)
The growth and granulocytopoietic support capacity of adherent stromal layers derived from the liver.
Journal of Anatomy **152**: p234
With Hosne Ara and D Brynmor Thomas

17) Briscoe CV (1987)
The growth of adherent stromal layers as an index of the haematopoietic support capacity of the liver.
Journal of Anatomy **155:** p230
With Hosne Ara and D Brynmor Thomas

18) Briscoe CV (1987)
Long term bone marrow culture of a murine myeloid leukaemia.
Med Sci Res **15:** p1461
With JN Chapman, M Hepburn and AC Riches

19) Briscoe CV (1990)
Regulation of haematopoietic stem cell proliferation by stimulatory factors produced by murine fetal and adult liver.
Journal of Anatomy **168**: p209
With KA Dawood, AC Riches and D Brynmor Thomas

20) Briscoe CV (1990)
Murine myeloid leukaemic cells in long term bone marrow cultures.
Experimental Hematology **18:** p640
With M Hepburn, J Melville and AC Riches.

21) Briscoe CV (1990)
The haematopietic support capacity of the liver in osteopetrotic microphthalmic mice.
IX th International Symposium on Morphological Sciences, Volume des Resumes, p141
With SC Marks and D Brynmor Thomas

22) Briscoe CV (1991)
Extramedullary haematopoiesis in osteopetrotic mice.
Journal of Anatomy **176:** p238
With SC Marks and D Brynmor Thomas

23) Briscoe CV (1991)
Persistence of murine myeloid leukaemic cells in long-term bone marrow cultures.
Bone Marrow Transplantation **7**: p329
With M Hepburn, J Melville and AC Riches

A THE DISCOVERY AND CHARACTERISATION OF BLOOD CELLS AND THEIR PRECURSORS

I Historical Introduction

The English word 'blood' is derived from the old English blod and related to modern German 'Blut', old German 'Bluot' and Old Norse 'Bloth'. This word, common to all the Germanic languages, is apparently derived from the same root as the Latin *flatus* (breath), and probably originally conveyed the meaning of 'life' or 'living'.

The earliest ideas on haematology were primarily concerned with the blood within the body, its loss and hence cessation of life. Although nothing was known about the origin and function of blood it was seen as ' vital fluid' . A relationship between blood and the heart was early recognised by observing that when the blood stopped flowing the heart ceased beating. This connection has first been demonstrated in a palaeolithic cave drawing of a mammoth in northern Spain. (Breuil, 1935) For centuries poets, healers, and philosophers saw and described the close link between blood and life.

The first written symbol for blood was used by the Sumerians in the third millenium B.C. It appears like the letter Y inverted and might suggest a branching blood vessel. (Majno, 1975) The ancient Hebrews identified blood with the soul, and the consumption of blood was strictly prohibited, as is still reflected in the Jewish laws regarding the slaughter of animals and the preparation of food. Spilt blood had to be covered with earth. (Leviticus) According to the Ebers Papyrus from Thebes (about 1550 B.C.) it was a widespread belief in Egypt that the heart would convert food in the stomach into blood. (Ebbell, 1937) In China in the Shang Yin dynasty (1532 - 1027 BC), blood was depicted by one to four strokes within the pictograph for illness (chi), which was itself composed of two other radicals representing 'man' and 'bed' (Unschuld,1985). In Egypt and in China the healing and magic powers of blood

were thought to cure and prevent diseases. Blood of fallen soldiers was drunk to gain their heroic strength, and blood of juveniles was supposed to give new life to the aged. Many ancient peoples believed milk and semen to be forms of blood, conveying 'life'.

The Greeks believed they could resurrect the shades of their dead by pouring blood into their graves. In some human communities the binding power of blood was called upon when two people (or more) would drink each others' blood and thus were tied together in 'blood-brotherhood'. Blood was also regarded as forming a link between Man and the gods. Altars would be covered with blood; and animal and human blood was shed to appease or feed the gods.

A theory which existed for about 1400 years had as its basis the idea that the four humours, blood, phlegm, black and yellow bile, made up the body. This originated circa 400 B.C. in one of the Hippocratic writings and was further conveyed by Galen (ca A.D. 130-200). (Siegel, 1968) It formed the basis of general medical thinking up to the seventeenth century. A proper balance between these four humours assured health, whereas any upset of their equilibrium was regarded as the cause of disease.

The contents of veins and arteries were the subject of greatly differing opinions over the centuries. Erasistratus (ca 310-250 B.C.) thought that only the veins held the blood, whereas the arteries contained air (pneuma) which converted into the "vital spirit" after leaving the lungs. (Garrison,1929) Galen believed that the venous blood was composed of a blend of humours and pneuma which he referred to as spirit, which together supplied the food throughout the body The blood flowing in the arteries was regarded as more refined, vaporous with a finer pneuma and responsible for vital activities and the maintenance of body heat. (Temkin, 1951)

Harvey (1578 - 1657) was the first to suggest that blood, whether venous or arterial, was one substance only, though he was unable to give an explanation for the difference in the colours. (Garrison, 1929) The beginnings of microscopy in the seventeenth century and revelations about chemical particles in the blood did not affect Harvey's view that "life...resides in the blood (as we are also informed in our sacred writings)...blood is the generative part, the fountain of life, the first to live, the last to die, and the primary seat of the soul." (Willis. 1847)

II The Development of the Compound Microscope

The invention of the optical lens and the combination of several convex lenses within a tube heralded the beginning of microscopy, and thus the fulfilment of the ancient dream of seeing beyond the limits of the human eye into the microcosmos. The learned thirteenth century monk and early scientist, Roger Bacon, appears to have been the first to describe the use of a single convex lens. (Haden, 1939) Sadly, his writings were lost when he was imprisoned for reasons which are also lost to posterity. In the following centuries lenses were only used as spectacles and magnifiers.

However, in 1590 two Dutchmen, the spectacle makers Zacharias Janssen and Hans Lippersley combined lenses in a tube and were able to magnify minute objects. They were, however, more interested in the telescope. A Jesuit priest named Kircher (Worvill, 1971) who lived in Germany in the middle of the seventeenth century listed six different kinds of microscope. They included glass bowls and hemispheres filled with water, and another made up of two convex glass lenses.

It was Antonij van Leeuwenhoek (1674), another Dutchman and spectacle maker, whose masterly and persistent skill in grinding lenses enabled him to construct a simple compound microscope. He observed his own blood which he had obtained with what he described as a very thin glass pipe and which was attached by saliva to the pin of his microscope. He writes: "The blood is composed of exceeding small particles, named globules, which in most animals are of a red color, swimming in a liquor, called by physicians, the serum. ... These particles or globules are so minute that hundred of them, placed side by side, would not equal the diameter of a common grain of sand; consequently, a grain of sand is above a million times the size of one such globule." He was thus the first scientist to describe blood cells. Although Jan Swammerdam (1637-1680) and Marcello Malpighi (1628-1694) had seen "ruddy globules" in blood preparations they failed to associate them with their flow within the blood-vessels.

A greatly improved compound microscope was described in 1665 by Robert Hooke, who was the curator of the Royal Society of London. (Bracegirdle, 1978) Hooke elucidated the principles of the microscope and described its use in the study of botanical specimens, discovering the cell. He can be regarded as the father of true microscopy. These early microscopes, however, were not very satisfactory and provided a magnification of only about 30 diameters. Due to chromatic and spherical aberrations the image presented was lacking in crispness and clarity, but it was adequate for botanical and entomological specimens.

The discovery of colourless or white blood cells seems to be difficult to credit to one person. Malpighi and Leeuwenhoek are likely candidates, but it was William Hewson (1773) who first described the leucocytes. He observed that they were less numerous than erythrocytes which he defined as "flat as a Guinea" and he diluted the blood in serum rather than water as all previous and

contemporary researchers did. His questioning about the site of blood cell production led him to the theory that they might originate in the lymphatic tissue which, as he saw it, fed the cells through the thoracic duct into the blood circulation.

However, despite Leeuwenhoek's significant breakthrough in haematological morphology and Hewson's (Hewson, 1774) contributions not only to microscopy but also to other haematological fields such as blood coagulation, and theories about the function of the thymus and the lymphatic system, development in microscopy progressed slowly over the next two centuries. It was thought that microscopical images were distorted versions of the real invisible world. Goethe (Schrimpf 1963) in his thoughts on science declared "Mikroskope und Fernrohre verwirren eigentlich den reinen Menschensinn" (Microscopes and telescopes in fact confuse the pure human judgment.)

The development of achromatic objectives by Carl Zeiss and others after 1830 resulted in improved observations of blood corpuscles. This enabled George Gulliver (1875) to carry out systematic investigations into blood of various animal species. Together with William Addison (1841) he succeeded in giving a first detailed account of granulocytes by describing them as "rough or granulated, colourless corpuscles"

Alfred Donné (1844) first recognised the importance of microscopy as a tool in investigating blood for the study of diseases. He established a 'Cours de Microscopie complimèntaire des Études medicals' in Paris. His compatriot Gabriel Andral (1845) published an 'Essai d'Hématologie pathologique' which contained chapters on anaemias, and in which he first commented on the small size of red corpuscles in chlorosis.

Donné and Andral were the first physicians to advocate the use of the microscope in clinical medicine. So far it had remained in the domain of natural scientists, and although Donné and Andral were mocked by their mistrusting and doubting medical colleagues, they persevered and thus established an important basis for medical diagnostics research and teaching.

III The Application of Dyes

It was not until the last quarter of the nineteenth century, however, after Perkin's discovery of aniline dyes and their subsequent manufacture in Germany, and Paul Ehrlich (1877) developed the first important analytical technique for recognising the different cell types, that blood cell morphology made a giant leap forward. Instead of looking at fresh blood samples he fixed blood and bone marrow smears by heating the slides. His new triacid stains and his experiments on dyes in acid, basic, and neutral conditions revealed structures in cells that had been hitherto unknown. Ehrlich (1879) was the first to show clearly cellular details such as nucleus, cytoplasma, and granules within the cytoplasma. This made it possible to classify the three groups of granulocytes and thus the differential blood count was born.

Amongst others Ehrlich also described a "primitive", large, basophilic, mononuclear cell with a vesicular nucleus and few or no granules which he called the 'Myelozyt', the marrow cell, and which he considered to be the precursor of granulocytes. This was probably the first attempt to define a haematopoietic stem cell, a cell type that can maintain its own numbers by cell division and yet can provide descendants which will mature into the various blood-cells.

Ehrlich (1879) had also observed that within a very short time large amounts of pus were produced in infections. This went along without the expected decrease of the leucocytosis in the blood of the patient. He put forward the theory that their reduction in numbers was "counterbalanced by an eminent capacity for regeneration". He bemoans the lack of knowledge about the background to these facts, and cites his colleague Rindfleisch, who lamented without realising the prophesy behind his words "die Leukozyten sind eine Art Omnibus, in welcher alles Mögliche fährt" (leukocytes are a kind of omnibus in which all sorts travel).

Ehrlich's observation on cell granula convinced him that they originated in the cells which contained them and that they could be regarded as products of specific secretory cell activity.

Many great names have been put forward as fathers of haematology. and there still seems to be some dispute as to the real paternity. Perhaps, if one looks upon Leeuwenhook and Hewson, for example, as forefathers Ehrlich ought quite justifiably to be regarded as the father in this field. But he is the father of several offspring, one of them cytochemistry, because he was the first to demonstrate chemical reactions on a microscopic level, the others being immunology and chemotherapy by his putting forward theories on body responses to infections and their treatment.

Other workers developed more refined staining methods and combination of stains, of which Romanowsky (1891) stands out by far and gave his name to the dual stains of blood cells in general. He originally published a paper on the staining of parasites of red blood cells and used an improved mixture of methylene blue and eosin. This blend produced bright colours, and especially a very vivid red.

Methylene blue in solution in water combines with eosin and produces the eosinate of methylene azure.

Leishman (1901) dissolved the dyes in methanol, May Grünwald (1902) produced a combination which would not stain the nucleus but would differentiate the granules especially distinctly, whilst Giemsa (1904) greatly simplified the staining process by composing mixtures of dyes which were stable and gave consistent results. Other names that must be mentioned are Pappenheim, Wright and Jenner, whose methods of blood cell staining are still applied all over the world. The simplest and quickest of all staining methods , however, appears to be the DIFF-QUIK technique which, based on the Pappenheim principles, fixes and dyes a perfect blood film within 30 seconds. (Merz)

Thus with the further development of the compound microscope and easily adaptable staining techniques the era of morphology in haematology began early in the present century.

B THE PERTURBATION

OF HAEMATOPOIEITC TISSUE

Introduction

Perturbations of haematopoietic tissue can be achieved experimentally by physical and bio-chemical means. Thus, pathological states at different degrees can be induced, studied and treated under measured conditions. A genetic perturbation, osteopetrosis, also provides some insight into the working of haematopoietic cell systems in mice afflicted with this disorder.

The effects of radiation on living tissue are well established, and patterns of X-irradiation damage to murine haematopoietic cell compartments in particular, have been the basis of some of the following experiments. Low doses of X-irradiation can cause permanent perturbation through leukaemogenesis. Whole body exposure to 8.2Gy damages the blood forming tissues irreversibly, and only treatment with haematopoietic cell suspensions can restore their blood forming capacity. This fact has been exploited variously by assessing the stem cell contents in these suspensions and in the haematopoietic tissue they derive from.

Mustine hydrochloride (HN2, nitrogen mustard), an alkylating agent, was found to damage bone marrow and lymphoid tissues in a similar way to X-irradiation. The cytotoxic effects on the lymphomyeloid complex present themselves in hypocellularity of the medullary cavity of the femoral diaphysis and atrophy of lymphatic tissues. Thereafter, a spontaneous granulocytic regeneration of bone marrow occurs initially, to be followed by erythroid hyperplasia and finally recovery of the secondary lymphoid tissues at about fifty days after injection. The non-synchronic timing of recovery in these cell lines was thought to throw some light upon proliferation and differentiation patterns of haematopoietic and lymphoid stem cells and their microenvironment.

Yoffey (1966) had suggested that staphylococci and streptococci exert both chemotactic and maturational effects on the bone marrow, whilst, according to him, typhoid organisms have only a chemotactic effect. A leukocytosis was artificially induced by administering TAB endotoxin intravenously. TAB is a purified bacterial lipopolysaccharide and causes the bone marrow to discharge granulocytes into the bloodstream. It is possible to measure this response with some precision and to utilize it in evaluating changes in the marrow granulocyte reserve of patients with various pathological conditions. It has also been employed to assess the production of granulocytic progeny of transplanted haematopoietic cells within lethally irradiated murine recipients.

Although the mode of action of the TAB vaccine on the interchange of cells between the bone marrow compartments and the peripheral blood was obscure at the time of my experiments, it was thought that it may act directly on the cells of the bone marrow or it may induce the production or liberation of an intermediary factor (Gordon et al, 1960) inducing some or all of the changes following intravenous injection.

Osteopetrotic mice are unable to establish adequate medullary cavities due to defective osteoclast differentiation. Recent experiments show that the duration of hepatic haematopoiesis during their neonatal period is very similar to that of their normal counterparts. Further studies confirmed that extramedullary haematopoiesis in liver and spleen was not increased, as expected, during the first four weeks of life in mice afflicted with osteopetrosis.

Whatever steps had been undertaken to perturb the dynamic equilibrium between blood cells and their precursors, whether by X-irradiation, mustine

hydrochloride, or endotoxin, some effects on committed or on pluripotent stem cells have thus been demonstrated.

The Perturbation of Haematopoietic Tissue

a) By Endotoxin
Publication: 4

C HAEMATOPOIETIC STEM CELLS

I The Stem Cell Compartment of the Haematopoietic Tissue

Scholars, especially nineteenth century scholars, were very much concerned with classifying the flora and fauna they were discovering and observing. They tried to put the species into categories and, furthermore, attempted to follow their family tree down to the stem establishing familial relationships on the way. It became fashionable to talk about evolution, especially after Darwin's then controversial theories on the evolution of species.

Once it became obvious that blood was not a simple fluid, but contained corpuscles, questions arose about their origin, role and renewal. With the improvement of the microscope and the development of stains it became possible to distinguish between different types of corpuscles, and first attempts at categorisation were made.

Initially the preoccupation with the cells was with their pattern of differentiation, and the approach was to obtain more and more precise definitions of cellular categories. Red blood corpuscles were, as earlier mentioned, observed by Leeuwenhook, and colourless or white blood corpuscles by Hewson and others. Ehrlich succeeded in classifying and sub-classifying the white blood corpuscles with the help of his stains, followed by many others. But this posed further questions about the origin and the site of origin of these cells.

Speculations on these questions persisted for a long time. Hewson thought the origin to be in the lymphatic tissue, and Neumann suggested the bone marrow as the location of haematopoiesis in the adult. Blood formation was known to shift from the yolk sac to the foetal liver and thence, postnatally, to the bone marrow, and Heuck proposed that in certain pathological conditions the adult

liver could again become the site of haematopoiesis. Although the location of the blood forming process was defined eventually, questions about the progenitor cells involved remained unanswered until the middle of this century.

Thomas (1974) cites Virchow *omnis cellula e cellula* in his introduction to a chapter on the production of new living cells. As a possible candidate for an ancestral cell of the granulocytic line Ehrlich had pointed out the "Myelozyt". This was probably the first attempt to suggest a hierarchical cellular order which had to lead somehow to a stem. It became possible to study and identify morphologically lineages of granulocytes and erythroblasts down to the myeloblast and proerythroblast respectively, and observe changes of the normal ranges in these compartments under pathological conditions. Earlier stages of megakaryocytes and their end product, the platelets, were identified clearly. It was more difficult to understand the development of monocytes and macrophages. However, the lymphocytes proved a greater problem, as microscopically detectable signs of a maturation process were non-existent and differences in sizes almost impossible to interpret in the early days of cellular haematology.

Ehrlich had earlier already proposed that there was a different non-myeloid route of development for the lymphocytes. A long-drawn-out argument over four decades involving eminent scientists like Maximow, Naegeli, Pappenheim and Türk, ensued about whether the lymphoid and myeloid cells shared a common parental cell, a cell capable of producing myeloid and lymphoid progeny. Ehrlich favoured a dualistic view whereas Maximow and others believed that both cell types shared one monophyletic route to a totipotential stem cell, often termed as haemocytoblast, or, by Maximow, a lymphoid wandering cell.

Lajtha et al (1962) defined the stem cells as "cells that can maintain their own number and give rise to differentiated cells". Metcalf and Moore expanded on this by saying that "stem cells are defined as primitive haematopietic cells capable of extensive self-replication and endowed with a multiple differentiating capacity". The properties expected of a stem cell were well known, and they could not be detected by morphological means alone; they had to reveal their identity by functional measures. The only way to study their functions was by perturbing the haematopoietic compartment containing them, under controlled conditions, and specifically, by stem cell transplantation to provide an answer.

Three main cell populations form the haematopoietic system:
the pluripotent or multipotent stem cells, the committed progenitor or precursor cells and the mature cells

II The *in vivo* Isolation of Haematopoietic Cell Clones

In vivo and *in vitro* assays have been devised over the years to study the haematopoietic stem cell compartments in animals and humans.

Till and McCulloch (1961) pioneered experiments in which lethally X-irradiated mice were treated by transplantation of normal haematopoietic cell suspensions. These suspensions were capable of repopulating and thus restoring haematopoietic and lymphoid tissues. The mice survived and on biopsy ten days later revealed macroscopic nodules on the spleen surface. The nodules, or colonies, the number of which depended upon the cell dose injected, contained more than a million cells and were

frequently composed of granulocytes or mixed cell types, occasionally of megakaryocytes and eosinophils, but mainly of erythroblasts. This variability would suggest that the transplanted cell suspensions contained pluripotent stem cells, capable of giving rise to any of these progeny lines. In effect, these colonies represented clones, isolated *in vivo* .

Cells obtained from single colonies and re-transplanted into lethally irradiated recipients would give rise to similar types of colonies and thus demonstrate that the single cell which established the initial colony was capable of producing a variety of new nodules in another spleen. (Thomas 1974) Until recently it was thought that the colony-forming unit in the spleen (CFU-S) was the pluripotent haematopoietic stem cell.

However, recent experiments by Ploemacher et al (1989) suggest that the CFU-S might not be regarded as the pluripotent haematopoietic stem cell. It was found that spleen colonies taken 12 days after transplantation failed to maintain haematopoiesis *in vitro* , nor did they produce further CFU-S-12 or provide radioprotection in lethally irradiated recipients. Ploemacher was able to distinguish between two types of CFU-S by using rhodamine-123, a fluorescent, supravital, cationic dye with an affinity for mitochondrial membranes. Quiescent stem cells with hardly any mitochondrial activity do not take up rhodamine-123 and hence appear not as intensely stained as the cycling stem cells, the majority of which are responsible for colony formation. Separation on the basis of fluorescence intensity of these cells was possible with a fluorescence - activated cell sorter (FACS).

Further studies showed that CFU-S-12 with low rhodamine-123 staining possess higher radioprotective and marrow repopulating abilities. One practical and vital extension of these findings would be to isolate and increase

these crucial cells prior to grafting into depopulated bone marrow.

III The Regulation of Isolated Haematopoietic Cell Clones

Cell production depends upon the interaction of humoral regulators and the supportive stromal cellular network of the microenvironment on the stem cell compartment. Under normal conditions the majority of pluripotent haematopoietic stem cells, measured as spleen colony forming units, appears in a slowly proliferating state. (Becker, 1965) This state can alter, however, as soon as a demand for cell production occurs. Depleted, regenerating bone marrow after radiation or cytotoxic damage, or antigens, for example, exerts a stimulating effect upon the quiescent stem cells which, in order to compensate for cell loss, start proliferating rapidly by entering the mitotic cycle to synthesize DNA . (Lahiri, 1972) Once the demand is met inhibitory factors will step in to prevent overproduction of haematopoietic cells and to maintain remaining stem cells at their low rate of proliferation.

An inhibitor and stimulator have been isolated and semi-purified from the supernatant of incubated bone marrow cells by fractionated ultrafiltration on Amicon Diaflo membranes. The inhibitor (Lord, 1976) is found in the molecular weight range of 50 000 to 100 000 daltons and the stimulator (Lord, 1977) in the 30 000 to 50 000 dalton fraction.

A factor produced by foetal liver in its haematopoietic phase during gestation also shows stimulatory properties.

Wright et al (1980, 1982) described the characteristics of the inhibitor and stimulator producing cells after density

fractionation experiments. Both series of experiments pointed to different types of mononuclear phagocytes as the source of these regulators. These phagocytes, or macrophages, form part of a cellular support network, a microenvironment, which is essential for haematopoietic cell growth.

Wdzieczak-Bakala et al (1984) characterised and purified a CFU-S proliferation inhibitor. The molecules responsible are tetrapeptides, which are not species-specific and can now be produced synthetically. (Lenfant, 1989)

Frindel and Guigon (1977) postulated the idea of administering stem cell inhibitor to patients prior to cytotoxic therapy. The inhibitor would prevent stem cells from entering the mitotic cycle, and thus shield them from the effects of drugs aimed at a fast dividing cell population. On completion of treatment the unaffected stem cells could then start proliferating to compensate for the incurred cell loss.

IV Haematopoiesis *in vitro*

Sophisticated systems have also been devised to grow colonies of early haematopoietic cells *in vitro* . These cells are precursor cells which have differentiated from the pluripotent stem cell status into a committed state and hold an intermediate position between stem cells and morphologically recognisable blood cells.

With the addition of purified growth factors it became possible to grow precursors and progeny cells of all haematopoietic cell lines. These specific humoral regulators, glycoprotein inducers, or colony stimulating factors (CSF) are of different molecular weights and can be utilised, in their purified form, to differentiate primitive

cells into restricted different lineages of cells on agar plates or in culture flasks. Erythropoietin, for example, will stimulate the growth of erythroid and megakaryocytic colonies, whereas the interleukins act on myeloid stem cells or their progenitor cells. GM-CSF promotes granulocytic and monocytic colonies *in vitro* . By combining some of these growth factors a synergistic effect can be achieved, which reflects itself in very large colonies on agar plates. Recent data suggest that these might be derived from a stem cell that was not yet committed. Leukaemic stem cells, too, can be successfully grown in *in vitro* colonies and thus provide a tool for kinetic and therapeutic experiments.

The potential clinical applications of these haematopoietic growth factors are manifold: for example, normal haematopoiesis could be encouraged in patients with bone marrow failure from different causes, the radioprotective properties of Interleukin I could be applied and a higher yield of peripheral blood stem cells achieved, and finally leukaemic stem cells could be induced into cycle prior to chemotherapy or brought to differentiation.

Metcalf (1971) welcomes the *in vitro* colony assays: "...they have broken the tyranny of classification of cells on dubious morphological criteria."

V The Haematopoietic Microenvironment

It has been established that a small number of haematopoietic stem cells are found circulating in the blood. In bone marrow transplantation, by definition cells are transfused into the system's circulation. Although they will pass through various organs and tissues in which suitable humoral stimulating factors are produced, they will only undergo proliferation and self-renewal in a few

pre-destined tissues which offer a suitable cellular environment. This microenvironment alone can provide special local conditions necessary for inducing the quiescent stem cells to commence the haematopoietic progress. It is formed by a network of stromal cells which can occupy regions of different dimensions within the haematopoietic tissues.

Thomas (1961) appeared to be the first to point to 'the rôle of environmental factors' in determining the differentiation patterns of haematopoietic cells. In transplantation experiments published in 1971 he attributed the differences in cellular composition of various haematopoietic donor tissues, such as foetal liver, spleen and bone marrow, to the stromal microenvironment they derived from, and established that the medullary cavities were more conducive to the granulocytic lineage of cells whereas extra-medullary locations of haematopoiesis were more favourable for erythroblastic descendants of stem cells.

Histological and morphological studies of bone marrow by Lichtman (1981) revealed that poorly characterised cell populations formed the seed bed for haematopoiesis *in vivo* . These cells included adventitial reticular cells, endothelial cells, macrophages and fat cells.

After Dexter's (1977) development of a tissue culture system, continuing haematopoiesis could be achieved *in vitro* . Mouse bone marrow cells were cultured in the presence of medium with added horse serum, and after a few weeks, formed an adherent layer of stroma cells on the bottom of the flask. This layer was able to support and produce pluripotent stem cells and to propagate microenvironmental cells, which included epithelial and endothelial cells, reticular cells, fat cells, macrophages, and the so-called 'blanket cells'. These cells have been so

named because haematopoietic cells are found tucked under their cytoplasmic cover.

Gartner (1980) was able to culture human cells successfully using a modification of the Dexter system. He thus provided an important tool for the study of the human haematopoietic microenvironment under normal and pathological conditions.

D RECOGNITION AND CLASSIFICATION OF THE LEUKAEMIAS

I Historical Introduction

The first author to refer to white blood as 'albus sanguis', seems to have been Haller (1760), some 80 years before Virchow. Virchow (1845) himself cited him in his publication "Weisses Blut" which must now be regarded as classical. Haller's findings about blood "which had lost its colour so totally that it was compared to milk, chylus, mucus or pus" were instantly dismissed by Virchow as "totally useless because they lack microscopical investigations".

Reports of patients with enlarged spleens and unusual blood existed in France as early as the beginning of the eighteenth century. It was, however, Virchow who in 1847, two years after he had described a condition in a patient as 'Weisses Blut' , first used the term leukaemia. John Hughes Bennett had also observed a similar disease, and his published report preceded Virchow's by six weeks. Thirteen pages ahead of Bennett's article, in the same issue of the Edinburgh Medical Surgery Journal, Craigie (1845) gave an account of a similar case he had seen several years before.

Craigie never entered the battle between the two giants, Bennett and Virchow, who fought publicly, fiercely and sometimes acrimoniously about who was to be credited with the discovery of leukaemia. Both had recognized the significance of their observations, and a dispute ensued about priority, interpretation and the name of the new condition.

Bennett (1845) had investigated the blood of the diseased patients microscopically, and made very accurate drawings of the cells he had seen. After observing the excess of colourless corpuscles he had previously seen associated with pus, he called the condition 'Pyaemia' (which he later

changed into Leucocythaemia). (Bennett, 1852) From his findings he reached the conclusion that "death took place from the presence of purulent matter in the blood". He speculated further, after establishing that there was no evidence of inflammation, that "pus was formed universally within the vascular system independent of any local purulent collection from which it could be derived".

He was not certain for how long the blood had contained the huge numbers of colourless corpuscles, and he thought that they were formed towards the close of life. Bennett later acknowledged this erroneous theory and, as Parker (1990) points out, "this fact is relevant in assessing his primacy in the description of leukaemia".

Virchow (1845), on the other hand, interpreted the abundance of colourless corpuscles as a reversal of the normal ratio of the coloured and colourless blood corpuscles; ..."if I therefore talk about white blood ...I think indeed about the blood in which the proportions between the red and colourless (white, if in abundance) blood corpuscles are reversed without noticeable addition of foreign chemical or morphological elements". In referring to Hippocrates, who thought that nose bleeds were often caused by diseases of the spleen, Virchow proposed a new aetiological sequence in connection with the patient he was describing: spleen tumour, nose bleeds, white blood.

Like Bennett, Virchow (1847), too, saw no signs of inflammation in the patient, but in contrast to Bennett he did not refer to the blood as containing pus but called this condition ' Weisses Blut' (White Blood), which was translated into Greek, and hence became known as leukaemia. Some years later, in 1856, Virchow wrote: "Wherever the colourless blood corpuscles may come from, that is whence those that are so immensely prevalent in leukaemia must also be derived." Although he initially thought that blood cells derived from plasma he was

certain by now that they could only stem from other cells. (Gunz, 1980))

There was one point, however, Virchow and Bennett agreed upon, and that was that neither could put forward any ideas about the cause of the condition.

The colourless or white corpuscles which were described in leukaemia and also seen in pus were thought by many authors to be derived from the lymph nodes and from there released into the bloodstream. However, it did appear to Virchow that not all lymph corpuscles were of the same type. He was able to distinguish between two major cell forms; one showed granules and a multiformity of nuclei, the other was agranular with a round, smooth nucleus.

He had also observed differences in the clinical symptoms of leukaemias, one presented itself with an enlargement of the spleen in which the granular corpuscles prevailed, the other showed enlargement of lymph nodes where the round, smoothly nucleated lymph corpuscles were predominant. He suggested subdividing the leukaemias into two groups on this basis, namely the splenic or lineal leukaemia and the lymphatic leukaemia (Gunz, 1980). This distinction is still in use at the present time.

Neumann (1870) described a case of leukaemia in which he had noticed that the bone marrow was involved, as well as the spleen. He suggested that the white corpuscles might, after all, be formed within the bone marrow, and that the bone marrow rather than the spleen was the source of the abundance of the colourless cells. Thereafter many authors reported about 'splenomedullary leukaemias', a term which eventually was shortened into 'myeloid leukaemia' implying the bone marrow origin.

Heuck (1879) presented a case history of a patient with 'high grade' leukaemia in which he describes, apart from the usual changes in liver, spleen, lymph glands and blood, evidence of nucleated red corpuscles in spleen fluids and in particular in the liver fluids. He could not recall having seen this cell type in the adult liver before under these circumstances. He recognised the importance of his findings because he saw no evidence of these cells in blood vessels leading to the liver, and assumed that they must have been formed within, " an assumption which does not seem so far fetched if one considers which important role the liver plays in embryonic life, how numerous nucleated red blood cells are found in their blood at that time and further, how in a constant way and to a high degree it is affected in leukaemia."

The term 'acute' leukaemia was first used by Ebstein in 1888. Although authors before him had described the symptoms of this particular course of the disease with some degree of precision, the outlook for patients who were diagnosed thus was poor, and it meant for most of them a sentence of death within days rather than weeks. Though sufferers from chronic leukaemias were under a similar threat of death, the path of their disease was slower and often temporarily relieved by some kind of treatment or cure.

Ebstein had written about Pseudoleukaemia in the same publication. It was, however, Cohnheim (1865) who first coined the phrase, describing a single case of what appeared to be an acute form of leukaemia with splenic enlargement but without the ensuing increase in white blood corpuscles. This term became like a flag of convenience, incorporating all sorts of conditions with enlargements of spleen and/or lymph nodes, but with no increase in white corpuscles in the peripheral blood.

Dameshek (1951) proposed the term 'myeloproliferative syndrome' for a variety of pathological, haematological conditions which included polycythaemia vera, essential thrombocythaemia, myelofibrosis, erythroleukaemia and chronic granulocytic leukaemia. As many symptoms of these clinical disorders appeared to be overlapping and related with one another it was suggested that they all evolved from one defective haematopoietic stem cell.

As already mentioned in the introduction, Ehrlich's development of triacid dyes facilitated classification of haematopoietic cells in normal, leukaemic and other haematological conditions to a great extent and laid the foundation for a multiplicity of histochemical procedures and resulting discoveries.

The disease process of murine myeloid leukaemia was studied under standardized conditions, thus it was made possible to observe morphological changes within the haematopoietic cell compartments.

II The Induction of Leukaemia by Ionizing Radiation

The first published report of a connection between X-irradiation and leukaemia was by Jacic (1911), a Viennese physician. He reported on the incidence of leukaemia amongst radiologists, who, in the early years of X-ray application were unaware of the dangers to themselves and therefore had used no protection. He believed that the effect of X-rays on blood was twofold, in that there was a direct destruction of cells in the irradiated organs as well as an indirect influence on the non-irradiated organs by a 'Roentgentoxin' causing a slow-down of cell formation. Further cases were described in American medical journals, mainly amongst radiologists who showed an eight-fold increase in the incidence of chronic myeloid leukaemia compared to physicians, but later too in patients who had received large doses of X-irradiation as a means of treatment for ankylosing spondylitis. (Muir, 1971)

The nuclear bombing of Hiroshima and Nagasaki provided more evidence of a relationship between ionizing radiation and resulting leukaemias and other cancers. Bizzozzero (1966) found that the increase in leukaemia cases began three years after exposure and peaked at six years, affecting mainly people who were at the point nearest to the explosion. Others who were at a distance of 2000m or more showed no higher incidence of the disease than unirradiated people. These findings clearly pointed to a dose-related effect on the incidence of leukaemia. If one considers, however, that altogether 'only' 250 victims out of a population of 183 000 had developed leukaemia one has to consider other factors responsible as well. Gunz (1980) refers to one of them as "personal susceptibility".

However, the established fact that ionizing radiation, and in particular, X-irradiation, was a causative agent of

leukaemia was exploited experimentally by exposing animals to X-irradiation. Upton (1958) was one of the first researchers to use irradiation of mice in order to induce myeloid and other leukaemias. He demonstrated a higher incidence of myeloid leukaemia in male mice and a raised rate of thymic lymphoma in females. These findings corresponded approximately to the dose given; a radiation dose of up to 3 Gy would yield myeloid leukaemias whereas doses from 3 to 4.5 Gy resulted in a higher incidence of thymic lymphoma. To explain the falling rate of myeloid leukaemia following increasing doses of X-irradiation Upton suggested that "this may conceivably have resulted from a differentially high radiosensitivity of leukemia-susceptible myelopoietic cells to killing by radiation so that after heavy irradiation there was a selective survival of leukemia-resistant stem cells".

Upton's results showed too that age played an important role in successful induction of leukaemias. His particular strain of mice (RF) was resistant to myeloid leukaemia in the neonatal phase of life, although there was a susceptibility to lymphomas. He compared it to the high incidence of lymphatic leukaemia in irradiated children and acute and chronic myeloid leukaemias in irradiated adults.

Major and Mole (1978) used male CBA/H mice and exposed them to a single dose of whole body irradiation to induce myeloid leukaemia. It had been established that 58% of the leukaemia cases in atomic bomb survivors were of the myeloid type (Ishimaru, 1966). Major and Mole (1978) confirmed that the optimum X-ray dose for inducing myeloid leukaemia was between 1.5 and 3 Gy. The first leukaemic mice appeared at 8 to 10 months after exposure, with new cases arising in a more or less constant manner until the mice had reached the end of their natural survival time at about 30 months. (Mole, 1983) In all, 21% of all irradiated mice developed a myeloid leukaemia after

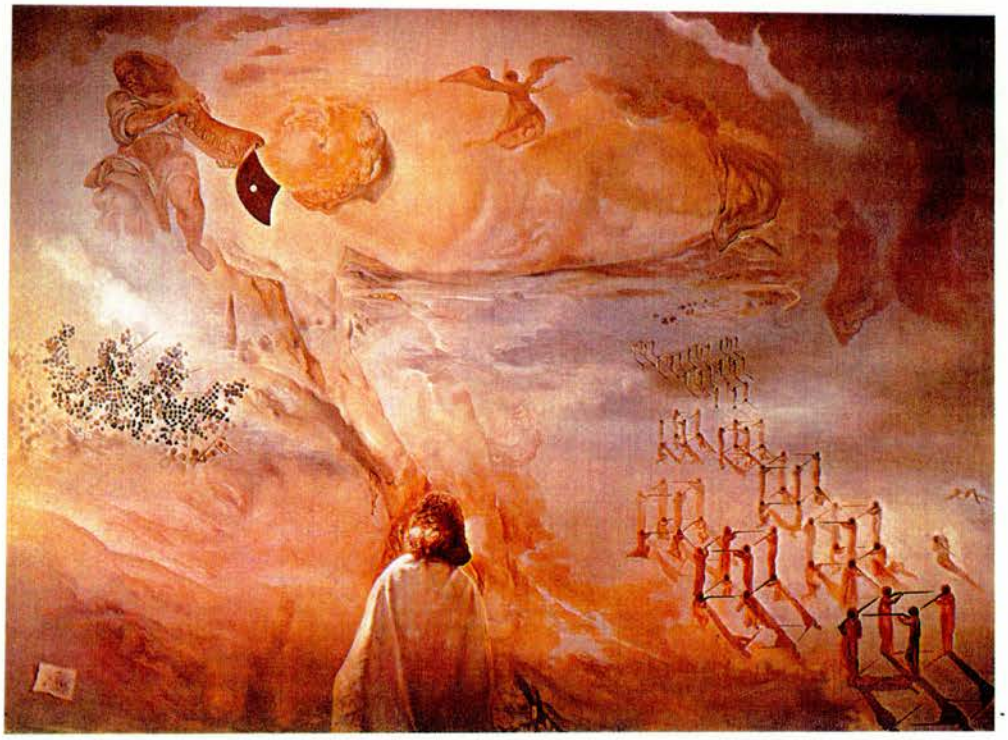
3 Gy of whole body X-irradiation. Contrary to Upton, whose mice showed a spontaneous leukaemia rate of between 2 and 6% in the unirradiated controls , there were no cases of leukaemia in Mole's control groups.

The expected higher incidence of myeloid leukaemia with increased X-irradiation doses of 4 to 6 Gy did not occur, which confirmed Upton's findings. Mole was also able to demonstrate an increase in what he referred to as "non-myeloid leukaemias" at higher doses up to 8 Gy. These leukaemias were mainly of a lymphoid nature. (1986) The difference in myeloid and non-myeloid leukaemogenesis did not fit in at all with the orthodox thinking that "initiation is a stable state and must be followed by multiple events over a period of time before cells express fully malignant behaviour". The main conclusion Mole would draw was "if specific cellular interactions are required to induce malignant disease, then these interactions must be basically different for these two categories of leukaemia and, by extension, that different categories of solid tumours would also have their specific pathogenesis".

SALVADOR DALI (1963)
Galacidalacidesoxyribonucleidacid

SALVADOR DALI (1963)
Galacidalacidesoxyribonucleidacid
Oil on Canvas
New England Merchants National Bank
Boston (Massachusetts)

(The molecular structures that were to appear in a number of his works are in this case those of desoxyribonucleic acid (DNA).



III Observations on Induced Myeloid Leukaemias

1.) Introduction

Apart from the differential counts of blood, bone marrow and spleen within the same animal, the findings of which are shown in the following results section, emphasis was put especially on one aspect of the kinetic state of each cell type and of the granulocytic group as a whole.

In nucleic acids the bases adenine, guanine and cytosine are present both in RNA and DNA but thymine in DNA alone. This was exploited in the autoradiographic labelling technique. (Rogers, 1967) Synthetically produced tritiated thymidine (3HTdR), the nucleoside of thymine, is taken up rapidly by sites within cells synthesizing DNA prior to mitosis. (S-phase) Thus cell suspensions incubated for a given amount of time with 3HTdR will reveal their percentage of labelling, the labelling Index (L.I.) at the time of fixing to a microscopical slide. The labelling itself is made visible through a photographic process where the beta-particles emitted by the 3HTdR react with silver bromide crystals on an emulsion applied to the slides. This process occurs within a certain period of time and in darkness, whereafter the slides are developed and fixed like photographic prints and stained to visualize the cells. The blackened silver grains are superimposed above the nucleus at the location where the DNA molecules had incorporated the 3HTdR.

The labelling index is taken as the fraction of labelled cells expressed as a percentage of the total population counted. On its own the labelling index is only a rough guide to the proliferative activity of cell populations, as it reflects the number of DNA synthesizing cells during the time the

different primary leukaemias and their morphological patterns together with the corresponding labelling indices, a diversity of results in spleen and bone marrow cell populations within the same leukaemic animal becomes apparent.

2.) Materials and Methods

Primary myeloid leukaemias were induced by exposing adult male inbred CBA/H mice to a whole-body X-irradiation dose of 3Gy at 250kVp, 14mA, 0.5mm Cu at a dose-rate of 0.8Gy per minute. Mice were checked regularly for signs of pallor, weight loss and ruffling of the fur. When these symptoms occurred the animal was killed by ether vapour and the thoracic cavity opened immediately. After incision of the heart, blood was either removed by Pasteur pipette and placed into heparinized tubes or collected directly into several heparinised Benjamin Haematokrit tubes which were emptied into heparinised tubes. Blood films were made with unheparinized blood. Several nucleated cell counts were performed per sample in a Neubauer haemocytometer. Packed cell volume (PCV) and haemoglobin values were determined, the latter by the HemoCue method (Aktiebolaget Leo Diagnostics, Helsingborg Sweden).

The spleen was removed, cleaned of fatty tissue, weighed and put into Fischers medium containing 10% of horse serum for optimal cytology. The spleen was then gently broken up by aspirating it a few times in and out of a 1ml syringe. A single cell suspension was obtained by syringing spleen cells through a No 25 gauge needle.

The femur was excised and scraped free of soft tissue. After removing both ends with a scalpel, the bone-marrow was flushed out of the diaphysis with 2ml of Fischers medium from a syringe with a No 23 gauge needle. The marrow cells were then aspirated through a No 25 gauge needle to give a single cell suspension. Using a Coulter Counter Model B, a nucleated cell count was performed to assess total femoral cellularity.

Cytospins were made from both cell suspensions using a Shandon Cytospin. Air-dried blood, spleen and bone-

marrow slides were fixed in methanol and stained using routine Jenner/Giemsa staining procedure.

Differential counts were performed on blood, bone-marrow and spleen cells. On a blood of normal or moderately increased leucocytic cellularity 100 cells were identified. If there was a large increase, however, 500 consecutive cells were scored, as were in the corresponding spleen and bone-marrow samples. Autoradiographic slides were counted in a similar manner with labelled and unlabelled cell types recorded separately to establish the number of haematopoietic cells in DNA synthesis at their different stages of proliferation.

Labelling Technique

Observing the required precautions in dealing with radioactive substances, 2ml of bone marrow and spleen single cell suspensions were labelled with 150KBq tritiated thymidine (Methyl-3 tritiated thymidine/3HTdR, specific activity 185GBq/mmol, Amersham International plc) and incubated in a water bath at 37 C. After 1 hour the cells were washed twice with serum-free Fischers medium by centrifuging at 800rpm for 10 minutes and replacing the supernatant. Pellets were reconstituted with Fischers, containing 10% horse serum, to a cellularity of about 10^6 cells per ml. Cytospin buckets loaded with alcohol-cleaned slides were filled with 0.4ml of the cell suspensions, and the Cytospin was operated as routinely at 800rpm for 10 minutes. Air-dried slides were fixed in fresh methanol for 15 minutes and kept dust free for the following procedure.

In a darkroom with Ilford 902 light brown safelight filter in a lamp fitted with a 15 Watt bulb, K2 Emulsion in gel form (Ilford Nuclear Research) was dissolved in a 50ml measuring cylinder in a waterbath at 45 C. The amount varied with the number of slides, in most cases a final diluted volume of 40 to 50ml was sufficient. After about

20 minutes, when the emulsion had liquefied, an equal volume of warmed distilled water containing 1% Glycerol was added and mixed with the emulsion by pouring it back and forth into a clean staining jar. To remove air bubbles which might have resulted thus, the staining jar containing the emulsion was put back into the waterbath for another 15 minutes. (It is advisable to place the jar into a glass beaker with warm water when working at the bench.)

The slides were dipped once, for 1 second, into the emulsion, the reverse side wiped clean with a tissue, and put upright on tissue paper against a wall for drying. After about 4 hours in the dark the emulsion had dried and the slides were transferred to a slidebox with inner sides lined with corrugated paper to keep them upright and separate from each other. A small container of silica gel was added to prevent moisture damaging the emulsion. The box was then sealed with tape and put into a small black polyethylene bag, sealed once more and put into another black bag to prevent any light contamination. Slides so wrapped were then kept in a refrigerator at 4 C for 4 days of exposure.

After this time slides were removed from the refrigerator to regain room temperature. The optimal temperature for Kodak D19 developer was at 20 C. Kodak Acid Fix (Hypo) at a dilution of 1+4 and tap water were brought to room temperature. The slides were developed for 3.5 minutes, rinsed for 30 seconds in distilled water and put into fixative for 5 minutes, by which time the emulsion should have been cleared from the slides. After gently rinsing for 20 minutes in running tap water, they were stained according to routine Jenner/Giemsa technique. Once dry, coverslips were mounted with Euparal.

The labelling index (L.I.) was determined by performing a differential count on about 500 consecutive cells and by recording the cells with a grain count of more than five

above the site of the nucleus in microscopic fields which were almost free from background grain contamination. The background count was uniformly low in all the processed slide preparations in our experiments, however, should there be an increased grain count, the threshold value for a cell classified a labelled should rise proportionally.

For the present studies only primary leukaemias were investigated. From a total of forty radiation-induced myeloid leukaemias fifteen primaries (SA7 - SA26) provided material for autoradiographic studies of spleen and bone-marrow cells.

3.) Results

In all, fifteen primary, radiation-induced myeloid leukaemias were used. Biopsies on leukaemic animals showed considerable differences in clinical and haematological findings. Body weight, spleen weight, haemoglobin, packed cell volume values (PCV), number of nucleated cells in peripheral blood and femoral bone marrow as well as differential cell counts on blood films, bone marrow and spleen cytospins all varied to a great extent. In comparison, however, with averaged values of normal mice, differences are detectable in all these compartments. Table 1 shows pooled results of normal and leukaemic mice.

The body weight was reduced in most cases and an increase in spleen weight was always taken as an indicator for established leukaemia. Animals with normal spleen weights were discarded. As expected, haemoglobin and PCV were reduced, and in most cases there was a sevenfold or higher increase in nucleated peripheral blood cells. Total femoral cellularity ranged from hypocellular to almost normal.

The main emphasis, however, was upon blood, bone marrow and spleen cell morphology. The cell distribution in conventional differential counts displayed typical trends for myeloproliferate disorders.

Table 2 shows the normal and leukaemic blood counts with an increase in myeloid cells above the normal range and incidences of blast cells up to 25% in the Primary Seven leukaemia (SA7). Low percentages of erythroblasts were observed in two cases (SA7 and SA12). Percentages of lymphocytes were reduced in eleven out of fifteen animals. There were no increases in monocytes or other cell types.

The trends in leukaemic bone marrow counts (Table 3) were more clearly defined compared to the normal ranges, with a considerable increase of myeloid cells in all but one case (SA7). The same leukaemia showed a particularly high blast count of 42%, although none of the other primaries rose above the 10% mark in this category. The erythroblastic compartment was suppressed throughout, with eleven leukaemias displaying only up to 1% erythroblasts. The lymphocytes were consistently below the normal 16% range, sometimes even lower than 1%. No dramatic changes were observed in the other remaining cell groups.

The spleen counts (Table 4) provided the definite proof of myeloid leukaemia in comparison with the normal mice. The 3% myeloid count in the normal murine spleen was dramatically multiplied more than twentyfold in most cases. The SA7 was the exception with a four fold increase only, but again this particular leukaemia displayed the highest blast count (33%), whereas the remaining blast counts ranged from 1.4% to 12%. Erythroblasts did not rise above the 3.7% mark. The lymphocytes decreased from 66% in the normal animal to 6.7%, with most of the other leukaemias in the 11% to 20% range. As in the previous tissues, other cell types did not seem to show any distinct variations.

The leukaemic picture intensified after computing the above differential blood, bone marrow and spleen counts according to FAB guidelines. This involved counting granulocytes and monocytes only, thus excluding erythroblasts, lymphocytes and others. In comparing normal spleen results, where an average 70% lymphocytic content was omitted, the relatively high percentages in the more mature neutrophilic compartments are misleading.

The blood films (Table 5) showed a considerable shift to the left with generalized blast cells ranging from 0 - 43%. In all leukaemias there was evidence of differentiation into the promyelocytic, myelocytic and metamyelocytic categories, together with the expected rise of these cell types. Varied degrees of maturation occurred towards the band and segmented neutrophil compartments.

Differential bone marrow counts (Table 6) reflected the same pattern as the blood films, with one primary leukaemia, the SA7, found to have 58% of generalized blast cells. Although some blast counts were identical to, or even lower than normal bone marrow values, the promyelocytic and myelocytic compartments provided conclusive evidence of myeloproliferative disorder. Metamyelocytes and band cells were greatly reduced throughout, with the exception of SA25, and proliferation into the final polymorphic stage occurred in all but one of the leukaemias presented here, with raised percentage values in some instances.

The spleen (Table 7), as before, gave the clearest indication and proof of myeloid leukaemia. Apart from the SA7, with a generalized blast count of 74%, there were only seven others where the values were higher than the normal 8.2%. As was the case in the bone marrow, the promyelocytes and myelocytes formed the largest group, with almost dramatic increases. Later stages of neutrophilic development cannot be compared here because of the distorted number in the normal spleens due to the FAB computation. Evidence of mature granulocytes, however, can be found in all spleens and is most certainly caused by contamination with peripheral blood.

Tables No 8 and 9 show cells in the state of DNA synthesis in bone marrow and spleen after one hour of

flash labelling with tritiated thymidine. The labelling index (L.I.) of each cell type capable of division had been determined. In the normal bone marrow almost half of generalised blasts and myelocytes and three quarters of promyelocytes seemed to be in S-phase (DNA synthesizing) after one hour of labelling. The L.I. of the entire granulocytic group, however, appears relatively low. This is probably due to the larger number of mature, undividing neutrophils in normal as compared to leukaemic animals.

The labelling indices of spleen and bone marrow within the same animal are compared in Table 10. About three quarters of all leukaemic animals displayed a higher L.I. in the spleen than in the corresponding bone marrow in the generalized blast, promyelocyte, myelocyte and metamyelocyte compartments. Nevertheless, it should be pointed out that since some of these compartments may contain small numbers of cells the precision of the L.I. measurement might be questionable. However, in the leukaemic animals, of course, the proportion of generalized blasts and promyelocytes is considerably increased in relation to the normal bone marrow.

With the exception of the SA25 all other leukaemias with an increased generalized blast count above the average 8.2% in the spleen had a raised L.I. over the same cell type in the matching bone marrow. All the other dividing cell groups expressed no regular pattern whatsoever in the tritiated thymidine uptake.

4.) Tables

TABLE 1: COMPARISON NORMAL / LEUKAEMIC MICE

	NORMAL MICE	LEUKAEMIC MICE
Body Weight (g)	27.00 ±0.20 (SE)	21.69 ±1.01
Spleen Weight (mg)	66.53 ±5.85	576.45 ±61.09
Haemoglobin (g/dl)	15.43 ±0.21	6.84±0.67
Packed Cell Volume (l/l)	0.43 ±0.0041	0.17 ±0.02
Nucleated Cells Blood (l)	$5.125 \times 10^9 \pm 0.033$	$3.175 \times 10^{10} \pm 5.62$
Nucleated Cells (Femur)	$1.96 \times 10^7 \pm 0.57$	$5.48 \times 10^6 \pm 0.90$

TABLE 2: NORMAL BLOOD (NBL) AND LEUKAEMIC BLOOD (SA7-26) DIFFERENTIAL COUNTS

%	NBL	±SE	SA 7	SA 8	SA10	SA11	SA12	SA13	SA14	SA15	SA17	SA18	SA19	SA21	SA24	SA25	SA26
GBL	0	0	25	16	2.0	0	2.0	11	1.0	4.0	1.0	0	2.0	2.0	13	0	0
MYL	23	3.2	28	49	46	60	71	24	60	37	79	43	51	57	39	35	32
MON	8.4	1.8	0.5	0	0	1.0	0	0	2.0	0	0	3.0	0	1.0	2.0	1.0	2.0
EBL	0	0	1.5	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0
LYM	50	2.8	23	18	29	30	6.0	38	30	45	15	49	32	25	37	45	38
DAM	5	1.2	8.0	6.0	15	5.0	18	17	2.0	11	2.0	3.0	11	10	2.0	5.0	8.0
UNC	0.4	0.2	5.0	5.0	1.0	4.0	1.0	3.0	5.0	1.0	2.0	2.0	4.0	2.0	5.0	3.0	4.0
OTH	0.6	0.6	4.0	6.0	1.0	0	0	5.0	0	0	1.0	0	0	0	0	0	6.0
TIS	13	1.8	5.5	0	6.0	0	1.0	2.0	0	2.0	0	0	0	3.0	2.0	11	10

GBL = Generalised Blasts, MYL = Myeloid Cells, MON = Monocytes, EBL = Erythroblasts, LYM = Lymphocytes, DAM = Damaged Cells, UNC = Unclassified Cells, OTH = Other Cells, TIS = Tissue Cells

TABLE 3: NORMAL BONE MARROW (NBM) AND LEUKAEMIC BONE MARROW (SA7-26) DIFFERENTIAL COUNTS

%	NBM	±SE	SA 7	SA 8	SA10	SA11	SA12	SA13	SA14	SA15	SA17	SA18	SA19	SA21	SA24	SA25	SA26
GBL	1.9	0.2	42	3.9	4.2	13	2.0	8.4	5.8	3.0	7.7	6.2	5.0	3.5	6.6	5.6	7.6
MYL	46	4.2	36	73	74	69	79	81	83	76	84	61	77	53	67	76	75
MON	2.0	0.3	0.2	0.4	0	0.2	0.2	0	0	2.0	0.4	1.9	3.0	0	2.6	2.2	1.1
EBL	20	1.7	0	0	0	0	0.2	0.4	0	0.2	0	3.6	1.7	11	0.7	2.2	0.8
LYM	16	1.3	10	1.2	0.4	5.0	4.4	0.8	2.6	6.4	1.4	14	6.3	3.9	2.6	5.6	7.3
DAM	2.2	0.3	1.8	11	17	3.2	3.6	4.1	3.6	5.6	3.4	8.1	3.7	18	13	3.7	6.5
UNC	0.4	0.1	1.6	1.4	2.0	1.6	1.0	1.2	1.6	1.2	0.4	2.6	1.7	2.6	1.0	0.9	0
OTH	1.0	0.2	6.0	3.7	0.8	0.6	3.4	1.2	0.8	1.0	0.6	0.5	1.0	0.3	1.6	1.2	0.6
TIS	9.7	2.1	3.0	6.1	1.8	7.5	6.4	3.5	3.0	5.2	2.6	2.4	1.0	7.1	4.6	2.8	1.1

GBL = Generalised Blasts, MYL = Myeloid Cells, MON = Monocytes, EBL = Erythroblasts, LYM = Lymphocytes, DAM = Damaged Cells, UNC = Unclassified Cells, OTH = Other Cells, TIS = Tissue Cells

TABLE 4: NORMAL SPLEEN (NSP) AND LEUKAEMIC SPLEEN (SA7-26) DIFFERENTIAL COUNTS

%	NSP	±SE	SA 7	SA 8	SA10	SA11	SA12	SA13	SA14	SA15	SA17	SA18	SA19	SA21	SA24	SA25	SA26
GBL	0.5	0.2	33	12	6.6	11	1.6	8.6	5.2	2.8	2.0	5.2	10	9.3	7.3	7.8	1.4
MYL	3.0	0.2	12	58	77	58	69	66	74	53	63	61	68	50	69	57	80
MON	2.6	0.7	0.2	0	0.6	0.6	0	0	0.6	0.8	0.6	1.8	0.6	0	2.3	3.2	1.8
EBL	1.0	0.4	0.4	0	1.0	0	2.2	0.8	2.2	2.6	0	3.9	1.2	3.4	3.3	3.7	2.1
LYM	66	3.6	16	7.3	4.2	11	14	12	8.8	24	17	3.9	15	19	9.2	24	6.7
DAM	1.9	0.6	2.2	8.2	3.4	6.5	5.3	5.4	1.6	10	7.3	15	4.0	4.5	6.9	3.2	4.9
UNC	0.5	0.1	1.8	1.1	2.0	0.8	1.6	2.0	3.2	1.6	1.2	0.5	0.9	0.8	0	0.6	0.7
OTH	0.9	0.1	7.6	4.3	1.6	0.8	1.2	0.6	1.6	0.4	0.4	1.3	0.3	0.5	0	1.1	1.4
TIS	24	3.1	26	9.5	3.2	12	5.5	4.6	3.2	5.2	8.7	8.0	1.2	11	2.0	0.9	1.4

GBL = Generalised Blasts, MYL = Myeloid Cells, MON = Monocytes, EBL = Erythroblasts, LYM = Lymphocytes, DAM = Damaged Cells, UNC = Unclassified Cells, OTH = Other Cells, TIS = Tissue Cells

TABLE 5: NORMAL BLOOD (NBL) AND LEUKAEMIC BLOOD (SA7-26) DIFFERENTIAL COUNTS (FAB - GUIDELINES)

	NBL	(%) ±SE	SA 7	SA 8	SA10	SA11	SA12	SA13	SA14	SA15	SA17	SA18	SA19	SA21	SA24	SA25	SA26
GBL	0		43	25	4.2	0	2.7	31	1.6	9.8	1.3	0	3.8	3.3	24	0	0
PRO	0		9	44	10	1.7	8.2	29	3.2	15	7.6	11	5.7	12	17	2.8	12
MYE	0		6.8	9.4	21	13	25	29	7.9	15	24	15	34	25	20	10	41
MET	0		2.3	6.3	33	25	30	8.6	9.5	20	18	30	15	33	19	19	32
BAN	19	2.27	6.8	3.1	17	37	25	0	22	15	8.9	20	19	20	31	33	8.8
SEG	49.25	3.82	32	13	15	22	9.6	2.9	52	27	41	17	23	5	1.9	25	0
EOSIN.	8.3	4.28	0	0	0	0	0	0	0	0	0	0	0	0	1.9	0	0
MONOC.	23.73	6.11	0	0	0	1.7	0	0	3.2	0	0	6.5	0	1.7	3.7	2.8	5.9

GBL = Generalised Blasts, PRO = Promyelocytes, MYE = Myelocytes, MET = Metamyelocytes, BAN = Band Cells, SEG = Segmented Cells, EOS = Eosinophils, MON = Monocytes

TABLE 6: NORMAL (NBM) AND LEUKAEMIC BONE MARROW (SA7-26) DIFFERENTIAL COUNTS (FAB - GUIDELINES)

	NBM	(%) ±SE	SA 7	SA 8	SA10	SA11	SA12	SA13	SA14	SA15	SA17	SA18	SA19	SA21	SA24	SA25	SA26
GBL	5.08	0.59	54	5.1	5.4	16	2.5	9.5	6.6	3.7	8.5	8.9	5.9	6.3	8.7	6.7	9.1
PRO	4.47	0.89	4.1	27	31	37	14	38	31	34	25	32	35	54	38	18	31
MYE	6.47	0.9	3.9	23	38	20	25	39	32	32	29	27	37	24	29	23	37
MET	43.33	2.02	6.4	28	22	17	30	11	14	23	26	21	14	14	15	18	19
BAN	24.56	0.94	18	12	1.8	8.7	15	2.2	7.5	3.5	9.6	6.2	2.4	1.1	6.1	22	2.4
SEG	6.22	0.85	13	4.6	0.3	2.2	13	0.2	8.8	1.2	2.6	2.4	2	0	0.4	9.3	0.3
EOS	5.53	0.55	0.5	0	0.3	0	0	0	0.2	0	0	0	0	0	0.4	0.4	0
MON	4.49	0.89	0.3	0.5	0	0.2	0.3	0	0	2.5	0.4	2.7	3.5	0	3.5	2.6	1.3

GBL = Generalised Blasts, PRO = Promyelocytes, MYE = Myelocytes, MET = Metamyelocytes, BAN = Band Cells, SEG = Segmented Cells, EOS = Eosinophils, MON = Monocytes

TABLE 7: NORMAL (NSPL) AND LEUKAEMIC SPLEENS (SA7-26) DIFFERENTIAL COUNTS
(FAB - GUIDELINES)

	NSPL	(%) ±SE	SA 7	SA 8	SA10	SA11	SA12	SA13	SA14	SA15	SA17	SA18	SA19	SA21	SA24	SA25	SA26
GBL	8.18	2.73	74	17	7.9	16	2.2	12	6.6	5	3	7.6	13	16	9.2	12	1.7
PRO	0		7.1	46	20	38	14	49	17	38	9.4	24	37	43	33	25	28
MYE	0		4.9	22	24	25	15	32	15	33	26	42	25	22	25	21	40
MET	1.48	0.98	4.5	11	42	17	52	5.4	26	20	30	17	14	16	21	20	23
BAN	19.83	2.09	4.9	3.2	4.5	3.2	13	1.1	18	29	20	3.4	8.6	3.6	7.6	14	4.7
SEG	21.28	4.72	3.6	0.8	0.2	0.6	3.9	0.3	16	0	9.7	2.7	1.6	0.4	0.4	3.6	0
EOS	10	3.38	0.4	0	0	0	0	0	0	0	0.3	0	0	0	1.3	0.5	0
MON	39.12	7.54	0.4	0	0.7	0.9	0	0	0.8	1.4	0.9	2.7	0.8	0	2.9	5	2.1

GBL = Generalised Blasts, PRO = Promyelocytes, MYE = Myelocytes, MET = Metamyelocytes, BAN = Band Cells,
SEG = Segmented Cells, EOS = Eosinophils, MON = Monocytes

TABLE 8: LABELLING INDICES BONE MARROW (%)

	NBM	±SE	SA 7	SA 8	SA10	SA11	SA12	SA13	SA14	SA15	SA17	SA18	SA19	SA21	SA24	SA25	SA26
GBL	47	12.2	47	45	4.8	14	0	19	24	40	62	69	6.7	9.1	25	22	33
PRO	73	10.7	56	48	30	25	26	40	38	40	61	50	32	13	25	43	43
MYE	48	7.9	33	40	21	12	27	17	24	33	46	39	14	16	16	40	36
MET	11	2.2	0	9.9	32	16	34	16	31	22	27	3.3	8.6	8	14	8.3	33
TOT GR.	13	0.8	29	27	25	16.3	20	26	25	31	40	35	18.8	12.5	18.9	21	37

GBL = Generalised Blasts, PRO = Promyelocytes, MYE = Myelocytes, MET = Metamyelocytes,
TOT GR = Total Granulocytes

TABLE 9: LABELLING INDICES SPLEEN (%)

	NSPL	SA 7	SA 8	SA10	SA11	SA12	SA13	SA14	SA15	SA17	SA18	SA19	SA21	SA24	SA25	SA26
GBL	N.A.	54	65	33	20	0	35	23	36	50	19	30	17	41	15	25
PRO	N.A.	75	45	48	30	52	42	44	45	58	47	41	36	55	31	53
MYE	N.A.	55	29	41	15	51	25	33	47	51	37	17	29	40	33	33
MET	N.A.	10	20	37	3.3	38	25	33	33	35	13	8.3	8.6	18	18	28
TOT.GRA	N.A.	49	40	38	19	39	33	23	41	31	32	25	26	37	21	36

GBL = Generalised Blasts, PRO = Promyelocytes, MYE = Myelocytes, MET = Metamyelocytes,
TOT GR = Total Granulocytes

TABLE 10: COMPARISON OF LABELLING INDICES OF SPLEEN AND BONE MARROW CELLS

(Differences expressed as Percentages)

SPL: BM	SA 7	SA 8	SA10	SA11	SA12	SA13	SA14	SA15	SA17	SA18	SA19	SA21	SA24	SA25	SA26
GBL	13	31	85	30	±0	46	-4.2	-10	-19	-72	78	46	39	-32	-24
PRO	25	-6.3	38	17	50	4.8	14	11	-4.9	-6	22	64	55	-28	19
MYE	40	-28	49	20	47	32	27	30	9.8	-5.1	18	45	60	-18	-8.3
MET	10	51	14	-79	11	36	6	33	20	75	-3.5	7.5	55	54	-15
TOT.GRAN	41	33	34	14	49	21	-8	24	-23	-8.6	25	14	25	±0	-2.7

GBL = Generalised Blasts, PRO = Promyelocytes, MYE = Myelocytes, MET = Metamyelocytes,
TOT.GR = Total Granulocytes

5.) Discussion

Biopsies on animals in their terminal stages of illness provided the first pointers towards myeloproliferative disorder with splenomegaly and occasional infiltration of the liver. Lymph nodes were only marginally enlarged in some instances. The increased nucleated cell count in the blood coincided with a reduction in haemoglobin and packed cell volume.

Most of the above results were based on morphological observations alone. Histochemical investigations on murine haematological material proved to be inconclusive. As there was hardly any neutrophilic granulation in the mature cells, and irregular granulation of promyelocytes and myelocytes, the possibility of confusing immature monocytes with early stages of granulocyte maturation cannot be excluded. Blast cells were consistently defined as such when - apart from the usual features - there were no granules at all displayed in the cytoplasm. These cells would correspond to Bennett's Type I blasts (Bennett, 1982) wheras the Type II blasts, containing granules, were classified as (early) promyelocytes.

The appearance of promyelocytes and myelocytes in the spleen which normally does not harbour these cells, was regarded as evidence of acute myeloid leukaemia. The spectrum of cell distribution in spleen and corresponding bone marrow of the same animal showed similar trends, although the pattern of the respective blood films differed in many aspects. This might have been overcome by using absolute numbers of the different cell types involved. However, this was omitted because - although absolute bone marrow cell numbers could be made available - it would nevertheless have been impracticable to try to establish absolute cell numbers in the spleens for comparison.

In all, blood, bone marrow, and - particularly - spleen differential counts gave a clear picture of myeloid leukaemia which was intensified when plotted according to FAB guidelines. Due to the uncertainty of possible involvement of early monocytic cells in some cases no attempts were made to categorize them in line with FAB recommendations, although most of the leukaemias presented here could be placed into the M2 category.

The labelling index (L.I.) provided some information about the kinetic status of each leukaemia. Although more than the flash label of one hour duration here applied is needed to get an insight into kinetic features of cell populations and (as in this case of leukaemic cell populations), it is nevertheless of great interest to compare these leukaemias and their different uptakes of tritiated thymidine with one another. As is evident from Tables 9 and 10 the incidence of labelled cells varied from one animal to the other. Indeed, the variation did not seem to follow any particular pattern and drawing conclusions or trying to interpret the differences within the cell compartments appeared fruitless. These findings seem to present a similar variability as the various types of leukaemia themselves. All that can be inferred is that if the L.I. is high the incidence of cells synthesizing DNA is high, which probably reflects a high rate of cell production.

The most interesting feature of this exercise, however, is revealed in Table 10. Over seventy percent of all granulocytic cells show a higher labelling index in the infiltrated spleen than in the bone marrow within one animal. It appears that the same leukaemic cell population displays different kinetic patterns within different haematopoietic tissues in the same mouse. Thomas (1971) pointed to the different patterns of haematopoietic activity in normal bone marrow, spleen and foetal liver cells and the post transplantational recovery pattern they display within medullary cavities and spleens of lethally X-

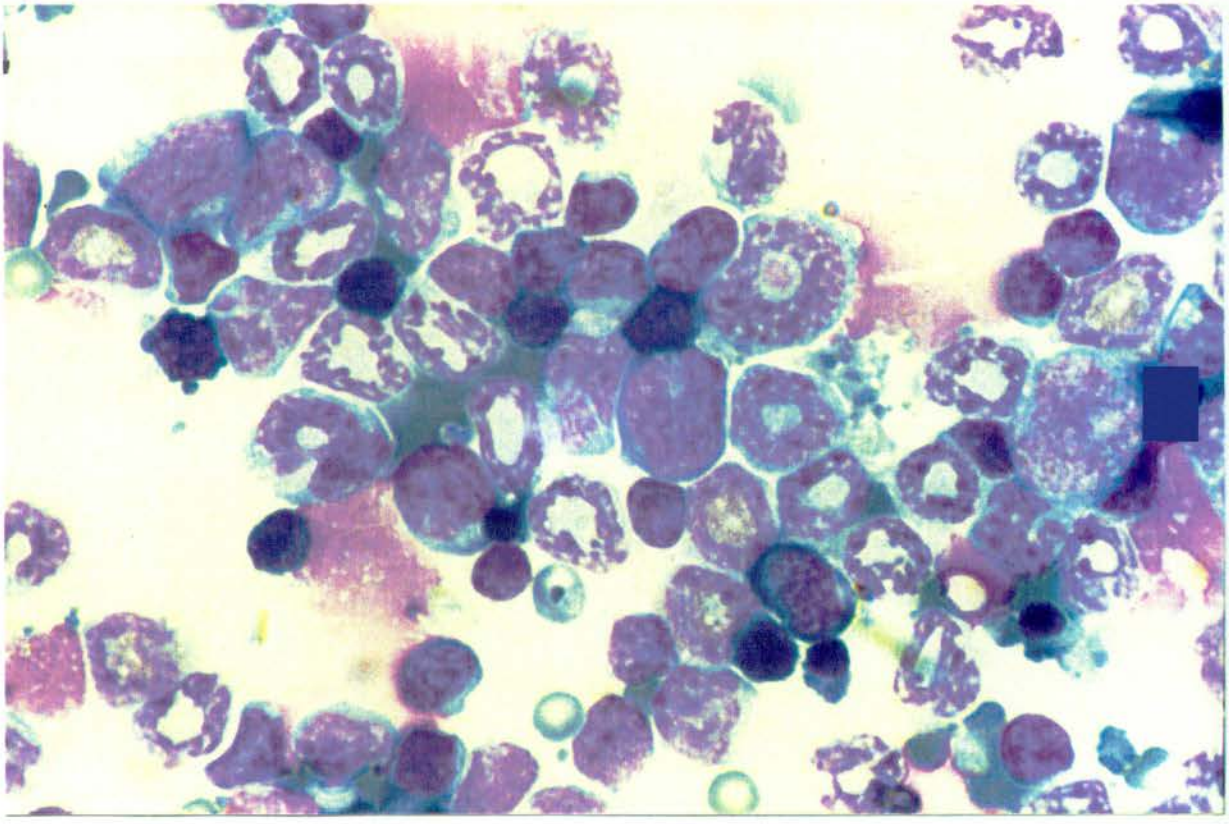
irradiated recipients. He concluded that the microenvironments of the donor tissues as well as that of the recipients are conducive to the predominance of different cell types.

It could also be possible that the various microenvironments might also influence the kinetic pattern of cellular proliferation. Baccarani (1978) investigated extramedullary haematopoiesis in chronic myeloid leukaemias and his findings illustrated a difference in cell composition, cell kinetic pattern, functional cell characters, and cell karyotype. He queries whether extramedullary haematopoiesis has different characters from bone marrow haematopoiesis and whether the abnormalities found in extramedullary tissues have any relevance to the course and prognosis of the disease. More experimental work might be indicated here.

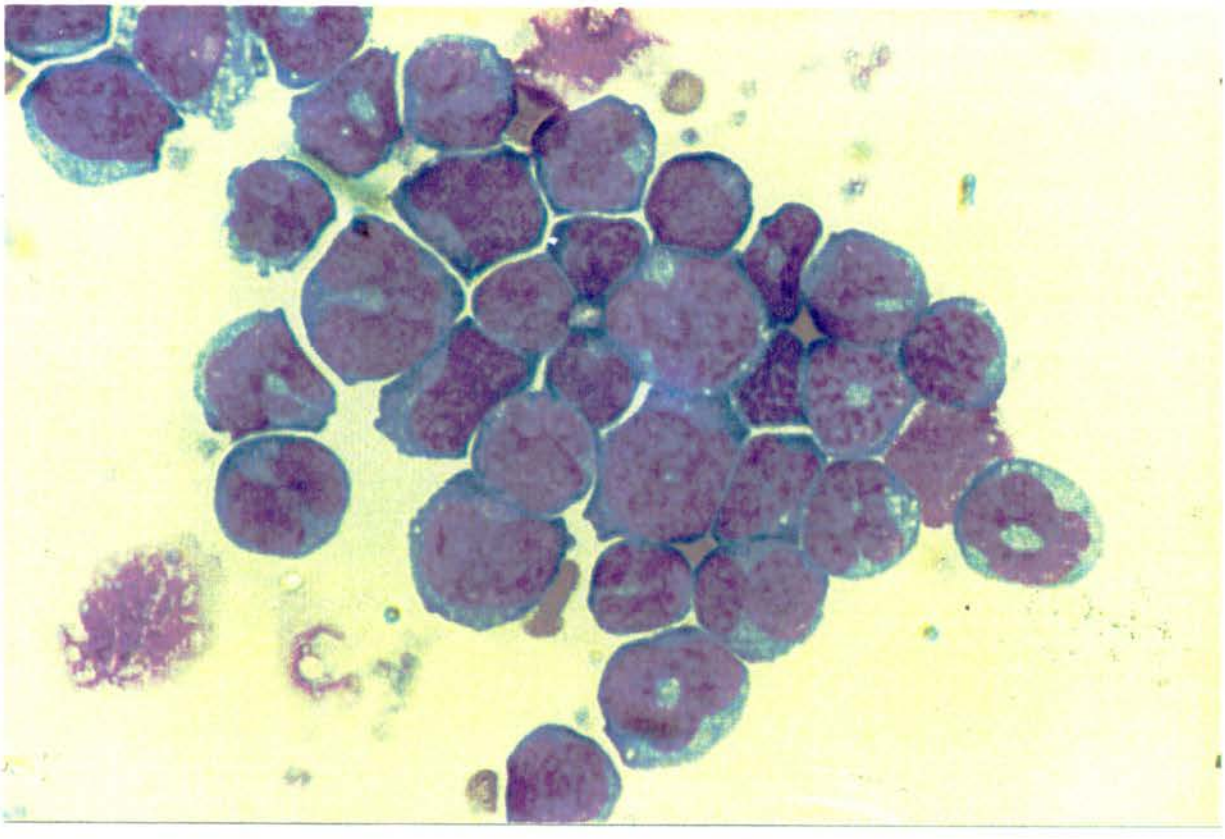
In conclusion it might be said that although there are now plenty of mechanistic investigations into the behavioural patterns of haematopoietic cells and their supporting tissues available, the descriptive disciplines of blood cell morphology are more important than ever now that more sophisticated techniques facilitate the definition of similar cell populations and allow their molecular pathology to be investigated.

ILLUSTRATIONS

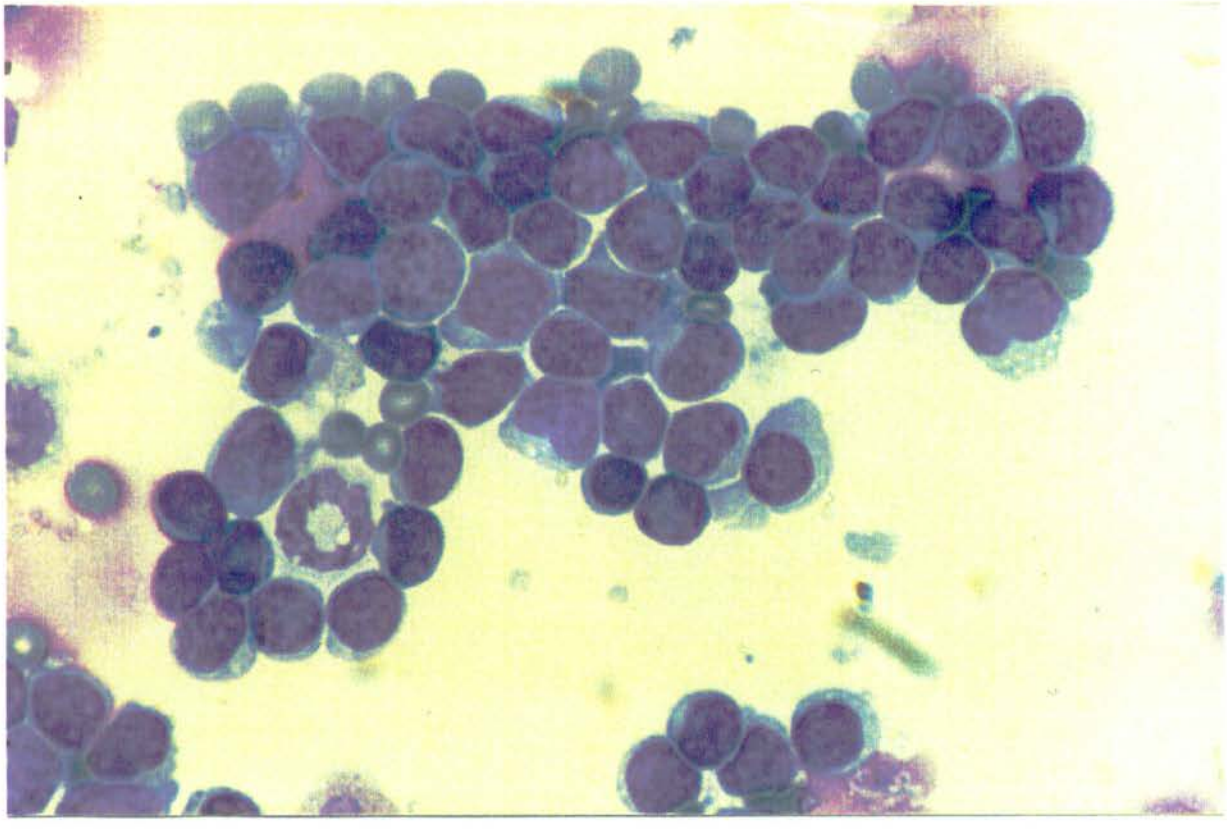
Normal Murine Bone Marrow (Cytospin Preparation)
(Magnification x 2000 Jenner/Giemsa Stain)



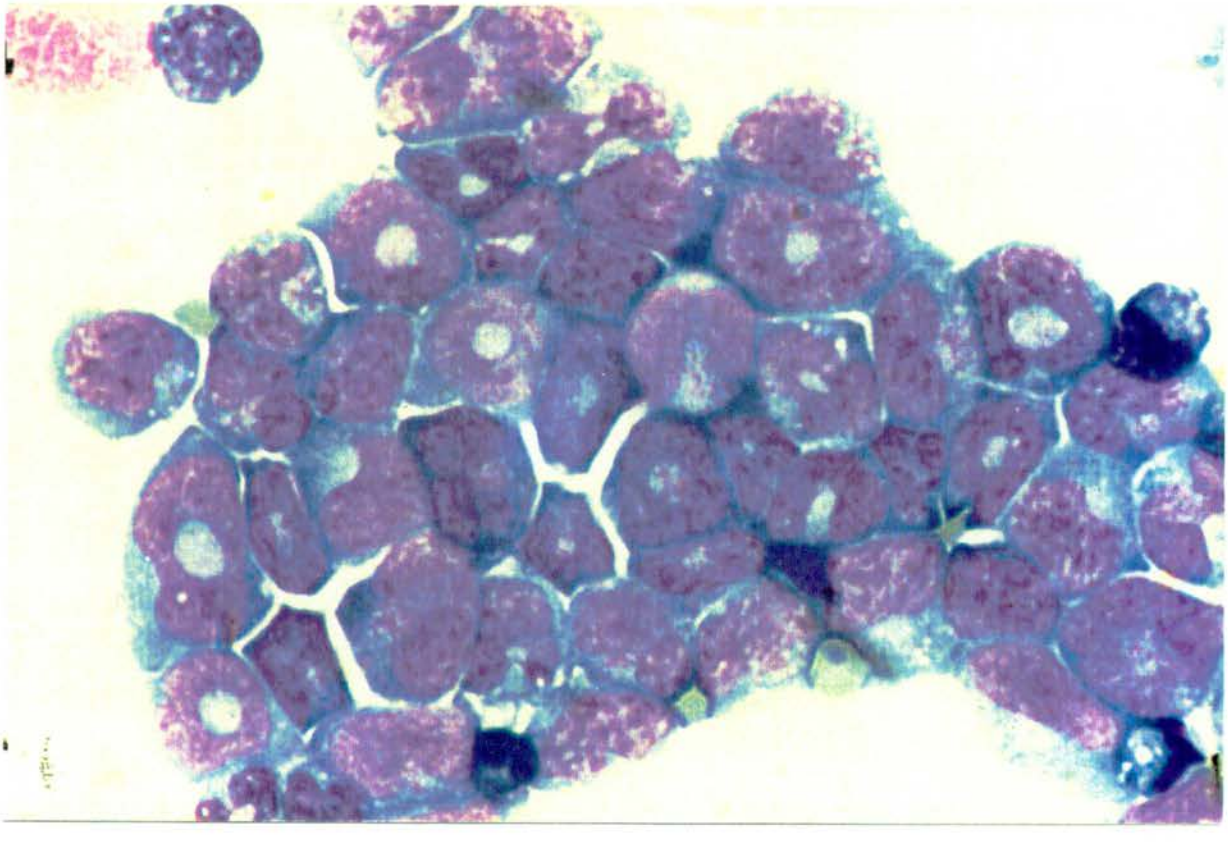
Leukaemic Murine Bone Marrow (Cytospin Preparation)
(Magnification x 2000 Jenner/Giemsa Stain)



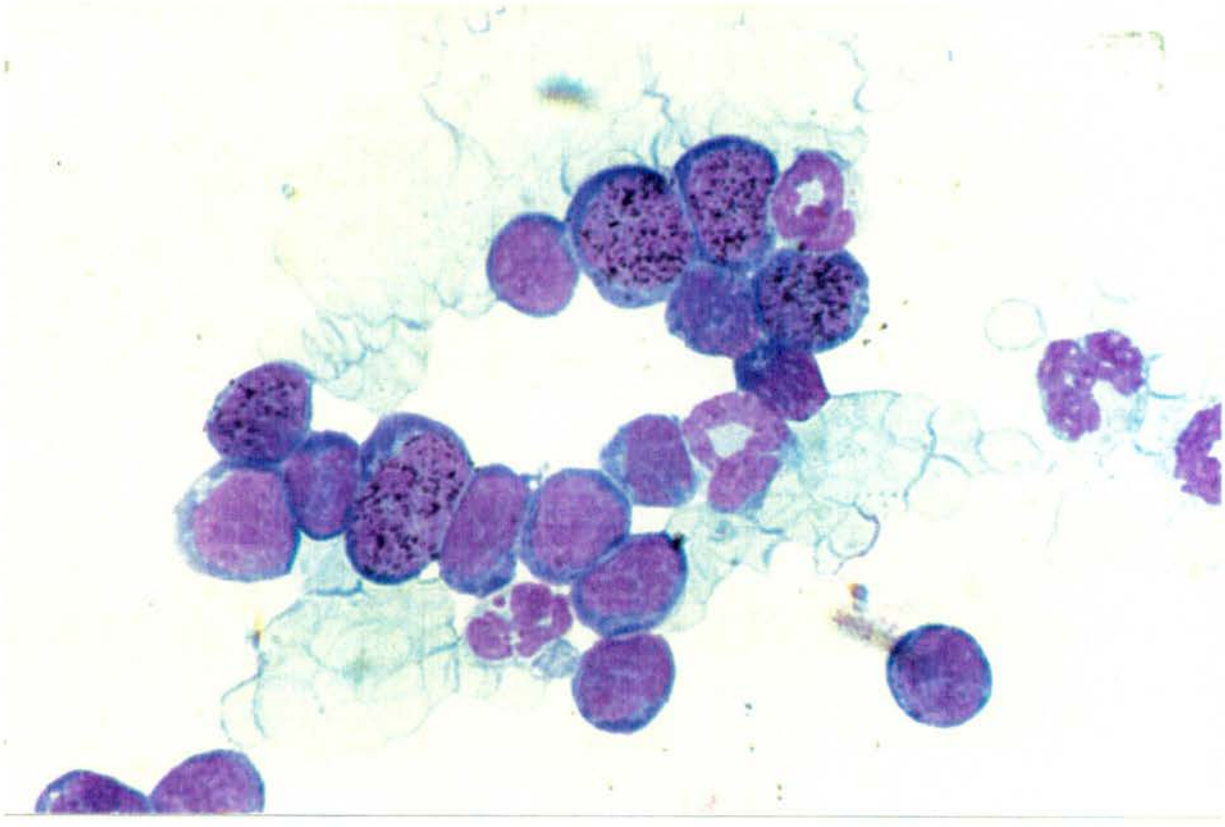
Normal Murine Spleen (Cytospin Preparation)
(Magnification x 2000 Jenner/Giemsa Stain)



Leukaemic Murine Spleen (Cytospin Preparation)
(Magnification x 2000 Jenner/Giemsa Stain)



Leukaemic Murine Bone Marrow (Cytospin Preparation)
Cells synthesizing DNA are labelled by Tritiated Thymidine
(Magnification x 2000 Jenner/Giemsa Stain)



Leukaemic Murine Spleen (Cytospin Preparation)
Cells synthesizing DNA are labelled by Tritiated Thymidine
(Magnification x 2000 Jenner/Giemsa Stain)