QUANTITATIVE CELLULAR HAEMATOLOGY

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AMERICAN LECTURE SERIES

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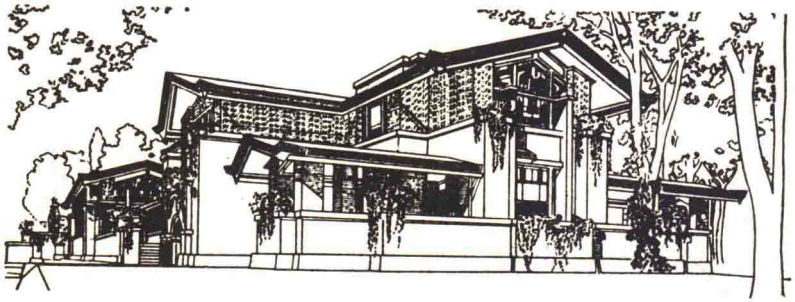

CHARLES C THOMAS · PUBLISHER

Springfield · Illinois · U.S.A.

CHARLES C THOMAS • PUBLISHER
BANNERSTONE HOUSE
301-327 East Lawrence Avenue, Springfield, Illinois, U.S.A.

Published simultaneously in the British Commonwealth of Nations by
BLACKWELL SCIENTIFIC PUBLICATIONS, LTD., OXFORD, ENGLAND

Published simultaneously in Canada by
THE RYERSON PRESS, TORONTO

Library of Congress Catalog Card Number: 60-12681

Printed in Cape Girardeau, Mo.
in the United States of America

Ehrlich, P. and Lazarus, A. (1898)

"Die physiologisch-experimentelle untersuchung der Knochenmarkfunctionen bereitet unüberwindliche Schwierigkeiten. Eine Ausschaltung des gesamten Knochenmarkes oder auch nur grösserer Partien desselben ist eine unmögliche Operation."

"The physiological and experimental investigation of the function of the bone marrow presents insuperable difficulties. The removal of all the bone marrow or even a large part of it is an impossible operation."

INTRODUCTION

THIS MONOGRAPH is an attempt to summarise the main trends in a series of haematological studies extending over a number of years. Much of the work would have been impossible without the enthusiastic co-operation of numerous colleagues whose names will be found throughout the text. It is with great pleasure that I place on record my warmest thanks for their invaluable collaboration.

The scope of the monograph is accurately described by its title. We have attempted to study the formation of blood cells by quantitative means. But in counting cells, it is essential to identify them accurately. Hence it has been important, especially in the case of the lymphocytes and the "blast" cells, to consider with some care the details of cellular morphology as an essential pre-requisite to reasonably accurate identification.

My first introduction to the problem took place many years ago when studying blood formation in fishes. Here, except for the spleen, and a rudimentary thymus, there is no separate lymphoid tissue. Blood formation occurs in the lympho-myeloid tissues, in which lymphoid and myeloid elements are freely intermingled. A spleen is present in fishes, but it too is essentially a lympho-myeloid organ, although its lymphoid tissue is beginning to form more discrete and well-defined masses. In a still more primitive vertebrate, such as the lamprey, there is not even a spleen. The lympho-myeloid tissue then is found immediately under the intestinal mucous membrane in the spiral valve, and among the tubules of the mesonephros. This primitive association of lympho-myeloid tissue with the kidney and intestine may possibly explain the persistence of chemical links between them long after, when they are no longer in juxtaposition.

As one ascends the vertebrate scale, to the mammalia, two major changes occur. There is a gradual development of a specific lymphoid tissue, both under the mucous membrane of the intestine, and in lymph nodes; at the same time the thymus is more in

evidence. While the lymphoid and myeloid tissues are dissociating, the myeloid tissues begin to occupy their final vertebrate domicile, the bone marrow, which is already beginning to develop in the amphibia. In mammals, the separation of lymphoid from myeloid tissues appears to be complete, and this curious phenomenon intrigued me greatly. Was the separation as complete as it appeared to be? Was the lymphoid complex merely the original lymphoid tissue which, for some reason or other, had separated from the myeloid, or was it a new development? If the former, why had the lymphoid tissues split off from the myeloid? And if they really had split off, was there still any functional connection between them?

Lymphoid tissue seemed in those days much more easily accessible than bone marrow, and it was here that I first began to look round for a point of attack. But what function should one study? There were then only two which had been seriously suggested. One was the relatively simple concept, advanced by Virchow, of a defence function for lymphoid tissue. This could only hold good for lymph glands, in which a lymph stream was flowing through lymph sinuses traversed by a network of cells, the sinus reticulum. These, it was true, could mechanically hold up foreign particles which had entered the lymph stream. But it was clear that it was only lymph glands which could be regarded in this way, and that other lymphoid tissues, without afferent and efferent lymph vessels and a sinus reticulum, could not function in like manner. It should be borne in mind that it was only later that we learned about plasma cells and their role in antibody formation.

The only other function, one which could presumably be shared by all the lymphoid tissues, seemed to be lymphocyte formation. This appeared at first to lend itself to ready experimental approach. Most of the lymph of the body returned to the blood through the thoracic duct, and in doing so conveyed to the blood large numbers of lymphocytes, which had presumably entered it in the lymph nodes through which it passed. The thoracic duct thus appeared to be a convenient natural bottleneck which made it possible to collect a large part of the body's lymph and count its lymphocytes. This was then regarded as a way of measuring lymphocyte production. It was not until a good deal later that we began to appreciate

that such might not be the case and that the study of lymphocyte production was a problem of much greater complexity.

This early work was performed in dogs, and as soon as sufficient data on thoracic duct lymphocytes had accumulated, we began to compare them with those present in the blood, and observed that the daily entry of these lymphocytes into the circulation was about two and one-half times the number normally present in the blood stream. This immediately posed the question of the fate of the blood lymphocytes. Since there was no evidence to suggest extensive destruction of lymphocytes in the blood stream, the choice seemed to lie between recirculation, or leaving the blood for some unknown destination.

On the evidence available—evidence which we thought we were later able to confirm—we ruled out massive recirculation, and then began to search for possible destinations. Continual excretion into the lumen of the intestine had been suggested, but on what appeared to be very unsatisfactory evidence. There seems to be no doubt that some lymphocytes, in virtue of their apparently random movement, may find their way into the lumen of the bowel, but the numbers which are lost in this way did not appear to be large. A possibility which we seriously considered was that of a steady drift into the connective tissues of the body. These may undoubtedly contain an appreciable number of lymphocytes, but we could not think of any way of studying them quantitatively, nor at the time could we think of any useful function which they might subserve in the connective tissues—though in recent years there has been renewed speculation on this topic. It may yet prove to be the case that migration through the various connective tissues and exposure to different metabolic environments may play an essential part in the life story of the lymphocyte.

The only other destination which seemed worth examining was the bone marrow, more especially in view of the original association of lymphoid and myeloid elements. The separation of the two components might then be more apparent than real. They could still be functionally connected by the migration of cells from one tissue to the other through the blood stream.

The first step was to devise a technique for the quantitative

study of the cells of the bone marrow. This was performed in the rabbit, not very effectively, but with sufficient accuracy to indicate that the matter was worth pursuing. At this stage it was felt that it was important to eliminate all possible sources of variation, whether due to age, species, or sex, and our later work has been carried out in a standard animal, a male guinea pig of the Dunklin-Hartley strain, weighing approximately 400 gms. Unless otherwise stated, all the bone marrow data refer to this standard animal.

Normal bone marrow contains about 1,800,000 nucleated cells per c.mm., and of these 430,000 are lymphocytes—nearly 25%. This is a considerably higher figure than one finds in a normal human adult where the lymphocytes are usually given as 10% or less. The next step was to estimate the total volume of red marrow, and so calculate the cell populations, lymphocyte and otherwise, in the marrow as a whole. The results were sufficiently interesting to make it worth studying the quantitative changes in the marrow cells in response to a variety of stimuli.

These studies which are still in progress seem to suggest more and more that the lymphocyte plays an essential part in cellular changes in the marrow, and that it can in fact function as a stem cell, as suggested by many early haematologists, notably Maximow. This, of course, is one of the classical controversies in haematology, and there are still many who look askance at such an interpretation. In the following pages we ourselves have come to adopt it, despite some cogent arguments which can still be levelled to the contrary. Again and again, in studying the response of the marrow to a variety of stimuli, when we expected that either granulocytes or erythrocytes would be primarily involved we find that lymphocyte changes also occur.

In an effort to keep this monograph short, I have perforce dealt sketchily with many matters and have kept down to a minimum the number of references cited. There are therefore many excellent papers which have not been quoted. I am sure the writers will both understand and forgive my sins of omission.

This monograph is directed primarily to the tissues in which the blood cells are being formed and is concerned only secondarily with the blood either as the final destination of these cells, or as the

channel along which cellular migration streams are moving between the various blood-forming tissues. But I cannot emphasize too strongly that this monograph is not intended as an exhaustive treatise on either lymphoid tissue or bone marrow.

I have merely endeavoured to gather together into as connected a story as possible the work which my colleagues and I have carried out over a number of years. The work is still far from complete, but as it gathers momentum its target is becoming more and more obviously "Quantitative Cellular Haematology."

I should like to record my thanks to my Secretary, Miss L. Lloyd, and to our Medical Librarian, Mr. A. E. S. Roberts, for the unstinting help they have given me throughout.

J. M. Y.

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Chapter 1

THE LYMPHOID COMPLEX

THE LYMPHOID TISSUES reach their most advanced development in mammals, where they form a complex which consists of: 1) Lymph glands, 2) Spleen, 3) Subepithelial lymphoid tissue such as the tonsils, Peyer's patches, solitary intestinal nodules and appendix 4) The thymus. In monkeys, apes and man the lymph nodes are smaller but more numerous than in lower animals. In most animals there is a large lymph gland at the root of the mesentery, the glandula mesenterica magna or Pancreas of Aselli. The members of the complex consists of mixed cell populations, which vary in the different parts of the complex, and also in the same parts in different functional conditions. But they have one cell in common, the lymphocyte. The lymphocytes are roughly divided, on the basis of size and structure, into three groups, large, medium and small, the latter being the most numerous.

In addition to lymphocytes there are three other major cellular constituents of lymphoid tissue, namely:—Primitive reticular cells, plasma cells, and macrophages. Of these, the most variable are the plasma cells. These are present sparingly in normal lymphoid tissue, but can appear in increased numbers when antibody production is stimulated. They are then most abundant in the spleen, and occur in moderate numbers in lymph glands, but practically never appear in the thymus.

HOW BIG IS THE LYMPHOID COMPLEX?

It is difficult because of the diffuseness of its distribution to obtain accurate information about the size of the lymphoid complex. Few investigators have gone to the trouble of dissecting out and weighing the scattered members of the complex. The position is made even more difficult by the fact that the lymphoid complex undergoes appreciable changes with age, reaching its maximum development at puberty, and then undergoing steady diminution, though never complete atrophy. Andreasen (1943) gives extensive

data for rats of various ages, and in the young adult estimates the total lymphoid tissue to be somewhere between 0.5-1.0% of the body weight. However, weight alone may be misleading. Lymph glands may contain much lymph in their sinuses: the splenic pulp may be distended with blood. Andreasen therefore suggested that the DNA content was perhaps a better way of measuring the cellular constituents, especially as the preponderant cell seemed to be the lymphocyte.

THE SCATTERED LYMPHOCYTE POPULATION

Scattered throughout the connective tissues of the body is a population of lymphocytes whose extent we are unable to measure. There appears to be a slow steady drift of cells from blood via the connective tissues to lymph. The bone marrow also contains a very large lymphocyte population, but this we are able to measure. The marrow lymphocytes raise problems of a different order from those present in connective tissue generally. We do not know to what extent lymphocytes in normal connective tissue may either multiply, or become transformed into other cells, as is believed to happen in a number of pathological conditions. Some interesting recent work, together with reviews of the literature, will be found in the papers of Rebuck and Crowley (1955) and Braunsteiner *et al.* (1958). Burnet (1959) has developed the concept of lymphocytes constantly "exploring" tissues in various parts of the body, and sooner or later meeting an antigenic stimulus which may then initiate a variety of changes, notably mutation and proliferation. The antigenic "activation" of lymphocytes postulated by Burnet is beyond the scope of this monograph, which is concerned primarily with normal blood cell formation. But here too, as will be seen, one appears to encounter the lymphocyte, in a resting or inactive phase suddenly becoming "activated" and then proliferating and differentiating into erythrocytic and granulocytic cells.

CLASSIFICATION OF LYMPHOCYTES

Though it is customary to describe lymphocytes as falling into three main groups, large, medium and small, the gradation of sizes between these cells makes an accurate separation difficult. Size measurements are made most conveniently in smear preparations,

where the size depends partly upon the speed with which the smear is made, partly upon the fluid in which the cells are suspended. Thus smears of thoracic duct lymph, with a lower protein content, give smaller cells than smears of blood which has more "body" due to its higher protein content. For many years it has been our practice therefore to prepare smears of thoracic duct lymph only after resuspending in serum. In the case of the medium-sized and smaller lymphocytes, a very distinctive feature is the high N:C (Nucleus to Cytoplasm) ratio, so that the cytoplasm may form only a small tuft at one pole of the cell. This is especially marked in the thymus and the bone marrow, where we frequently describe the small lymphocyte as being "polar" since it possesses only a tuft of cytoplasm at one pole of the cell.

THE PRIMITIVE RETICULAR CELL

The heteroplastic formation of lymphocytes from reticular cells was first established by Downey and Weidenreich (1912; see also Sundberg and Downey 1942). Maximow (1927) has particularly emphasized the importance of these cells as stem cells, the primitive undifferentiated precursors of the lymphoid cells. Marshall and White (1950) have recently reviewed the problem, and come to the same general conclusion. In the case of the thymus Sainte-Marie and Leblond (1958) have calculated that there are eight mitoses between the reticular cell and the small thymocyte, the first giving rise to a reticular cell and a lymphoblast (cf. Grundmann 1959). The reticular cell is believed to develop under normal conditions into either a macrophage or a lymphocyte. If it is proceeding along the lymphocytic line of development, it becomes first the large basophilic cell known as the lymphoblast, and this in turn through repeated divisions becomes successively large, medium and small lymphocytes. For convenience in description one may term this series of divisions the Reduction Pathway.

The primitive reticular cell itself represents potentially 128 small lymphocytes, if eight mitoses take place before the small lymphocyte stage has been reached, as Sainte-Marie and Leblond have calculated for the thymus. Grundmann (1958), working with rat lymph nodes, believes there are only six mitoses. When differ-