Disorders of Blood and Blood-Forming Organs in Childhood

H. S. BAAR
Director of Pathology, Pineland Hospital, Pownal, Maine
late Consultant Pathologist, Children's Hospital, Birmingham, England

STELLA BAAR
Biochemist, Medical Research Council, Unit for Burns and Industrial Injuries
Birmingham, England

K. B. ROGERS
Consultant Clinical Pathologist, Children's Hospital, Birmingham, England

E. STRANSKY
Professor of Paediatrics, Department of Paediatrics, Philippine General Hospital
Manila, Philippines

With 130 figures and 135 tables

HAFNER PUBLISHING COMPANY, INC.
NEW YORK

1963

Originally published by S. Karger AG, Basel, Switzerland

Printed in Switzerland by Buchdruckerei Stäfa AG, Stäfa
Clichés: Aberegg-Steiner & Cie. AG, Bern

Preface

In 1928 the senior authors have published a "Clinical Haematology of Childhood". Since that time spectacular discoveries have been made in the science of blood diseases. A clinical and morphological discipline has been replaced by one which comprises in addition biochemistry, histochemistry, immunology, as well humoral as cellular, and electronmicroscopy. It was attempted in this book to do justice to all these developments. In addition, a number of observations and experiments which have not been published elsewhere is included in this volume. Already in the book published in 1928 the authors have been working independently. Now a complete independence became a necessity. Dr. STRANSKY continued clinical work and became particularly involved in the study of tropical diseases, while myself confined my work for the past 23 years to the laboratory. While collaboration was close between the Birmingham group of workers the chapters from the Philippines were written quite independently. In the years 1945–1951 in collaboration with Dr. SMALLWOOD I have prepared the section on "Disorders of Blood and Blood-Forming Organs" for Parsons' and Barling's Diseases of Childhood. For technical reasons only excerpts of the original have been used in the definite publication. Use has been made of the original manuscript in this book. The delay in the publication of the present book made it necessary to record additional information in the form of footnotes. No such supplements were possible in the chapters written by Dr. STRANSKY.

It was attempted to make a clear distinction between statements based on own experience and those based on reports in the literature only.

Thanks are due to Miss ZELDA CUSHNER who checked all references and prepared the author-index and to Mrs. P. MUNDY for all the other secretarial work.

The authors are very grateful to the members of the S. Karger Publishing Company for their kind cooperation.

H. S. BAAR

Pineland Hospital, December, 1962.

Contents

CHAPTER I

The Formation of Red Blood Cells, their Life Span and Destruction

Synthesis and Katabolism of Haemoglobin

The Metabolism of Erythrocytes

H. S. Baar

The first free blood cells appear at about 3½ weeks of intrauterine life in extra-embryonic areas, namely the area vasculosa of the yolk sac, the chorionic villi and the body stalk. Undifferentiated mesenchymal cells of stellate shape form aggregates which are called "blood islands". The peripheral cells of such an island become differentiated into vascular endothelium, while in the centre the mass of the cells is hollowed out and thus the earliest blood cells become separated and float in the blood plasma. These earliest blood cells are nucleated red blood corpuscles of a special type, namely, megaloblasts. Leucopoiesis soon follows erythropoiesis and blood formation first scattered throughout the embryonic mesenchyma becomes localised at certain organs. With five (Osgood) to six (Arey) weeks the liver is the principal organ of haematopoiesis. Later, in the second to third month of embryonic life, the spleen and the lymph nodes and finally at the beginning of the fourth month the bone marrow show marked haematopoietic activity. The sites of activity show much overlapping. In the full term newborn infants the formation of erythrocytes is largely limited to the bone marrow with little erythropoietic activity in the spleen and even less in the liver. Production of the erythrocytes and the leucocytes of the myeloid series in these extramedullary foci ceases almost immediately after birth, so that after the age of five days only a very occasional haematopoietic focus may be seen in a dilated sinusoidal liver capillary and a few scattered erythroblasts in the splenic pulp. In immature newborn infants extensive blood formation is found in the spleen and the sinusoidal liver capillaries and not infrequently also in the periportal triads of the liver, the interstitial tissue of pancreas, the salivary glands, and in the submucosa of renal pelvis. The number and size of intracapillary haematopoietic foci in the liver of a small premature infant may be as great as in haemolytic disease of the newborn. Their postnatal disappearance is, however, almost as rapid as that in the full term baby.

The bone marrow of young infants is red and haematopoietically active throughout. Between the ages of six months and two years there is not only an absolute but also a relative increase in the amount of active bone marrow tissue. This is due to the formation of a marrow cavity. In the newborn there is cancellous bone throughout

the whole length of the shaft of a long bone and the trabeculae occupy a comparatively large space. Later the trabeculae in the middle of the shaft become resorbed and thus a cavity is formed which is filled with red haematopoietic bone marrow. Between the ages of two and five years there occurs a gradual replacement of red marrow by fat marrow which proceeds then more rapidly so that by the time puberty is reached haematopoiesis is limited to the metaphyses of long bones and to the small bones, mainly the sternum, ribs, iliac crest, and vertebrae.*

In the older children and in adults the fat marrow is the principal site of potential erythropoiesis and becomes easily reconverted into red marrow whenever increased blood destruction necessitates increased formation. The infant whose bones are normally full to capacity with red marrow has no such reserves. His main "shadow factories", the sites of extremadullary haematopoiesis, are liver and spleen and occasionally the lymph nodes, thymus, suprarenals, pancreas and kidney, which normally become inactive shortly after birth yet retain their capacity for renewed activity in times of emergency such as may follow severe haemorrhage or haemolysis. This extramedullary erythrogenesis is therefore a phenomenon which in children comes into action more frequently than in adults.

The megaloblast and the macroblast. The first or megaloblastic generation of nucleated red blood corpuscles is rapidly replaced during the second month of foetal life by a new and different generation—the normoblastic (KNOLL). Recent work by ISRAELS, GILMOUR, THOMSON and in particular of FRUHLING and his associates lends strong support to the teaching of EHRLICH AND NAEGELI that the megaloblast is not a precursor of the normoblast, as has been assumed by many American haematologists, but that the two cells represent different types of erythrogenesis. Thus, megaloblastic blood formation is physiological only in early embryonic life. It is always pathological in extrauterine life. The first nucleated red blood corpuscles of the normoblastic series to appear in the embryo are large cells which are called macronormoblasts or macroblasts in contradistinction to megaloblasts. Their differentiation is of great importance in the pathology of blood-forming organs. Thus the megaloblast as found in the young embryo and in the sternal marrow of a case of untreated pernicious anaemia is a large cell with a nucleus which is characterised by a fine, but fairly dense and uniformly distributed, network of chromatin threads without chromatin condensations. Its cytoplasm is considerably greater in amount than that of the macroblasts and becomes haemoglobinised more rapidly. This explains the presence of haemoglobin in cells which still show an immature nucleus with a fine reticular chromatin structure and the frequent finding of large fully haemoglobinised erythroblasts with an eccentrically situated pycnotic but not yet karyorrhectic nucleus. Megaloblastic erythropoiesis, particularly that of pernicious anaemia, is characterised by a discrepancy between nuclear maturation and haemoglobinisation of the cytoplasm. Although embryonic megaloblasts are morphologically very similar to those of pernicious anaemia in relapse, these two should not be called identical. The haemoglobin of embryonic megaloblasts is of the foetal type, that of pernicious anaemia of the adult type (HAUROWITZ). In addition the bone marrow in pernicious anaemia shows a series of other pathological changes, such as polyploidy of the megaloblasts (E. SCHWARZ) and multipolar mitoses which are foreign to embryonic haematopoiesis. During recent years there have been many references to the frequency of megaloblastic blood and bone marrow pictures in early life, but in the writer's ex-

* The absolute weight of active bone marrow is the same in childhood as in adults, namely 1½ kg.

perience it is but rarely that megaloblasts are found in the circulating blood or bone marrow of children.

The macro- and normoblast. The macroblast as seen in large numbers in the liver of a three to four months old foetus or in the sternal marrow during or shortly after an acute haemolytic crisis is a large cell with a comparatively narrow rim of cytoplasm, which stains deep blue with Giemsa's stain, and with a nucleus, the chromatin threads of which are thicker but somewhat more loosely arranged than those of the megaloblast. Its haemoglobinisation runs parallel with or lags behind the maturation of the nucleus. In subsequent mitoses the cells become smaller, the nucleus shows condensation of the chromatin, pyknosis and finally karyorrhexis. The cytoplasm becomes greyish-blue, polychromatic and finally pink orthochromatic. A so-called "intermediate" erythropoiesis between megaloblastic and normoblastic has been described by many authors. This conception is based mainly on the study of bone marrow films in early remission of Addisonian anaemia, and interpreted as "maturation of megaloblasts into normoblasts". Caution appears justified in the cytological interpretation of sternal marrow 24 hours after commencing liver-or B_{12} therapy. However, the abrupt replacement of megaloblastic by normoblastic erythropoiesis in the embryo (KNOLL) does not favour the idea of any intermediate erythropoiesis. FRUHLING, ROGER, and SPEHLER have recently demonstrated quite clearly that the antianaemic liver principle does not convert megaloblasts into normoblasts, but initiates a rapid macro-normoblastic hyperplasia, while the megaloblasts undergo degeneration. This lysis of megaloblasts is evidenced not only by morphological findings, but also by a rapid increase of urinary purine-excretion which follows therapy with folic acid (FRUHLING). Disturbance of nucleic acid metabolism in the megaloblast has been claimed by several authors, but the reports are contradictional (see LAJTHA). However, megaloblastic and normoblastic erythropoiesis may occur simultaneously. This is seen in the megaloblastic anaemia of infancy and occasionally in coeliac disease. One may conclude that megaloblastic erythropoiesis is not a "maturation arrest" but a pathological blood formation.*

The haemocytoblast and the proerythroblast. In the bone marrow which is the site of great erythropoietic activity and occasionally in normal bone marrow large cells are seen which have a pale blue cytoplasm, more abundant than that of basophil macroblasts, and a pale finely reticular nucleus with several nucleoli. Where intermediate forms between these cells and typical deeply basophil macroblasts are found, such cells may be considered as their precursors, namely the most immature nucleated red cells, the proerythroblasts. It is probable, however, that under special conditions they may develop into megaloblasts and that they are also precursors of the myeloblasts and thus of the myeloid series of white blood cells (JONES). Therefore, they may be considered as common "stem cell" of the erythroid and myeloid series, as haemocytoblasts.**

* JONES (in MACFARLANE and ROBB-SMITH' Functions of the Blood. Acad. Press, New York and London 1961) came to the same conclusion stating: "The lack of liver principle inhibits normoblastic development and causes proliferation of the pathologic red cells (megaloblasts) and neutrophils".

** In the haematological literature there exists some confusion in the use of the word "haemocytoplast". Ferrata's haemocytoblast is identical with Naegeli's myeloblast. Maximow's haemocytoblast corresponds to the cell described above and the difference between this description and that of MAXIMOW is due to the fact that he used histological sections for the morphological characterisation while the writer's description is based on Giemsa-stained bone marrow films. Maximow's claim that these cells are also precursors of the lymphatic series is not shared by the writer.

The diagnosis of haemocytoblast or proerythroblast which is based on the morphology of a single cell without consideration of the whole marrow picture is in the writer's opinion not practicable.

The activity of erythropoietic tissue may be estimated on the basis of morphological or of histochemical findings. The "mitotic index" i. e. the percentage of cells in mitotic division seen in a bone marrow film is not a reliable guide, if the duration of karyokinesis and of the intermitotic phase is unknown. A definite advance in the analysis of the maturation—and division-problems in erythropoiesis was achieved by EMIL SCHWARZ in 1951 who calculated separately the mitotic indices of the proerythroblastic stage and of the following erythroblastic generations. Twenty percent of all proerythroblasts were found in mitotic division and 2% of all other erythroblast-generations. The relation between proerythroblasts and all other nucleated red cells of the marrow was found to be constant in normal and a variety of pathological conditions. A further advance in our knowledge is due to the work of LEIBETSEDER and of WEICKER. These authors applied the principles developed by JACOBJ for a variety of tissue cells, mainly the liver, to the study of erythropoietic tissue. JACOBJ measured the diameters of nuclei and calculated their volumes assuming the shape of a sphere (this includes an error, if applied to bone marrow films, but this error appears to be not significant). He found that the nuclear volumes are either distributed according to GAUSS' probability curve or show several peaks which correspond to a geometrical series: $aq^0 : aq^1 : aq^2 : aq^3 \ldots$ A mitotic division of a nucleated red cell may be heteroplastic resulting in two cells, the nuclei of which have half of the volume of the mother cells (halfing division) or it may be homoplastic in which the nuclei of the daughter cells have the same volume as those of the mother cells, and the division is therefore preceded by growth with duplication of the nuclear volume. The presence of homoplastic mitoses may often be concluded from the histological examination of bone marrow sections by the presence of nests of erythroblasts of equal size. Such nests have been described already more than half a century ago by HELLY as "Erythrogonien-Nester". They are frequently seen in the bone marrow of experimental iron deficiency anaemia.

All nucleated red cells of a bone marrow may be divided in 5 classes: K_2, K_1, $K^1/_2$, $K^1/_4$, and $K^1/_8$. Fig. I. 1 taken from LEIBETSEDER's work shows a graphical pres-

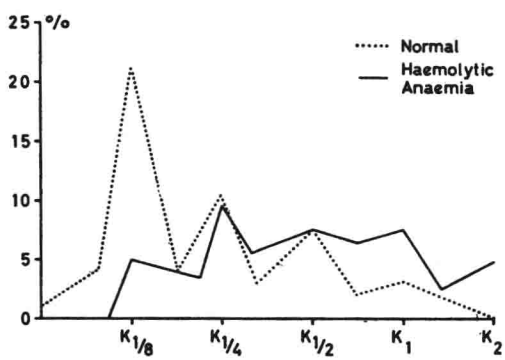

Fig. I. 1 Distribution curve of nuclear volumes of erythroblasts in a normal person and a case of haemolytic anaemia (from LEIBETSEDER).

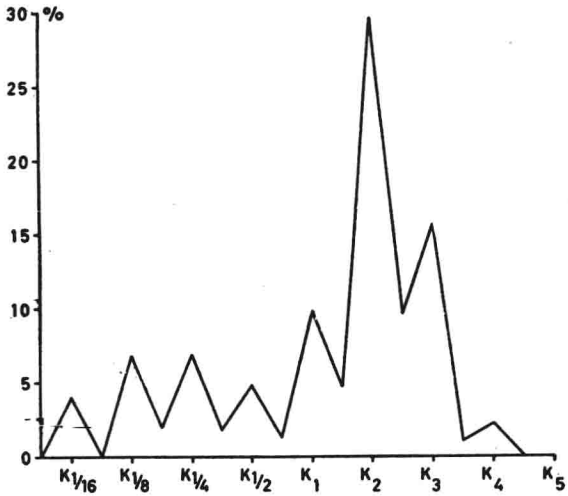

Fig. I. 2 Distribution curve of nuclear volumes in pernicious anaemia (from LEIBETSEDER).

entation of nuclear volumes in normal bone marrow and in haemolytic anaemia with a shift of the maximum towards a higher class and the presence of homoplastic mitosis. Fig. I. 2 shows that of pernicious anaemia with 8 classes, maximum peaks for K2 and K4 and an abnormal peak corresponding to $K^1/_{16}$

In addition to the nuclear volumes the ratio nucleus to cytoplasm is a useful measure of erythroblastic maturation. This figure is fairly constant in the basophilic generation of erythroblasts, but alters in favour of the cytoplasm as haemoglobinisation proceeds.

Equally important is the estimation of nucleolar size. BUCHNER recently published a series of karyometric investigations on tissue cultures using tissues of different intensity of growth, addition of growth stimulating substances and of growth inhibitors. He found that with the rapidity of growth nucleolar size (site of nuclear ribonucleic acid) increases more than that of nuclei and the nucleus to nucleolus quotient therefore decreases while the reverse is found with growth inhibition.

The histochemical study of erythropoiesis was first carried out by THORELL and presented in a classical monograph. All young marrow cells which are growing and multiplying have a basophil cytoplasm. This basophilia is due to the presence of ribonucleic acid (BRACHET, THORELL, WHITE) and this is related to the synthesis of proteins. The deep basophilia of plasma cells is due to the same chemical property and is related to the hyperproteinaemia which accompanies a general increase of these cells in tissues and to the synthesis of immune globulins which is a function of the plasma cells. THORELL's investigations were based on the microspectrophotometric estimation of ribonucleic acid, a method inaugurated by CASPERSON. Ribonucleic acid has an ultraviolet absorption maximum at 2570 Å while the unspecific absorption maximum of proteins due to their content of tryptophane and to a lesser degree of tyrosine is at 2750 Å. The relation E^{257}/E^{275} gives therefore an estimate of ribose-nucleic acid. In the nuclei of the youngest cells of the erythroblastic series, the proerythroblasts, the

ribonucleic acid is concentrated in the nucleoli and spreads towards the nuclear membrane, forming the parachromatin or as THORELL calls it the "nucleolus associated chromatin". The amount of ribonucleic acid is a reliable measure of the rate of protein synthesis. THORELL divides the cytoplasmatic maturation of normoblasts (including the proerythroblasts) into four phases: (1), growth with maximal concentration of ribonucleic acid, (2), declining growth with diminution of the concentration of ribonucleic acid, (3), differentiation with rapid formation of haemoglobin and (4), declining differentiation. Figures I. 3 and I. 4 from THORELL's monograph show in the normal bone marrow haemoglobinisation beginning when ribonucleic acid is almost at its minimum contrasted with pernicious anaemia where early haemoglobinisation is associated with an almost unchanged content in ribonucleic acid. Some ribonucleic acid is still present after expulsion of the pyknotic nucleus in the substantia reticulofilamentosa. A few modifications must be nowadays introduced into THORELL's scheme.

His studies suggested that globin is formed prior to haem and then linked to the latter. However, recent investigations by MUIR and NEUBERGER and by NIZET who studied haemoglobin synthesis in vitro from porphobilinogen using marked amino acids and by RIMINGTON showed conclusively that globin and haem are

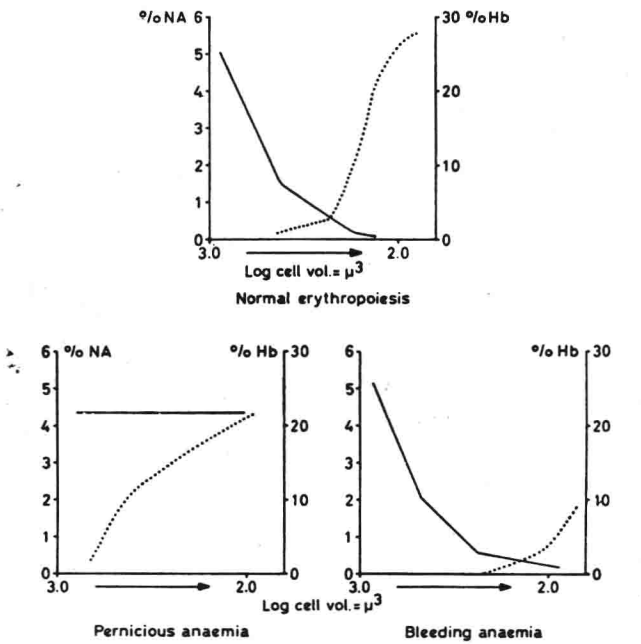

Fig. I. 3 showing the concentrations of ribose-nucleic acid and haemoglobin during erythropoiesis in normal individuals, in pernicious anaemia and bleeding anaemia.
Legend: % PNA = Concentration of cytoplasmic Ribose Polynucleotides
% Hb = Concentration of Haemoglobin in the cytoplasm

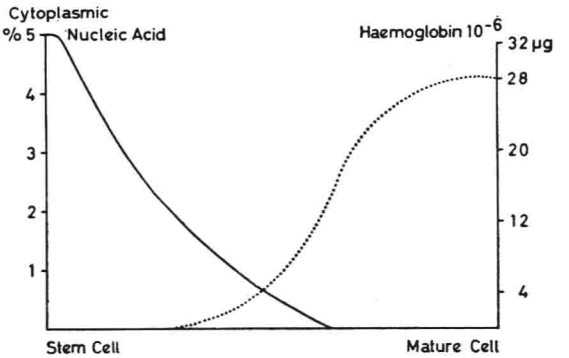

Fig. I. 4 after THORELL, showing that rapid synthesis of haemoglobin coincides with very low ribonucleic acid content.

synthesised simultaneously. The latter controls the synthesis of globin.* The findings of THORELL apparently indicate the synthesis of ribonucleic acid containing precursors. This is a feasable assumption since a ribonucleoprotein intermediate of globin synthesis has been actually demonstrated (RABINOVITZ AND OLSON). Furthermore a considerable haemoglobin synthesis takes place in the anuclear reticulocyte stage. Haemoglobin synthesis appears first simultaneously in the nucleus and in the cytoplasm, but disappears from the nucleus in the orthochromatic stage, while the cytoplasmatic haemoglobin-synthesis continues after denucleation (SENO *et al.*).

In the past few years the measurement of the plasma iron turnover** with the help of ^{59}Fe has been used for the assessment of erythropoietic activity. In this method plasma iron binding protein (see page 149) is tagged with a tracer amount of radioiron and the rate of disappearance of radioactivity is followed for several hours. BOTHWELL *et al.* studied the plasma iron turnover by this method in normal persons and in a variety of haematological disorders and compared the turnover with the

$$\text{"erythropoietic index"} = \frac{\text{subjects reticulocytes/cu mm}}{\text{normal reticulocytes/cu mm}} \times 100$$

and found that the plasma iron turnover is almost entirely determined by the rate of erythropoiesis.

In recent years the synthetic processes in erythroblasts have been studied with the help of radioactive aminoacids, determining their fate or incorporation into the proteins of bone marrow cells. This work has been reviewed by LAJTHA. Generally the younger the cell the higher its uptake of ^{35}S-methionine in vitro. It is almost absent in orthochromatic normoblasts.

Some haemoglobin is already present in the basophil macroblast and is located partly in the nucleus (SENO *et al.*). Iron in the nuclei has been demonstrated already by MCCALLUM and by RIECKER, and SENO *et al.* showed that this iron is in the form of

* However, DRABKIN and WISE claim independent biosynthesis of haem and globin (Science, *132:* 1491, 1960).

* Clear definitions of the terms turnover, turnover rate and time are given in a paper by ZILVERSMIT (Am. J. Med. *29:* 832, 1960).

haem. CARVALHO found that basophil normoblasts contain $2–6 \times 10^{-6}$ micrograms of haemoglobin. THORELL found in early normoblasts 1×10^{-6} micrograms haemoglobin, but according to CARVALHO it is 16×10^{-6} micrograms.

The mechanism of the transition from the nucleated red blood cell to the anuclear reticulocyte was for a long time subject to controversy. While some authors assumed dissolution of the nuclear substance (karyolysis) in the cytoplasm, others postulated fragmentation (karyorrhexis) followed by expulsion of nuclear fragments. The problem appears to be settled in favour of the latter theory by the findings of MALKIN and DENSTEDT. These authors reported that diphosphopyridine-nucleotide-pyroposphorylase is present in nucleated avian erythrocytes, but absent in rabbit's and human red blood corpuscles and reticulocytes. Expulsion of the nucleus is also more compatible with the decrease in the diameter when the late erythroblast changes into a young reticulocyte. According to PAOLINO this decrease varies between 0.7 and 2.4 microns and is in most instances 1.5 microns.

After extrusion of the nucleus an erythroblast becomes a reticulocyte.* This is a red blood corpuscle which contains a granular and filamentous substance, demonstrable by supravital staining, preferably with brillant cresyl blue, the so-called substantia reticulo-filamentosa. Contrary to earlier statements this substance is not an artifact. It is present in the native cell and demonstrable by electron-microscopy (JUNG, ASEN, and WOLPERS), although its appearance in the form of filaments appears to be an artifact. Electronmicroscopic investigations (SENO *et al.*) indicated that the substantia reticulo-filamentosa is the coagulated endoplasmic reticulum which is demonstrable in reticulocytes apart from mitochondria. It is considered as the basophilic component of the cytoplasm which surrounds the mitochondria. This substance was already known to EHRLICH but its significance as evidence of blood regeneration was first recognised by CESARIS-DEMEL in 1907. The younger a reticulocyte the more of the substantia reticulo-filamentosa it contains. This substance which consists of ribose-nucleotides (LAVEA AND THOMA, DUSTIN) is already present in nucleated red blood cells. It is not a derivative of the nuclear substances. HEILMEYER AND WESTHÄUSER distinguished 5 classes of reticulocytes according to their content of reticulofilamentous substance: Class 0 is the erythroblast with a reticulum, in class I this material forms a dense cluster while in class IV, the oldest reticulocyte on the verge to become a mature erythrocyte only a few vital staining granules and/or filaments are seen. The polychromatophil-erythrocyte is a reticulocyte in which the reticular substance becomes diffusely dissolved in the process of fixation and staining. The number of polychromatophil-erythrocytes is usually lower than that of reticulocytes, but the agreement becomes closer as the number of reticulocytes increases (BAAR AND LLOYD). A peculiar form of reticulocytes—the achromoreticulocytes, has recently been studied by HEILMEYER and his school. These are large reticulocytes, the haemoglobinised body of which is demonstrable only after 6–12 hours Giemsa-staining (EILERS). They are absent from normal blood, but present in a variety of anaemias, particularly in pernicious anaemia where their number may exceed that of normal reticulocytes. Free supravital staining filaments are frequently seen in films with high reticulocyte-counts. They are partly achromoreticulocytes and partly definite artifacts. The achromoreticulocytes mature to achromocytes which are identical with the "gigantocytes" or a "corps en demi-lune" of SERGENT AND BRUMPT.

* A recent review of studies on the reticulocyte is that of LOWENSTEIN (Intern. Rev. Cytol. *8:* 135, 1959).

The transition from the reticulocyte to the mature erythrocyte is associated with a decrease in the diameter. Studies of WEICKER resulted in the surprising, but by measurements supported, statement that this is due to a single division and not to shrinking of the cell.

In many pathological conditions red blood cells are seen which contain iron granules giving a positive Prussian blue reaction. These have been discovered by GRÜNEBERG in a hereditary anaemia of "flexed tail mice". They were studied in humans by DONIACH, GRÜNEBERG, and PEARSON; DOUGLAS AND DACIE; CASE; KAPLAN AND ZUELZER a. o. The iron in haemoglobin is firmly bound by ionic and covalent bonds in the haem molecule and is not demonstrable as Prussian blue or by any other colour reaction given by Fe ions—such as the ferrous dipyridyl complex. GRÜNEBERG named these cells "iron cells" or siderocytes. Nucleated red cells containing iron granules are called sideroblasts. The literature on sideroblasts has recently been reviewed by MOURIQUAND. GRÜNEBERG considered the following possibilities for the origin of siderocytes in the anaemia af flexed tail mice. (1) The flexed anaemic mice are unable to complete the haemoglobin synthesis and they furnish their red cells with a hitherto unknown haemoglobin precursor, the iron of which is still demonstrable by the Prussian blue reaction or (2) the iron of siderocytes is the result of a hitherto unknown process of haemoglobin breakdown within the red cells. In hereditary anaemia of mice the presence of siderocytes is determined by a gene which behaves as a simple recessive factor, determines an early and transitory anaemia, a nearly normal viability before and an increased mortality after birth. In addition it causes flexures of the tail and the presence of a belly spot. In man they are frequent in lead poisoning and particularly abundant after splenectomy with persistent reticulocytosis. CASE thought that siderocytes are evidence of intracellular breakdown of haemoglobin and believed that the index reticulocytes to siderocytes gives a good representation of the formation of red cells, particularly in lead poisoning. However, DOUGLAS AND DACIE showed clearly that the siderocytes are young erythrocytes. KAPLAN AND ZUELZER and MOURIQUAND found iron granula in normal erythroblasts. Their number is increased in many anaemias but they are absent in iron deficiency anaemia prior to iron therapy. They rise rapidly after the administration of iron. They are usually in normal numbers in haemolytic anaemias. The iron of siderocytes is not that of verdohaemoglobin (see page 25) or BARKAN's "easily detachable iron" which is probably identical with the latter. It is probably a loosely protein-bound iron which is on the verge of being incorporated into the protoporphyrin. Siderocytes indicate, therefore, that the last stage of haemoglobin synthesis lags behind the erythrocyte formation. The iron granules of siderocytes are often associated with a basophil protein particle and then demonstrable by the Romanowski stains as "Pappenheimer bodies" (DACIE AND DONIACH).

In pathological conditions also other forms of young erythrocytes are found. Basophil stippling which is abundant in lead poisoning and also in the Rietti-Greppi-Micheli type of Mediterranean anaemia is a pathological manifestation of the same material as substantia reticulo-filamentosa and polychromasia. This has been shown by WHITTBY AND BRITTON. Polychromasia is the appearance of immature erythrocytes in Romanowski-stained preparations. The agreement between the number of reticulocytes and of polychromatophil erythrocytes is the better the more numerous they are (BAAR AND LLOYD). The polychromatophil erythrocytes appear blue blue-grey or reddish-grey. This depends on the quantitative relationship between basophil substance and haemoglobin. A separation of blue polychromatophils from grey