ERYTHROPOIESIS WITH PARTICULAR REFERENCE TO ITS STUDY BY BIOPSY OF HUMAN BONE MARROW: A REVIEW

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BY

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In this review we are attempting to present a picture of the morphology, physiology, and pathology of erythropoiesis in man. The ease with which the blood cells and their precursors may be examined provides an almost unique opportunity for their study and greatly adds to the charm and interest of haematology; and the results obtained thereby are probably significant for the growth of human tissues as a whole. Despite great progress in recent years, however, many gaps in knowledge and anomalies still exist, particularly in respect of the factors

underlying the growth and differentiation of blood cells and the effects of pathological states upon them.

Because of the very great importance of technique, we are prefacing the main discussion with sections on the methods of bone marrow biopsy.

Methods of Bone Marrow Biopsy

The haemopoietic function of the red marrow has been realized since the description by Neumann in 1868 of the origin therein of the erythrocytes. The early studies on human material were carried out

on post-mortem tissue, however, and it is only comparatively recently that biopsy of marrow has been widely employed and has thus rendered possible the examination of fresh material in stained smears. A big step forward in the technique of examination was made by Seyfarth (1923), who introduced a method of trephining the sternum by means of a small 3 mm. straight trephine. The preparation of histological sections from such material was admirably described by Custer (1933). However, although trephine biopsy provides excellent material for sections and smears, it has disadvantages; it is a minor surgical operation, which can be safely carried out only under aseptic conditions in the operating theatre, and which may be dangerous in the presence of a bleeding tendency or of granulocytopenia. In addition, the skin incision takes several days to heal, and the trephining can seldom be repeated. The introduction by Arinkin (1929) of the method of needle puncture biopsy of the sternum has, therefore, given a great impetus to the study of the marrow tissues during life. The relative values of the trephine technique and of needle biopsy are discussed by Dameshek and others (1937) and by Osgood and Seaman (1944). Stained smears of material aspirated after death are generally unsatisfactory, for the degenerating cells are easily broken up by the process of spreading (Rohr and Hafter, 1937; Leitner (Leitner and others, 1949a)).

Sternal puncture.—Arinkin's method of sternal puncture provides material which can not only be spread on slides, dried in the air, and stained like blood films, but which may also be examined supravitaly by various optical techniques, and in addition provides marrow particles which may be sectioned or subjected to chemical micro-analysis. The results obtained by Arinkin's technique in health and in many blood diseases have appeared in numerous papers and several monographs (for example, Segerdahl, 1935; Bodley Scott, 1939; Leitner, 1941; Rastelli, 1943).

A variety of needles exists. Some sort of adjustable guard is essential, and the needle designed by Salah (1934) is widely used in this country. In addition, needle-trephines have been designed; that of Türkkel and Bethel (1943) is useful, yielding a small piece of cortical bone and underlying marrow. The trephine is passed through a hollow needle, and no skin incision is required.

In careful hands and using an aseptic technique sternal puncture is a safe procedure in the great majority of patients and may often be performed on out-patients. However, fatalities have resulted, mostly through complete transfixion of the sternum (*Lancet*, April 10, 1948), but this should not occur

if a guarded needle is employed. It should not be lightly undertaken in the presence of a bleeding tendency. Although, as a rule, little haemorrhage results in patients with a prolonged bleeding time, it should never be performed in haemophilia or in patients with similar coagulation defects.

The usual site for sternal puncture is the manubrium sterni or the first or second piece of the body of the bone; in our experience rather more cellular marrow with a smaller admixture of blood is obtained from the manubrium, although in this site it is rather difficult to be sure that the needle has entered the medullary cavity. The sternum may be repeatedly punctured; a different site should be selected for each puncture in order to avoid marrow possibly disorganized by haemorrhage resulting from previous punctures.

Other sites for needle biopsy; the tibia in childhood, the iliac crest, and vertebral spines.—In the youngest children, from birth to four or five years, the medial aspect of the head of the tibia may be punctured and active marrow withdrawn. This procedure is certainly less dangerous and less upsetting to the child than is sternal puncture. In older children the tibial cortical bone is usually too dense and the marrow within normally less active. Better samples are obtained by sternal puncture (Kato, 1937).

Recently the iliac crest (Rubinstein, 1948) and spinous processes of the vertebrae have been punctured and found in adults to yield good samples of marrow. Puncture of the spinous processes of the vertebrae is particularly convenient, as they lie superficially and the overlying tissues are not highly sensitive. The needle is passed vertically into the spines of the lumbar or lower thoracic vertebrae of the sitting or reclining patient (Loge, 1948). Rather more pressure is required than for sternal puncture. An added advantage of spinous process puncture is that the patient cannot see what is happening and that several attempts at puncture and aspiration can be made in the same anaesthetized area if necessary.

It is an advantage to have a choice of several sites, both in the same region, for example the sternum and vertebral spines, and between these areas, as repeatedly "dry" or non-cellular punctures are sometimes encountered.* In these cases, selection of a different site may either yield a cellular marrow or strengthen suspicion of a widespread pathological change affecting the marrow. The questions of different sites and of various forms of puncture needles are discussed at length by Rastelli (1943a).

* Repeated failure to withdraw marrow is disconcerting. In our experience "dry" punctures or aspirates containing only blood or very few marrow cells have been due, in cases where we have been confident that the needle has penetrated the cortical bone, to marrow aplasia or hypoplasia, to marrow fibrosis, to secondary carcinoma-tosis, and sometimes to leukaemia, particularly of primitive cell types (see also page 4). Dameshek and others (1937) and Dameshek (1948) have had similar experiences. In these cases a surgical trephine operation may be required before the diagnosis can be made with certainty.

Although there is normally a considerable variation in the composition of cellular marrow withdrawn from adjacent or different sites, the general trend and type and maturity of haemopoiesis and the balance between erythropoietic and leucopoietic activity is similar (Pizzolato and Stasney, 1947). This essentially similar yet variable pattern is well shown when sections of aspirated material are studied (J. N. Davidson and others, 1948).

The Examination of Aspirated Bone Marrow

The preparation and staining of bone marrow films.—The volume of marrow which may be aspirated by puncture is limited, and the more material aspirated the greater the proportion of contaminating blood. Osgood and Seaman (1944) recommend that 1 ml. should be withdrawn as a routine, but we prefer not to take more than 0.2 ml.

Most, if not all, workers employ a Romanowsky dye for the routine staining of bone marrow films; many use a panoptic method, at a controlled pH (about 6.8) using May-Grunwald-Giemsa or Jenner-Giemsa stains. Both these techniques give excellent results, but in our experience a relatively long fixation with methanol (3–5 min.) is essential (Turnbull, 1948).*

A well spread and well stained cellular film of marrow can be a delightful object, but the method of preparation of the film is as important as the staining technique. It is desirable to concentrate the marrow cells at the expense of the blood by which they are inevitably diluted, and various methods of concentration have been suggested. Davidson (1941) for instance expresses the contents of the aspiration syringe into a watch glass, picks out with forceps the fragments of marrow which adhere to the glass surface, and spreads them individually on slides. This technique provides marrow fragments with little contaminating blood and gives a better idea of the cellularity of the tissue than can be obtained by merely spreading drops of the aspirated material directly on to slides. Other authors (Limarzi, 1947) use more complicated methods (*vide infra*). We ourselves employ a very simple concentrating device which is generally satisfactory. A series of drops of syringe contents is delivered on to slides and most of the blood is then sucked off with a fine Pasteur pipette applied to one edge of the drop; the irregularly shaped

marrow fragments tend to adhere to the slide and are left behind. A film is then made of the marrow fragments and the remaining blood by means of a smooth-edged glass spreader of not more than 2 cm. in width; the marrow fragments are dragged behind the spreader and leave a trail of cells behind them. It is on these trails that differential counts should be done, commencing from the marrow fragment and working back towards the head of the film; in this way smaller numbers of cells from the peripheral blood become incorporated in the differential count.

Concentration of marrow by centrifugation.—Several workers have used centrifugation techniques in an attempt to assess the relative distribution of marrow cells, peripheral blood, and fat in aspirated material. Limarzi (1947) has devised a method for centrifuging heparinized aspirated marrow in a Wintrobe haematocrit tube. Yellow fat, red fat, plasma, marrow cells, and mature erythrocytes separate from above downwards. The marrow cell layer is used for preparing films and for differential cell counts. Limarzi (1947) claims that his method gives a relatively accurate indication of marrow hypo- or hyperplasia and that the amount of fat present can be gauged. He also claims that the separation of fat from the marrow cells facilitates their proper staining and that numbers of uniform preparations can be obtained from the sample. Berman and Axelrod (1947) have described a comprehensive method for treating aspirated marrow so as to obtain volumetric readings of the constituent fractions, as well as smears, imprints, and histological sections.

The data obtained by concentration techniques employing centrifugation are useful in group studies, but are less valuable in individual cases on account of the wide range of values encountered even in the normal.

Quantitative cell counts on aspirated marrow.—A number of figures for the cell content of aspirated normal marrow have been given in the literature (Osgood and Seaman, 1944; Vaughan and Brockmyre, 1947). The variation is extremely wide; this is hardly surprising in view of the uncontrollable factor of dilution with peripheral blood and the tendency of the marrow cells to adhere together in clumps of varying size. Perhaps the best method of enumeration is that of Isaacs (1937a), in which the particles of aspirated marrow are mixed with and broken up in normal serum. He attributed the dispersing action of serum to enzyme activity (Isaacs, 1930).

Tentative normal standards are given by Osgood and Seaman (1944). Fieschi, quoted by Rastelli (1943b), with whom we largely agree, states that no information is to be obtained from cell counts on the marrow that cannot be derived from assessment of simultaneously prepared films and sections. Clearly, the value of the quantitative method depends upon details of technique and is of greatest use in serial studies (see Stasney and Pizzolato, 1942).

* Although Romanowsky staining is undoubtedly the best single staining method, there are simple supplementary techniques of value. By wet-fixing films in Susa fluid, pyronin-methyl green may be used to demonstrate nucleoli and cytoplasmic basophilia (White, 1947). Similarly Feulgen's staining reaction gives a good picture of the chromatin and chromosomes; light green yellowish S is a particularly suitable counter-stain. Phase-contrast techniques on unstained cells mounted in formalin have been used by Jones (1948) in the study of mitochondria.

The preparation of histological sections of aspirated bone marrow.—Although the operation of trephining the sternum provides excellent material for imprint preparations and for sections, the latter require decalcification which impairs staining, particularly by the Romanowsky dyes. It is, however, possible to prepare sections of the small fragments of marrow, free from bone, which may be obtained by simple aspiration through an ordinary puncture needle. These fragments of marrow, which Schleicher (1944a) has termed "marrow units," are naturally much smaller than the material obtained by surgical trephining, but they can be stained readily and they then provide a picture in miniature of the cellularity, proportion of fat spaces, and general architecture of the marrow. It is their small size, usually up to 1 mm. in diameter, which limits the value of the technique, for there is always some uncertainty as to how representative of the marrow the fragments are.

A number of techniques for sectioning these fragments have been described (for example, Davidson, 1941; Mertens, 1945; White and others, 1946; Cappell, 1947; Weisberger and Heinle, 1948), which differ in the details of handling the fragments and fixing and embedding. In none of these methods is it necessary to decalcify the tissue. These histological sections are quite easy to prepare; do they yield reliable and helpful information? The doubt as to whether the small fragments are really representative has already been referred to. In practice we find that although they are a useful adjunct to the examination of films, and reveal details of structure otherwise not easily appreciated, they do not often reveal more of diagnostic importance than do film preparations; indeed the latter are made from fragments similar to those sectioned. They certainly are less satisfactory for the study of subtle differences in haemopoiesis than are well stained films, particularly as the cytoplasmic structures are less well preserved. On the other hand they do indicate the relationships of the marrow cells one to another and to the parent reticulum cells and they show the proportion of the marrow occupied by fat and the relationships between sinusoids and the marrow cells. In addition it is easy to recognize cells such as phagocytic reticulum cells, which are difficult to identify in smears, and easily broken in the spreading of the film. Similarly megakaryocytes, which are only seen in smears in small numbers, are more prominent in sections, and occasionally a satisfactory and decisive view of tumour cells may be obtained.

Whilst relatively large fragments of marrow are obtained by simple aspiration on most occasions, we have sometimes been unsuccessful just when it seemed most important to obtain material. In these patients, mostly suffering from marrow aplasia or hypoplasia, marrow fibrosis ("myelosclerosis"), secondary carcinomatosis, or aleukaemic leukaemia, the films are similarly uninformative. In myelosclerosis and in carcinomatous infiltration the tissue is presumably too firmly organized, with a thickened reticulum and perhaps an increase in collagen also, to be disrupted by suction. In the leukaemias and in "aplastic" anaemia it is more difficult to understand why aspiration should fail; sometimes it is

difficult even to obtain blood. Possibly the ease with which material is withdrawn depends not only on the texture of the marrow, in particular the volume of developing marrow cells compared with the basic reticular structure, but also on its blood supply. If the blood circulating through the bone marrow is restricted, as it might be, due to proliferation of leukaemic cells or perhaps to marrow aplasia, difficulty in aspiration might result; for it is clear that, when one is aspirating from a small enclosed area, blood must enter to replace the material withdrawn. In practice, if the operator fails to obtain marrow from the sternum, it is advisable to try another site such as the lumbar spine. If aspiration of the spine is unsatisfactory, trephining through an introducer needle (Türkel and Bethel, 1943) or surgical trephining of the sternum may be needed.

In Plate I are illustrated photomicrographs of sections of fragments of marrow from patients suffering from a variety of blood disorders. The specimens were obtained by aspiration of the manubrium of the sternum and prepared by the method of White and others (1946).

The Extent of the Normal Marrow at Different Ages

As the normal distribution of the bone marrow has a bearing on the selection of sites for biopsy, this question will be briefly considered. At birth, active red marrow is found throughout all the ossification centres in the skeleton, and puncture of the tibia medial to the tubercle will yield active marrow in young children. The regression of active marrow from the long bones in later childhood and adolescence and the partial regression in the flat bones is well illustrated by Custer and Ahlfeldt (1932). The active red marrow is replaced by fat in which the potentially haemopoietic reticulum cells normally lie dormant. Under abnormal conditions, such as in many anaemias and in the leukaemias, these reticulum cells once more become active and form haemopoietic stem cells; the fat cells may almost completely disappear. There does not seem to be any striking difference between the extent of the marrow once the growth period is completed. There is no significant difference in distribution and cellularity between the marrows of elderly subjects and normal adults (Reich and others, 1944; Leitner and others, 1949b).

The cause of the apparent increase in volume of the marrow in childhood is uncertain; it may in fact be more apparent than real. It is not certain that the volume of marrow related to the child's total blood-cell volume, which it constantly maintains, is any greater than the marrow-total-blood-cell volume relationship in the adult. A possible cause of a hyperplastic marrow would be that the erythrocytes (and leucocytes) in childhood had a shorter life than in the adult. Mollison's (1948)

observations on the survival of the blood of newborn infants in other normal infants suggests, however, that postnatal erythrocytes are likely to survive for a normal length of time. Moreover, the total granulocyte count in childhood and adolescence is also very similar to that of the adult, which suggests that the demand on the marrow for granulocytes is similar throughout life and is not likely to be a cause of marrow hyperplasia.

Normal erythropoiesis: site of development.—The exact site of erythropoiesis in the normal marrow and the problems connected with the haemopoietic potencies of the more primitive marrow cells have been a source of controversy for many years. Although it is not our intention in this paper to review in detail the many theories of haemopoiesis that have been suggested, a limited treatment is required. A cardinal point in the polyphyletic theories of Sabin, Cunningham, and Doan and co-workers has been that, whereas granulocytes are formed extravascularly, the erythrocytes arise intravascularly from the lining endothelium of intersinusoidal capillaries (Doan, 1923; Doan and others, 1925). Their evidence was based partly upon the study of regeneration occurring in the hypoplastic fatty marrow of pigeons previously starved. However, granulocytes and erythrocytes do not seem to develop in this way in man and in other mammals, and an extravascular origin for both has been claimed by the majority of workers (Bunting, 1906; Maximov, 1910; Drinker and others, 1922; Bloom, 1938a). More recently Hamre (1947) has described the extravascular formation of erythrocytes and granulocytes in rats and the subsequent migration of the more mature cells across the intact sinusoidal endothelium.

Study of our own sectioned material has convinced us of the correctness of the conception of extravascular haemopoiesis in man. The more primitive haemopoietic cells in both normal and abnormal marrows always seem to be in the stroma of the marrow and outside the marrow sinusoids and capillaries.* In the sinusoids, however, there may be seen a few partly mature forms, such as polychromatic normoblasts and metamyelocytes, in addition to mature erythrocytes and leucocytes.

If it is admitted that haemopoiesis is extravascular, the problem of the migration of the developing blood cells from the marrow stroma into the blood stream remains to be solved. In the case of granulocytes, with their known capacity for amoeboid movement, the migration of the more mature forms across an endothelial barrier presents little difficulty and is generally admitted to occur, but the movement of the non-motile normoblasts is more difficult to understand. In Drinker and others' (1922) view it was the pressure of expanding collections of maturing normoblasts that forced the latter through the sinusoidal endothelium. Maximov (1910) and Key (1921) postulated temporary deficiencies in the continuous endothelium but did not produce conclusive evidence of this. Hamre (1947) has failed to demonstrate in rats any such breaches in continuity. He

observed the passage of both granulocytes and erythrocytes through the sinusoidal walls, and considered that the cells passed between the margins of the lining endothelial cells and that pressure from the growing mass of cells in the stroma might well help them to do so. Our evidence from human material points in the same direction; in hyperplastic marrows such as are seen in Addisonian pernicious anaemia we have observed groups of cells bulging into the lumina of the sinusoids. Isaacs (1933) has called attention to the mutual adhesiveness of the more primitive marrow cells. As the cells mature they become free from one another through liquefaction of the intercellular material and come to lie in "lake-like" spaces. This separation is a necessary preliminary to a cell's entry into the vascular sinusoids proper. The relationship between erythrocyte or normoblast migration through the sinusoidal walls and the formation of poikilocytes will be considered in a later section. Although it can be accepted that the erythrocytes and granulocytes are normally formed extravascularly in the marrow, this is not necessarily true of haemopoiesis in other situations. In the spleen, for instance, active haemopoiesis in dilated sinusoids may sometimes be seen in pathological material (see also the section on foetal erythropoiesis).

The Morphology of Normal Erythropoiesis

Nomenclature of the erythrocyte precursors.—For the sake of simplicity the vexed question of nomenclature* has so far not been considered, but the subject cannot be entirely avoided. The normal form of erythropoiesis gives rise to the normal erythrocyte (the normocyte) by a process best referred to as normoblastic erythropoiesis. The earliest definite erythrocyte precursor is the pronormoblast, and later nucleated forms are normoblasts. In later sections we shall refer to and describe other types of formation, megaloblastic, intermediate, and macronormoblastic, etc.

The term "erythroblast" should be reserved for any nucleated erythrocyte precursor without reference to the form of development, in accordance with the original usage of Ehrlich (Ehrlich and Lazarus, 1898, 1900).

Synonyms for the pronormoblast and normoblast.—Ferrata (Ferrata and Storti, 1948) called the earliest recognizable erythrocyte precursor the pro-erythroblast, derived from the haemocytoblast and developing through erythroblast stages to the erythrocyte. This terminology is used by the Italian school of haematology generally (Ferrata and Storti, 1948; Rastelli, 1943c). Naegeli (1931) has used the term macroblast (makroblast) to refer to the younger and larger erythroblasts, particularly in embryonal organs and in the marrow in states of rapid

* Silver impregnation of the marrow, by demonstrating the reticular framework, helps to differentiate the marrow stroma with its network of fine fibrils from the sinusoids which lie on a layer of longitudinal argyrophil fibrils (see Plate I, Figs. 5 and 6).

* In this paper we discuss the orthodox and traditional nomenclature only. Osgood and Ashworth (1937) introduced an entirely novel system which has not been generally accepted. The whole question is now once more under review by the International Society of Haematology.

blood regeneration, and Swiss authors such as Rohr (1940) and Leitner (1941) have used the term similarly, but this usage is confusing and better avoided (see the discussion on macronormoblasts in a later section). Similarly, the use by Sabin and co-workers of the term "megaloblast" for young basophilic red cell precursors under normal conditions (Doan and others, 1925) has no justification and has generally been abandoned.

Schulten (1937) derived the normal erythrocyte from the pro-erythroblast through macroblast and normoblast stages, and Dameshek and Valentine (1937) a series of normoblasts, A, B, and C, from haemohistioblasts through the erythrocyte. Israëls (1939), whilst deriving the erythrocytes from multi-potent haemocytoblasts, called the earliest recognizable stage the pro-erythroblast, and the subsequent stages of normoblasts A, B, and C, in increasing order of maturity.

Turnbull (1936a), basing his observations on sectioned material, described the derivation of normoblasts from pluripotent haemocytoblasts through a series of "primary erythroblasts." He recognized three stages of normoblasts of similar size; the earliest with basophilic cytoplasm, then the polychromatic stage, and finally ripe orthochromatic normoblasts of the final stage.

Formation in intra-uterine life.—Details of the formation of the erythrocytes in intra-uterine life are of more than academic interest, for there are certain interesting contrasts and analogies between foetal erythropoiesis and that of the adult in health and in disease. Ehrlich (1880; Ehrlich and Lazarus, 1898, 1900) was the first to call attention to the similarity between the megaloblasts of pernicious anaemia and the haemoglobinized* primitive erythroblasts developing within the primitive blood vessels of the presumptive human embryo of 2 to 5 mm. in length. These primitive foetal erythroblasts do not lose their nuclei and are very large cells. They are replaced by the first "definitive" erythroblasts, which in size and nuclear structure are intermediate between the cells of the primitive generation and those of adult life.† These cells lose their nuclei when mature and give rise to erythrocytes (macrocytes) whose volume is greater than those of the normal adult. The nucleated precursors may be termed macronormoblasts‡ (Jones, 1943). The liver is the chief site of their formation in mid-foetal life.§ From the fifth month of intra-uterine life onwards erythropoiesis commences within the marrow cavities of the ossification centres and at birth intramedullary formation is of prime importance, extramedullary foci having almost entirely disappeared by this time. The type of formation at birth is normoblastic and similar to that of the adult.

* It appears that the type of haemoglobin produced at all stages of intra-uterine life is the same—that known as foetal haemoglobin (H. M. Jope, 1948; Hoch and others, 1949).

† The primitive generation of erythroblasts is formed intravascularly, as are the definitive erythroblasts (macronormoblasts) in early foetal life. Later, erythropoiesis in the liver is at first both intravascular and extravascular, but eventually is entirely extravascular, as it is in the bone marrow from the start (Gilmour, 1941).

‡ Some details of macronormoblastic development are given later in this paper.

§ Gilmour's (1941) paper is an important contribution to the study of intra-uterine haemopoiesis. It is based on 57 human embryos and foetuses. See also Bloom and Bartelmez (1940).

The cause of this variation in cell morphology at different age periods is far from clear. There is a gradual diminution in erythrocyte diameter and volume as the foetus matures,* but there is little evidence to prove that the macrocytosis is dependent upon an insufficient supply of anti-anaemia factors (Wintrobe, 1946a). Possibly the presence of the primitive persistently nucleated erythroblasts recalls phylogenetic development. That foetal macrocytes may have a shortened life *in vivo* is suggested by Mollison's (1948) transfusion experiments. In this connexion it is interesting to recall that macrocytosis (and macronormoblasts) are seen in adult life in certain anaemias associated with rapid blood formation.

It is well known that foci of extramedullary haemopoiesis may be met with in adults in a variety of blood diseases, particularly where the bone marrow is widely invaded by carcinoma or where the marrow is undergoing fibrosis as in myelosclerosis. Less frequently extramedullary haemopoiesis is seen in haemolytic anaemia or even in pernicious anaemia. The foci occur most frequently in the liver and spleen, sites of vigorous intra-uterine formation, but are occasionally found in lymph glands or as heterotopic foci (see Vaughan, 1936a and b).

Normoblastic erythropoiesis in the adult.—The irregular lattice-like pattern of cellular and fatty areas in the adult human marrow has already been referred to, and close inspection of the cellular areas shows that focal areas of erythropoiesis are surrounded by larger zones of leucopoiesis.

The basic potentially haemopoietic cell in the marrow is a reticulum cell. Maximov (1927) has described the appearance of these cells in sections as flattened, pale-staining, and inconspicuous, and he considered them to be less differentiated than the dye-storing and phagocytic cells of the marrow stroma or the littoral cells of the marrow sinusoids. The question as to whether these more differentiated phagocytic cells can form haemocytoblasts or more mature forms of haemopoietic cells has not been settled and is discussed by Bloom (1938b). Ferrata and Storti (1948) recognized the haemohistioblast as an intermediate form between the primitive reticulum cells and haemocytoblasts. Clearly, irrespective of nomenclature, there is continuity of development between the most primitive and the more mature forms. Normally, these primitive cell types are present in small numbers and are somewhat difficult to identify in marrow smears. The cells are fragile and easily broken up by spreading, and cytoplasmic outlines may be indistinct. In pathological states such as Addisonian pernicious anaemia, and in certain refractory anaemias where there is hyperplasia of primitive cells, they are more frequent and may be seen in groups or even as syncytia (see Schleicher, 1945).

* Wintrobe (1946, p. 32) gives interesting data from thirty human foetuses; see also Wintrobe and Shumacker, 1935.

In film preparations the marrow reticulum cells vary between 20 and 30 μ . The cytoplasm is palely basophilic and abundant, and may contain a few azurophil granules; fine spherical mitochondria are numerous and evenly distributed. The nucleus is large, round, or oval, with a pale-staining* fine chromatin pattern and one or more round or oval nucleoli.

The *haemocytoblast*† is a large cell of somewhat variable outline, in size averaging about 20 μ in diameter. The cytoplasm is moderately deeply basophilic, and contains numerous mitochondria but no granules. The nucleus is round, averaging about 15 μ in diameter; it stains palely with Romanowsky dyes and presents a finely stranded and stippled (leptochromatic) chromatin pattern. Multiple nucleoli are present or a single elongated nucleolus of complex form bounded by a very fine layer of nucleolus-associated chromatin.

The *pronormoblast* is a slightly smaller cell than the haemocytoblast and usually of a rounded outline, although often showing fine pointed processes at the periphery of the cytoplasm. The average diameter is less than 20 μ and the nucleus less than 15 μ . The cytoplasm is more basophilic but less extensive than in the haemocytoblast; mitochondria are numerous. The chromatin pattern of the nucleus is more condensed and the nucleoli are smaller and are less easily seen, being surrounded by denser nucleolus-associated chromatin. The term *normoblast* refers to the developing erythroblast from the stage when the nucleoli have disappeared to the loss of the nucleus itself. As the cell matures there is a progressive shrinkage in size to about 8 μ and a parallel decrease in size of the nucleus. The cytoplasm becomes less basophilic as the cell matures, and increasingly acidophilic, this latter property being considered to be due to the formation of globin or haemoglobin.

Thus normoblasts may be described as basophilic, polychromatic, or orthochromatic, although in our experience fully orthochromatic cells are rarely

encountered in normal subjects—that is to say, cells in which the cytoplasm is as acidophilic as that of adult erythrocytes. Such cells are, however, often illustrated (Wintrobe, 1946, plate 2; Whitby and Britton, 1946, plate 4). Everything depends upon the staining. It is possible to stain films to show orthochromatic normoblasts, but they are seldom seen if staining is controlled in such a way that polychromasia is well demonstrated. Indeed it is difficult to imagine an orthochromatic normoblast (having presumably lost its cytoplasmic basophilia) becoming a polychromatic reticulocyte and regaining cytoplasmic basophilia when its nucleus is disposed of. This would only be possible if the desoxyribonucleic acid of the normoblast nucleus contributed to the basophilic staining of the cytoplasm of the non-nucleated cell which developed from it, and for this there is no evidence. Brachet (1946) states, however, that Dustin suggested such a possibility. Reticulocytes are Feulgen-negative. If ribonucleic acid had been formed from desoxyribonucleic acid, an increase of both the reticular-filamentous material and diffuse basophilia removable by ribonuclease would be expected in the reticulocytes as compared with the normoblasts, but this does not seem to be so. A point that should be stressed is that the progressive diminution in size and pyknosis of a cell's nucleus is a more reliable guide to that cell's maturity than is the state of ripening of the cytoplasm, and that even in the normal the relative progress of nuclear and cytoplasmic ripening varies appreciably from cell to cell.

The areas of deeply staining chromatin, which first appear in the vicinity of the dwindling nucleoli and near the nuclear membrane, are brought together by shrinkage of the nucleus and ultimately fuse. In its final form the nucleus is entirely pyknotic and about 4–6 μ in size. That the changing pattern of the nuclear chromatin is not an artifact produced by fixation has been shown by studies with the electron microscope (Rebuck and Woods, 1948).

A few additional details as to the cytoplasm of erythroblasts may be added. Although the earlier forms possess a cytocentrum, Golgi apparatus, and mitochondria (Maximov and Bloom, 1948), these structures are lost in the course of differentiation. Mitochondria may be demonstrated by the supravital use of Janus green (Key, 1921; Sabin, 1928), and Jones (1947, 1948) has shown that, due to their content of lipoids, they can be stained by Sudan black by the method of Baker (1945) in air-dried films fixed in formol-calcium chloride (see Plate IV, Fig. 5). Jones has also used phase contrast techniques. He has demonstrated that in air-dried unstained films mounted in formalin, mitochondria lie above and below the nucleus, and that in this way they must contribute to the chromatin pattern of the nucleus

* The descriptions given hereafter refer to Romanowsky-stained methanol-fixed dry film preparations. The appearances in sections or in wet fixed films differ due to the preservation of a more spherical shape of the cell and to the use of different fixatives. A description is outside the scope of this article.

† The haemocytoblast of Ferrata is the marrow stem cell of the erythrocytes, granulocytes, and megakaryocytes; it is the most organized stem cell capable of developing and differentiating to more than one type of blood cell (Ferrata and Storti, 1948; Rastelli, 1943c). Downey (1938a) discusses at length the potency of the haemocytoblast. He agrees that it is multi-potent, but unfortunately confuses the issue by referring to it as a "myeloblast." Helly's (1910) "erythrogon" is probably the same cell (see also Dameshek and Valentine, 1937). The multi-potency of the haemocytoblast is not universally accepted. Rohr (1940), for instance, derives the erythrogon (uni-potent) directly from the reticulum.

The development of the granulocytes from haemocytoblasts proceeds in a parallel fashion to that of the erythroblast. Mitosis occurs only in the earlier stages, and as the cell matures the degree of cytoplasmic and nucleolar basophilia diminishes and at the same time the azurophil and later the specific granulations appear.

as revealed by staining with Romanowsky dyes. He has also shown that the clear perinuclear area represents the unstained negative images of mitochondria and not hyaloplasm (see Wilson, 1928). More cytoplasmic particles are revealed by phase contrast microscopy than can be stained supravivally by Janus green.

Differential cell counts on marrow films.—Most workers perform differential cell counts on marrow films, and by presenting the data in the form of a "myelogram" express the incidence of

the various cell types as percentages. Such figures, unfortunately, are less accurate than they appear to be at first sight. Not only is there an unknown admixture with cells from the peripheral blood in which the marrow cells are diluted, but in addition there is a tendency for the more primitive cells and relatively fixed marrow cells such as megakaryocytes to remain behind in the marrow. Ideally, differential counts should be performed on sections of aspirated material, but here, unfortunately, there is

TEXT FIG. 1.—Series of scale drawings of maturing erythroblasts. Series A shows representative megakaryoblasts (Figs. 1–5) from a patient with severe Addisonian anaemia, erythrocytes 1,300,000 per c.mm. In series B (Figs. 6–10) are illustrated intermediate megakaryoblasts of comparable maturity from the marrow of a patient with steatorrhoea, erythrocytes 3,500,000 per c.mm. In series C (Figs. 11–15) the cells (normoblasts) were from the marrow of a normal adult man. Series D (Figs. 16–20) shows cells (macronormoblasts) of similar age groups from a patient with a macrocytic haemolytic anaemia (nocturnal haemoglobinuria), erythrocytes 1,500,000 per c.mm. In series E (Figs. 21–25) the cells were drawn from the marrow of a patient with a severe iron deficiency anaemia, erythrocytes 3,000,000 per c.mm.

In each series the topmost cell is a primitive cell (promegakaryoblast or pronormoblast).

TEXT FIG. 2.—Curves of maturation and mitotic activity during erythropoiesis.

Upper figure: Maturation curves.

— Maturation curve from calculated means of figures from 15 normal adult marrows ($6 \times 1,000$; 9×500 cell counts). The observed range is indicated in the stippled area.

— Maturation curve for erythropoiesis in Addisonian pernicious anaemia.

—○—○— Normoblastic development.

—○—○— Megaloblastic development.

— Maturation curve in iron deficiency anaemia.

..... Maturation curve in congenital haemolytic anaemia.

Ordinates: Per cent of cell types.

Abscissae: Stages of erythropoiesis: RE, reticulum cells. HAE, haemocytoblasts. Pr.N., pronormoblasts. B.N., basophilic normoblasts. P.N. early and late, early and late polychromatic normoblasts. N., pyknotic normoblasts.

Lower figure: Mitosis during erythropoiesis.

Distribution by developmental stages of 67 erythroblasts in mitosis, encountered in 100 mitoses from normal marrows.

Ordinates: Per cent of the mitotic cells.

Abscissae: Stages of erythropoiesis, as in upper figure.

difficulty in the proper identification of cells. Dameshek and others (1937) give illuminating figures for parallel observations made on sections and on smears made from puncture fluid, and other observations are reviewed by Osgood and Seaman (1944) and by Leitner and others (1949). Osgood and Seaman, in an excellent review, also discuss the unavoidable statistical errors involved in making differential counts and the necessity for counting large numbers of cells, particularly if an attempt is being made to assess the frequency of cells present in only small numbers. A further difficulty is the impossibility of accurately dividing into arbitrary classes cells of which every gradation of development

may be seen; and the fact that different authors naturally use different criteria to separate, say, "early" from "late" normoblasts makes comparison between their figures still more difficult.

For all the above reasons the figures given in the literature for the "normal" myelogram differ widely, as is illustrated in the tables of Bodley Scott (1939), Osgood and Seaman (1944), and Leitner and others (1949c).

Pontoni (1936) and Rastelli (1943d) have also discussed the analysis of differential cell counts. Pontoni correctly uses the term "haemomyelogram" for the ordinary differential count, and restricts the use of the term "myelogram" to counts

made on the residuum of cells after excluding the fully ripened ones, that is the segmented granulocytes, lymphocytes, and monocytes, etc. The commonly employed leucocyte-erythroblast or leucocyte-erythroid ratio, being based on the "haemomyelogram," is of limited usefulness; in practice it gives a very wide range in normal subjects (2:1 to 12:1). That dilution with peripheral blood is a major factor in leading to a high leucocyte-erythroid ratio is shown by our lymphocyte values in a series of fifteen normal volunteers. The lymphocyte percentage varied between 13 and 31 per cent, and although some of these cells may have been derived from isolated lymph follicles, in most instances it is likely they came from the peripheral blood.

Pontoni's "leuco-erythrogenetic" ratio is a better expression of the relative proportions of leucopoiesis to erythropoiesis. Mature leucocytes are excluded from the count, and in our series of normal subjects the ratio varied from 0.56:1 to 2.67:1. In most anaemias the ratio is below unity.

Fortunately, the factor of dilution with peripheral blood does not affect differential counts on marrow erythroblasts, unless there is a marked peripheral erythroblastaemia. These differential counts are more satisfactorily expressed by relating the numbers of the different classes of erythroblasts to the total number of erythroblasts rather than expressing them as percentages of the total nucleated cells present.

Because of differences in classification it is difficult to find from the literature normal ranges for the proportion of erythroblasts of different maturities. There is, however, wide agreement that in health the pronormoblasts and basophil normoblasts are few in number and that normoblasts of medium maturity with various grades of acidophilia of their cytoplasm and increasing density of the nucleus predominate. Fully pyknotic cells are less numerous, and pyknotic cells with fully orthochromatic cytoplasm are rarely met with. In our series of fifteen normal subjects, 2 per cent of the erythroblasts were pronormoblasts, 5 per cent basophilic normoblasts, 51 per cent early and late polychromatic normoblasts, and 42 per cent pyknotic normoblasts. In disease these proportions may be much altered.

Maturation curves (Pontoni, 1936; Baserga, 1939) have been used to illustrate the changes in proportion of the various classes of cells in different diseases or at different stages in the course of an illness (Text Fig. 2). Cotti and others (1938) have used a rather different way of expressing the proportion of primitive to maturing cells, and by their maturation index indicate the numbers of

TEXT FIG. 3.—Densitometer records across spectrogram of frog red cell at three wavelengths, obtained from reflecting microscope. (Figure kindly supplied by Drs. R. Barer, E. R. Holiday, and E. M. Jope.)

N., cell nucleus. C., cytoplasm.

405 μ . In the intense Soret band absorption due to haemoglobin.

312 μ . A wavelength at which little specific absorption occurs.

265 μ . In the strong absorption band due to purine and pyrimidine residues of the nucleic acids.

polychromatic and orthochromatic erythroblasts relative to the basophilic erythroblasts. We have employed a similar device to express the marrow immaturity in Addisonian and other megalocytic anaemias (Text Fig. 4).

Growth and Differentiation of Erythroblasts

In the previous sections we have described the stages in development from the haemocytoblast to the pyknotic normoblast. It is almost superfluous to state that this separation into stages is artificial;

TEXT FIG. 4.—Relationship between the peripheral erythrocyte counts and the erythroid-leucocyte ratios (left-hand chart) and the primitive cell-maturing cell ratios (right-hand chart) in thirty-five patients who had megaloblastic marrows. Twenty-six of the patients had Addisonian pernicious anaemia, ●; four patients had steatorrhoea, ○; in three patients the anaemia was associated with pregnancy, □; in two with malnutrition, ■; and in one patient, ◆, anaemia had followed a gastro-enterostomy.

in reality there is a smooth gradation from the most primitive to the most mature forms. This process of development is a dual one, involving growth and cell division and differentiation. According to Cowdry's (1942) classification of levels of cellular activity the haemopoietic reticulum cells and haemocytoblasts are classed as vegetative intermitotic cells and the earlier erythroblasts as progressively differentiated intermitotic cells. In the final stages of development, when differentiation is completed, the cells are fixed post-mitotic cells.

Mitosis during erythropoiesis.—A proportion of the haemopoietic cells are always in mitosis. Japa (1942) studied the sternal marrows from three non-anaemic subjects and found 0.5 per cent of mitoses in fixed and stained films, but in aceto-carmine squash preparations from the same material there were 1.5 per cent, about 45 cells of the 100 in mitosis being erythroblasts. He thought that some of the dividing cells were disrupted when films were made. Mitoses may be seen at all stages, from haemopoietic reticulum cells to the polychromatic erythroblasts. Mitoses are not observed in latest polychromatic and pyknotic normoblasts, which is not surprising in view of the nuclear structure. Normally the absolute numbers of mitoses increase from the more primitive to the more mature stages, and it is rare to find a primitive cell in mitosis without extensive search. However, as the numbers of intermitotic cells also increase at each stage, the relative number of cells in mitosis at each stage is not so variable (see Text Fig. 2). This suggests an orderly increase in cell numbers by

mitosis throughout the growth and earlier differentiation phases of erythropoiesis. The products of the final mitoses then complete differentiation irreversibly. Abnormalities in mitosis leading to the formation of multinucleated cells are not infrequently found, even in normal erythroblasts and their precursors (see p. 30).

Fieschi (1938) represented the incidence of each stage of the mitotic cycle graphically and found that metaphases were the most numerous in normal erythropoiesis, and our results and those of Japa (1942) also indicate this. Increased erythropoiesis, however, leads to a higher proportion of prophases.

Life span of the erythroblasts.—The exact number of mitoses that take place in the development of a normal erythrocyte and the length of the mitotic and inter-

mitotic periods at each stage of development are unknown. The whole process probably occupies several days. Ponder (1948) calculates that in the rat the lifetimes of the haemocytoblast, "erythroblast," normoblast, and reticulocyte are 0.23, 0.35, 2.3, and 3.6 days respectively. Hevesey and Ottesen (1945) have shown that if phosphates containing P^{32} isotope are fed to hens, isotope appears in the desoxyribonucleic acid of the nuclei of circulating erythrocytes after five days (see also Hevesey, 1948a). Shemin and Rittenberg (1946) have shown that in normal man N^{15} -containing haem appears in circulating erythrocytes within two days of the ingestion of N^{15} -glycine. This represents the time between the formation of haem and the delivery of the erythrocytes into the blood stream, and is only part of the total time required for erythropoiesis (see also p. 21).

In man there is other indirect evidence. The response to potent liver extract in a severe case of Addisonian pernicious anaemia indicates that the reticulocytes are delivered into the peripheral blood stream at the maximum rate at about four to six days after an injection of liver. This period of maximum reticulocyte production corresponds presumably to the final maturation of the predominating age group of marrow cells, which in a severe case are the abnormal haemocytoblasts and basophilic promegaloblasts. Owren's (1948) studies on the mechanism of the production of crises in congenital haemolytic anaemia also provide some evidence about the rate of development of the maturing erythroblast. In his case 4, regeneration of "pro-erythroblasts" (pronormoblasts) in the marrow had started on the ninth day of the crisis. Reticulocytes were appearing in the

NORMAL AND ABNORMAL ERYTHROPOIESIS

Figs. 1 to 14: Normoblastic Erythropoiesis in Normal Sternal Marrow

Fig. 1.—Haemohistioblast (haemopoietic reticulum cell). Fine azurophil granulation of palely basophilic cytoplasm. $22.7 \times 13.2 \mu$; nucleus $13.7 \times 10.5 \mu$.

Fig. 2.—Haemocytoblast, $19.2 \times 17.5 \mu$; nucleus $15.0 \times 13.4 \mu$.

Fig. 3.—Pronormoblast, $13.3 \times 12.5 \mu$; nucleus $12.5 \times 10.8 \mu$.

Fig. 4.—Basophilic normoblast, $12.5 \times 11.7 \mu$; nucleus $10.8 \times 10 \mu$.

Fig. 5.—Early polychromatic normoblast, $10.8 \times 10.8 \mu$; nucleus $8.3 \times 7.5 \mu$.

Fig. 6.—Late polychromatic normoblast, $9.2 \times 8.3 \mu$; nucleus $6.6 \times 6.6 \mu$.

Fig. 7.—Pyknotic normoblast, $8.3 \times 7.3 \mu$; nucleus $5 \times 4 \mu$.

Fig. 8.—Mature erythrocyte, $7.8 \times 7.2 \mu$.

Fig. 9.—Haemocytoblast, $13 \times 10.4 \mu$; nucleus $9.1 \times 7.8 \mu$. Film wet-fixed in Susa; pyronin-methyl green staining after one hour in veronal-acetate buffer, pH 6.85. Deep cytoplasmic and nucleolar basophilia attributable to content of ribonucleic acid.

Fig. 10.—Polychromatic normoblast ($7 \times 7 \mu$; nucleus $6.3 \times 5.6 \mu$) from wet-fixed film in Susa stained by Feulgen method. Chromatin stains purple. Cytoplasm is acidophilic and stains with light green yellowish.

Fig. 11.—Polychromatic normoblast ($8.4 \times 6.3 \mu$) from same film as Fig. 9. Cytoplasmic basophilia is much less at this stage; nucleoli have disappeared and chromatin nodes stained by both dyes are prominent.

Fig. 12.—Polychromatic normoblast ($7.7 \times 7.7 \mu$) from similar film treated with ribonuclease in veronal-acetate buffer, pH 6.85, for one hour before staining. Basophilic material stainable by pyronin extensively removed from cytoplasm by enzyme. Chromatin stains more markedly with methyl green through removal of pyronophil component by enzyme.

Fig. 13.—Late polychromatic normoblast ($7.0 \times 7.0 \mu$) from brilliant cresyl blue preparation of normal marrow; wet-fixed film in Susa treated with pH 6.85 buffer and stained by pyronin-methyl green. The basophilic material of the cytoplasm is aggregated by action of the vital dye.

Fig. 14.—Similar cell from parallel preparation treated with ribonuclease at pH 6.85; this has removed the aggregated pyronophil material.

Figs. 15 to 21: Intermediate Erythropoiesis in Sternal Marrow of a Patient with Mild Addisonian Pernicious Anaemia

Fig. 15.—Haemopoietic reticulum cell developing to haemocytoblast, $31.7 \times 21.7 \mu$; nucleus $20 \times 18.4 \mu$.

Fig. 16.—Intermediate promegaloblast, $20 \times 18.4 \mu$; nucleus $15.5 \times 15.5 \mu$.

Fig. 17.—Basophilic intermediate megaloblast, $16.7 \times 16.7 \mu$; nucleus $13.4 \times 13.4 \mu$.

Fig. 18.—Early polychromatic intermediate megaloblast, $16.7 \times 14.2 \mu$; nucleus $11.7 \times 11.7 \mu$.

Fig. 19.—Late polychromatic intermediate megaloblast, $11.7 \times 10 \mu$; nucleus $6.7 \times 6.7 \mu$.

Fig. 20.—Pyknotic intermediate megaloblast, $11.3 \times 11.3 \mu$; nucleus $3.3 \times 3.3 \mu$.

Fig. 21.—Intermediate megalocyte, $9.6 \times 8 \mu$.

Figs. 22 to 34: Megaloblastic Erythropoiesis in Sternal Marrow of a Patient with Severe Addisonian Pernicious Anaemia

Fig. 22.—Haemopoietic reticulum cell (haemohistioblast), $32.5 \times 25 \mu$; nucleus $21.7 \times 16.7 \mu$.

Fig. 23.—Haemocytoblast, $21.7 \times 20.7 \mu$; nucleus $18.4 \times 16.7 \mu$.

Fig. 24.—Promegaloblast, $23.4 \times 23.4 \mu$; nucleus $18.4 \times 18.4 \mu$.

Fig. 25.—Basophilic megaloblast, $20.0 \times 16.7 \mu$; nucleus $15.0 \times 14.3 \mu$.

Fig. 26.—Early polychromatic megaloblast, $21.7 \times 21.7 \mu$; nucleus $15.8 \times 12.5 \mu$.

Fig. 27.—Late polychromatic megaloblast, $19.2 \times 19.2 \mu$; nucleus $13.2 \times 10 \mu$.

Fig. 28.—Pyknotic megaloblast, $16 \times 13 \mu$; nucleus $6.7 \times 5 \mu$.

Fig. 29.—Megalocyte, $10.2 \times 9.6 \mu$.

Figs. 30 to 34: Cells from Films of Sternal Marrow in Addisonian Pernicious Anaemia, wet-fixed in Susa and stained by Pyronin-methyl Green after Treatment with Veronal-Acetate Buffer, pH 6.85, or Buffer-ribonuclease for one hour

Fig. 30.—Haemocytoblast, $15.6 \times 11.7 \mu$; nucleus $10.4 \times 9.7 \mu$. Control slide in buffer. Deep pyronin staining of cytoplasm and nucleoli attributable to ribonucleic acid.

Fig. 31.—Haemocytoblast, $13.0 \times 12.3 \mu$; nucleus $11.0 \times 9.1 \mu$. Slide treated with ribonuclease. Extensive removal of pyronophil material by enzyme.

Fig. 32.—Early polychromatic megaloblast, $18.2 \times 9.1 \mu$; nucleus $9.1 \times 7.8 \mu$. Control slide in buffer. Nucleoli still present. Pyronophil basophilic material in cytoplasm is smaller in amount at this stage.

Fig. 33.—An older polychromatic megaloblast, $14.3 \times 14 \mu$; nucleus $10.4 \times 8.4 \mu$ from same slide as Fig. 32.

Fig. 34.—A late megaloblast, $11.7 \times 10.4 \mu$; nucleus $6.5 \times 6.5 \mu$. Nucleoli absent and cytoplasm only slightly pyronophil.

Figs. 1 to 8 and 15 to 29 are drawn from air-dried films fixed in methanol and stained by May-Grunwald-Giemsa method.

All cells drawn using Zeiss 2 mm. Homog. Immers. Apochromat. (aperi. 1.40) and $\times 8$ compensating ocular, with 250 mm. tube length.

peripheral blood at a maximum rate between the twelfth and fourteenth days, giving a maturation time of three to five days.

The orthodox view that a single normoblast gives rise to a single erythrocyte has not passed unchallenged. Plum (1947) has maintained that the numbers of erythroblasts in mitosis are too small to yield the requisite daily quota of erythrocytes necessary to maintain equilibrium, and has published details of studies *in vitro* in support of his contention. He believes that several erythrocytes may be derived from each normoblast by budding off (gemmation) from the cytoplasm. Boström (1948) supports Plum's view and illustrates schematically how the budding process is supposed to take place (see also p. 30). These interesting and revolutionary views need confirmation.* Ponder (1948) on theoretical grounds does not see the necessity for gemmation, and it cannot necessarily be assumed that "erythroblasts" behave *in vivo* as they do *in vitro* in artificial culture.

In our experience study of biopsy films of normal or hyperplastic normoblastic marrows yields no support for the mechanism of gemmation. The general appearance of films seems to indicate that ripe normoblasts become reticulocytes after the nucleus is lost. If, for instance, an actively erythropoietic marrow film from a patient with haemolytic anaemia is examined, satisfactory evidence of budding from the cytoplasm is extremely difficult to find. On the other hand pyknotic normoblasts with well formed evenly rounded polychromatic cytoplasm are frequent. Some normoblasts with scanty irregularly contoured polychromatic or slightly basophilic cytoplasm may be seen, and at first sight it would appear possible that these were cells which had already budded off cytoplasm. Against this hypothesis is the serious objection that the only non-nucleated masses of cytoplasm usually seen are fully formed polychromatic erythrocytes, which seem to be clearly derived from pyknotic nucleated precursors. Basophilic and irregularly formed cytoplasmic masses are rarely found in normal or hyperplastic normoblastic marrows. What happens to the normoblasts with scanty cytoplasm is far from clear: possibly they never mature at all. The possibility of limited gemmation appears more plausible in marrow films from patients with severe megalocytic anaemias. Promegaloblasts and polychromatic megaloblasts of irregular contour are not infrequent, and separated fragments of cytoplasm may be found; whether these fragments can develop into mature erythrocytes is unknown.

Derivation of reticulocytes from normoblasts.—It is generally considered that reticulocytes are direct successors of ripe normoblasts, after loss of the nuclei. The evidence for reticulocytes being younger than the bulk of the circulating erythrocytes is firmly based on the constant finding of increased numbers of reticulocytes whenever other evidence indicates an increased production of erythrocytes, as well as upon the fact that blood from the bone marrow contains a higher proportion of reticulocytes than is found in the peripheral

blood (Unglicht, 1938). Moreover, culture *in vitro* (Jacobsen, 1947) and survival studies on transfused blood containing a large number of reticulocytes have demonstrated loss of basophilic material and transformation into non-reticulated erythrocytes (Young and Lawrence, 1945).

Reticulocytes are on the average probably a little larger in diameter, and possibly a little thinner, than are adult erythrocytes, and it is presumed, therefore, that a slight alteration in corpuscular shape takes place after the cells have left the bone marrow. It is possible that it is the spleen which is largely responsible for the modification of corpuscular shape (Miller and others, 1942); however, the evidence can be interpreted in more than one way.*

The characteristic feature of reticulocytes is their ability to react supravitality with certain basic dyes; they contain a component which is precipitated in unfixed corpuscles by brilliant cresyl blue as a blue granular network, and which in methanol-fixed air-dried films gives a diffuse basophilic staining and an appearance of "polychromasia" to the whole field of corpuscles. Dustin (1944, 1946) has shown that this basophilic material contains ribonucleic acid, and it seems very likely that it represents the last remnants of the basophilic ribonucleoproteins so abundantly present in the primitive cells from which the reticulocyte has been derived. Indeed, if material aspirated from the bone marrow is treated with brilliant cresyl blue before smears are made and fixed, staining with Romanowsky dyes will then show that the basophilic material in the primitive cells and in the developing normoblasts has been aggregated by the dye in much the same way as in the reticulocytes themselves. Another apparent difference between reticulocytes and adult erythrocytes is that reticulocytes contain more protoporphyrin than do the adult corpuscles† (Watson and Clarke, 1937; Watson and others, 1944). Haemoglobinizing normoblasts also appear to contain free protoporphyrin (Stasney and McCord, 1942).

It is as yet uncertain whether all the erythrocytes that enter the blood stream are unripened reticulocytes or whether a proportion are fully ripened adult erythrocytes. Heath and Daland (1930), Young and Lawrence (1945), and Wintrobe (1946) believe that a proportion of mature corpuscles are delivered. Baar and Lloyd (1943) and Nizet (1946) disagree with this. Nizet's view that all the cells delivered are reticulocytes is based upon a consideration of the constant proportion of the most mature type of reticulocyte in the blood stream compared with more immature types, and upon cross-circulation experiments with marked Heinz-body-containing

* There is little question that reticulocytes seen in pathological states, such as in recovery from haemorrhage and in some haemolytic anaemias, are macrocytes with an increased diameter and volume (Dameshek and Schwartz, 1940; Wintrobe, 1946b). It is probably a fair inference that normal reticulocytes are also a little larger than adult corpuscles, but more evidence is required on this point. The controversial question as to how the spleen influences erythropoiesis is discussed by Doan and Wright (1946) and Dameshek and Estren (1947).

† The reticulocyte protoporphyrin cannot be held responsible for the reaction with cresyl blue as suggested earlier by Watson and Clarke (1937).

* Several other heterodox hypotheses have been put forward (see *Lancet*, March 29, 1947).

erythrocytes. The question is important in relation to calculations of the longevity of the erythrocytes based on reticulocyte ripening times, and cannot yet be considered as finally settled.

Loss of the nucleus of the normoblast.—All authors agree that the nucleus of the normoblast disappears after it becomes pyknotic* and the cell is ripe. The mechanism of its disappearance has been the subject of much discussion. There is no unanimity; loss by expulsion, karyorrhexis, or karyolysis have from time to time been thought to play a part. Naegeli (1931) considered that karyolysis took place, and states that this also was the view of Koellicker and Neumann. Amongst others in agreement with this conception are Cooke (1930), Davidson (1930), Habelman (1940), Kracke (1941), and Ferrata and Storti (1948). Howell (1890) thought on the other hand that expulsion was the mechanism, and more recently Maximov (1927), Turnbull (1936b), and Wintrobe (1946) support this point of view. Fieschi and Astaldi (1946) claim to have observed this in their marrow cultures.

It has been argued that the formation of Howell-Jolly bodies indicates that karyorrhexis does in fact take place, and that in certain circumstances it is incomplete and that a remnant of the nucleus persists. However, study of the bone marrow in those cases where Howell-Jolly bodies are being formed shows that they are separated off from the nucleus at a relatively early stage, before the nucleus as a whole becomes pyknotic. Moreover, this form of karyorrhexis is not normally seen, and it seems, therefore, unlikely that the nucleus of the normal normoblast is disposed of by this mechanism.

La Cour (1944) and Discombe (1946) have observed budding of nuclear material into the cytoplasm of animal and human polymorphonuclear leucocytes, and La Cour has suggested that the normoblast nucleus may lose its capacity to stain owing to loss of desoxyribonucleic acid during haemoglobin formation. It is interesting to note that in 1912 Schilling-Torgau suggested that it was only the chromatin of the normoblast nucleus that was lost. It seems almost unnecessary to point out that the presence or absence of a nucleus is usually judged by the presence or absence of chromatin staining and that loss of the nucleus itself would be inferred if for any reason its characteristic staining reactions were lost. *In vitro*, for example, depolymerization of nuclear desoxyribonucleic acid by the enzyme, desoxyribonuclease, and loss of staining has been shown by Brachet and Shaver (1948) to take place very rapidly.

It is true that in most smears of bone marrow cells naked pyknotic nuclei are occasionally found. Because it is impossible to exclude mechanical trauma as the cause, this observation by itself cannot be used to support the view that loss by expulsion occurs *in vivo*, especially in view of the small number of free nuclei encountered.

It is clear that no answer can yet be given as to how the normoblast loses its nucleus. The absence of transi-

tion stages between pyknotic normoblasts and reticulocytes seems to us significant; whatever the process, it is clearly a rapid one.

Regulation of erythropoiesis.—In health, erythropoiesis is nicely balanced to compensate for the normal daily loss of corpuscles, amounting to about 1/120 of the total of the circulating erythrocytes. Biopsy of the bone marrow gives a still picture of this normal compensatory erythropoiesis, but tells little of the mechanisms by which it is controlled and adjusted to the needs of the body. The very many haemopoietic factors which seem to be required are well reviewed by Cartwright (1947). These include several metallic elements (Schultze, 1940), amino-acids, vitamins, and growth factors, many of them being nutrients in a general sense. Knowledge of their importance is based upon critical experiments with laboratory animals and observations on man. A more mysterious control may be effected by endocrine glands and growth or maturation substances of intrinsic origin. Clinical evidence suggests that anaemia may develop in hypothyroidism (Bomford, 1938), in hypopituitarism (Snapper and others, 1937; Watkinson and others, 1947), in hypogonadism (McCullagh and Jones, 1942), and in Addison's disease (Wintrobe, 1946c). In rats, hypophysectomy regularly results in anaemia (Crafts, 1946). Finkelstein and others (1944) have claimed that male hormones stimulate and female hormones depress erythropoiesis. It seems reasonable to conclude that the endocrine glands control erythropoiesis to some extent, perhaps largely by their action on the growth and metabolism of the body as a whole. The subject is reviewed by Daughaday and others (1948).

The reticulocyte-ripening factor of Plum (1942) has already been mentioned, and Jacobsen (1947) refers to unpublished work suggesting that the ripening of normoblasts may be controlled by similar humoral factors. These interesting studies require confirmation.

A lowered oxygen tension has for long been considered an important stimulus to erythropoiesis, and the polycythaemia which develops at high altitudes is evidence of this relationship. The recent studies of Hurtado and others (1945) indicate that although there is a wide variation in response, the majority of people residing at high altitudes develop polycythaemia. There seems a direct relationship between the degree, duration, and continuity of the anoxia and the rise in the erythrocyte count.

The exact way in which oxygen-lack stimulates erythropoiesis is obscure. Grant and Root (1947), working with dogs, observed increased erythropoiesis to last for three weeks following loss of 30 per cent

* Pyknosis of the nucleus denotes in general an injured or dying cell. M. J. D. White (1947), discussing pyknosis, suggests that this process may be a modified non-functional mitosis in which the chromosomes have become fused.

of the blood volume, but the lowered oxygen tension and saturation in the bone marrow blood lasted three to five hours only; and the same degree of increased erythropoiesis resulted when the blood loss was brought about by smaller successive bleedings, which did not result in any diminution in the oxygen tension (see also Grant, 1948; Berk and others, 1948). Again the studies made by Rosin and Rachmilewitz (1948) on marrow cultures *in vitro* indicated depression of erythropoiesis at oxygen percentages below 12 per cent and increased activity when the oxygen percentage was raised to 50 per cent. These results are not, however, supported by the clinical observations of Reinhard and others (1944), who observed a diminution in erythropoiesis, resulting in a fall in reticulocyte percentage and a fall in the erythrocyte count, when patients with sickle-cell anaemia inhaled 70 to 100 per cent of oxygen for eight to 20 days. Tinsley and Moore (1948) reported an extension of this work. They observed depression of erythropoiesis in patients with sickle-cell anaemia and with congenital haemolytic anaemia who inhaled 50 to 95 per cent oxygen, and a rapid rise in reticulocytes when oxygen inhalation was discontinued. The reticulocyte response of patients with Addisonian pernicious anaemia treated with liver and inhaling 70 to 80 per cent oxygen was also partly suppressed; a second and higher response followed the cessation of oxygen inhalation.

The picture is thus complex; reduced oxygen tension stimulates and increased oxygen tension inhibits erythropoiesis. Increased erythropoiesis regularly follows haemorrhage, although (in dogs) no reduction in oxygen tension may be demonstrable, and in marrow cultures reduced oxygen tension is a depressant rather than a stimulant.

It is obvious that further information is required on the relationship between plasma oxygen tension, the respiration of erythroblasts, and erythropoiesis. Possibly the actual flow of blood through the marrow is a factor which affects erythropoiesis, by controlling the delivery of erythrocytes into the circulation from the sinusoids, and perhaps even by affecting erythropoietic activity itself.

Cytochemistry of Erythrocyte Development

Before discussing what is known of the chemical changes which take place in the developing normoblast, it is necessary to refer briefly to recent knowledge in general cytochemistry, for in many respects the developing haemopoietic marrow behaves in the same way as do other actively growing tissues.

Although the factors which control the growth and differentiation* of cells remain obscure, more is known of some of the chemical substances involved, and of the chemical changes which take place during differentiation. The nucleic acids† play an important role in this process. They are biologically distributed in two chemical forms, the desoxyribose polynucleotides or "desoxyribonucleic acid" and pentose polynucleotides or "ribonucleic acid." Caspersson and his school (consult Caspersson and Santesson, 1942; Caspersson, 1946; Thorell, 1947a) have studied the distribution of these two forms of nucleic acids by means of their ultra-violet spectral absorption. This is revealed from a sequence of monochromatic photographs or photometer readings obtained from a quartz microscope illuminated through a monochromator.

Both cell nuclei and the cytoplasm of young, actively growing cells can be shown to contain nucleic acids in high concentrations (Caspersson and Schultz, 1939, 1940). Both forms of nucleic acid are found. The nucleolus and cytoplasm contain ribonucleic acids associated with proteins; the Feulgen reaction* is negative in these areas of the cell. The nucleolus may also contain protein of the histone type.† The chromatin and chromosomes contain, however, desoxyribonucleic acid, and are Feulgen-positive; they also contain proteins.

Caspersson (1946) has suggested a number of principles which he believes to be of wide applicability: that protein synthesis needs the presence of nucleic acids; that the nucleus is the centre of the cell for the formation of proteins and that the nucleolus-associated chromatin secretes into the nucleolus substances of protein nature which diffuse outwards to the nuclear membrane; and that it is on the outer surface of the nuclear membrane that ribonucleoproteins are synthesized.‡§

Brachet and his school have approached the subject independently from a different angle. They have utilized enzymes (nucleases) to depolymerize nucleic acids from fixed cells and have judged their effects by alterations in the staining reactions, particularly with pyronin-methyl green. Purified ribonuclease has been used by Brachet (1942, 1946) and Brachet and Shaver (1948), and desoxyribonuclease (McCarty, 1946) has been employed to depolymerize desoxyribonucleic acid (Brachet, 1942; Sanders, 1946; Brachet and Shaver, 1948; Catchside and

* The Shorter Oxford Dictionary defines "differentiation" (biol.) as: "The process, or the result of the process, by which in the course of development a part, organ, etc., is modified into a special form, or for a special function; specialization; also the gradual production of differences between the descendants of the same ancestral types." (See also Bloom, 1937.)

† Extensive treatment of the whole subject of the role of the nucleic acids is given in two recent symposia (1946, 1947) and in the reviews of Greenstein (1944) and Hevesey (1948b).

* The Feulgen reaction (Feulgen and Rossenbeck, 1924) is generally held to be a specific cytochemical test for desoxyribonucleic acid (Dodson, 1946; Stowell, 1946), although this has been questioned (Carr, 1945; Stedman and Stedman, 1947). The chemistry of the reaction has been investigated by Stacey and others (1946).

† The view that histones are important nuclear constituents has recently been challenged (Pollister and Ris, 1947; see also Stedman and Stedman, 1947).

‡ Spiegelman and Kamen (1947) have warned against necessarily accepting the conception of a particularly close association between the formation of protein and the metabolism of nucleic acids.

§ Hinshelwood (1946) also reviews the conditions under which proteins are synthesized.

LEGENDS, PLATES I TO III

PLATE I

3 μ SECTIONS OF ASPIRATED STERNAL MARROW

FIG. 1.—Normal adult man. Iron haematoxylin and eosin, $\times 580$.

FIG. 2.—Aplastic anaemia in man, with severe normocytic anaemia and thrombocytopenia purpura. Marrow hypoplastic, with many fat spaces and few cellular areas. Iron haematoxylin and eosin, $\times 280$.

FIG. 3.—Idiopathic hypochromic anaemia in female. General hyperplasia, with numerous erythroblasts. Delafield's haematoxylin and Giemsa, $\times 580$.

FIG. 4.—Addisonian pernicious anaemia. Very hyperplastic marrow. Iron haematoxylin and eosin, $\times 580$.

FIG. 5.—Subacute combined degeneration. No anaemia. Marrow of normal structure. Gordon and Sweet's modification of Wilder's silver impregnation, to show argyrophil reticular fibrils outlining sinusoids and fat spaces and permeating stroma, $\times 250$.

FIG. 6.—Addisonian pernicious anaemia. Hyperplastic, megaloblastic marrow; stained by silver impregnation to show outlines of dilated sinusoids and increase in reticulin of stroma, $\times 250$.

PLATE II

FIGS. 1 AND 2.—Bone marrow from two patients with severe Addisonian pernicious anaemia. Many primitive promegaloblasts are present. Erythrocyte count approximately 1,000,000 per c.mm.

FIG. 3.—Typical megaloblasts from the bone marrow of a patient with Addisonian pernicious anaemia. Erythrocyte count 1,500,000 per c.mm.

FIG. 4.—Intermediate megaloblasts from the bone marrow of a patient with mild Addisonian pernicious anaemia. Erythrocyte count 3,400,000 per c.mm.

FIG. 5.—Intermediate megaloblasts from the bone marrow of a patient with steatorrhoea. Erythrocyte count 4,300,000 per c.mm.

FIG. 6.—A primitive marrow from a patient with tropical nutritional anaemia. Erythrocyte count 1,600,000 per c.mm.

FIG. 7.—The megaloblastic bone marrow of a patient with pernicious anaemia of pregnancy. Erythrocyte count 2,800,000 per c.mm.

All the preparations in Plates II and III were made from bone marrow aspirated from the sternum. Stained by May-Grünwald-Giemsa, $\times 800$.

PLATE III

FIG. 1.—Normoblasts from the bone marrow of a normal adult man.

FIG. 2.—Micronormoblasts from an iron-deficient bone marrow.

FIG. 3.—Macronormoblasts from the bone marrow of a patient with a macrocytic haemolytic anaemia (nocturnal haemoglobinuria).

FIG. 4.—Abnormal megaloblasts from the bone marrow of a patient with pernicious anaemia of pregnancy.

FIG. 5.—Megaloblast-like cells from the bone marrow of a patient with leukaemia. (We are indebted to Dr. Sheila Newstead for this film.)

FIG. 6.—A megaloblast-like cell amongst normoblasts in a bone marrow film from a patient with carcinoma of the bone marrow.

Holmes, 1946). The results of this work are very similar to those of Caspersson. Brachet (1946) considers, however, that chromatin, especially the heterochromatin,* contains a proportion of ribonucleic acid (see also Kauffman and others, 1948) and that the nucleus is of minor importance in relation to the synthesis of cytoplasmic protein compared with the "cytoplasmic particles" (mitochondria and microsomes) with which the ribonucleoproteins are largely associated (Bensley, 1942; Claude, 1943; Brachet, 1946).

The cytochemistry of the developing bone marrow cells has been studied by several authors. Thorell (1947b, etc.) has developed Caspersson's techniques of ultra-violet absorption microspectrophotometry. Brachet (1942) has applied his ribonuclease test to various amphibian and mammalian bone marrows. White (1947) has extended Brachet's work to normal and pathological human marrow cells, and J. N. Davidson and others (1948) have used the ribonuclease test as well as chemical microanalysis of marrow nucleic acids. Dustin (1946, etc.) has em-

ployed ribonuclease in his researches on reticulocytes and Wislocki and Dempsey (1946) have subjected samples of Rhesus monkey marrow to ribonuclease in addition to other histochemical techniques.

Thorell's work, recently summarized in a monograph (Thorell, 1947b), deserves further consideration. He worked with the marrow of rats and rabbits, and with normal and pathological human marrow. Individual living cells were subjected to analysis at different growth stages and the concentrations and proportions of nucleic acids, proteins, and haemoglobin were determined by means of their characteristic absorption maxima at particular wavelengths of the ultra-violet.*

Thorell's work has provided examples of the more general views of Caspersson. The primitive pronormoblasts contain the maximum amount (5 per cent approximately) of ribose polynucleotides in the cytoplasm and nucleoli. Desoxyribonucleic acid is confined to the nuclear chromatin and nucleolus-associated chromatin. The concentration of ribose

* Heterochromatin (Heitz, 1929) is chromatin closely associated with the nucleolus and is thought to possess important metabolic functions (see Schultz, 1947). It is probably identical with the nucleolus-associated chromatin of Caspersson, which Thorell (1947b) believes may regulate the growth of marrow cells, as it does of cellular growth in general.

* The nucleic acids give a maximum extinction with light of wavelength 257 $m\mu$. Proteins were estimated by absorption maxima at 280 $m\mu$, attributable to their content of tyrosine and tryptophane (Holiday, 1936), and haemoglobin was estimated by absorption maxima at 404.7 $m\mu$ and 435.8 $m\mu$ (the Soret band).

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

PLATE I.

B

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

FIGS. 1-4.—Bone marrow obtained by sternal puncture biopsy from patients who died of severe refractory anaemia. In Fig. 1 the marrow cells are particularly large and primitive. Fig. 2 shows maturing megaloblasts as well as primitive cells, some of them megaloblasts; also cells with pyknotic nuclei and degenerating cytoplasm. Fig. 4 shows primitive cells and degenerating cells with basophilic cytoplasm.

FIG. 5.—Megaloblasts from the bone marrow of a patient with Addisonian pernicious anaemia, stained by Sudan black by the method of Baker (1945). The mitochondria are chiefly found in the perinuclear zone. Note the deeply stained granulocytes at the top of the figure.

polynucleotides in the cytoplasm diminishes moderately in partially differentiated cells but markedly so in the nucleolus. By the time the cytoplasm is polychromatic the concentration of ribonucleic acid has decreased to one-tenth of its maximum value and has completely disappeared in orthochromatic cells. This work, therefore, supports the impression that ribonucleic acid concentration is paralleled by the depth of cytoplasmic basophilia.

Absorption attributable to substances of porphyrin type was not observed in the cytoplasm of the most primitive cells; the earliest cells in which it was first detected were of moderate maturity with polychromatic cytoplasm, at a concentration corresponding to 3 $\mu\mu\text{g}$. of haemoglobin per cell. The haemoglobin content increases to 28 $\mu\mu\text{g}$. in the most ripened cells with fully pyknotic nuclei, at a concentration of 20 to 25 per cent. The final rise in concentration of haemoglobin to 33 per cent is attributed by Thorell to shrinkage of the cell when it changes from a rounded form as a normoblast to a disc-like erythrocyte.

On the basis of his cytochemical analysis Thorell refers to four phases of activity in the development of the normoblast: (1) a phase of growth where the concentration of ribonucleic acid is maximal, (2) a phase of declining growth where the ribonucleic acids are diminishing and basic cellular proteins like globin are being synthesized, (3) a phase of differentiation when haemoglobin is being formed rapidly, and (4) a phase of declining differentiation.

Although the globin necessary for haemoglobin synthesis is probably formed at an early stage in the life of the erythroblast (through participation of ribose polynucleotides as for protein synthesis generally) the formation of haem compounds and their coupling with globin appears to take place at a later stage, after the ribopolynucleotides have substantially disappeared (see also Shemin and Rittenberg, 1946).

The complexity of the apparatus and methods used by the Caspersson school is a disadvantage. Recently E. M. Jope (1949) and Barer and others (1949) have outlined a simplified technique using the reflecting microscope (Burch, 1947). By this method simultaneous monochromatic cross-sections of the cell structures may be studied (Text Fig. 3). The intracorpuseular haemoglobin of individual erythrocytes produces the same intensity of absorption at the Soret band as was expected from studies of haemoglobin in simple solution.

Formation of haemoglobin.—Knowledge of the mechanism of haemoglobin synthesis is still very incomplete. Duesberg in 1938 published a good review of the position up to that time, and Bur-

mester (1937), in a chemical study of pigment formation in avian erythroblasts, included figures for the iron content of cells at different stages of development. The precursors of the porphyrin ring have for long remained mysterious, but recently Shemin and Rittenberg (1946) have shown that in rat and man glycine labelled with N^{15} -isotope is built into protoporphyrin and ultimately appears in the haemoglobin of the mature erythrocytes. A similar synthesis has been demonstrated *in vitro* when duck erythrocytes and erythrocytes from patients with sickle-cell anaemia were incubated with N^{15} -containing glycine (London and others, 1948). Glycine containing C^{14} -isotope in the α -position has also been used in similar experiments; it is incorporated into both haem and globin moieties of haemoglobin (Altman and others, 1948).

Thorell's work, already referred to, indicates the relatively early diminution in cytoplasmic ribopolynucleotides in polychromatic erythroblasts before the start of haemoglobin formation. H. M. Jope (1948) points out that at this stage the cytoplasmic protein is probably related to the globin of haemoglobin, and that, later, haemoglobin itself is elaborated from this globin, which either forms haem groups on its surface or combines with them after their formation. The cytoplasmic acidophilia of the early polychromatic normoblast is thus due to its content of globin rather than of haemoglobin, which is developed later.

Siderocytes.—An interesting abnormality of the distribution of iron in erythrocytes and their precursors has recently been described. Grüneberg (1941, 1942) observed siderocytes, or erythrocytes with granules giving the Prussian-blue reaction for iron in the blood of normal rat, mouse, and human embryos, and in large numbers in mice with a congenital anaemia. Later they were observed in adult human blood by Doniach and others (1943), and further details have been provided by Pappenheimer and others (1945), Case (1946), McFadzean and Davis (1947), and Dacie and Doniach (1947). Siderotic granules are to be found also in polychromatic and pyknotic normoblasts, usually as a few isolated granules, but sometimes confined to a zone around the nuclear membrane (Dacie and Doniach, 1947).

Siderotic granules of the type described by Grüneberg seem not to be formed in damaged or aged erythrocytes as has been claimed by Case* (1945), but seem rather to be produced at the same time as haemoglobin is elaborated. Possibly a proportion of the iron which normally enters the haem molecule is concentrated in loci in the cytoplasm of the developing normoblast in a relatively free form. It is noteworthy that when many granules

* Case (1946) has used either $\alpha\alpha'$ -dipyridyl-thiocyanate or acid-potassium ferrocyanide after a preliminary unmasking treatment with ammonium sulphide to demonstrate siderocytes. By these techniques, siderocytes (Case) are present in normal blood and increase in numbers in stored blood. Case believes siderotic granules indicate an ageing cell.

are present the rest of the cytoplasm of the cell stains palely with acid dyes. When stained with basic dyes the siderotic granules appear as basophilic granules (Pappenheimer bodies). The incidence of siderocytes in human pathology has not yet been fully worked out. They are not present in the peripheral blood of normal adults, but constantly appear in small numbers after splenectomy; in certain haemolytic anaemias almost all the corpuscles may be affected.

Abnormal Erythropoiesis

Megaloblastic erythropoiesis.—The term "megaloblast" was used by Ehrlich (1880) to describe the type of nucleated erythroblast found in the bone marrow of patients with pernicious anaemia in relapse. Since that time the terms "megaloblast" and "megaloblastic erythropoiesis" have been widely used in descriptive haematology, but their correct usage and limits, and their significance, have proved to be a controversial problem.

Morphology of the megaloblasts.—Megaloblasts differ from normoblasts in several ways; they are larger cells with increased cytoplasm and nuclear size and at every stage in development they have a different and more open nuclear chromatin pattern, and a tendency in the later types for the cytoplasm to manufacture haemoglobin at stages when the chromatin of the nucleus is still arranged in an open manner. We should like to emphasize several general points before describing the cells themselves. First, that the "megaloblastic" change may be appreciated at all stages in development from the most primitive types to the most mature orthochromatic megaloblasts with pyknosis of the nucleus; secondly, that the degree of abnormality varies greatly between cells of the same apparent age and that all grades of change may be seen between grossly abnormal cells through intermediate types to cells which are almost if not quite normal; and thirdly, that "megaloblasts" are not normally present in the marrow. However, although they are essentially pathological cells, megaloblasts are not a race of cells apart but a type of abnormality* affecting cell growth and maturation, which develops to a greater or lesser extent as the result of deficiencies in growth factors of which the anti-pernicious anaemia factor is an important example.

These points will now be considered in more detail. The earliest megaloblasts, the *promegaloblasts* (Ferrata and Negri-Rinaldi, 1914), are larger cells than the pronormoblasts, which are their normal counterparts. In size, they range from 20 to

25 μ in diameter in stained dried films. The cytoplasm is deeply basophilic and relatively abundant, with numerous mitochondria. The chromatin of the nucleus is arranged in a characteristically well defined finely stippled manner, and one or more basophilic nucleoli can be identified. From these basophilic promegaloblasts a series of *ripening megaloblasts* develop. These cells remain larger than normal throughout the ripening process, and the cytoplasmic outline is usually less round than in the normal series. When the cytoplasm attains a polychromatic staining reaction, this is often far in advance of that expected on the basis of nuclear structure, and mitochondria are still numerous. Thus polychromatic megaloblasts with small nucleoli are often observed, and the cytoplasm may even become orthochromatic before pyknosis of the nucleus has been completed. As the cells mature the nuclei become smaller and the chromatin more condensed; nevertheless, the chromatin pattern remains more open than in developing normoblasts. The chromatin takes the form of short strands and small nodes, but there is sufficient "parachromatin" to give an overall mottled effect. The nucleolus-associated chromatin remains finer and more discrete from the rest of the chromatin than in normal development. As the cell matures further the chromatin strands thicken and the nodes enlarge and tend to fuse, but complete homogeneous pyknosis is rarely seen. The nucleus is at first evenly rounded or slightly oval in shape, but often becomes distorted and has an irregular outline before it is fully pyknotic. The mitochondria finally disappear. Abnormal mitosis and nuclear fragments (Howell-Jolly bodies*) are quite frequently seen. The difference between cells developing normally and those with well marked megaloblastic changes is illustrated in Figs. 1-8 and 22-29 of the Coloured Plate and in Text Fig. 1.

Intermediate megaloblasts.—The above description and the illustrations apply to megaloblasts as seen in well stained smears from patients with severe Addisonian pernicious anaemia. The degree of the abnormalities and the extent to which the cells deviate from the normal are closely linked with the severity of the lack of haemopoietic factors. This is illustrated in the photomicrographs of Plate II and the Coloured Plate, and in the Text Fig. 1, in which are shown a series of scale drawings.

* Erythrocyte precursors are not the only developing cells affected by deficiency of the anti-pernicious anaemia factor. Abnormally large and atypical granulocytes are quite as characteristic, but their description is beyond the scope of this article. As in the megaloblasts a characteristic feature is an asynchronism between the growth and development of the nucleus and the cytoplasm.

* Howell-Jolly bodies are small round Feulgen-positive bodies, approximately 0.5 to 2 μ in size. They are derived from nuclear chromatin and may be seen in polychromatic and orthochromatic erythroblasts. It is not certain whether they are produced by nuclear karyorrhexis or are formed from chromosomes, which have been isolated during mitosis. They are found in many types of anaemia and are especially frequent in Addisonian pernicious anaemia, in severe haemolytic anaemias, in idiopathic steatorrhoea (Engel, 1939), and after splenectomy (Singer and others, 1941).

of megaloblasts and a series of normoblasts for comparison. The megaloblasts in the four photomicrographs of marrow films (Plate II, Figs. 1-4) differ not only in size but in the extent to which they deviate from normal cells. These four patients A, B, C, and D had anaemia of varying severity; A and B were the most severe cases, with approximately 1,000,000 erythrocytes per c.mm.; case C was less severe, with 1,800,000 erythrocytes per c.mm.; and case D the least anaemic with 3,400,000 erythrocytes per c.mm. It is evident that not only is the degree of megaloblastic change intimately linked with the seriousness of the lack of haemopoietic factors and that it is hence proportional to the severity of the anaemia in the patient, but that the relative proportion of primitive to maturing megaloblasts is also affected. It is in the severely anaemic patient that the primitive cells are most abundant, and in the mildly anaemic the least abundant. Bilaki-Pasquier (1948) refers to stained marrow containing many primitive megaloblasts as "*moelle bleue*," and notes that the primitive cells are most numerous when the peripheral erythrocyte count is lowest. See also Leitner (Leitner and others, 1949d).

The nature of the megaloblastic change.—Megaloblasts are produced when there is a deficiency of certain haemopoietic factors, which normally are necessary for effective growth and division and for normal differentiation. These factors may resemble enzymes or coenzymes in their action, and only minute amounts may be required; in the case of the recently purified liver factor (Lester Smith, 1948), it has been calculated that 1 μ g. per day is sufficient (Rickes and others, 1948).* The effect of a deficiency of the liver factor is that haemopoiesis becomes disordered and ineffective. The exact details of how this is brought about are still obscure; certainly mitosis is deranged to some extent (Japa, 1945), and may occasionally be multipolar or incomplete, with variation of chromosome numbers in the daughter cells (La Cour, 1944). There seems to be no deficiency in the synthesis of ribonucleic acid as judged by the amount of cytoplasmic and nucleolar basophilia removable by ribonuclease (White, 1947), or by microanalysis (J. N. Davidson and others, 1948)—indeed, the content of both types of nucleic acid in the marrow is increased. The derangement is

a complex one affecting both growth and differentiation and has important effects on cell size, chromatin arrangement, and cytoplasmic ripening, and typically results in an asynchronism between nuclear and cytoplasmic maturation, a point recently emphasized by Bessis (1946, 1948). It has already been mentioned that similar changes may be observed in the developing leucocytes. Thorell (1947b) has studied the distribution of ribose polynucleotides and of haemoglobin in the megaloblasts of marrow from two patients with pernicious anaemia by the micro-spectrophotometric method. He found haemoglobin present in cells that still possessed the same high concentration of cytoplasmic and nucleolar ribose polynucleotides that normally characterize only the more primitive erythrocyte precursors. Use of the ribonuclease test shows, however, that in those megaloblasts which actually develop to erythrocytes there is a steady decrease of both cytoplasmic and nucleolar ribonucleic acid content. In view of this, it seems possible that Thorell has omitted to analyse the most mature forms of megaloblasts.

The effects on erythropoiesis of a deficiency of the liver principle are mainly twofold: primitive promegaloblasts are present in far larger proportion than are primitive cells in normal marrow, and may total 50 per cent or more of the erythroblasts. In addition there is a variable proportion of maturing abnormal cells (megaloblasts) from which evolve the megalocytes and poikilocytes of the peripheral blood. In addition to primitive cells which definitely have the characters of promegaloblasts, there are also found increased numbers of undifferentiated reticulum cells and transitional stages between the most primitive cells and promegaloblasts and myeloblasts, such cells corresponding to the haemocyto blasts of normal marrows.

As has been mentioned, it is in the most severe cases that the higher proportion of primitive cells is found, and it seems clear that in these patients there is a severe impediment to successful cellular differentiation. It is not so clear what happens to the primitive cells if they fail to mature; in certain of our marrows it is possible to trace stages in shrinkage of cell size and pyknosis of the nucleus, without cytoplasmic ripening, until a small indeterminate remnant results. In the less severely affected marrows the proportion of primitive cells is less, and degenerating forms are inconspicuous; many more maturing megaloblasts are present, and it is obvious that whilst erythropoiesis is abnormal and relatively ineffective, it is at least proceeding. This is borne out by the fact that in these cases the peripheral blood count may remain in equilibrium

* It is outside the scope of this article to consider in detail whether Vitamin B₁₂ is but one of several factors, absence of which will cause megaloblastic change. It is probable that more than one factor is concerned and that the normal chain of processes can be broken at several places. It is generally held that in patients with the sprue syndrome, in certain relatively refractory megaloblastic anaemias, in pernicious anaemia of pregnancy, and in nutritional megalocytic anaemia, liver alone, even in large doses, may not be wholly effective (Wills, 1948). It may be added that pteroylglutamic acid is not exclusively concerned with erythropoiesis, but is a growth factor in a wide biological sense.

for long periods at a moderate level, or at least that it only falls slowly.

The variable nature of megaloblastic marrow.—In the preceding sections on megaloblastic erythropoiesis we have attempted to describe the morphology of megaloblasts and their causation, and have developed the thesis that all grades of change may be recognized between extremely abnormal cells and cells almost indistinguishable from the normal. In this section we shall present further evidence as to the variability of the megaloblasts and of the marrow picture as a whole in patients with Addisonian pernicious anaemia, and will indicate our views as to the abnormal types of erythropoiesis met with in the sprue syndrome and in pernicious anaemia of pregnancy. In the following section we shall compare our own viewpoint with that of other workers.

It is well known that in Addisonian pernicious anaemia the volume of red marrow increases and extends out into the long bones of the skeleton and that there is a diminution or complete loss of the fat cells normally so abundant in the marrow of an adult (see Plate I), and the same is true to a greater or lesser extent in the other allied megalocytic anaemias. Sternal puncture usually provides very cellular material in which marrow particles are readily seen. If differential cell counts are performed an increased erythrocytic-leucocytic ratio will be found (Schartum-Hansen, 1937). This is illustrated from our material in Text Fig. 4; there is a general correlation between the severity of the anaemia and the predominance of erythrocytic cells, but the picture is complicated because of the associated disturbance in leucopoiesis. These marrows are in fact not as one-sidedly erythrocytic as in chronic haemolytic anaemias with similar degrees of anaemia. In Text Fig. 4 are also shown the results of plotting the degree of anaemia of the patient (a rough indication of the severity of the lack of haemopoietic factors) against the proportion of primitive nucleated cells with basophilic cytoplasm* amongst the whole population of nucleated erythrocytic cells. There is an obvious positive correlation between the degree of anaemia and the proportion of primitive cells, and, as has been mentioned before, the megaloblasts become less and less abnormal and more "intermediate" *pari passu* with the falling proportion of primitive cells and reduction in anaemia (Plate II).

Of the thirty-five patients whose data are plotted in Text Fig. 4, nine were suffering from non-

Addisonian megalocytic anaemias, and it is particularly interesting to note that the data from these cases fall into line with those obtained from the more numerous patients with typical Addisonian anaemia. In the two severely anaemic patients, one an example of tropical nutritional megalocytic anaemia and the other a patient with pernicious anaemia of pregnancy, there was a high proportion of primitive cells and well marked megaloblastic change; in the less anaemic patients there were fewer primitive cells and the megaloblasts were much more intermediate in type. As far as our experience goes the megaloblastic picture of the non-Addisonian megalocytic anaemias is similar to that of typical untreated Addisonian pernicious anaemia of comparable severity.

Effect on megaloblastic bone marrow of treatment with the liver anti-anaemic principle.—It is well known that the bone marrow of a typical case of Addisonian pernicious anaemia rapidly loses its megaloblastic character and becomes normoblastic if effective doses of liver are given. The exact details and mechanism of this change have been a matter of some controversy (Jones, 1943; Limarzi and Levinson, 1943; Leitner and others, 1949e). It is probable that the ripening megaloblasts complete their transformation into megalocytes if they have lost the capacity for further divisions, but that the primitive nucleated cells respond to the presence of the liver haemopoietic factor by undergoing normal divisions and differentiating as normoblasts.

The earliest changes are appreciable within twenty-four hours of an injection of liver extract, when basophilic pronormoblasts and normoblasts at an early stage of development may be seen in addition to ripening megaloblasts. During the next forty-eight hours many more ripening polychromatic normoblasts appear and the megaloblasts are less conspicuous, but the latter do not entirely disappear for at least five days, even if the dose of liver produces a maximal response.* If the liver extract is not fully potent a very mixed picture results, "intermediate" megaloblasts replacing to a greater or less extent the expected normoblasts. The completeness or otherwise of the transformation towards normoblastic formation (and the disappearance of abnormal leucocytes) is, naturally, the most delicate index of the adequacy of the liver extract.

A discussion on the megaloblast problem.—The preceding sections have been a presentation of our own point of view, based on our own material. A point of great controversy is the question of "intermediate" forms of megaloblasts; whether they occur at all, what is their significance, and what is their relationship to macronormoblasts. The conception of "intermediate" forms is by no means

* Reticulum cells and slightly more mature types corresponding to haemocytoblasts which we could not definitely identify as erythrocyte precursors were not counted.

* The change from an inhibited marrow with a high proportion of primitive cells to an actively maturing erythrocytic one is accompanied by striking reductions in the content of both ribonucleic and desoxyribonucleic acids (J. N. Davidson and others, 1948).

new, but relatively few authors have paid much attention to it. In pre-war Continental literature these forms were referred to under a variety of names, as "macroblasts" by Scharf-Hansen (1937), as "megaloblasts" by Hotz and Rohr (1938) in cases of sprue, and as "intermediate erythroblasts" by Lambin and de Weert (1938, 1939). They have been described by Trowell (1942-3) in tropical dimorphic anaemia and by Zuelzer and others (1947) in megaloblastic anaemia in infancy. Bessis (1946, 1948) in his review of the megaloblast question admits their existence, and Davidson and others (1947) refer to their occurrence in two patients with sprue, and they have been described as "atypical normoblasts" by Cooke and others (1948) in cases of idiopathic steatorrhoea, and as "intermediate erythroblasts" by Innes (1948) in sprue. Our own views have been set out in two previous preliminary communications (Dacie and White, 1947; Dacie, 1948). Other workers have not considered the problem at all or have decided against the existence of intermediate forms (Jones, 1943), and have stressed the striking differences between normoblastic erythropoiesis and gross megaloblastic change rather than the similarities which may be seen between the two series (Israëls, 1939; Leitner and others, 1949f). Most authors, however, including Dameshek and Valentine (1937) and Jones (1938), admit that mixed pictures occur and that a variable proportion of normoblasts may be found in a predominantly megaloblastic marrow.

Not only are we convinced that intermediate forms occur, but we believe that they have a special significance; they appear when the marrow suffers minor deficiencies of haemopoietic principles. They are seen, in our experience, in mild Addisonian anaemia before liver treatment, in more severe examples of Addisonian anaemia that have received suboptimal doses of liver, and in cases of the sprue syndrome and other megalocytic anaemias, where anaemia is relatively mild and response to liver unsatisfactory; presumably in these cases some substance other than the purified liver principle is required, or utilization by the marrow is impaired. We feel that these types of cell deserve a wider recognition and should be referred to as "intermediate megaloblasts" rather than as atypical or intermediate erythroblasts or normoblasts, because their pathogenesis seems to be the same as that of more typical megaloblasts. The difference is merely quantitative and the cells are less definitely abnormal. The question of their relation to macronormoblasts and heteroplastic erythroblasts is discussed in later sections.

Other Types of Abnormal Erythropoiesis

Macronormoblastic erythropoiesis.—The term "macronormoblastic erythropoiesis" has been employed by Jones (1943) to describe the developing erythroblasts in conditions where there is a peripheral macrocytosis not dependent upon a deficiency of the liver principle. This type of blood picture is seen in some chronic haemolytic anaemias (Dameshek and Schwartz, 1940), in liver disease (Bodley Scott, 1939), recovery from haemorrhage (Wintrobe, 1946d; Leitner and others, 1949g), and in patients responding to iron therapy where there has been iron deficiency. It is also found in late foetal life. The term macronormoblast refers to the nucleated precursors of the peripheral macrocytes. Jones, unfortunately, includes an increased proportion of "early forms (pronormoblasts and basophilic normoblasts)" in his conception of a macronormoblastic marrow. To us it seems preferable to use the term solely to describe normoblasts which are larger in size than their normal counterparts of similar age, and not to complicate matters by referring to an increased proportion of earlier types which, even if perfectly normal cells, would increase the average size of the normoblasts. Few authors seem, however, to have demonstrated the abnormal size of "macronormoblasts" by direct measurement. Dameshek and Schwartz (1940) measured the diameters of the erythroblasts in the marrow film of a patient with acute haemolytic anaemia and found the cells to be from 1 to 1.3 μ larger than normal cells of the same age group. We have also made some measurements, and contrasted the maturing polychromatic and pyknotic normoblasts in normal subjects with those from patients with macrocytic haemolytic anaemia and iron deficiency anaemia (Text Fig. 5). The results of such measurements as these are, however, open to question, as an increased proportion of more primitive cells produces an apparent macrocytosis, and it is very difficult to measure only cells of strictly comparable age groups. Our figures show, however, that whereas in the five normal marrows 11 to 26 per cent of the cells measure 8 μ or less, in the macronormoblastic marrow only 2 per cent of the cells are within this range and in the iron-deficient marrow there are as many as 70 per cent. As these small cells are all mature or almost mature types it seems reasonably certain that real differences exist (see also Plate III, Figs. 1-3).

Do macronormoblasts differ from normoblasts in any respect other than size? Their nuclear structure resembles that of normoblasts and thus differs from that of intermediate megaloblasts. Israëls (1941) has stated that premature haemoglo-

binization of the cytoplasm of erythroblasts (presumably macronormoblasts) often occurs in hyperplastic marrows with normal maturation. This change seems to us to be a slight one and unreliable as a diagnostic aid. We often experience some difficulty in the recognition of individual macronormoblasts, but believe that this is only to be expected because both macronormoblasts and intermediate megaloblasts merge into the normal. The general trend of both abnormal types is, however, quite distinctive if the abnormalities are marked.

Little is known as to why macronormoblasts develop or about the mechanism of their formation. In most instances they are found when haemopoiesis is proceeding rapidly and when there is a raised proportion of reticulocytes in the peripheral blood. Possibly in a hyperplastic marrow unripened cells are forced into the blood stream because the active growth of the marrow as a whole outstrips the maturation of individual cells. Abnormal size and occasionally the persistence of a nucleus, as well as cytoplasmic basophilia, may thus all be signs of immaturity. An alternative hypothesis is that large ripened normoblasts are produced as the result of a diminished number of cell divisions through which they have been derived from the pronormoblasts. The differences between the abnormal and normal are relatively slight, however, and it seems unlikely that a stimulated, actively erythrogenetic marrow

would respond to the demand for more erythrocytes by reducing the number of cell divisions.

Another hypothetical possibility is that there is normally some humoral mechanism which controls the development and maturation of the erythroblasts, similar to that described as controlling the maturation of reticulocytes (Jacobsen, 1947), and that in conditions of rapid erythropoiesis there may be a relative deficiency of ripening factors. At the moment all that can be said is that none of these hypotheses has been proved.

Erythropoiesis in iron deficiency (Micronormoblastic erythropoiesis).—The effect of iron deficiency on the erythrocytes of the peripheral blood is well recognized, but rather less is known about the morphology of the erythrocyte precursors in the marrow, and as to how deficiency of iron results in disordered erythropoiesis. Bodley Scott (1939) has reviewed the early literature and described his own findings in twenty-three patients. He found an increased cellularity of the marrow and an increased proportion of erythrogenetic cells roughly parallel to the severity of the anaemia. He described the predominant cells as polychromatic normoblasts "with an irregular and jagged cell outline and only a small rim of slate-grey cytoplasm around the pyknotic nucleus." Since Bodley Scott's paper the disordered erythropoiesis of iron deficiency has received wider recognition (Wintrobe, 1946e; Whitby

TEXT FIG. 5.—Diameter distribution curves of 100 polychromatic and pyknotic normoblasts, measured in sternal marrow films from five normal subjects x—x, a patient with a macrocytic haemolytic anaemia ●—●, and from a patient with an iron deficiency anaemia ○—○. (For interpretation, see text.)

and Britton, 1946), and might well be referred to as "micronormoblastic" in type (Plate III, Fig. 2, and Text Fig. 1). The haemocytoblasts and pro-erythroblasts in iron deficiency seem normal in size and appearance and are usually present in a normal or slightly increased relative proportion, but there is usually an increase in the numbers of basophilic normoblasts (Leitner and others, 1949h). Our own observations and measurements (Text Fig. 5) confirm the small size of the more mature normoblasts and are in agreement with Bodley Scott's description. Cytoplasmic ripening seems to lag behind nuclear condensation, and fully orthochromatic cells are not seen.

The presence of a hyperplastic erythrocytic marrow in association with peripheral anaemia has led to the view that there is an arrest of maturation similar to that found in Addisonian pernicious anaemia. However, there is little obvious morphological evidence for this; not only are more normoblasts present than in the normal, but a high proportion seem to be maturing. It might be thought that the malformed hypochromic erythrocytes and poikilocytes are unduly sensitive to the normal process of wear and tear and that this contributes significantly to the anaemia, which is in effect partly haemolytic* in type, and that the presence of so many normoblasts indicated a rapid cell production. However, the level of reticulocytes in the peripheral blood in a case of untreated anaemia due to iron deficiency is rarely above 2 or 3 per cent, and the serum bilirubin is low. The fact that effective iron therapy leads to an outpouring of reticulocytes is in favour of the previous existence of some sort of retardation of development. It is possible that the effect of a lack of a sufficient level of plasma iron is a failure of the last stages of normoblast differentiation in addition to inadequate synthesis of haem, and that the ragged-bordered polychromatic but almost pyknotic normoblasts are dying cells, the most severely affected of which never become erythrocytes at all. The reduced size of these normoblasts is presumably directly due to the reduced synthesis of haemoglobin, resulting in decreased globin formations from its precursors, and hence a small cytoplasmic mass.

Erythropoiesis in refractory anaemia.—The refractory anaemias (Bomford and Rhoads, 1941) are dyshaemopoietic anaemias often referred to by the descriptive titles aplastic, hypoplastic, or pseudo-aplastic, or as panmyelophthisic anaemias. For convenience we are also including "refractory

megaloblastic" and achrestic anaemias. The whole group is probably heterogeneous, but has in common a resistance, more or less complete, to any treatment now known. It is because we do not think that the descriptive titles mentioned above necessarily indicate distinct entities that we hesitate to discard the more general term "refractory anaemia." As a rule leucopenia or thrombocytopenia accompanies the anaemia, which is normocytic or macrocytic in type.

The only aspect of this group that we are now considering is the cytology of erythropoiesis. There have been several important studies in recent years. Israëls and Wilkinson (1940) described as suffering from "achrestic anaemia" six patients in whom the biopsied marrow was found to be megaloblastic or mixed megaloblastic and normoblastic. Bomford and Rhoads (1941), basing their account on sectioned biopsy or autopsy material, reported the marrow to be partly mature and cellular, or hypoplastic, or immature and cellular; and Davidson and others (1943) in a smaller series of cases reported the marrow, as studied by sternal puncture, to be hypocellular and normoblastic, or hypercellular and megaloblastic. Other patients in whom the marrow appearances greatly varied are described by Leitner and others (1949i).

In our own material we have observed great variation in the type of erythropoiesis. This variability is illustrated by the details of the following seven patients. In the first case there was a primitive marrow containing numerous haemocytoblasts and increased numbers of reticulum cells, with but few ripening cells, mostly intermediate megaloblasts, and a normocytic peripheral picture (Plate III, Fig. 4). In the second patient the marrow was extremely primitive, with some differentiation to typical megaloblasts, and a megalocytic peripheral blood picture (Plate III, Fig. 1). In the third patient the marrow was hyperplastic; primitive cells were present in moderately large numbers, and there was a moderate proportion of differentiating cells, some being megaloblasts. In addition, degenerating cells with pyknosis of the nuclei but without cytoplasmic ripening were conspicuous. The peripheral blood picture was of a megalocytic anaemia (Plate III, Fig. 3). In the fourth patient the marrow, although hypoplastic at autopsy, was shown during life by sternal puncture to be primitive, but to contain differentiating cells definitely megaloblastic in type (Plate III, Fig. 2). In the marrow of the fifth patient found to be hypoplastic at autopsy, there was in life, on the other hand, very little evidence of a megaloblastic change; most of the nucleated erythroblasts were normoblasts, a few were intermediate megaloblasts. The sixth patient, less anaemic than the foregoing, had a moderately hyperplastic and partly primitive marrow; once again the differentiating cells were mostly megaloblasts of intermediate type. In the seventh patient the marrow at biopsy appeared to be hyperplastic but not

* It is possible that this mechanism may contribute to the anaemia of Mediterranean anaemia (Cooley's anaemia). The hypochromic erythrocytes are extremely deformed, and there is evidence suggesting increased haemolysis (Dameshek, 1943).

primitive; erythropoiesis was normoblastic and normal except for some macronormoblasts with almost pyknotic but distorted nuclei. Anaemia was, however, severe and slightly macrocytic in type.

It is difficult to understand the mechanism of anaemia in those patients who have a hyperplastic and differentiating marrow (pseudo-aplastic anaemia). Presumably in these cases the final maturation of the normoblasts is unusually slow or the ripe cells may die before reaching the circulation. Possibly also, in some cases, hyperplastic areas alternate with areas of hypoplasia. In the patients whose marrows are predominately primitive in type, the mechanism of the failure to deliver adequate numbers of erythrocytes into the peripheral circulation is probably similar to that which operates in Addisonian pernicious anaemia. The primitive cells fail, to a greater or less extent, to complete their differentiation, and may be seen to undergo pyknosis of the nucleus and to degenerate without differentiating at all. Of the maturing cells a variable proportion have characters more or less typical of megaloblasts. As with our own series of patients with typical Addisonian anaemia, the more seriously anaemic the patient the more inhibited and primitive the marrow is likely to be.

Where aplasia seems to follow a proliferating marrow of primitive type, this change may well be due to an intensification of the factors causing the anaemia, whether they be "toxins," lack of growth factors, or the presence of a growth antagonist. This change from hyperplasia to hypoplasia of the marrow was observed by Bomford and Rhoads (1941) in some of their patients. Marrow cellularity, diminishing as the disease progressed, seemed to be a characteristic feature of the group of patients whose marrows were shown to be hypercellular and "partly mature" at biopsy; in five patients, however, the change was from a "partly mature" marrow to an immature one.

Erythropoiesis in haemolytic anaemia.—Increased haemolysis leads almost invariably to increased erythropoiesis,* and, in marrow biopsy smears of patients with an active haemolytic anaemia, erythropoietic cells are usually extremely numerous. The type of erythropoiesis has almost always been described as normoblastic (Leitner and others, 1949j). Turnbull (1936c) has, however, identified megaloblasts in addition to normoblasts in post-mortem sections of marrows from several patients with acholuric jaundice who died of haemolytic crises, and he has suggested that in these cases

there may have been a deficiency of haemopoietic factors. We have not yet encountered typical megaloblasts in marrow smears from our own patients; the normoblasts, however, tend to be a little larger than normal and may thus be described as macronormoblasts. This increase in size is most easy to recognize in the most mature cells. In addition to this we have noted, in patients where haemolysis is severe, that complete nuclear pyknosis is infrequent and that the nucleus of the almost mature cells with polychromatic cytoplasm may be slightly more "open" than in normal normoblasts, in relation to the acidophilia of the cytoplasm. Occasionally, the most mature nuclei may be irregular in shape. The whole picture is, however, unmistakably normoblastic rather than megaloblastic.

Erythropoiesis in leukaemia and allied disorders.—The association of anaemia with leukaemia is almost invariable, except in the earliest stages. In the peripheral blood the presence of undue anisocytosis and poikilocytosis and of erythroblasts suggests that erythropoiesis itself is disordered. Occasionally the disorder of erythropoiesis is dominant and the leucocyte abnormalities subsidiary or even subsequent; such cases, in which a severe macrocytic anaemia with evidence of abnormal erythropoiesis precedes the development of indubitable leukaemia, have been referred to as "leukanaemia" (Leube, 1900; Forkner, 1938; Foy and others, 1946). In addition to this variety of leukaemia there are patients in whom the *proliferative* process predominately and persistently affects erythrocytic cells. This rare disorder was first described by di Guglielmo* as acute erythraemia or erythraemic myelosis. "Leukanaemia" seems to occupy an intermediate position between erythraemia and leukaemia.

In myelogenous, lymphogenous, and monocytic leukaemia, erythropoiesis is predominately normoblastic, but a small proportion of cells simulating intermediate megaloblasts may be found; occasionally, and often towards the termination of the illness, these cells are present in higher proportions. "Megaloblasts" have been reported more frequently in cases of leukanaemia (Penati, 1937; Foy and others, 1946; Collins and Rose, 1948). In acute erythraemia, di Guglielmo has described as "paraerythroblasts" the atypical cells which are frequently encountered, and whose origin in some cases may be traced from the proliferating reticulum cells (haemohistioblasts). Atypical mitoses and irregular and multilobed nuclei are not infrequently

* Owren (1948) has demonstrated that *hypoplasia* of the erythropoietic marrow cells may be a cause of severe crises in congenital haemolytic anaemia; lack of maturation of primitive erythroblasts may also be a cause (Dameshek and Bloom, 1948).

* In di Guglielmo's (1946) review in French there is a full bibliography of his earlier papers.

seen in more mature forms of these cells, and there may also be unusually early acidophilia of the cytoplasm. In general, however, the type of erythropoiesis has been described as normoblastic, although amongst the "paraerythroblasts" there are some cells which closely resemble megaloblasts; these have been called "megaloblastiform" cells by Heilmeyer and Schoener (1941), and this term has been used by di Guglielmo himself and by Bessis (1948). Presumably also the patient described by Downey (1938b) as suffering from leukaemic reticulo-endotheliosis, in which megaloblast-like cells were observed to be developing heterotopically from reticulum cells, and the first patient described in Schleicher's (1944b) paper represent examples of a further rare variant of the leukaemia-erythraemia group.

We have observed cells rather similar to intermediate megaloblasts in leukaemia (Plate III, Fig. 5) and in erythraemia. These cells are rather larger than normal for their apparent maturity, and the acidophilia of the cytoplasm is in advance of the form of the nucleus. In general, we believe that this change towards a "megaloblastiform" type of erythropoiesis cannot be influenced by liver or folic acid therapy. In all probability it indicates more than a deprivation of growth factors by the rapid growth of leukaemic tissues, and is rather a reflection of the disordered growth caused by the "leukaemic" stimulus itself affecting erythropoiesis, and resulting in changes in morphology not unlike those caused by minor deficiencies in the liver haemopoietic principle (see also Schwarz, 1946).

Erythropoiesis in carcinomatosis of the bone marrow.—The leuco-erythroblastic anaemia of carcinomatosis of the bone marrow has been well described by Vaughan (1936a and b) and Turnbull (1936d). They have reported the presence of megaloblasts of Ehrlich with premature ripening of the cytoplasm in the bone marrow and in the peripheral blood. Both Vaughan and Turnbull remark, however, on the small size of the megaloblasts; in Turnbull's description of the marrow findings "most are relatively small megaloblasts with scanty cytoplasm and some are not appreciably larger than normoblasts. . . . Erythropoiesis is, therefore, partly megaloblastic, partly normoblastic, the normoblastic usually predominating." Bessis (1946) has also referred to intermediate types of "erythroblasts" when the bone marrow is invaded by metastatic carcinoma. In our view it is uncertain whether these abnormal cells should be considered as macronormoblasts or megaloblasts; further study is required. In a recent case erythropoiesis was predominantly normoblastic, but isolated cells

resembling intermediate megaloblasts could be found (Plate III, Fig. 6). Possibly both types of reaction occur. The cause of these changes is obscure. Vaughan concluded that the growing tumour cells interfered with the metabolism of the erythrocytic cells; this may well be the case.

Cellular gigantism in human erythropoiesis.—The occurrence of cellular gigantism has recently been reviewed by Schwarz (1946) and by Berman (1947). Binucleated "erythroblasts" may be found in both normal and abnormal bone marrows and they probably arise by endomitosis.* Schwarz (1946) discusses at length the abnormalities in mitosis which give rise to these multinucleated cells and describes the very large erythrocytes (gigantocytes), 18–25 μ in size, to which they give rise. He stresses the fact that no intermediary links are found between normal cells and gigantocytes and their multinucleated precursors. He does not consider that these giant cells are ever produced by amitosis. Berman found in eight normal subjects from 1 to 5.1 binucleated cells per 1,000 normal erythroblasts. Multinucleated erythroblasts produced by multipolar mitosis are, however, not found in health; not one example was seen by Berman out of 53,167 erythroblasts examined in his eight normal marrows. In disease, however, they occur not infrequently. Limarzi and Levinson (1943) have described three types of multinucleated erythroblasts: the first is produced as the result of multipolar mitosis without cytoplasmic separation; the second arises by folding and lobulation of the nucleus and finally by separation of the nuclear fragments; and the third by complete or incomplete amitosis—a mechanism which has been questioned (Schwarz). The resulting giant cells are well illustrated by Limarzi and Levinson (1943), Schleicher (1944b), Schwarz (1946), and Berman (1947).

Multinucleated erythroblasts have been observed in a variety of blood disorders; in Berman's series, in cases of leukaemia, lymphoblastoma, pernicious anaemia, congenital haemolytic anaemia, thrombocytopenic purpura, and liver disease. Schleicher (1944b) reported large multinucleated cells in a patient with reticulum-celled sarcoma (reticuloendotheliosis) of the bone marrow and in pernicious anaemia. Limarzi and Levinson's case was considered to be an example of erythroblastoma, and we have recently encountered a good example in a patient with pernicious anaemia of pregnancy (Plate III, Fig. 4).

Berman has stressed the fact that these giant cells are not specific for any particular disease process, that the process may be reversible, as in pernicious anaemia, and that the nuclear and cytoplasmic patterns conform to the type of the accompanying uninucleated cells. Thus multinucleated normoblasts or megaloblasts may be found, or giant dysplastic types with some of the characters of megaloblasts, as in Limarzi and Levinson's case.

* Endomitosis is complete nuclear division without division of the cytoplasm.

Although, therefore, the exact cause of the aberrant nuclear divisions remains obscure, it is clear that these processes do not necessarily indicate a malignant ("leukaemic") process. The position seems to be very similar to that for the occurrence of giant or multinucleated cells in other sites throughout the body. In the marrow they are most frequent during active erythropoiesis, as they are in other tissues during rapid growth phases (for example, in the testis of the normal mouse—Howard, 1948).

Abnormalities of mature erythrocytes.—An almost constant feature of anaemia is an increased variability in the size (anisocytosis) of the mature erythrocytes over and above the normal cell to cell variation. In addition, in most anaemias, corpuscles of abnormal shape and not strictly round in contour (poikilocytes) are found. The normal variation in cell diameters has been well worked out by Price-Jones (1933), who has shown that it conforms to a normal biological distribution, and similar variations in volume and thickness, etc., also probably exist. The cause of the exaggerated variation in anaemia is so far unsettled; anisocytosis and poikilocytosis are marked in dyshaemopoietic anaemias, particularly in Addisonian pernicious anaemia in severe relapse, in Mediterranean anaemia, and in some examples of leukaemia and myeloid leukaemia, and to a lesser extent in iron deficiency anaemia. In haemolytic anaemias and other anaemias with active regeneration, poikilocytosis is much less obvious, and variation in size is partly due to the large size of the polychromatic corpuscles. Spherocytic microcytes* may also be present and may add to the range of cell diameters. Poikilocytosis may thus be taken to indicate disordered erythropoiesis, and this is probably also true of marked anisocytosis, although in this case the production of macrocytes may be due to rapid rather than to abnormal formation.

To authors such as Plum (1947) and Boström (1948), who believe that erythrocytes are produced by budding from the cytoplasm of erythroblasts, the problem of anisocytosis and poikilocytosis presents no difficulty. The budding process is held to be deranged so that buds of different sizes are formed. Boström considers that poikilocytes are produced by cytoplasmic budding taking place through pathologically thickened walls of medullary sinusoids, and schematically illustrates this. However, as already mentioned, these unusual conceptions are not easily acceptable. Nevertheless, it is possible that the small poikilocytes of pernicious anaemia are cytoplasmic fragments (schistocytes: see Rous, 1928), but whether they are formed in the marrow (Habelmann, 1940) or represent fragments broken off larger cells in the peripheral blood stream is uncertain. In general we feel that the solution of the problem of the formation of poikilocytes depends on an understanding of the forces

which convert the cytoplasm* surrounding the nucleus of the normoblast into the disc-like form of the reticulocyte. It is easy to imagine that when erythropoiesis is abnormal this process is deranged and that imperfect erythrocytes result. Anisocytosis is likely to be a reflection of variations in the size of the normoblasts of the same apparent age. That this variation exists is illustrated in the normoblast diameter distribution curves (Text Fig. 5) and in Plate III, Figs. 1-3. Very large erythrocytes (gigantocytes) are occasionally encountered. They arise from multinucleated erythroblasts produced by abnormal mitosis (Schwarz, 1946).

Conclusions

In this review we have attempted a survey of erythropoiesis in man. The subject has many ramifications and a vast literature. While mentioning, therefore, some publications of historical interest, we have mostly referred to modern work. As our aim has been to present as wide a picture of erythropoiesis as possible, we have not confined ourselves to morphology and cytogenesis; we have included sections on the growth and differentiation of the erythropoietic tissue and a discussion of the cytochemical aspects of erythropoiesis, for not only is the mature erythrocyte a perfect example of the differentiation of a cell for a highly specialized function, but the sequence of changes accompanying development affords good examples of the processes of tissue growth as a whole.

The general pattern of erythropoiesis has been described in health and in disease. In health, a bone marrow biopsy gives a momentary but still picture of what is really a carefully regulated dynamic process, but even here much of the mechanism of normal erythropoiesis is ill-understood. In disease, the pattern of erythropoiesis may be greatly altered; accelerated, abortive, and aberrant formation may all be seen, sometimes in combination. Here, too, the changes are dynamic. All grades of change may be seen between the most abnormal bone marrows and a marrow scarcely distinguishable from the normal.

Bone marrow biopsy provides an almost unique opportunity for the examination of living human tissue. It is an indispensable adjunct to the proper understanding of the diseases of the blood.

We are indebted to our medical colleagues for freedom to investigate patients under their care, to Mr. E. V. Willmott, F.R.P.S., for the photomicrographs, to our laboratory and clerical staff for their assistance, and to other friends for helpful discussions.

* "Microspherocytosis," is a change which takes place after the erythrocytes have been delivered from the bone marrow. The marrow normoblasts are not abnormal; neither, as a rule, are the reticulocytes. The same is true of elliptical corpuscles (see Leitner and others, 1949k) and of sickle cells (Wintrobe, 1946f). The antithesis of spherocytosis target-cell formation seems, in Mediterranean anaemia at least, to depend upon an abnormality of formation.

* Pronormoblasts and ripening normoblasts are probably almost spherical cells. As the nucleus shrinks and the cytoplasm becomes more abundant relatively, the cell is roughly elliptical in vertical cross-section.

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