Aplastic Anaemia

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BAILLIÈRE TINDALL · LONDON

A BAILLIÈRE TINDALL book published by
Cassell Ltd
35 Red Lion Square, London WC1R 4SG
and at Sydney, Auckland, Toronto, Johannesburg
an affiliate of
Macmillan Publishing Co. Inc.
New York

First published 1979

ISBN 0 7020 0698 X

Printed in Great Britain by Spottiswoode Ballantyne Ltd. Colchester and London

British Library Cataloguing in Publication Data

Aplastic anaemia.
1. Aplastic anemia
I. GEARY, C G
616.1'52 RC641.7.A6

ISBN 0-7020-0698-X

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Preface

Although aplastic anaemia is a rarer disease than leukaemia, the clinical picture of bone marrow failure presented by severe aplasia is the more poignant when one recalls that at least 50% of all cases are a result of modern man's contact with his environment: indeed, as Dr Benestad points out on page 26, marrow damage is the most feared of all drug reactions since it is, so frequently, irreversible.

Ninety years have elapsed since Ehrlich's first graphic clinical and morphological description of aplastic anaemia, yet only in the last ten years have haematologists begun systematically to investigate and so comprehend the pathophysiology of the disease. Techniques which enable marrow progenitor cells to be grown in culture in the laboratory have permitted at least a semiquantitative analysis of the defect which exists in chronic marrow aplasia, while application of immunological methods is now providing clues as to how damage to stemcells might be provoked and sustained. While the enigma of the marrow defect in aplastic anaemia is slowly unravelled, management, whether simply supportive, or aimed at selecting patients for immunosuppressive therapy or bone marrow transplantation, is gradually becoming more rational and effective.

This book represents a synthesis of the experience of eight experimental and clinical haematologists who have a particular interest in the disease in both adult and paediatric practice. The sections on red cell aplasia and the aplasia–leukaemia syndrome are included because the first, an intriguing 'partial' variant of aplastic anaemia itself, may yet provide us with understanding of immunological mechanisms at work in some cases of panhypoplasia, while the second is of importance in an era when marrow depression is increasingly frequently seen in patients treated with cytoxic immunosuppressant drugs, for many different conditions.

We hope that this detailed account of aplastic anaemia will provide an up-to-date and comprehensive account of the disease for all who are involved in its diagnosis and management.

October 1978

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Pathophysiology of Marrow Hypoplasia

C. G. GEARY and NYDIA G. TESTA

The association of fatty atrophy of the red marrow with cytopenia in the blood was first described by Ehrlich in 1888, though the term 'aplastic anaemia' was probably not used until Chauffard coined it to describe the clinical syndrome in 1904. Although these early descriptions, based on autopsy findings, emphasized hypoplasia of the marrow as a cardinal diagnostic feature, the terminology later became confused, because 'aplastic anaemia' became virtually synonymous with blood pancytopenia. Benzene—the first substance recognized to cause marrow damage—is indeed unusual in producing pancytopenia with both hypocellular and hypercellular marrows, and even extramedullary erythropoiesis (Hunter 1969; Saita 1973), but the main reason for confusion was the lack of a method for examining marrow cellularity during life, marrow puncture not becoming a routine procedure until the late 1930s. Patients with pancytopenia and cellular or hypercellular marrow pictures were subsequently classified as having hypersplenism, 'achrestic anaemia', 'refractory anaemia with cellular marrow' or, in kinetic terms, 'functional aplasia'. Although it is now recognized that many patients with aplastic anaemia have islands of intense, though frequently abnormal, haemopoietic activity, called 'hot pockets' (Kansu & Erslev 1976) (indeed, complete marrow 'aplasia' would be incompatible with life) there still seem to be good clinical grounds for distinguishing between pancytopenic patients with predominantly hypocellular marrows and those with cellular marrows. Thus isolated cytopenias are more common in the 'cellular' group, and the natural history is different: leukaemia may be a more common sequel in the latter group (Vilter et al. 1967), whilst others respond to immunosuppressants. Also, the aetiology probably differs. Apart from benzene, some antimetabolites and possibly chloramphenicol (Bithell & Wintrobe 1967), no substances are known to cause chronic marrow failure with both the hypercellular and hypocellular pictures; X-irradiation may produce the syndrome of pancytopenia with maturation arrest in a normocellular marrow, but this appears often to

be a pre-leukaemic picture (Wendt 1971). Although drugs known to cause panhypoplasia also cause selective aplasias of one or other cell lines, the latter are more often reversible on stopping the drug.

Nevertheless, similar qualitative abnormalities of erythropoiesis are found in patients with hypoplastic marrows and those with hyperplastic, ineffective, marrows, while some children with constitutional aplasia present initially with pancytopenia and cellular marrows, and later develop hypoplasia (Fanconi 1967) (see Chapter 8), suggesting that too rigid a distinction should not be made.

DEFINITION AND CLASSIFICATION

Definition. Aplastic anaemia can be defined in morphological or in kinetic terms. The following definition is modified from one suggested by Heimpel and Kubanek (1975): 'a syndrome of peripheral blood pancytopenia, without dominant peripheral blood cell destruction, associated with hypocellularity of haemopoietic tissue, in both intramedullary and extramedullary sites, and without bone marrow fibrosis or invasion by malignant cells'. In kinetic terms, it can be defined as the failure of the early haemopoietic precursor cells (probably multipotential stem cells) to provide sufficient progeny for the maturing, morphologically identifiable cell compartments of the marrow. Dose-dependent transient aplasia does, of course, occur after irradiation or cytotoxic therapy, but, provided stem-cell damage is not profound, the haemopoietic tissue will regenerate and chronic aplasia will not occur (see p. 18). Heimpel and Kubanek have suggested, therefore, that these inevitable but usually rapidly reversible cases of iatrogenic aplasia should be excluded in defining aplastic anaemia, which is usually an 'unpredictable' disease.

Table 1.1. Classification of aplastic anaemia

I Ionizing radiation Cytotoxic chemicals	dose-dependent
II Chemicals, drugs conditional immunological	largely dose-independent
III Viral cytotoxicity direct immunological	
IV Autoimmune cytotoxicity	
V Hereditary defect	

After Benestad (1974).

Table 1.2. Kinetic classification of aplasia

Pluripotent stem cells
(*a*) reduced numbers
(*b*) defective function
Environmental influences
(*a*) defects in the bone marrow environment
(*b*) abnormalities of short or long-range humoral factors influencing stem-cell growth
(*c*) inhibitors of cell growth

Classification. No scheme for the classification of aplastic anaemia is completely satisfactory because the precise way in which marrow damage occurs in aplasia is unknown. In the majority, the disease arises as the result of an apparently idiosyncratic (genetically determined?) reaction to the environmental agent. The possible ways in which drugs might provoke this idiosyncratic response, and damage marrow cells, are discussed in Chapter 2. Table 1.1 summarizes possible pathophysiological mechanisms, and Table 1.2 shows a kinetic classification of aplastic anaemia.

HAEMOPOIETIC STEM CELLS

Stem cells occur in all rapidly dividing tissues and are customarily defined as those that can both maintain their own numbers and give rise to differentiated cells. The haemopoietic system, in fact, is a cell-renewal system in which a continued supply of differentiated non-dividing blood cells is sustained by the proliferation and differentiation of such ancestral, pluripotent cells (Fig. 1.1). The mouse spleen colony assay (Till & McCulloch 1961) detects cells, operationally defined as colony-forming units (CFU-S), which have the properties defined above: they have extensive self-renewal and differentiation capacity and give rise to clonal colonies which contain cells of the granulocytic, erythroid and megakaryocytic series (Siminovitch et al. 1963). Although there is, as yet, no assay available for human haemopoietic stem cells, recent reports of mixed colonies of unicellular origin derived from cultured mouse cells, and containing erythroid, granulocytic and megakaryocytic cells (Johnson & Metcalf 1977), suggest that clonal stem cell growth from human bone marrow haemopoietic tissue may be achieved in the near future.

There are, however, colony assays originally developed for mouse cells, which can be applied to the study of human haemopoiesis. Two different populations of cells give rise to *in vitro* colonies composed of recognizable erythroid cells. One type of colony-forming cell has been

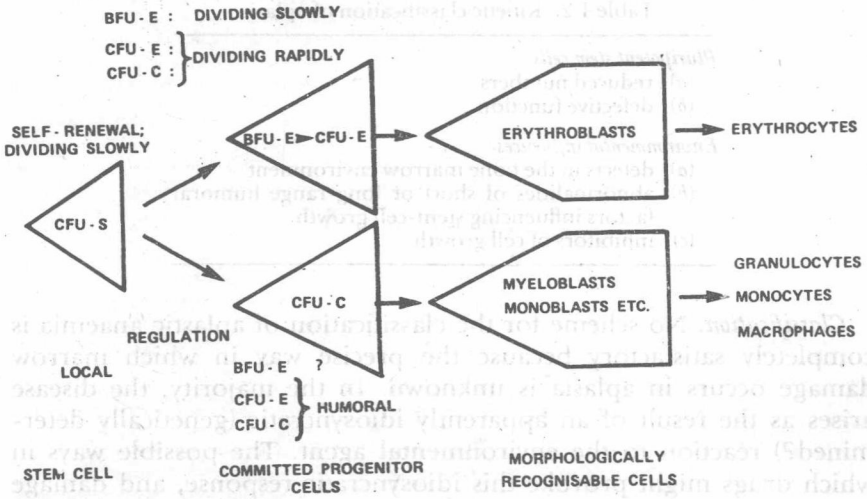

Fig. 1.1. The haemopoietic stem cell and some of its progeny. For an explanation of the abbreviations, see text. Populations of non-T lymphocytes are derived from a common 'myelolymphoid' stem cell, at least in some myeloproliferative syndromes (Fralkow 1978). This may explain the lymphopenia seen in severe aplastic anaemia.

named 'burst-forming unit, erythroid' (BFU-E) due to the characteristic arrangement of the cells in the fully developed colonies (Axelrad et al. 1974). A more mature cell, CFU-E ('colony forming unit, erythroid'), progeny of the former (Gregory & Heubelman 1977) gives rise to small colonies (Stephenson et al. 1971; Iscove et al. 1974). These two assays require the presence in the cultures of erythropoietin (Epo), a humoral regulator of erythropoiesis. Although, in contrast to the CFU-E, the BFU-E themselves do not appear to be regulated by Epo *in vivo*, their progeny became sensitive to Epo at some time during the growth of the bursts (Iscove 1977*a*).

Another method, also based on colony formation *in vitro*, permits the study of a cell population (CFU-C) which gives rise to colonies of granulocytes and/or macrophages when molecules with colony-stimulating activity (CSA) are present in the cultures (Bradley & Metcalf 1966; Pike & Robinson 1970). Examples of these different types of colonies are seen in Fig. 1.2.

In the generally accepted sequence of differentiation in the haemopoietic system, the cell populations which give rise to these colonies are considered to be committed progenitor cells, subject to different regulatory mechanisms from those acting at the pluripotent stem-cell level (McCulloch et al. 1965; Curry & Trentin 1967; Lajtha & Schofield 1974). An enumeration and brief description of the assays available for the study of stem cells and committed progenitor cells is presented in

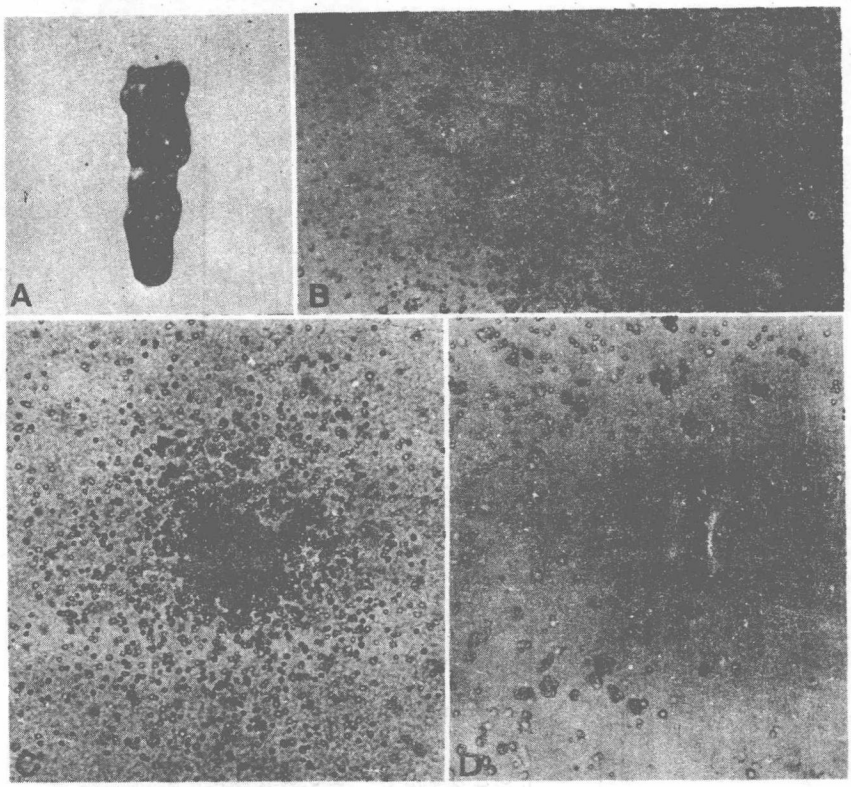

Fig. 1.2. A, Mouse spleen with surface colonies (CFU-S assay). B, Portion of an erythroid burst grown in methylcellulose (BFU-E assay). C, Granulocyte–macrophage colony grown in agar (CFU-C assay). D, Erythroid colonies grown in methylcellulose (CFU-E assay).

Table 1.3. Such colony assays have shown that bone marrow from patients with aplastic anaemia possesses reduced or undetectable numbers of progenitor cells, both for the granulocyte/macrophage series (CFU-C) (Kurnick et al. 1971; Greenberg & Schrier 1973; Kern et al. 1977) and for the erythroid series at both the CFU-E and the BFU-E levels (Hansi et al. 1977; Moriyama 1978). Other *in vitro* colony assays which detect progenitors of megakaryocytes, B-lymphocytes and T-lymphocytes have been developed recently (Metcalf et al. 1975*a*, *b*; Rozenszajn et al. 1975), but will not be discussed here.

Much clinical and experimental evidence indicates that the haemopoietic system is capable of responding to a high demand for increased cell production, even over a whole lifetime, without failing. For example, aplastic anaemia is not more common in the elderly, nor in patients with chronic haemolytic anaemia, such as hereditary spherocytosis, in which the system is under continued stress. CFU-S are

Table 1.3. Assay system for stem and progenitor cells in haematopoieses

Cells	*Nomenclature*	*Method*	*End point*	*Regulation*	*Applicable to man*
Stem	CFU-S	Injection into potentially lethally irradiated mouse	Spleen colony	Local regulatory	—
Granulocyte-macrophage progenitor	CFU-C	Culture in agar or viscid medium in the presence of CSA	*In vitro* colony	Colony-stimulating activity (CSA)	Yes
Erythroid progenitors	BFU-E	Culture in viscid medium in the presence of Epo	*In vitro* colony	?	Yes
	CFU-E	Same	*In vitro* colony	Erythropoietin (Epo)	Yes
Megakaryocyte progenitor	CFU-Meg	Culture in agar in the presence of spleen conditioned medium		?	—
B-Lymphocyte progenitor	CFU-B	Culture in agar in the presence of β-mercaptoethanol	*In vitro* colony	?	—
T-Lymphocyte progenitor	CFU-T	Culture in agar in the presence of phytohaemagglutinin	*In vitro* colony	?	Yes

Factors present in serum, necessary for the growth of 'bursts' *in vitro* (Iscove) may be specific regulators.

capable of increasing their turnover, as evidenced by the high proportion undergoing DNA synthesis in experimental situations which require an increased rate of cell production: for example, during development, or regeneration after injury (Becker et al. 1965; Lajtha et al. 1969; Metcalf & Moore 1971). This property, together with an increased number of cell divisions in the more differentiated cell populations, producing an 'amplifying' effect (Lord 1965), makes it possible to maintain comparatively normal production of mature cells with substantially decreased numbers of stem cells. Although a compartment size exceeding 10% of the normal number of stem cells has been postulated as necessary before stem cells differentiate (Boggs et al. 1972; Boggs & Boggs 1976), experiments in which mice were exposed to continuous irradiation at a low dose rate indicate that 1–2% of the normal CFU-S and CFU-C numbers can still maintain femoral bone marrow cellularity at about 50% of the normal value (Testa et al. 1973).

Clearly, the condition necessary to avoid stem-cell exhaustion is that, in situations which provide maximum demand for differentiation, the proportion of stem cells that differentiate, as opposed to those which produce more stem cells, is no higher than 50%, irrespective of the absolute number of stem cells. In fact, it has been calculated that in bone marrow undergoing regeneration only about 40% of CFU-S per cell cycle are removed for differentiation (Lajtha et al. 1971). Physiological stem-cell 'exhaustion' therefore seems an unlikely cause of marrow aplasia if the stem cells are qualitatively normal, although it has been postulated in aplasia due to benzene toxicity (Benestad 1974) (see p. 39). If progressive stem-cell depletion leading to 'exhaustion' of the progenitor cell compartment were a common mechanism of aplastic anaemia, it would be anticipated that many patients with hypoplasia would proceed, inexorably, to total aplasia (Boggs & Boggs 1976), which does not, in fact, occur (see p. 108). However, the stem cell (CFU-S) population in mice is known to be very heterogeneous with regard to self-reproduction (Worton et al. 1969). Any selective damage to the most immature (i.e. those with the highest self-reproductive capacity), would be of more serious consequences than damage that affects either the most mature stem cells or the whole population in a random fashion.

Another example of the remarkable functional reserve of the marrow stem-cell compartment is provided by the work of Morley and Blake (1974*a*,*b*) who gave mice small doses of busulphan over long periods. Although marrow CFU-S and CFU-C were severely reduced, with CFU-S and CFU-C at less than 5% of their starting value (Morley

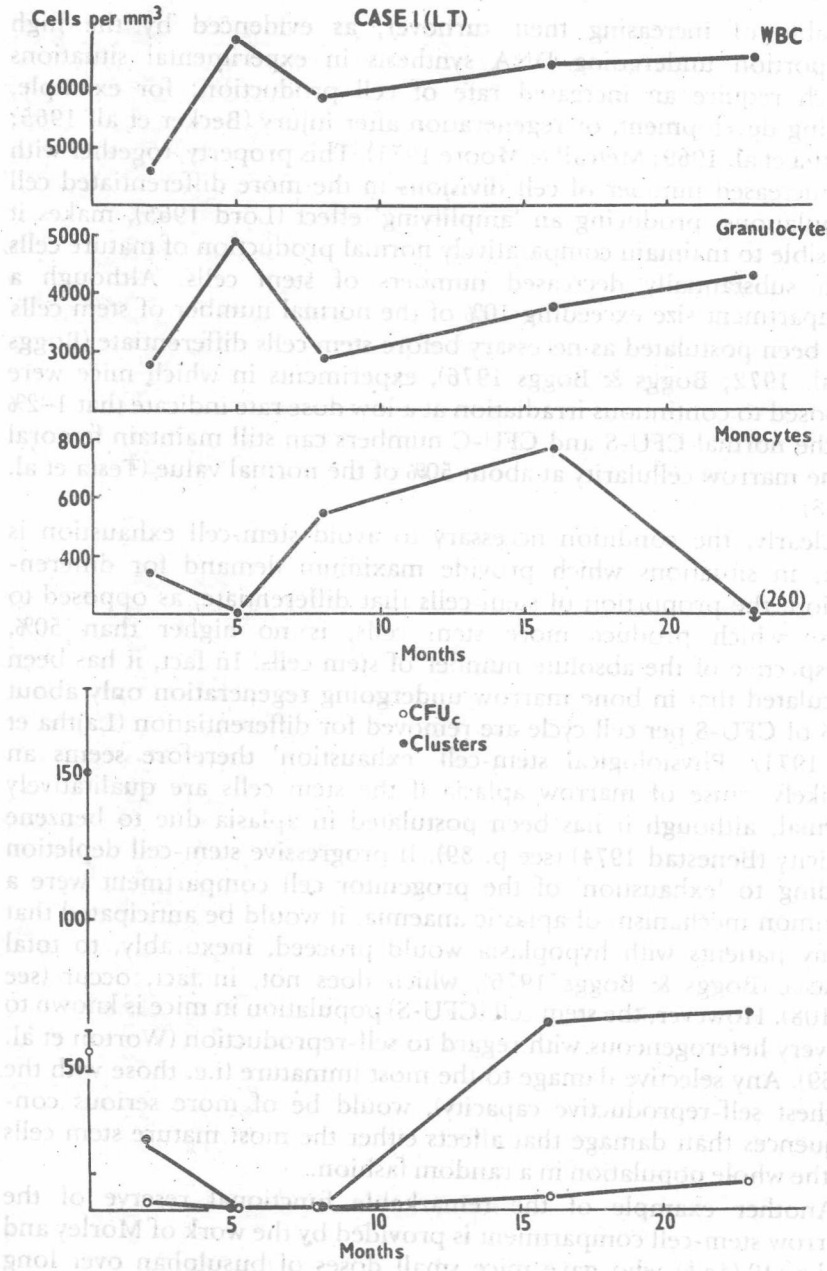

Fig. 1.8. Peripheral blood cell counts. CFU-C (more than 50 cells per colony and clusters (5 to 50 cells) in a patient who presented with thrombocytopenia, normal erythropoiesis, no sideroblasts and apparently normal granulopoiesis 3 years before these determinations. Values in ordinate represent the mean ± 1 SD in normal controls.

et al. 1975) the production of differentiated cells was maintained until haemopoiesis failed after a long period of up to 300 days. A similar situation exists in the W/W^v strain of mice in which the number of CFU-S is about 0·1% of that in normal mice (Russell & Bernstein 1966), yet the numbers of CFU-C and CFU-E are virtually normal (Bennett et al. 1968; Gregory et al. 1974). Evidently, then, a substantially reduced CFU-S compartment can sustain haemopoiesis at normal or nearly normal levels, for long periods. This concept may be of clinical importance, because it suggests that a latent bone marrow failure could exist for a long time without being detected clinically (Morley & Blake 1974*a*). Certainly some patients *without* granulocytopenia show a persistent defect in CFU-C numbers in the marrow (Fig. 1.3). This unrecognized defect may be of importance in patients undergoing treatment with cytotoxic agents, and could explain apparently idiosyncratic reactions to certain drugs (see Chapter 2).

APLASTIC ANAEMIA AS A DISORDER OF STEM CELLS

The evidence that aplastic anaemia is a disorder of haemopoietic stem cells derives from both clinical and experimental sources. The fact that marrow transplants can be achieved in many patients with aplastic anaemia (Storb & Thomas 1975) suggests a deficit which can be corrected by repopulation of marrow with normal haemopoietic stem cells. It has been shown in at least one case of successful marrow transplantation that, while the haemopoietic cells were identified as those of the donor, the stromal fibroblasts remained those of the patient (Wilson et al. 1978). Nevertheless, the possibility exists that a regulatory cell population (see below) is also transplanted and may correct a marrow defect in some cases. Moreover, the immunosuppressive treatment given to prepare the recipient for the graft might remove inhibitory factors, although this is not true of grafts between identical twins, which may be successful without such preparation (Pillow et al. 1966).

Other largely circumstantial evidence is as follows: the latent period, sometimes of several weeks, between exposure to the suspected toxic agent and onset of clinical aplasia suggests damage to a slowly replicating ancestral cell rather than its more mature progeny (Heimpel & Kubanek 1975) (though injury to a slowly dividing stromal cell might give a similar pattern); the presence of cytogenetic abnormalities in both lymphocytes and marrow cells from some children with constitutional aplasia and in marrow cells from occasional cases of acquired aplasia (El-Alfi et al. 1965; Castaldi &

Mitus 1968); the knowledge that some agents, such as chloramphenicol, benzene and the hepatitis virus, which undoubtedly produce aplasia, can cause chromosomal abnormalities in all marrow cells (Pollini & Combi 1964; Mitus & Coleman 1970); the observation that paroxysmal nocturnal haemoglobinuria and, less commonly, acute leukaemia develop in chronic aplasia, suggesting sequential mutations in an unstable clone. Finally, although it is not yet possible to quantify the ancestral stem cells in human marrow, most patients with aplasia show drastically reduced numbers of their closely related progeny, CFU-C (Table 1.4), and these have been shown to recover

Table 1.4. CFU-C numbers in aplastic anaemia

Reference	*No. of cases*	*CFU-C/10^5 bone marrow cells**	
		Patients	Controls
Kurnick et al. (1971)	5	7 (4–8)	25 (16–34)
Greenberg and Shrier (1973)	5	7 (4–9)	26 (14–36)
Kern et al. (1977)	22	3 (0–18)	36 (16–52)

* Mean and range.

after successful marrow transplant (Haak et al. 1977) but rarely after other treatments (Kern et al. 1977), though CFU-E recover more readily (Hansi et al. 1977). Very low or undetectable CFU-C numbers are also observed in refractory cytopenias with cellular bone marrow (with or without increased blast cells) and pre-leukaemias (Greenberg et al. 1971; Senn & Pinkerton 1972; Moore 1974; Sultan et al. 1974; Milner et al. 1977).

The defect in the capacity of the stem-cell compartment to produce its differentiated progeny could arise in several ways. In addition to direct damage to stem cells (Heimpel & Kubanek 1975) defects in the marrow stroma (Knospe & Crosby 1971), regulatory disturbances (Stohlman 1972) or autoimmune damage to the marrow (Ascensao et al. 1976) have been postulated.

Marrow Environment

Aplastic anaemia could arise as a result of damage to the supporting marrow matrix, or 'microenvironment', rather than the haemopoietic stem cell itself. Although the influence of the cellular and humoral environment on the proliferation of developing blood cells is not well understood, there is abundant evidence of its importance (McCulloch et al. 1965; Russell & Bernstein 1966; Trentin 1970; Gidali & Lajtha 1972). The distribution of haemopoiesis in marrow and spleen of adult mice shows that these tissues possess specialized conditions essential

for growth and differentiation of haemopoietic cells. Bone marrow, at least in mice, appears to be a highly organized tissue where the distribution of CFU-S and CFU-C is not random (Lord et al. 1975). Moreover, there is evidence that the microenvironment may exert different regulating influences on stem cells or may induce them to develop along one or other line of differentiation (Trentin 1970). The importance of the environment is illustrated by the defect in a genetically anaemic mouse, the S1/S1^d (Steel) mouse, which possesses normal stem cells, as defined by the spleen colony assay, and by the capacity of its marrow to reconstitute haemopoiesis when injected into lethally irradiated mice, but appears to have a defect in its haemopoietic environment which impairs the proliferation of normal stem cells. The anaemia of this mouse is cured by an implant of spleen tissue which provides a normal environment into which the stem cells can migrate and differentiate (Russell & Bernstein 1966).

Microvasculature

Aplastic anaemia could arise because of direct damage to the marrow sinusoids: the only agent regularly causing aplasia in man which is known to injure blood vessels is X-irradiation (Kistler 1934) and this has been used as a tool to induce experimental aplasia in the marrow of normal rats, using gradually increasing doses (Knospe & Crosby 1971) (Fig. 1.4).

With the smallest dose (below 1000 rads), morphological aplasia occurred at 4 days but regeneration occurred within 14 days. This was interpreted as death of differentiated cells, and a proportion of stem cells; however, a prompt recovery occurred as a result of regeneration of undamaged stem cells in the irradiated area, migration of healthy cells from neighbouring unirradiated marrow, or possibly by heterotypic conversion of uncommitted cells normally resident in the cortex of the bone (Rubin et al. 1977). The microvasculature was presumably left relatively unscathed by such doses. However, at doses of 2000 rads or more, initial recovery was followed, 3 months later, by secondary aplasia: this was ascribed to damage to sinusoidal vascular cells, which have a much longer life cycle than the haemopoietic cells. Haemopoietic cells could not regenerate in this damaged environment.

Nevertheless, some recovery of the sinusoidal microcirculation eventually occurred with doses of less than 4000 rads, so that haemopoiesis was re-established in the irradiated site after about 6 months. When doses of 4000–6000 rads were used, however, aplasia appeared to be permanent, as is often found clinically in the sternal marrow of women receiving irradiation for breast carcinoma (p. 18). Nevertheless, regeneration was still possible, even in heavily irradiated areas, if