HANDBOOK OF
HAEMATOLOGICAL
AND
BLOOD TRANSFUSION
TECHNIQUE

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Early, intermediate and late megaloblasts (pernicious anaemia)
Intermediate megaloblast with Howell-Jolly body (pernicious anaemia)
Later megaloblast with three Howell-Jolly bodies (pernicious anaemia)
Megaloblast in metaphase (pernicious anaemia)
Early normoblast
Intermediate normoblast
Late-intermediate normoblasts (iron deficiency anaemia)
Twinning deformity intermediate normoblast (haemolytic anaemia)
Leukaemic monocyte
L.E. cell
Heinz bodies (Phenacetin poisoning—methyl violet and eosin)
Feulgen appearance of micro-myeloblasts
Pappenheimer bodies (lymphosarcoma)
Mononuclear of glandular fever
Plasma cell (myeloma)
Cabot ring (pernicious anaemia)
Leukaemic myeloblast
Basophilic stippling (lead poisoning)
Hypersegmented neutrophil (pernicious anaemia)
Nuclear appendages in neutrophil (type found in both sexes, more common in males)
Megakaryocyte

FOREWORD

A LABORATORY is as good as its technical staff. One of the principal tasks of chief and senior technicians is to see that junior technicians are properly trained, so that their benchwork is accurate and reliable and they reach the standards required at the various examinations they have to take. Mr. Delaney is well known for his clear and concise teaching in the lecture room and at the bench, and many technicians have reason to be grateful to him. He has now collected in book form the essence of current teaching on haematology and blood transfusion, and has set it out in such a way that it will prove valuable both to those engaged in practical work in the laboratory and to those working for examinations.

In these days when so many books tend to be compilations of the views of other workers it is refreshing to find one whose author has clearly a great deal of practical experience behind his writing. It should prove of great value and I wish it every success.

IAN DAWSON

PREFACE

IT WOULD not be possible to compress into a volume of this size all the accumulated knowledge of haematology and blood transfusion. Instead I have tried to produce a book which will assist candidates to pass the examination of the I.M.L.T., but with the best book in the world I do feel that much bench work and a qualified instructor are also essential for examination success.

It is certain that by the time this book is published some sections will be out of date, but that is inevitable in a subject of which our knowledge increases daily. I have tried therefore to teach basic techniques and make no apology for the length of Chapter 1, which I feel is essential for the understanding of the aetiology of blood disorders, nor for the inclusion of Chapter 7, which is an introduction to the study of genetics. The chapter dealing with statistics is necessarily incomplete because of the nature of the subject, but I hope it will stimulate the reader to further study.

I must thank Dr. J. Humble for kindly reading the first section of this book, his chief technician, Mr. N. Thacker, F.I.M.L.T., for valuable advice and reading the proofs, and Dr. J. P. Nicholson for reading the chapter on radioisotopes. Mr. L. Marsh read the second section and provided many useful suggestions, as did Dr. K. L. Goldsmith, and I am grateful to them both. My thanks must also go to Dr. W. Bloom for permission to reproduce Fig. 2 from *A Textbook of Histology*, by A. M. Maximow and W. Bloom, to Professor D. D. Van Slyke for kindly allowing me to use his technique and line chart for the copper sulphate haemoglobin method, to Professor L. J. Witts for allowing me to use his classification of the megaloblastic anaemias, and to Dr. G. I. M. Ross for much of the B_{12} material, and I am also grateful to Dr. Rosemary Biggs for permission to reproduce graphs from her works on coagulation. If I have failed to give credit in the text for any technique or original hypothesis I must apologize in advance.

The photomicrographs are due to Mr. R. Sandison, F.I.M.L.T., and Mr. E. Pittock, and the other photographic work was done by the Westminster Medical Photographic Unit. Only I can take the blame for the drawings. Last, but not least, I must thank my sister, Mrs. A. Davis, and Miss B. Southey, who so patiently typed and retyped the manuscript and I hope learned some haematology while doing so.

J. D

SECTION I

HAEMATOLOGY

CONTENTS

CHAPTER 1

THE ORIGIN, LIFE AND DEATH OF THE BLOOD CELLS

EMBRYOLOGICAL BLOOD FORMATION

THE STUDY of human embryological blood formation is hindered by the lack of material. It is therefore necessary to study the processes as they occur in the chick and compare them with those known to occur in man. In this way gaps in the understanding of these processes may be filled by reasonable assumptions. The following description will be termed embryological blood formation, without reference to animal or species.

THE OVUM: FERTILIZATION

The mature ovum is fertilized, usually in the fallopian tube. Immediately the two pro-nuclei—represented by the ovum nucleus and the head of the sperm—have fused, segmentation of the fused mass begins. By the time the ovum has reached the uterus, it consists of a cyst-like structure containing a mass of cells, and is called the morula (Fig. 1 (*a*)). The cells which form the outer wall of the cyst exert a lytic action on the lining of the uterus, and thus a nest is excavated for the ovum. The uterine membrane then grows over the ovum, sealing it away from harm. The hormones which make this implantation possible are not within the province of this book and may be conveniently ignored.

STRUCTURE OF THE EMBRYO

The embryo at this stage may be represented diagrammatically as a sphere enclosing two smaller spheres (Fig. 1 (*b*)). The outer cyst-like structure is the chorion, the inner two are the amnion and the yolk sac. The cells forming the amnion are known as ectoderm, and those of the yolk sac as entoderm. Where these two structures touch, a third layer of cells arises from the ectoderm, and is called mesoderm (Figs. 1 (*c*) and (*d*)). The mesoderm is laid down between ectoderm and entoderm, and by numerous processes eventually forms the connective tissues of the body. The area of the yolk sac outside the point of contact of the two primary layers is called the area vasculosa.

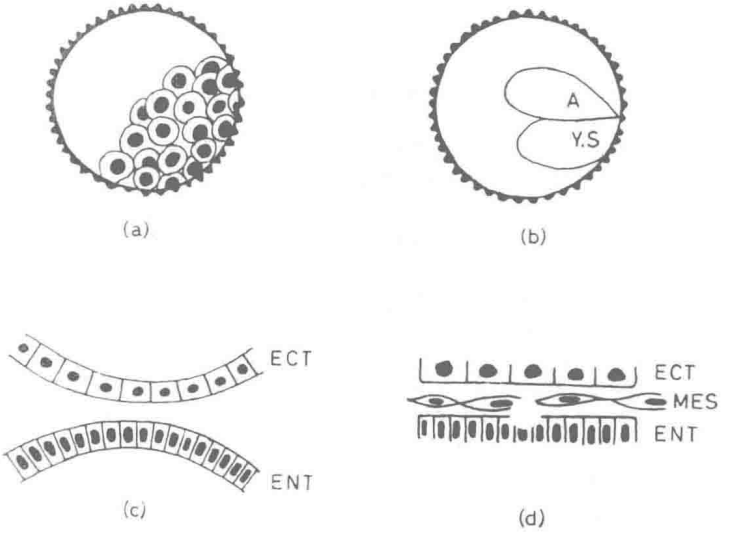

FIG. 1.—Diagrammatic representation of cells. (*a*) Morula stage; (*b*) " three-cyst " stage—A: amnion, Y.S: yolk sac; (*c*) primary layers—ECT: ectoderm, ENT: entoderm; (*d*) mesoderm arising between ectoderm and entoderm.

MESOBLASTIC STAGE OF BLOOD FORMATION

At this stage blood formation begins and is known as the mesoblastic stage. Mesodermal cells migrate out to the area vasculosa of the volk sac and form collections of cells called blood islands. The mesoderm proper is a syncytium, that is, a sheet of nuclei with no obvious cell boundaries, and it forms the body mesenchyme, which is the forerunner of the connective tissues. The syncytial mesoderm, by a hollowing-out process, forms tubes, one cell thick, which are primitive blood vessels. They become filled with primitive blood plasma secreted by the cells lining the walls. Some of the cells which take part in the hollowing-out process float away in the plasma as the first blood cells. Individual cells lining the vessels also sometimes break off and float away and become blood cells (Fig. 2). The cells now forming the vessels no longer exist as a syncytium, cell membranes having appeared, but have differentiated to become primitive endothelium. They retain the power to differentiate to perform other functions. The nucleated cells which are now present in the plasma are termed the megaloblasts of Ehrlich (Fig. 3), and although having the same name as cells seen in pernicious and other

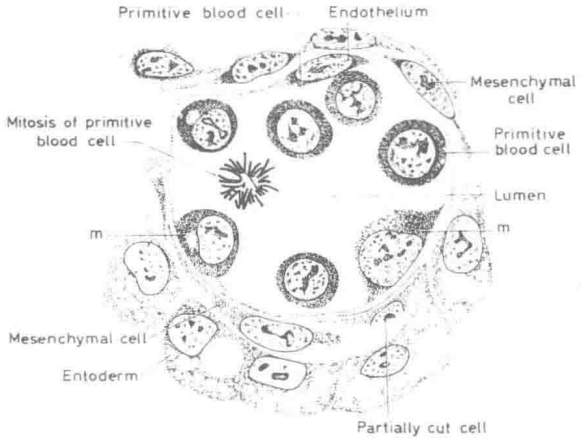

FIG. 2.—Cross-section of a vessel of the area vasculosa of a rabbit embryo 8½ days; m: rounding off of endothelial cells and their transformation into primitive blood cells. (*Reproduced by courtesy of the authors and publishers* (*Saunders; Philadelphia*) *of A Textbook of Histology*, *4th edn.*, 1942).

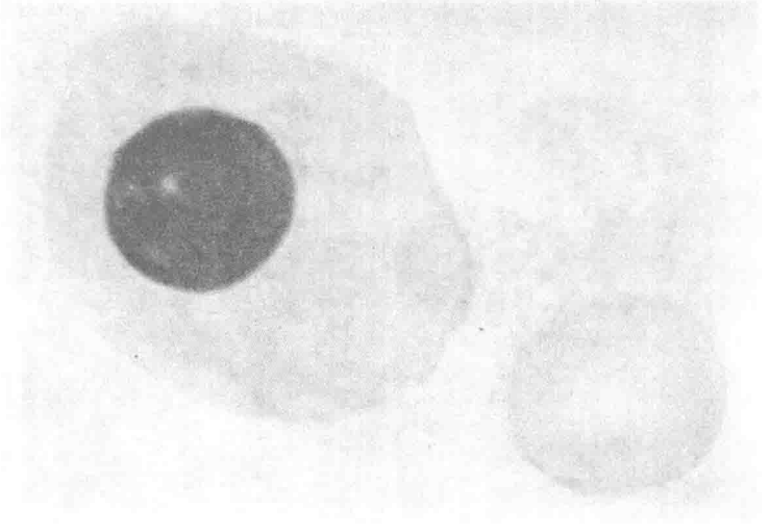

FIG. 3.—Megaloblast of Ehrlich (May-Grunwald-Giemsa); a normal size red cell is shown for comparison (× 750).

anaemias they are not pathological but embryological cells. All the cells of the embryo at this stage are rapidly dividing and differentiating, but true blood cells, as known, have not yet appeared. The cells described as megaloblasts have differentiated purely to perform the essential work of carrying oxygen to the tissues and to this end have built up a primitive haemoglobin from precursor substances already present in their cytoplasm. However, they have differentiated only to do this work, and as soon as more suitable cells have been developed they disappear. The primitive white cells are developed from the mesenchyme cells at this same stage of development. These cells, which are recognizable as leucocytes, make their way to the vessels and pass through the interstices between the endothelial cells, thus entering the vessels.

HEPATO-SPLENIC STAGE OF BLOOD FORMATION

The growing embryo (Fig. 4) needs more and more blood as development proceeds. Mesenchymal cells are found in the primitive liver, spleen, thymus and other sites, so that at about 6–8 weeks of foetal life the hepatosplenic stage of blood formation has begun (Fig. 5). The primitive stem cells are still producing blood cells but in different sites.

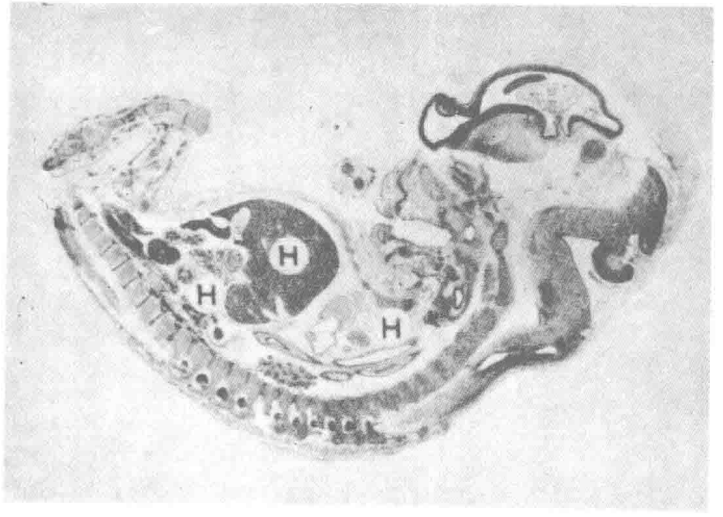

FIG. 4.—Section of 11-week human embryo (H_2E); H: Sites of haemopoiesis, thymus, liver and spleen.

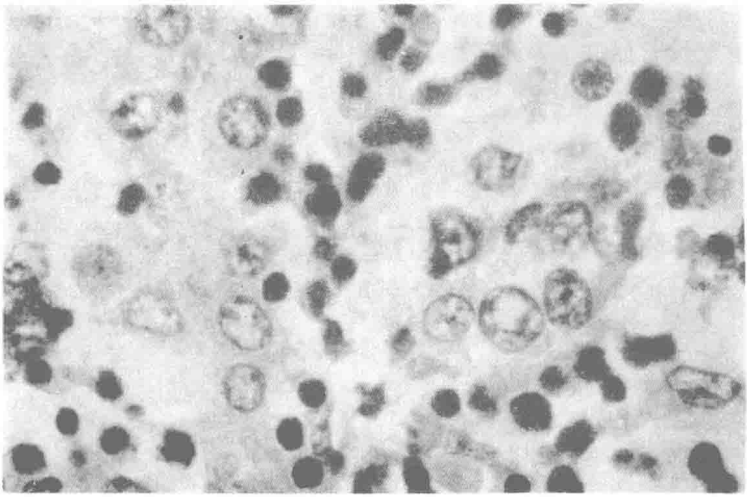

FIG. 5.—High-power view of liver from embryo shown in Fig. 4 (H and E); islands of haemopoiesis are seen between the large parenchymal cells (× 425).

MYELOID STAGE OF BLOOD FORMATION

At about middle foetal life the bone marrow is formed and the myeloid stage of blood formation is begun, to continue throughout adult life. The mesenchymal cells, however, although their activity is restricted, are still present in the other sites and, given a stimulus, will resume their embryonic function. Thus, if a newborn child loses blood rapidly and continuously, either from haemorrhage or haemolysis, areas of new blood formation reform in the liver, spleen, muscle, subcutaneous tissue or in any area where there is a mesenchymal rest. Histological sections of such tissue show very active centres of haemopoiesis. They are referred to as ectopic or heterotopic areas of bone marrow. In blood diseases such as leukaemia, nodules of the characteristic cells appear in the skin. This may not be an invasion of tissue, like a cancerous growth, but the stimulus, which has been applied to the white cell production centres, operates equally well on the rest of the persisting mesenchymal cells scattered throughout the body no matter where they are. The stimulus causes the stem cells to differentiate rapidly so that a nodule of leucocytes is produced. Again, in the disease myelosclerosis the bone marrow becomes replaced with fibres, and

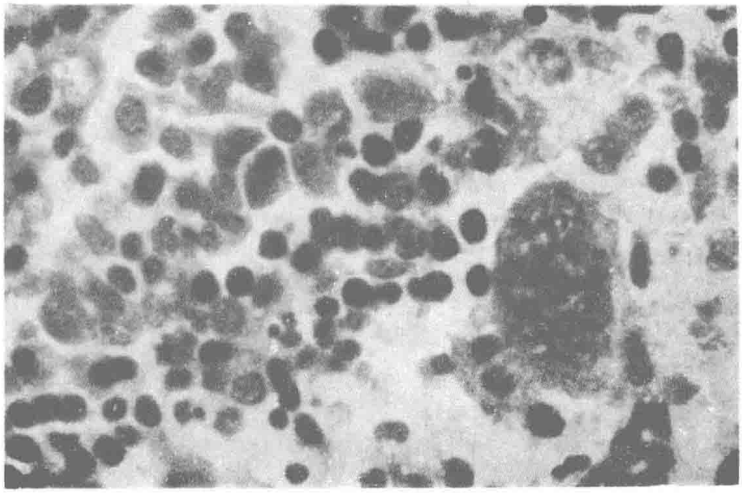

FIG. 6.—Section of spleen from a case of myelosclerosis (H and E); haemopoietic activity includes megakaryocytic production (× 425).

the spleen and liver revert to their embryonic function of producing the cells the marrow is failing to manufacture. Sections of such tissue show active haemopoiesis (Fig. 6).

BLOOD FORMATION IN THE ADULT

Undifferentiated mesenchyme still exists in the adult, as a syncytium scattered through the body. On appropriate stimulation cell boundaries appear and fine argyrophil fibrils running through the cytoplasm can be demonstrated. These cells were first called by Aschoff " the reticulo-endothelial system ". This is a system of cells found in many organs and performing many vital physiological functions. The system consists of cells, which are phagocytic and dye storing, in a ticulum of silver-salt reducing fibres. Some of the cells may become freed from the reticulum and are then known as wandering histiocytes, macrophages or clasmatocytes. The resemblance of such cells to the monocyte of the blood is striking enough to assume a direct descent of the monocyte from the reticulum cell and, indeed, some authorities hold this or a similar view. The freed reticulum cell may become a fibroblast or littoral cell, but the concept that cells of the mesenchyme are the ultimate precursor still existing in the adult body gives a wider system which includes Aschoff's reticulo-endothelial system and is known as the reticular or lymphoreticular system of Maximow.