

Hematology

PENGAD-Byonne, N. J.

PLAINTIFF'S
EXHIBIT

1343

3rd
Edition

Hematology

WILLIAM J. WILLIAMS, M.D.

*Edward C. Reifstein Professor of
Medicine and Chairman of the De-
partment of Medicine, Upstate Med-
ical Center, State University of New
York*

5th Edition

ERNEST BEUTLER, M.D.

*Chairman, Department of Basic and
Clinical Research, Head, Division of
Hematology and Oncology, and
Clinical Professor of Medicine,
University of California at
San Diego*

ALLAN J. ERSLEV, M.D.

*Cardeza Research Professor of Medi-
cine, Jefferson Medical College of
Thomas Jefferson University. Director,
Cardeza Foundation for Hematologic
Research*

MARSHALL A. LICHTMAN

*Professor of Medicine and Radiation
Biology and Biophysics, Co-Chief,
Hematology Unit, and Senior Associate
Dean for Academic Affairs and
Research, University of Rochester
School of Medicine and Dentistry*

McGRAW-HILL BOOK COMPANY

*New York St. Louis San Francisco Auckland Bogotá Guatemala Hamburg
Johannesburg Lisbon London Madrid Mexico Montreal New Delhi
Panama Paris San Juan São Paulo Singapore Sydney Tokyo Toronto*

Hemopoietic stem cell disorders — aplastic

CHAPTER 20

Aplastic anemia:

ALLAN J. ERSLEV

Aplastic anemia is a stem cell disorder characterized by fatty replacement of hemopoietic tissue and pancytopenia. The reduction in functional marrow mass is believed to be caused by toxic, radiant, or immunologic injury to marrow stem cells or their microenvironment, reducing their capacity for normal cellular renewal. The remaining active marrow is usually scattered uniformly throughout the marrow cavities but occasionally may be confined to small pockets surrounded by fatty tissue. These pockets can be extremely cellular, but the overall functional capacity of the marrow, as assessed by blood counts and iron clearance studies, is reduced.

In 1888, Paul Ehrlich reported the case of a 21-year-old woman who had died from severe anemia and neutropenia and on postmortem examination was found to have a yellow, hypocellular marrow [1]. This may have been the first description of an illness which subsequently was given the name aplastic anemia by Chauffard in 1904 [2]. During the next few decades, numerous cases of so-called aplastic anemia were reported, and there was a mounting realization of the possible relation between x-rays or certain chemicals and marrow failure. Since in vivo marrow examination did not become routine until the 1930s, the term aplastic anemia was used interchangeably with other terms for marrow failure, such as primary refractory anemia, aregenerative anemia, aleukemia hemorrhagica, panmyelophthisis, toxic paralytic anemia, Fanconi's anemia, Estren-Dameshek anemia, Diamond-Blackfan anemia, and, of course, hypoplastic anemia. In 1941, Bomford and Rhoads [3] made a major attempt to classify pancytopenia according to marrow morphology. Unfortunately, this attempt did not resolve the problem of terminology, and aplastic anemia continued to be used as a synonym for diseases as disparate as pure red cell aplasia, aplastic crisis, refractory normoblastic anemia, pancytopenia with hyperactive marrow, and pancytopenia with hypoactive marrow. Despite our still limited knowledge of the pathophysiology of marrow failure, it seems appropriate now to split rather than to lump, and in this chapter the term aplastic anemia will be restricted to pancy-

topenia caused by the decreased functional capacity of a hypoplastic, fatty marrow.

Etiology and pathogenesis

The development of a fatty, inactive marrow and pancytopenia has been associated with prior exposure to drugs and chemicals, to radiation, and to a variety of diseases (Table 20-1). Unfortunately, establishment of a cause is in most cases merely an educated guess supported by statistical correlations or suggestive temporal relationships. When it is not possible to hazard even a guess, the aplastic anemia is designated as idiopathic. a confession of ignorance we have to make in about 50 percent of the cases. Obviously, even in these cases there must be some constitutional or environmental etiologic factor, and in industrial society the possibilities for unrecognized exposure to potentially toxic compounds seem almost limitless. Many of the chemicals used in the household and in the cosmetic industry contain complex benzene radicals, and the widespread use of insecticides, fertilizers, and food supplements makes even our "daily bread" suspect.

Despite difficulties in pinpointing a cause in patients with aplastic anemia, it is mandatory that a serious attempt be made in each case. The patients should particularly be queried about model building, painting, and other hobbies, daily activities, and medications, and a thorough history of occupational exposures to chemicals or to radiant energy should be obtained. A great deal of ingenuity and persistence may be demanded of the physician [4]. It is essential to remember that aspirin, sleeping tablets, laxatives, and antihistamines are frequently not considered medications by the patient and that the term **drug** today often is interpreted to mean an illegal mood-altering chemical rather than a therapeutic agent.

Regardless of exact etiology, aplastic anemia is proba-

TABLE 20-1 Etiologic classification of aplastic anemia

IDIOPATHIC	
Constitutional (Fanconi's anemia)	
Acquired	
SECONDARY	
Chemical and physical agents:	
Drugs	
Nonpharmacologic chemicals	
Radiation	
Infectious:	
Viral: hepatitis	
Bacterial: miliary tuberculosis	
Metabolic:	
Pancreatitis	
Pregnancy	
Immunologic:	
Humoral	
Cellular	
Paroxysmal nocturnal hemoglobinuria	

bly caused by failure of pluripotential stem cells with secondary depletion of hemopoietic precursor cells and fatty marrow replacement [5-8]. The early committed stem cells, i.e., CN-C, BN-E, and CN-M, are believed to be capable of limited self-renewal in addition to continuous proliferation and differentiation. However, if impaired or depleted, they must be replenished from a compartment of pluripotential stem cells (CFU-S). If this compartment fails to respond appropriately to emergency signals from the committed stem cells, marrow hypoplasia and sustained pancytopenia ensue.

Failure of the pluripotential stem cell compartment may be caused by either stem cell dysfunction or stem cell depletion. Stem cell dysfunction could occur if the microenvironment were damaged or if the stem cells themselves were altered. Although the success of marrow transplantation appears to rule out the presence of a detrimental microenvironment, the possible cotransplantation of a missing environmental helper cell keeps this option open [9]. A stem cell alteration which selectively would impede stem cell renewal could also lead to aplastic anemia. However, such an alteration would probably have to be clonal, and it is difficult to envision a clone capable of suppressing normal clones but permitting engraftment of stem cells from an identical twin.

Stem cell depletion, however, could explain sustained underproduction of normal cells as well as successful transplantation of compatible stem cells. Early studies involving retransplantation of CN-S through generations of irradiated mice had suggested that the normal CN-S compartment is nearly inexhaustible [10]. However, more recent studies have shown that regeneration after repeated depletions actually becomes less and less efficient until it fails altogether [11]. Furthermore, in humans, repeated exposure to non-cycle-active chemotherapeutic agents such as busulfan or nitrosourea leads to prolonged and often irreversible reductions in the number of stem cells [12]. Similarly, the number of CFU-S in mice exposed to busulfan can be shown to be permanently reduced even though pancytopenia is not present, so-called latent aplasia [13]. Consequently, it is possible that the capacity for stem cell regeneration is finite and that aplastic anemia develops when injury to pluripotential stem cells has reduced their number to a level inadequate for the sustained maintenance of committed stem cell compartments. Overactivity of the remaining stem cells with rapid cellular transit of their progeny could explain the appearance of small CFU-C colonies [14] and the production of macrocytic "stress erythrocytes" with increased amounts of fetal hemoglobin [15-18].

In order to explain that exposure to concentrations of drugs or chemicals generally considered innocuous in some individuals causes stem cell failure and aplastic anemia in others, it appears necessary to invoke an element of hypersensitivity. This vulnerability may be caused by a genetic or acquired defect in drug elimination or detoxification or by a genetic or acquired vulner-

ability of stem cells. Heterozygotes for the gene for Fanconi's constitutional aplastic anemia [19] or for the genes controlling cellular uptake and processing of folic acid [20] are hematologically normal but could represent an important subpopulation with vulnerable stem cells. Acquired "latent aplasia" with predisposition to the development of further stem cell damage may also be quite common but not easily demonstrable in humans. In mice it has been shown convincingly that animals with busulfan-induced "latent aplasia" are, for example, excessively vulnerable to chloramphenicol [21].

Immunologic rejection of stem cells can undoubtedly cause aplastic anemia in some patients, but the frequency of this mechanism is still hotly debated. Coculture of marrow or blood lymphocytes from patients with aplastic anemia and normal individuals have revealed the presence of suppressor or killer lymphocytes [22,23]. However, careful review of the cases and the techniques employed has led to the conclusion that in many instances the suppressor or killer lymphocytes were not primary but rather secondary to transfusions of HLA-incompatible blood [24,25]. Nevertheless, in the few patients with no preceding transfusions and in the patients in whom the lymphocytes suppress autologous marrow [26] the evidence for a pathogenic role of cellular immunity is very strong. Furthermore, immunosuppressive therapy has caused complete hemopoietic recovery in some patients [27], and even transplantation between identical twins has in a few instances required concurrent immunosuppression in order to be successful [28,29]. Although circulating anti-stem cell antibodies have been demonstrated [30,31], immunologic rejection, when it occurs, is generally believed to be due to cellular rejection.

A defect in the marrow microenvironment leading to inadequate stem cell function has been found to cause pancytopenia in strains of inbred mice [32] and has been postulated to be of pathogenetic significance in humans as well. The high initial take of transplanted marrow in patients with aplastic anemia suggests that an abnormal microenvironment is of only occasional pathogenetic significance.

DRUGS AND CHEMICALS

CHLORAMPHENICOL (CHLOROMYCETIN)

Among the many drugs and chemicals which have been associated with the development of aplastic anemia, chloramphenicol is undoubtedly the one regarded with the greatest suspicion. This drug was introduced in 1948, and because of its wide antibiotic spectrum and ease of administration, it rapidly became one of the most popular antibiotics. In 1952, after a few unheeded warnings, a number of reports suddenly appeared strongly associating chloramphenicol treatment with the onset of aplastic anemia [33]. Since then there have been hundreds of additional reports which, abetted by malpractice suits and senatorial interest, have led to the acceptance of chloramphenicol as a potentially danger-

ous antibiotic [34-36] whether administered by mouth or intravenously [320]. Data accumulated by the American Medical Association in 1967 [37] show that of 771 cases of pancytopenia suspected of being drug-related and reported to the AMA, 338 cases, or 44 percent, followed the use of chloramphenicol. Of even greater importance was the finding that of these 338 cases, 154 had received chloramphenicol as the only drug administered in the previous 6 months. In a tabulation of 787 cases of aplastic anemia reported between 1970 and 1977, 164, or 21 percent, followed the use of chloramphenicol [38]. However, a bad reputation leads to increased suspicion, and any patients with pancytopenia and the slightest previous exposure to chloramphenicol will be designated as suffering from chloramphenicol-induced aplastic anemia. Nevertheless, Chloramphenicol has achieved its bad reputation with good reason, and it should be used with great caution and respect. The actual risk of developing fatal aplastic anemia after being treated with chloramphenicol is low, about 1 in 20,000 to 30,000 [39,40], but this is still 13 times the risk of developing fatal idiopathic aplastic anemia [41].

Concomitantly with the realization that chloramphenicol can induce prolonged, self-sustaining marrow hypoplasia, it was found that it also can cause a brief, reversible marrow suppression in many, if not all, exposed patients [42,43]. This drug-dependent marrow failure is associated with an increase in serum iron (Fig. 20-1) [44] and with vacuolization of marrow cells (Fig. 20-2) [45,46].

FIGURE 20-1 The effect of a chloramphenicol analog on serum iron and blood counts. Similar effects are seen with chloramphenicol itself. (Weisberger [41].)

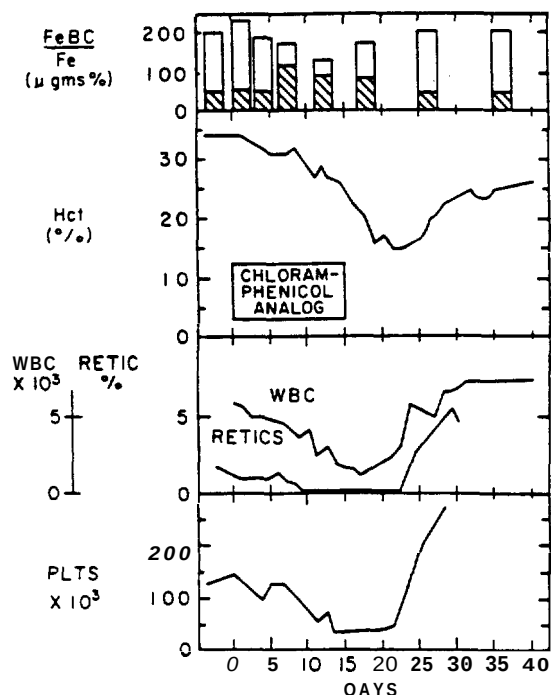


FIGURE 20-2 Vacuolization of marrow cells in a patient treated with chloramphenicol. (Courtesy of R. W. Rundles.)

It has been tempting to consider this marrow suppression as being an early, still reversible manifestation of impending chloramphenicol-induced aplastic anemia. Consequently, it has been recommended that marrow suppression heralded by an increase in serum iron, decrease in reticulocytes, and vacuolization of marrow cells should be an absolute indication for the discontinuation of the drug [47]. However, some physicians have continued to use chloramphenicol despite these early warnings and apparently have not encountered serious consequences [48,49]. Furthermore, an analysis of 94 cases of chloramphenicol-induced marrow failure led to the conclusion that the two conditions are independent [50]. One is described as a reversible marrow suppression occurring during drug administration and directly related to length of drug exposure and to amount of drug used. It is uncertain whether or not this suppression is of clinical consequence, but it presumably does not lead to marrow aplasia. The other is described as a frequently irreversible type of marrow aplasia occurring some time after the drug has been discontinued and not related to the amount or length of drug exposure. The basic difference between the pathogenesis of these two disorders is presumed to be that in one chloramphenicol affects the maturation and proliferation of differentiated cells, rendering them temporarily ineffective, and in the other chloramphenicol changes the genetic structure of the stem cells rendering them permanently incapable of differentiation. However, the similarities between the two disorders cannot be disregarded. An analysis of 408 cases reported to the American Medical

Association Registry did not permit separation of the cases into two distinct groups [34]. Until further information has been provided, it seems prudent to consider the early drug-induced suppression of the marrow a warning which, whenever clinically appropriate, should be heeded in order to reduce the possibility that a benign, reversible suppression might become a dangerous, irreversible marrow aplasia.

Chloramphenicol is a nitrobenzene compound with a dichloracetamide side chain. Such chemical compounds have been regarded with great suspicion since Kracke and Parker in the early 1930s pointed out that they were often responsible for agranulocytosis [51]. Although there is no experimental support for this suspicion, almost all hydrocarbons involved etiologically in aplastic anemia contain the benzene ring. In addition, the spatial configuration of chloramphenicol resembles the pyrimidine nucleotide **uridine-5-phosphate**, a challenging similarity [52] since it could explain a proposed competitive inhibition of chloramphenicol on messenger RNA formation. In the bacterial cell this competition appears to lead to impaired formation of peptide bonds and reduced protein synthesis [53], but the mode of action in the mammalian marrow cell is still unknown. In this cell, impaired protein synthesis appears to be restricted to the protein synthesis which takes place independently in the mitochondria [54,55]. The synthesis of mitochondrial ferrochelatase has been singled out as particularly vulnerable to chloramphenicol [56].

In suspension cultures of intact marrow cells, chloramphenicol has been found to decrease iron uptake, amino acid incorporation, heme synthesis, and synthesis of DNA and RNA [57-59]. This inhibitory action occurs only when marrow cells or reticulocytes are exposed to chloramphenicol in concentrations 5 to 10 times those observed in vivo. At more clinically important concentrations, however, chloramphenicol has been demonstrated to inhibit colony growth of **CN-C** [60]. The physiologic relevance of the in vitro test is supported by the fact that glucuronidation of chloramphenicol renders the drug inert both in vivo and in vitro [58]. Nevertheless, it is probably unrealistic to equate drug-induced alterations of the metabolism of differentiated cells in vitro with the stem cell injury which must underlie the development of aplastic anemia. Possibly of more relevance is the observation [61] that chloramphenicol can cause vacuolization of chromosomes. Such chromosomal change could lead to irreversible and self-perpetuating changes in the stem cells [62], resulting in aplastic anemia, acute leukemia [63-65], or paroxysmal nocturnal hemoglobinuria [66].

Although it is possible that the toxic alteration of the genome is a chance phenomenon, it seems more likely that there is an underlying genetic or acquired stem cell hypersensitivity to chloramphenicol. Some, but not all, studies have suggested that marrow from patients who have recovered from chloramphenicol-induced marrow hypoplasia or marrow from relatives of chloramphenicol victims may be more sensitive in vitro to the inhibitory

action of chloramphenicol than normal marrow [31,67,68]. Furthermore, latent marrow damage induced in mice by busulfan renders the marrow excessively sensitive to the effect of chloramphenicol [21]. There is also some reason [44] for believing that individual differences in the rate or extent of chloramphenicol detoxification play a role [69,70]. An immunologic mechanism has been proposed with rejection of the marrow either by anti-stem cell antibodies or by sensitized lymphocytes. However, the only evidence for this idea is the fact that chloramphenicol can act as a haptene and cause the production of specific humoral antibodies [71] and that steroids or splenectomy occasionally can induce a remission. Unfortunately, a suitable animal model for the study of chloramphenicol toxicity has not been developed; so we have to accept its capricious toxic actions as another example of "pharmacogenetic polymorphism" [72].

BENZENE (BENZOL)

Since the turn of the century it has been recognized that benzene and benzene derivatives are of potential toxicity to the marrow [73]. The extensive use of benzene as a solvent in industry has led to numerous attempts to define its toxicity and to establish safe limits. The early regulations defined safe limits as less than 100 parts per million, but present federal regulations limit the safe industrial level to 10 parts per million [74]. A recent attempt by the Occupational Safety and Health Administration (OSHA) to reduce the acceptable limit to 1 part per million was, however, not accepted by the U.S. Supreme Court [75]. Unfortunately, the manufacture of many solvents used in the home is not carefully controlled, and these may contain significant amounts of benzene. Although benzene, **C₆H₆**, is highly volatile, with a boiling point of 80°C, the distillation process used in the preparation of many petroleum products, such as paint removers, kerosene, degreasers. Stoddard's solvent, etc., is often incomplete, and uncertain quantities of benzene may remain in these popular household solvents.

Benzene will cause marrow suppression and occasionally leukemia in laboratory animals [76,77]. In mice it appears that benzene acts both by suppressing DNA synthesis of differentiated marrow cells [78] and by damaging the colony-forming stem cells [79]. Studies in rats have suggested that the hematologically toxic compounds in benzene poisoning are various phenolic breakdown products, especially the diphenol pyrocatechin (pyrocatechol) [80]. In humans, benzene exposure has been associated with a confusing array of hematologic abnormalities including hemolytic anemia, marrow hyperplasia, myeloid metaplasia, lymphopenia, and acute myelogenous leukemia, but the most common toxic result is pancytopenia due to a hypoplastic marrow or to a hyperplastic but ineffective marrow [81-85].

Pancytopenia may occur years after actual exposure to benzene, but such delayed reactions should always be suspected of being coincidental rather than related. In

most cases, the marrow depression appears shortly after exposure to the chemical, with a close relation between amounts and duration of exposure and degree of marrow suppression. The subsequent development of leukemia in patients with pancytopenia due to a hyperplastic but ineffective marrow has occurred frequently enough to consider benzene a potential leukemogenic agent [81-85].

OTHER CHEMICALS AND DRUGS

A great number of chemicals structurally related to benzene are seen in industry, on the farm, and at home [86], but although they all should be suspect, only a few have convincingly been associated with the development of aplastic anemia. The insecticides pentachlorophenol, lindane, and DDT [87-89] have been shown to have potential marrow-toxic properties, and exposure to trinitrotoluene [90] in industry or toluene among glue sniffers [91] or glue users [92] is also hazardous.

Table 20-2 lists drugs and key references [93-177] to studies which have shown a suggestive cause-effect relation between drug administration and the development of aplastic anemia. Spurred on by the sudden realization of the potential toxicity of chloramphenicol, the American Medical Association Council on Drugs in 1955 established a Sub-Committee on Drug-Induced Blood Dyscrasias. This committee was charged with developing a reporting system which would give the medical profession early warning of possible hematologic side effects of new drugs. As such, the system seems to have failed, since almost all information about toxic side effects has been provided through other channels, such as from manufacturers, clinical trials, and case reports. However, thousands of reports sent in to the AMA from this country and abroad [37] have provided useful data in the assessment of the relative toxicity of drugs. In order to evaluate these data, it is of importance that they be correlated with a rough estimate of the total consumption of the individual drugs and that the appropriate adjustment be made. In the majority of patients, several drugs had been administered prior to the onset of aplastic anemia; so the cases in which only one drug had been administered take on an added significance. Similarly, drugs administered with other medication considered "innocent" should be regarded with more suspicion than drugs administered along with other drugs already suspected of causing marrow damage. An attempt has been made in Table 20-2 to separate these categories. However, it should be emphasized that a drug generally considered innocent, such as aspirin, has been reported by some to cause aplastic anemia [94,95], and that current conclusions about potential marrow toxicity still rest on judgment and experience—hal- lowed but very vulnerable criteria.

From the many reports of single cases, it is difficult to gain much information except that most drugs on occasion have been associated with the development of marrow hypoplasia and that some, such as the hydantoins, pyrazolones, sulfonamides, and gold preparations, ap-

pear to have an unusual affinity for this association. The only drug for which statistically significant toxicity data are available is quinacrine (Atabrine). During World War II [164], the incidence of fatal aplastic anemia was found to be 3 for 100,000 quinacrine-treated soldiers as compared with 0.2 for 100,000 untreated soldiers. This incidence of about 1 in 30,000 is not far from what has been suggested for the incidence of fatal chloramphenicol-induced aplastic anemia [35,178], although the pathogenesis may well be quite different. Quinacrine-induced aplastic anemia, for example, occurs while the drug is being administered and in about 50 percent of the cases is associated with skin lesions, suggesting a hypersensitivity reaction.

RADIATION

The biologic effect of radiation depends on the amount of radiant energy absorbed by the tissues (measured in rads) and the specific radiosensitivity of the tissue. The highly penetrating radiant energy delivered by x-rays, y-rays, or neutrons is effective at a distance, but the pathway of α and β particles is so short that it is necessary to introduce the radiant source directly into the tissues. Here the radiant energy generates electrons and causes a wave of ionizations and further energy release. One rad is defined as the radiation dose which causes 10^{12} primary ionizations per gram of tissue or releases 100 ergs of energy per gram of tissue. The absorbed energy, when greater than the energy of chemical bonds, will cause random molecular changes with the formation of ions, peroxides, and other free radicals which in turn may transfer the absorbed energy to large, critical macromolecules [179-181]. As a guide to radiation exposure and its potential dangers, Table 20-3 lists some published figures [182-185] relating to whole-body radiation.

Extremely high radiant energies such as those released in laboratory or reactor accidents or by extracorporeal irradiation of blood can cause damage to mature, differentiated cells [186,187], but otherwise only organ systems with a rapid cellular turnover are vulnerable. The radiosensitivity of these systems can be ranked in the following order [188]: (1) germinal epithelium of the testes, (2) hemopoietic cells, (3) intestinal epithelium, and (4) basal layers of the skin.

Exposure to lethal or sublethal amounts of whole-body radiation results in extensive cell death in the two critical cellular systems, marrow and intestine, and the patients may succumb from the combined effect of acute marrow aplasia and intestinal ulcerations. If the patient lives through the first crucial 3- to 6-week period, surviving stem cells will effect slow marrow regeneration. This regeneration may completely restore marrow function to normal, but in some cases the stem cells have become permanently damaged and ineffective. Such damage can lead to chronic marrow hypoplasia with various degrees of pancytopenia, or it can cause the formation of a hyperplastic but ineffective marrow displaying multinucleated giant cells, asymmetric mitotic