Scientific Basis of Clinical Practice

Red Cell Production

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Red blood cells (erythrocytes) are normally produced only in the bone marrow of adults, but during embryonic and fetal life erythropoiesis occurs in the liver, and during the second to the fifth month of fetal life it occurs in the spleen. From the fifth month after gestation marrow production increases gradually, having begun to take over from the liver during late fetal and early neonatal life. During the early years of life erythropoiesis occurs in the marrow of most bones but as age progresses active marrow is replaced by fat cells, starting from the distal bones of the limbs and proceeding towards the trunk, so that by adult life activity is confined to the vertebrae, ribs, sternum, skull, and iliac crests.

Normal Production and Development

The identity of the erythroid stem cell remains unsettled and may be different in the fetus and the adult. According to the monophyletic school it is identical morphologically with a common blood lymphocyte, but the polyphyletic school of Sabin holds that it is derived from the endothelial cells lining the sinusoids of the bone marrow. Possibly there is a multipotential cell producing several unipotential cells which are dependent for differentiation upon their microenvironment, one group being erythropoietin-responsive cells totally committed for erythroblast production. Such stem cells must maintain their own number and also supply cells capable of maturation. Therefore there are probably two types of division, one symmetrical, producing two stem cells, and the other asymmetrical, producing one stem cell and one daughter cell of finite life-span. The steady state is then maintained by asymmetrical division while regeneration after injury occurs by symmetrical division.

The rate of multiplication of erythroid cells in adults is probably controlled by erythropoietin, while an entirely unknown but different mechanism operates in the fetus and neonate. Erythropoietin is a mucopolysaccharide of plasma. It probably arises from the activation of a plasma protein substrate produced by the liver by an enzyme erythropoietin produced by the kidney and other tissues, particularly the spleen and endocrine glands. The renal factor is highly sensitive to oxygen deficiency in the blood. Other agents affecting multiplication of erythroid cells are androgens, adrenocorticosteroids, a thyroid hormone, and the nervous system. These may well all act through the erythropoietin-responsive cell.

With the aid of radioactive-labelled DNA, RNA, protein, and haemoglobin the mitotic cycle of each red cell precursor has been measured and the cytoplasmic content of each type of cell studied. From these a model of erythron kinetics can be constructed which reasonably resembles human red cell production (see Figure). The morphology of the precursor cells at each stage of production is as follows.

![Erythron Kinetics Model](http://www.bmj.com/)

Cell Ia (Pronormoblast).—A round cell 15-20 μ in diameter with a thin rim of basophilic cytoplasm devoid of granules and a large round nucleus composed of finely reticulated chromatin with a tendency to form minute triangular masses arranged roughly radially and having indistinct nucleoli. Incorporation into the cell occurs by small surface invaginations into which iron-siderophyllin is drawn. The iron is released from the protein and the invagination becomes a vacuole within the cell cytoplasm.

Cells Ib and Ic (Early and Late Basophilic Normoblasts).—Round cells 10-14 μ in diameter with a deeper basophilic cytoplasm than the pronormoblast and having a nucleus with chromatin coarsened by coalescence of the previous network pattern but maintaining the radial arrangement. Nucleoli are absent. During basophilic normoblast maturation iron enters into the mitochondria of the cytoplasm where it is incorporated into protoporphyrin to form haem. This may be catalyzed enzymatically (haem synthetase) and requires pyridoxine. Globin synthesis from four polypeptide chains, whose production is controlled genetically, occurs in the ribosomes. Globin and haem synthesis occur simultaneously at roughly similar rates, production of one being a control of the other.

Cell II (Polychromatric Normoblast).—Irregularly shaped cells 8-12 μ in diameter with polychromatic cytoplasm owing to the increasing content of haemoglobin. The nucleus is a condensed mass of chromatin in cartwheel arrangement.

Cell III (Orthochromatic Normoblast or Late Polychromatic Normoblast).—Increasing haemoglobin content of the cytoplasm
leads to increased eosinophilic staining. Completely ortho-
chomatic cells may not always be stainable in the marrow
smears. The attainment of a critical haemoglobin level may
serve as a negative feed-back to further DNA synthesis and cell
division. When the nucleus can no longer synthesize DNA it
undergoes pyknotic degeneration, appearing either as a homo-

genous mass or assuming buds, rosettes, clover leaves, double
spheres, or a faint ring. Finally it is lost either by extrusion or
intracellular karyolysis.

Cell IV (Marrow Reticuloctyes).—Even after the nucleus has
been lost remnants of cytoplasmic basophilic-staining RNA
persist for a short time either uniformly (diffuse basophilia),
tinged with eosinophilic cytoplasm (polychromasia), or
as fine or coarse granules (punctate basophilia, basophilic
stippling). Supravital staining shows the reticulum as a meshwork
(reticuloctye). The appearances depend mainly upon the
technical manipulations of the red cell in preparation for
microscopy. Reticuloctyes are more adhesive than mature red
cells and under normal conditions tend to remain in the bone
marrow. Their maturation time in the bone marrow is from
36-44 hours and 24-29 hours when released into the peripheral
circulation.1

Nuclear maturation is not always simultaneous with cyto-
plasmic changes, so that some reticuloctyes contain a pyknotic
nucleus and some orthochromatic normoblasts mature without
passing through the reticuloctye stage. During maturation
10-15% of normoblasts die (ineffective erythropoiesis) and
some miss a mitotic division, becoming macroctye reticulo-
cytes. These cells have a shortened life span.

Haemopoietic tissue in marrow lies in cords between vascular
sinuses which drain into a central longitudinal vein. The
haemopoietic cells probably enter a sinus through an aperture
in the wall, the adventitial cells possibly playing some part.
The sinusoid vasculature is a closed system with continual
reroighting and change of flow rates which are related to the state
of haemopoiesis and possibly influenced by humoral agents.
Periodic localized floodings of the parenchyma occur by
extravasations of cells which may have an effect on cell release.8

Assessment of Production

Much of our knowledge of red cell production has been gained
from study of animal experiments, particularly by the use of
radioisotope techniques. In man study is dependent on examina-
tion of specimens of bone marrow. Indirect assessment can
be made by counting immature cells such as reticuloctyes in the
peripheral blood and by measuring the rapidity of removal of
radioiron from the plasma (plasma iron clearance) and its
reappearance in the red cells (red cell utilization). The activity
of the marrow can also be assessed by radioactive counting.8

Disturbances of Production

A stimulus for an increase in red cell production may come from
a rapid depletion of red cells from haemorrhage or haemolysis
or from a need to replace deficient substances such as folates or
vitamin B12. Irrespective of origin of the stimulus, the response,
by a mechanism unknown, is a release of immature erythroid
cells from the bone marrow. They are larger than normal,
contain RNA (polychromatophilic reticuloctyes), and have a
shorter life span. Nucleated red cells may be released under
severe stress.

The first reticuloctyes released contain large amounts of
reticulum, which decreases later. Once the demand for red
cells has ceased the release of reticuloctyes falls to normal.
The secondary response is an increase in red cell production with
more normoblasts in the bone marrow, premature attainment of
the critical haemoglobin concentration, inhibition of cell
divisions, and macroctyosis.8

Apart from in fetal life, erythropoiesis outside the bone
marrow occurs only in abnormal circumstances. Severe anaemia
of infancy may lead to erythropoiesis in liver, spleen, lymph
nodes, suprarenals, and kidneys. Extramedullary erythropoiesis
is rare in adults except in connexion with myelofibrosis and
myeloid leukaemia, but has been reported in most forms of severe
haemolytic and megaloblastic anaemias.

Decreased Multiplication

A decrease in multiplication of erythroid cells is associated with
marrow hypoplasia. The plasma iron clearance is slow, red cell
utilization is decreased, and radioactive counts over the marrow
after intravenous injection of 57Fe are low.

There are a number of causative factors which may lead to a
lowered production of red cells. There may be mitotic abnormali-
ties leading to exhaustion of daughter cells or an intrinsic cellular
abnormality leading to increased intramedullary destruction,
supported by chromosomal abnormalities in the Fanconi type of
congenital hypoplasia. Congenital anomalies of the skin and the
kidney usually coexist. Irradiation of the marrow in the adult or
in the fetus results in a higher incidence of hypoplasia.

Another cause may be destruction of the marrow micro-
circulation.9 Marrow sinusoidal structures have an incomplete
or no basement membrane, and this may allow immune
mechanisms to mount antigen-antibody or cellular immune
reactions restricted to marrow sinusoidal structures. This
would be consistent with the frequent history of drug admini-
stration in cases of hypoplasia.

Disturbance of the intercellular surface relationships when the
marrow is invaded by “foreign” cells, resulting in cell autolysis,
is a possible explanation of hypoplasia associated with carcinoma
metastases, myelomatisos, lymphosarcoma, megaloblastic or lympho-
blastic proliferation (acute leukaemia), or the presence of miliary
tubercles. The belief that marrow is replaced by the foreign
tissue is no longer tenable.9

Deficiency of erythropoietin is rare, and in most cases of
hypoplasia the level is raised. Low levels have been found in
cases of insufficiency but though the anaemia temporarily
improves after haemodialysis erythropoietin levels remain low.
Another inhibitor of erythropoiesis may be active in renal
failure.10

Hypoplasia has been reported after destruction of the anterior
pituitary gland (Simmonds's disease), probably owing to a
deficiency in an “end organ” hormone. Hypoplastic anaemia
occurs after orchidecmy and responds to androgen therapy,
and some cases of idiopathic hypoplasia respond to testosterone
or its derivatives (oxydemethalone).11 Hypoplasia is sometimes
associated with hypothyroidism, but it does not respond to
thyroxine. The response to corticosteroid therapy in a number
of cases, particularly those associated with drug hypersensitivity,
is more likely to be due to its immunosuppressive action.

With protein deficiency of high order, as in kwashiorkor, a
hypoplastic anaemia develops which disappears spontaneously
on feeding. The view that protein deficiency may be a cause
of marrow hypoplasia is supported by finding that starvation
in animals leads to depression of erythropoiesis and diminished
erythropoietin formation as a result of low amino-acid substrate.

Toxic agents are present in about half of the cases of marrow
hypoplasia. These are either directly cytotoxic (for example,
benzene and its derivatives, and cytotoxic drugs) or are sub-
stances to which the individual is hypersensitive (for example,
chloramphenicol, phenylbutazone, and the hydantoin group of
drugs).

In severe haemolytic crises of both sickle-cell disease and
hereditary spherocytosis the marrow may temporarily become
hypoplastic. The pathogenesis is not understood.

In myeloproliferative disorders when there is an increase in
fibroblast activity marrow may be replaced by fibrous tissue.
This usually occurs in association with extramedullary haemo-
poiesis.
Finally, there may be pure red cell aplasia associated with a benign thymoma and sometimes hypogammaglobulinaemia. There is presumably an immunological basis for the disorder which at present remains obscure.\textsuperscript{13}

**Increased Multiplication**

Over production of red cells by the bone marrow usually leads to an erythrocytosis with an increase in red cell mass in the peripheral blood. There is often an associated over production of granulocytes and thrombocytes. The stimulus for over production is sometimes an increase in circulating erythropoietin (secondary erythrocytosis) but in many patients the aetiology is unknown. Polycythaemia vera is a disorder related to myeloid leukaemia.

Increased formation of erythropoietin may be due to hyperplasia of erythrogenin-producing cells or secondary to deficiency in oxygen saturation of the blood. Anoxia associated with erythrocytosis occurs in patients with right to left shunts due to pulmonary arteriovenous aneurysm or to a congenital heart disease such as pulmonary stenosis usually with defective ventricular or auricular septum, patent foramen ovale, patent ductus arteriosus, persistent truncus arteriosus, complete transposition of the great vessels, or Fallot's tetralogy. In these conditions the arterial saturation may be only 30-35\textdegree C.

Erythrocytosis also occurs in poikilothermic disease causing inadequate oxygenation of the blood. Emphysema is the most common of these but pneumoconiosis may also be associated with erythrocytosis, as may chronic cor pulmonale and primary disease of the pulmonary arteries.

Hyperventilation of primary origin or associated with obesity, the Pickwickian syndrome, may give rise to erythrocytosis, and so may living for long periods at high altitudes, where oxygen tension is low. Haemoglobin levels of 21 g/100 ml have been recorded in people living at 19,000 ft (5,776 m). The degree of erythrocytosis is not proportional to the altitude and it may be secondary to changes in pulmonary arteries.

Erythrocytosis may be due to a deficiency of haemoglobin pigment arising from oxidation of reduced haemoglobin to methaemoglobin or sulphaemoglobin. This is caused by a variety of drugs, and there is a hereditary form. Poisoning by aniline and its derivatives and by toluidine, atoxyl, and nitrobenzol has been reported to cause erythrocytosis. The administration of cobalt in excess to animals results in erythrocytosis with increased erythropoietic activity in the liver and spleen. This may be due to local anoxia caused by cobalt inhibiting the enzyme activities that deal with the transport of oxygen to the tissues. Cobalt has been used for the treatment of erythrocytotic hypoplasia.\textsuperscript{12}

Increased erythropoietin secretion with erythrocytosis has been described in association with infiltratentary vascular tumours (particularly cerebellar haemangioblastoma), hepatomas, uterine fibroids, and undifferentiated carcinoma of the lung. Removal of the tumours relieves the erythrocytosis. Renal lesions such as hypernephroma, renal cysts, Wilms' tumour, hydronephrosis, poly cystic kidneys, renal ischaemia due to disease of the extra renal vasculature, and renal transplantation have all been reported in association with erythrocytosis. Extracts of tumour and cyst fluid have been shown to contain erythropoietin. Erythrocytosis is well recognized in Cushing's syndrome with adrenal hyperplasia and in patients with a phaeochromocytoma. It also follows adrenocorticosteroid therapy in large doses.

**Disordered Production (Dysplastic Erythropoiesis)**

Disturbances of multiplication, maturation, and release all lead to an increased cellularity of the bone marrow and a decrease in the number of circulating erythrocytes (ineffective erythrocytosis). Radioiron studies show a rapid iron clearance, reduced red cell utilization, and a varied organ-scanning pattern.

**Disorders of Cell Division (Endomitosis)**

Abnormalities of nuclear mitosis may generate abnormal multinucleated normoblasts. These are seen in a congenital dysplastic anaemia\textsuperscript{14} and in erythraemic myelosis. In the latter there are changes in erythroblast chromosomal structure and arrangement, and the disorder behaves like a form of acute leukaemia.

Erythroblasts with structural abnormalities (spherocytosis, elliptocytosis) are produced either as the result of a hereditary disorder or by somatic mutation. Mutations may account for the abnormalities found in the erythrocytes of patients with paroxysmal nocturnal haemoglobinuria\textsuperscript{15} or red cell polyagglutination,\textsuperscript{16} and they could also be the cause of the dysplastic anaemias sometimes seen in association with kwashiorkor, liver disease, tuberculosis, and after irradiation.

**Disorders of Maturation**

Disorders of maturation either disturb DNA synthesis in the nucleus or cause a deficiency in haemoglobin synthesis by the erythrocyte cytoplasm.

**Disturbances of DNA Synthesis**

Disturbances of DNA synthesis (megaloblastosis) are due to a deficiency of coenzymes essential in nuclear acid synthesis. This may be congenital or acquired. An example of the former is the deficiency of orotidylid pyrophorylase or of orotidylid decarboxylase, both of which are rare hereditary autosomal recessive defects. Acquired deficiencies may be seen in the condition characterized from a disturbance of pyridoxine metabolism, particularly if this is associated with deficiency of riboflavin or administration of isonicotinic acid hydrate. Another group of acquired deficiencies is associated with disturbance of folate, vitamin \textit{B}_12, or ascorbic acid metabolism. This may result either from dietary deficiency, intestinal malabsorption, or increased utilization or from interaction of drugs such as anticonvulsants, cytotoxic agents, or alcohol. Ascorbic acid acts indirectly through the maintenance of folates in the reduced state.\textsuperscript{17}

Delays in DNA synthesis result in many cells simultaneously being near the stage of division; at the same time the cytoplasms matures, resulting in the accumulation of RNA. This combination gives rise to megaloblastosis: the cell nuclei in the promegablast and basophilic megaloblast show uniform distribution of nuclear chromatin without any tendency to clumping. At the orthochromatic stage the nucleus may show clumping of chromatin, later becoming shrunken and eccentric with pyknotic budding. The maturation time of the red cell series is prolonged and some of the early polychromatic megaloblasts may not complete this last division. An increased proportion of the cells die during maturation. Reticulocytes produced after skipped division are macrocytic, have an increased tendency to adhere together (so remaining in the marrow), and on release into the peripheral circulation have a shortened life span.

**Deficiency of Haemoglobin Synthesis**

\textbf{Iron Deficiency}.—This occurs as a result of intestinal deficiency, malabsorption, or chronic haemorrhage. There is sideropenia and inadequate iron for haem production. Because synthesis is reduced there is also a diminished formation of globin by the
cells. The presence of copper may be important in maintaining availability of iron to the erythropoietic cells.

**Disturbance of Iron Storage.**—For reasons not yet understood, iron may be stored in the macrophages, which should normally be available to the erythroblasts for the synthesis of haem. This availability may be sharply reduced in rheumatoid arthritis, reticuloses, and in some infections. The bone marrow normoblasts are increased but there is no increase of intracellular iron.16

**Disturbance of Haem Synthesis.**—This may be due to a deficiency of an essential enzyme, pyridoxine, or pantothenic acid at the cellular level. This deficiency may be hereditary or due to an acquired disorder such as lead poisoning. There is hyperplasia of the erythroid cells in the marrow with accumulation of intracellular iron which cannot be utilized (sideroblasts). The mature erythrocytes also contain iron (siderocytes) and are retained in the bone marrow; hence there is a reduction in the number of red blood cells in the peripheral blood.16

**Deficiency of Globin Synthesis.**—This is the underlying abnormality in the hereditary disorder thalassaemia. Globin synthesis is genetically controlled, and while in thalassaemia the predominant deficiency may be of a specific polypeptide chain, there is also a deficiency of the total globin formed. Characteristically iron accumulates in the marrow cells and other storage sites.17

The effect of any deficiency in haemoglobin production, whatever the cause, is slow attainment of the critical haemoglobin concentration. This leads to extra cell divisions and microcytosis with red cells of low haemoglobin content.

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**Any Questions?**

We publish below a selection of questions and answers of general interest.

**E.C.G. Changes in Shock**

*What E.C.G. changes occur in shock? Can they be differentiated from myocardial infarction?*

The common causes of non-cardiogenic shock such as trauma, haemorrhage, sepsis, pancreatitis, and anaphylaxis are usually clinically obvious and differentiation from myocardial infarction hardly arises. However, I can find no recent references in the world literature to what E.C.G. changes occur in shock. Probably they would chiefly be ST segment depression and abnormal T waves. It would seem unlikely that pathological Q waves and ST elevation would be seen.

Of more interest is the differentiation of Gram-negative bacteraemic shock from massive pulmonary embolism, since both conditions occur postoperatively. Here the E.C.G. can be helpful if it shows the typical pattern of pulmonary embolism—right bundle branch block and T wave inversion in the right precordial leads (a deep S wave in lead I, an inverted T wave in lead III, and the presence of a Q wave in lead III).

**Allaying Anxiety before Exams**

*Is there any drug which can safely be used to allay anxiety before examinations, games, stage appearances, and the like?*

A safe and predictable tranquilizer suitable for use on a single but critical occasion has yet to be found. The advent of the phenothiazines with their differential action on the central nervous system producing more hypotonic than cortical activity raised hopes that an ideal drug would be discovered. However, notwithstanding the development of such preparations as thioridazine, chlordiazepoxide, diazepam, and oxazepam it has proved impossible to predict whether there will be any action upon the patient's cortex, with resultant drowsiness, even in small doses. Consequently their use on a single occasion without a trial beforehand is unwise.

When a patient seeks advice it is preferable to discuss the management of his anxiety with him. By helping him to cope with his anxiety the individual may work through what is, after all, a potentially healthy reaction. There is a use for drugs in certain special situations—such as important examinations—under strict psychiatric supervision either through regular outpatient consultation or by admission of the student to a psychiatric unit.

So generally drugs are not helpful, since those that are pharmacologically effective are likely to be drugs of dependency, whereas non-dependent drugs can have unpredictable side effects. If drugs are used at all it must be under medical supervision. Self medication in any form is to be deprecated.

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**Notes and Comments**

**Loss of Libido in Depression.**—Dr. M. ROBURN (Vancouver, British Columbia) writes: In the answer to this question ('Any Questions', 16 October, p. 160) you Expert advised a thorough investigation of a man of 47 with mild depression who had responded to amitriptyline treatment but still suffered from lack of libido and difficulties with ejaculation. The question did not specify whether amitriptyline has been discontinued. Amitriptyline, like other tricyclic antidepressants and phenothiazines has parasympatholytic side effects, which can include disturbance of sexual function especially of ejaculation. Therefore, first of all, amitriptyline should be stopped.