The Lymphocytes

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PREFACE TO SECOND EDITION

Between the mid-1950's and the mid-1960's, our knowledge of the small lymphocytes expanded rapidly in several directions. These, then new, developments caused haematologists, pathologists, anatomists, and immunologists to renew their interests in these cells which had come to be regarded by most as end cells which were not really worthy of study in depth. Due to the efforts of a relatively small number of workers it soon became clear that this was far from the truth. This period saw the discovery of a number of new concepts concerning the life history of these cells. The four most important innovations were probably the realisation that some lymphocytes could be extremely long-lived whilst others had a relatively short life span; the establishment of the phenomenon of lymphocyte recirculation; the demonstration that some small lymphocytes could transform into other cell types under certain *in vivo* and *in vitro* conditions; and the central role of the thymus in lymphocyte production.

During the six years since the appearance of the first edition of this monograph there has been immense study of the lymphocyte by many workers in a number of areas. The result of this has been the firm establishment of the four concepts outlined above, and their further amplification. It is now quite clear that there is no such entity as the small lymphocyte, and that we are in fact dealing with a range of cells with different functional potentialities. It is the immunologists who have been particularly active in lymphocyte research recently. Not only has the importance of the thymus in the development of immunocompetence been firmly established, but it has also become clear that the production of antibody following an antigenic stimulus is the result of complex interaction between lymphocytes of different origin and functions. The precise details of these interactions are as yet not clear, but it may be hoped that within the next five years or so they may be elucidated.

The purpose of this edition of this monograph, remains as it was in the case of the first edition, to attempt a comprehensive review of the entire field of study of the lymphocyte. Again it is hoped that this review will serve as an introduction to this fascinating group of cells for those newly entering the field, and also to those workers in other fields whose studies bring them into the boundaries of the lymphocyte's realm.

In some areas, particularly those covered by the first four chapters, the main progress has taken the form of consolidation and some extension of our existing knowledge. These chapters have therefore been revised but largely maintain their original format. It is in the immunological sphere that the most rapid expansion of knowledge has taken place and therefore the chapters covering these aspects have been in the main re-written and two new chapters, covering *in vitro* studies of the homograft reaction and cell co-operation in the immune responses, are now included.

Again it has been impossible to include all contributions to our knowledge of the lymphocyte, but I hope that sufficient of the literature is cited to enable the reader to gain an overall picture of the field to lead him into the finer aspects of those areas in which he is interested through the bibliographies given in the papers which are quoted.

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PREFACE TO FIRST EDITION

The small lymphocyte, which is so insignificant in its morphology and yet so ubiquitous in its distribution in the body, has for long posed riddles, the answers to which have been sought since the eighteenth century at least. As soon as one answer seems to be obtained, many other problems come to light. Many fields of medical research have been occupied in the solution of the enigma of the lymphocyte; immunologists, haematologists, pathologists, radiobiologists and many others have been engaged in the task. The fascination that these cells arouse is almost unique in medical research and as evidence to this point one needs only scan the literature to see the names of a number of authors, each of whose work spans several decades: the lymphocyte creates a state of lymphomania in its adherents such as few other cells can.

The lymphocyte although so morphologically unstriking, nevertheless seems to play vital roles in many processes of the body. A recent concept even goes so far as to ascribe to the lymphocyte the role of "controller-in-chief" of the whole growth of the body and its various organs (Burwell, R. G., 1963: *Lancet*, **2**, 69). An exciting prospect.

In the ten years which have elapsed since the comprehensive monograph on the lymphocyte by Yoffey and Courtice was published, great advances have been made in the study of this enigmatic cell. As a result of work carried out in many centres over the last few years, the field of the lymphocyte has expanded considerably. Concomitant with expansion has come the "fragmentation" of the small lymphocyte into many aspects. Its function, its production, its relationship to other lymphoid cells, its connection with the thymus are but a few of the topics currently under study. The point has now been reached where very often workers interested in one of these aspects of the lymphocyte are unaware of the results of others studying the cell from a different angle. One of the purposes of this monograph is therefore to attempt to draw the strings together as far as possible and obtain a more unified view of these different aspects. One point which emerges from such a review is the heterogeneity of the cells which bear the name "small lymphocyte". It is now clear that lymphocytes fall into groups differing in life span and function. It is hoped that the future will see a more close study of these different types of cells and some correlation between form, life-span, site of production and function. The primary object of this book is therefore to attempt such a correlation, as far as is possible, on the data available and to examine the theories of lymphocyte function.

This monograph is concerned largely with the normal mammalian lymphocyte. Only when the behaviour and function of lymphocytes under normal conditions is better understood can we hope to start to unravel that interesting group of malignancies, the reticuloses or lymphoma. Are these diseases, in their earliest stages, a normal reaction of the lymphocyte to unknown stimuli, which leads via the process of somatic mutation to malignancy?

The second object is to condense the literature on the subject of the small lymphocyte into a form which may be of value to those not directly concerned with this fascinating cell but who require some knowledge of it.

In any attempt at comprehensive review of a field as large as that of the small lymphocyte it is inevitable that there will be some omissions through lack of time and space. It has been impossible to include every contribution to this field, but it is hoped that the main "signpost" papers have been included and will lead the reader to the more detailed aspects of the various topics discussed.

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CHAPTER I

ANATOMY AND PHYSIOLOGY OF THE LYMPHOCYTE

HISTORICAL INTRODUCTION

Post-mortem study of the lymphatic system is difficult due to the fact that the vessels soon collapse and are difficult to trace. However the presence of lymphatic vessels was well known to the physicians of the Alexandrian school as early as 300–200 B.C.[202] Asellius, in the early seventeenth century, studied the mesenteric lymphatics of well-fed dogs, and made observations on the milky fluid which escaped when these vessels were pricked. He suggested that this fluid was carried to the liver and there made into blood. In the same century Pecquet described the thoracic duct through which the contents of the lymphatics or "Milkie veins" were thought to pass into the heart. The association of the lymphatic system and the lymph with the blood system was thus early recognised.

The discovery of the cellular elements of the lymph was however necessarily dependent upon the discovery of the microscope and its subsequent improvement from the middle of the seventeenth century. When it is realised what poor instruments were available to the early microscopists, it is to their credit that they were able to glean as much information as they did about the cells of the blood.

Most prominent in the field of the lymphatic system in this early period was undoubtedly William Hewson whose life was cut short by a fatal infection acquired in the post-mortem room during the course of his studies.

During the 1770's Hewson wrote a series of papers, which were published by the Royal Society, in which he described the lymphocyte in the blood and recognised its origin from the lymphatic system. Hewson was well qualified to discuss the lymphatic system as he had made extensive studies of it in birds, fish and amphibia as well as in the mammals, including man. In a final treatise, published after his death, he described the structure of the organs of the lymphatic system, the lymph glands, spleen and thymus; and was probably one of the first to attempt to relate structure to function.

He described lymphocytes in the ligated lymph ducts and suggested that they resembled the central part of the "vesicles of the blood", or the red blood cells. Particularly remarkable were his ideas about the thymus and its function. It was, he thought, a lymphocyte distributing organ, and that its regression in adult life was correlated with a decreased need for red blood cell formation. Many of his ideas would not be out of place in the pages of the present day *Lancet*.

Hewson's theory of the role of the lymphocyte in erythropoiesis was held by others for many years until, in 1845, Zimmerman described the blood platelets and ascribed to them this role. The lymphocyte was first defined as a distinct cell type by James in 1846 after a study of the blood and lymph.

Modern ideas on the lymphocyte and its function stem from the last quarter of the nineteenth century with the introduction by Ehrlich of staining methods. These methods, together with the improvement of the microscope, phase contrast microscopy of living cells, and the more recent histochemical techniques have led to a vast accumulation of information about this cell but its functional significance, its mode of origin, and its capacity is still not clearly understood. However, very soon after its morphology had been studied, theories of lymphocyte function began to spring up until at the present time the world literature is flooded with them. Two main schools of thought have been prominent: the first supposes the cell to be a multipotential cell which may act as a stem-cell and give rise to other cell types. The second school regarded the lymphocyte as a futureless end-cell; a subdivision of this group are those who regard it as a trephocytic cell which contains nutrient material for use by other cells in conditions of tissue repair after the breakdown of the lymphocyte. The morphology of the cell favours this theory as it is essentially a nucleus, with desoxyribose nucleic acid (DNA), and very little cytoplasm; it is also in a form in which it may be readily transported about the body. This enormous rift has existed right up to recent years and probably owes its origin to the Franco-Prussian war when two of the leading histologists of the time, Ranvier and Ehrlich, quarrelled and "fell out". The object of this monograph is to review the wealth of information which has now accumulated and, by so doing, to gain a fuller understanding of the lymphocyte.

THE EVOLUTION AND DEVELOPMENT OF LYMPHOID TISSUES

Although this monograph is concerned chiefly with the lymphocyte and lymphoid organs of the mammal, a brief survey of the evolutionary aspects of the lymphoid system will not come amiss. Comparative studies of the anatomy of the lymphoid system in the vertebrates have been undertaken since the end of the last century. These studies have been reviewed, and considerably extended, by the work of Good and his colleagues[64, 72, 73] and it is mainly their work which is summarised here.

The lowest vertebrates in the evolutionary scale comprise two groups; the hagfishes and the lampreys. In the two species of hagfishes studied, haemopoiesis is carried out in two sites; in the lamina propria of the gut, and in an organ derived from the anterior kidney. These tissues are, however, concerned with erythropoiesis and no lymphoid foci were seen. Neither species of hagfish has a thymus. It is of interest that these animals do possess, in their blood, a small cell which is morphologically similar to the mammalian small lymphocyte. This cell is, however, not involved in immune responses, which are absent in this animal, but may possibly participate in inflammatory responses.[72] Various transitional cells were seen in the blood which suggested that these small lymphocyte-like cells were stem cells for erythrocytes, protogranulocytes and thrombocytes. Hagfishes have no plasma cells.

Higher up the evolutionary scale in the lamprey haemopoiesis is carried out in the gill region, in primitive marrow in the protovertebral arch region, and by the spleen which is located in an infolding of tissue in the anterior gut region. Lymphocytes are found in the blood and in foci in the spleen and in the marrow; these lymphoid cells will respond to antigenic stimulation by proliferation.[72] No plasma cells are found. It is at this evolutionary level that a rudimentary thymus first appears as foci of lymphoid cells proliferating from the epithelial cells.

In primitive elasmobranches the thymus is a well-developed lymphoid organ located in the gill region. It arises, as in the lamprey, from the epithelium of the pharyngeal pouches. In these fish the spleen is a relatively well-developed lymphoid organ and is organised into red and white pulp. Lymphoid tissue is also found in the lamina propria of the gut and in the parenchyma of the kidneys. In the embryonic rays the thymus is the first lymphoid organ to appear and even at birth the spleen is entirely concerned with erythropoiesis and myelopoiesis, and is lacking in lymphoid elements which appear a few weeks after birth. No members of the plasma cell series are found. The lymphoid tissues are very similar in the higher sharks and rays, but in both of these groups pyroninophilic cells, similar to plasma cell precursors, are found in the spleen, and in the sharks mature plasma cells could be found.

In the bony fishes, the chondrostean paddlefish, representative holosteans, and some teleost fishes, all possess thymic tissue, a well-developed spleen, which contains members of the plasma cell series, and gut-associated lymphoid foci. The marine teleosts, although possessing thymus, spleen and gut-associated lymphoid tissues, seem to have a sparcity of the plasma cell line.

In the amphibia the thymus and spleen are well developed organs; the latter may contain plasma cells after antigenic stimulation. Good and his colleagues have observed primitive lymphoid nodules in the sublingual regions in the case of two species of amphibia (mudpuppy and bullfrog).[72] These were simply collections of lymphocytes which lacked any lympho-epithelial organisation. More recently however lymph node-like organs have been described in certain Anuran amphibia. Kent and his co-workers describe lymph node analogues in the adult of a species of toad; these were situated in the neck, axillary region, and at the base of the heart.[103] These structures could take up injected india ink particles, and on antigenic stimulation cells of the plasma cell series were seen.

Cooper has made a detailed study of the lymph node analogues in both the larval and adult forms of the bullfrog.[43] In the larval stage a nodular lymphoid organ (gland) is present bilaterally in the region of the developing anterior limb bud. In their microscopic structure these organs were found to be surrounded by a capsule of connective tissue, and to consist of aggregations of lymphocytes of various sizes. The lymphoid aggregates are surrounded by a blood-filled sinus which is lined by phagocytic cells. The larvae also possess three smaller paired bodies—the ventral cavity bodies—which are situated near the gills. These bodies, although consisting mainly of lymphoid cells, are unlike the lymph gland; they do not have a phagocytic sinusoidal structure and they possess fewer lymphoid cells. In the adult bullfrog four pairs of lymphomyeloid organs are present in the neck region. Three of these pairs resemble the larval lymph gland, being made up of aggregations of lymphoid cells and phagocyte-lined sinuses. Lymphocytes, of all sizes, and cells of the plasma cell series are both present. The fourth pair—the epithelial bodies—are less complex, lacking a phagocytic sinus and having fewer lymphoid cells. The relationships between these adult and larval lymph node analogues are not yet clear. One major difference between the amphibian lymphoid organs and the mammalian lymph node is that the former lack the division into cortex and medulla.

In the reptiles, such as the alligator, the tonsils are well developed lympho-epithelial structures. By the time that the evolutionary level of the amphibia and reptiles is reached, the plasma cell series is well established, and for the first time in the evolutionary scale plasma cells are found in the lamina propria of the gut.[72]

The major evolutionary development in the birds is the development of the bursa Fabricius which is a gut associated lympho-epithelial organ. Like the thymus it has been shown that some of its lymphocytes are generated from epithelial precursors in the bursa.[6] Like the thymus the bursa is "central lymphoid tissue" and its presence is essential for the development of the ability to produce antibodies.[44] In the embryo however the thymus is the first to appear, followed after three or four days by the bursa.

In most birds the lymphoid tissue is in the form of diffuse deposits although in some swimming birds simple lymph node-like structures are found. These organs consist of collections of lymphocytes around a lymph vessel and surrounded by connective tissue capsule, but germinal centres may also be seen in some of these "glands".

In the mammal there is no bursa, but it is possible, in the light of recent studies, that other gut-associated lymphoid tissue may be "central lymphoid tissue", and analogous to the bursa (see Chapter III). The most primitive mammals are the monotremes. A study of the lymphoid organs of the Echidna has shown the existence of a tri-radiate spleen, which is very similar in its micro-anatomy to that of the higher mammals, a thymus, an appendix, Peyer's patches, and small lymphoid nodules.[51] The latter are associated with blood vessels and are situated within the lumen of the lymphatic vessels. In their structure these nodules are intermediate between the lymphoid tissue of the amphibian and the highly developed lymph node of the eutherian mammal. Each lymphoid nodule seems to be equivalent to the lymphoid follicle in the node of higher mammals. The nodule can be divided into two zones. The cortical area is well vascularised, possessing post-capillary venules, and it contains many small lymphocytes. Clusters of large pyroninophilic cells may be seen among the lymphocytes but there is however no evidence for the existence of primary follicles. The medulla is a central pale-staining area which contains primitive, DNA synthesising, cells and also macrophages; some of the latter contain "tingible bodies". This medulla may be analogous to the germinal centre but is not the equivalent of the medulla of the lymph node of higher mammals because it has no apparent medullary cords nor lymphoid sinuses. The whole nodule is surrounded by a circular sinus which represents the space between the nodule and the wall of the lymphatic vessel in which it is situated.

The appendix of the Echidna consists of a collection of lymphoid follicles similar to those already described except that some of them contained epithelial cells which are either dispersed or aggregated to form a central epithelial mass.[50] These epithelial cells may indicate that the appendix originated as a collection of lymphoid cells around epithelial crypts of the gut. Diener and Ealey are not convinced that any homology exists between the appendix of Echidna and the bursa Fabricius on the grounds of both its anatomical location and the evolutionary histories of the birds and mammals.[50] A functional analogy cannot however be excluded on these grounds.

The lymphoid tissue reaches its highest development in the eutherian mammals. The lymph nodes are well-developed organs, and, in addition, masses of lymphoid tissue are found under the mucous membrane of the gut—the Peyer's patches, tonsillar glands, etc. These lymphoid organs are supplied by lymphatic vessels in contrast to the spleen which may be regarded as collections of lymphoid tissue within the blood stream. Islets of lymphoid tissue may be found in the bone marrow. The enigmatic thymus is present in all mammals, either cervical or thoracic in location, and is seen at the height of its development in the young animal. This organ, composed as it is of masses of small lymphocytes, plays a central role in the lymphocyte story, and will be discussed in detail, in later chapters.

THE MAMMALIAN LYMPHATIC SYSTEM

The lymphatic system is largely composed of lymph nodes and patches of lymphoid tissue which are all connected by a system of

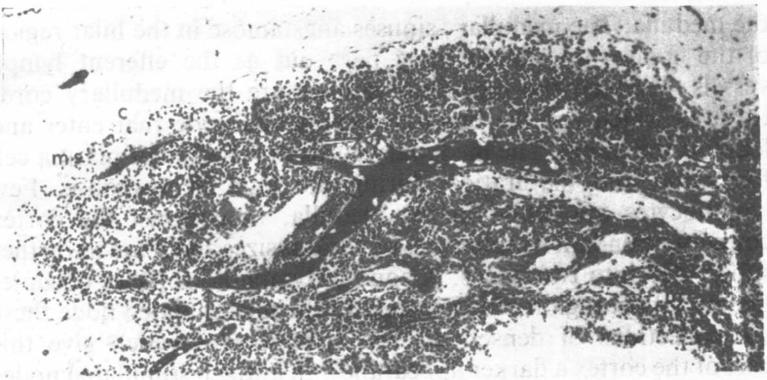

FIG. 1(*a*).—6μ section through rabbit lymph node showing cortex (cort), medulla (med), marginal sinus (ms), and capsule (c). The node has been perfused with Indian ink to outline the blood vessels. Post-capillary venules are marked by arrows. (Methyl green-pyronin $\times$160.) (From Burwell[35]; by permission of the author and the New York Academy of Sciences.)

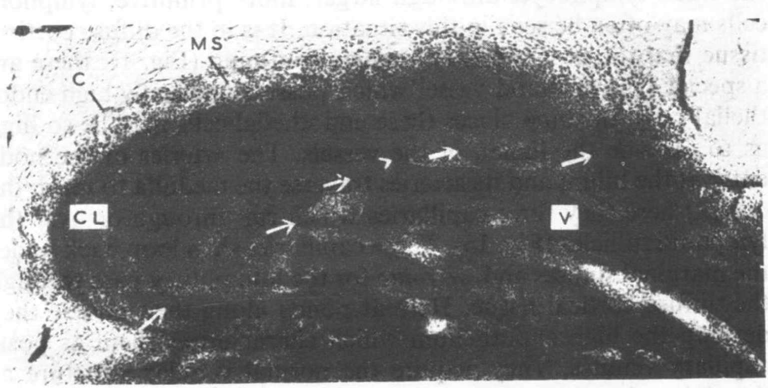

FIG. 1(*b*).—Thick section (100μ) adjacent to section in Fig. 1(*a*) showing blood vessels in relation to lymph node architecture. Capillary loops (CL) lie under the sub-marginal sinus and drain into post-capillary venules (arrowed). The PCVs drain into the larger vein. (MS = Marginal Sinus, C = Capsule; Safranin $\times$160.) (From Burwell[35]; by permission of the author and the New York Academy of Sciences.)

lymph vessels. The mammalian lymph node is made up of an outer cortex and a central medulla and has recently been described in detail by Sainte-Marie and Sin[156] (Fig. 1). Between the cortex and the capsule of the node is a phagocytic sinus (marginal sinus) into which the afferent lymph vessels open. Branches of the marginal sinus (intermediary sinuses) extend down to the sinus system of the medulla. The medullary sinuses anastamose in the hilar region of the node from whence they pass out as the efferent lymph vessels. Between the medullary sinuses are the medullary cords through which run the blood vessels of the node that enter and leave at the hilum. The medullary cords are the site of plasma cell formation and maturation during the immune response. Few lymphocytes are found in the medulla. In contrast the cortex contains abundant lymphocytes of various sizes and it can be further subdivided into two zones. A zone of lymphoid follicles occupies the outermost region of the cortex. In the unstimulated node these are collections of densely packed lymphocytes which give this part of the cortex a darker appearance. In antigen stimulated nodes germinal centres may appear in association with these follicles (Chapter V). The lymphoid follicles are separated from the medulla by the diffuse cortical area (= paracortical area, or extrafollicular zone). The predominating cell type in this zone is the small lymphocyte although larger, more primitive, lymphoid cells may often be seen in this situation. It is in the diffuse cortical tissue that the post-capillary venules are found (Fig. 1): these are a special type of blood vessel which possess unusually high endothelial cells. In some places these endothelial cells may be so high as to occlude the lumen of the vessels. The arteries of the node enter at the hilum and its arteries traverse the medulla to reach the cortex; here they form capillaries which run through or over the lymphoid follicles (Fig. 1*b*). These capillaries then loop back under the marginal sinuses and, *en route* for the hilum, they pass through the diffuse cortical tissue. Here at points along their course they develop the high endothelium which characterises them as post-capillary venules. They acquire the normal venular structure as they leave the cortex and pass down through the medulla to leave the node.

Thus the lymph node can be regarded as having three main structurally distinct units each of which is also functionally distinct as will become apparent in later chapters.

The lymphoid system originates in the embryo from six primary lymph sacs which bud off from the veins to form two paired—one pair in the jugular region and one pair in the iliac region—and two unpaired sacs. From these the lymphatic system grows out to cover all areas. The primary sacs become lymph nodes and other lymph nodes develop along the duct system. In the fully developed animal, the lymphatic vessels form a fine network of tubes which permeate almost every organ of the body, the only exception being the central nervous system which is devoid of lymphatic vessels. The fine lymphatic capillaries in the tissues possess only a single layer of endothelial cells but as they fuse, forming larger collecting ducts and eventually the large main lymphatic trunks, they become surrounded by connective tissue and in some cases muscle fibres.

The lymphatic system gains access to the blood stream via the thoracic duct which fuses with the cervical and subclavian ducts to enter the left jugular vein. This is probably the major site of lymph entry into the blood but the smaller right lymph duct may play a small but significant role (see Chapter II). Experiments in which the thoracic duct is ligated have indicated that minor "blood-lymph" anastomoses may exist;[67] X-ray studies of the lymphatic system have, however, failed to reveal them.[36] During experiments in which the thoracic duct of an animal was ligated, it was found that the number of lymphocytes in the blood returned to normal after an initial fall—indicating development of anastomoses.[34, 111] There is also a marked difference between the contents of the thoracic duct, which is milky, and that of the right duct which is clear; the lymph in the right duct may, however, become cloudy under certain experimental conditions.[45]

MORPHOLOGY AND STRUCTURE OF THE LYMPHOCYTE

There are a number of different cell types seen in lymphoid and other tissues to which the name "lymphocyte" has been given (Fig. 2). The small lymphocyte, which is the most ubiquitous cell in the body, is 6–10 microns in size, and has a very high nucleus/cytoplasmic ratio. In Romanowsky stained blood films the nucleus is usually dark with condensed clumps of chromatin. The presence of a nucleolus in the small lymphocyte is not usually obvious in routinely stained blood films, except occasionally in damaged cells. This apparent absence is due largely to the dense clumps of chromatin in these preparations. In wet smears and histological sections, however, the nucleolus is often visible.[56] In films stained with methylene blue at pH 4·9, or cells stained supra-vitally with Brilliant Cresyl Blue, the presence of this organelle in the small lymphocyte can be well demonstrated.[173] Perry and Reynolds have shown the presence of small amounts of pyroninophilic

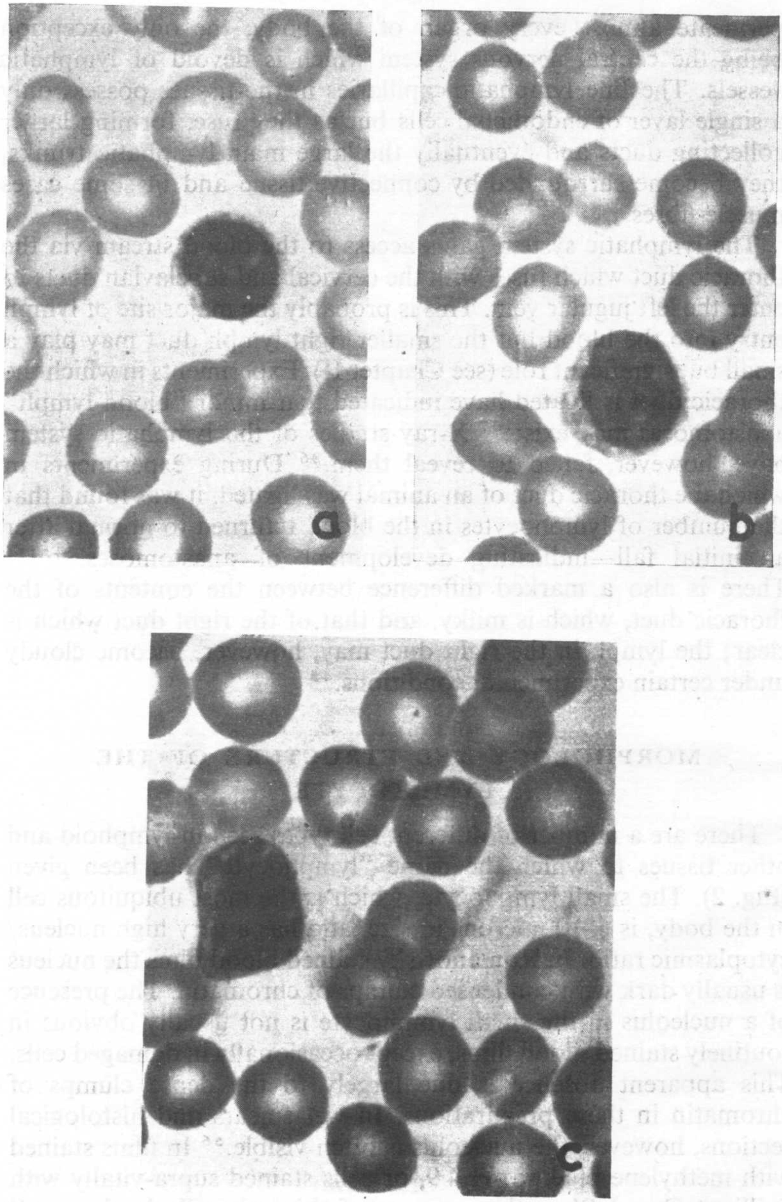

FIG. 2