Proposed Revised Criteria for the Classification of Acute Myeloid Leukemia

A Report of the French-American-British Cooperative Group


The first proposals for the morphologic classification of the acute leukemias by the French-American-British (FAB) group (1) were put forward in the hope that they might serve as a basis for a generally acceptable system of classification. Such a system would permit comparisons between series of cases, show prognostic differences between different categories, and provide a framework of reference for persons working in different biological disciplines who use material from patients with leukemia.

In the last 7 years the FAB proposals have been accepted by many groups involved in cooperative clinical trials with both acute myeloid leukemia and acute lymphoblastic leukemia and by the International Workshops on Cytogenetics (2-4). The FAB group has introduced some objectivity in what was traditionally regarded as an essentially subjective exercise and has had a beneficial effect in improving the degree of uniformity of recording diagnoses. The group's aim was to formulate diagnostic guidelines from cytologic material likely to be readily available in most hematologic laboratories, namely, Romanovsky-stained films of peripheral blood and aspirated bone marrow, and a few essential cytochemical reactions. Because of the remarkable morphologic heterogeneity of the acute myeloid leukemias and the overlap between the commonly recognized categories, a quantitative element was introduced, whereby the boundaries between different categories were arbitrarily specified by defining limits for the percentages of the predominant cell types in each category. It was hoped that the quantitative element would reduce the subjectivity of diagnosis and help in separating from the acute leukemias the group of myelodysplastic conditions, which were generally regarded at the time as unsuitable for chemotherapy.

Experience has shown that the original proposals helped to achieve the aims of the group but that some aspects were inadequate. In general, it was expected that the proportions of the different categories would be similar in series reported from different centers, but this has not always been the case. Sometimes, differences have arisen because of inadequacy in the original descriptions, and sometimes this inadequacy or ambiguity in the interpretation of the descriptions led workers to modify the definitions while retaining the FAB nomenclature (5). Some suggestions for improving the proposals have been published by van Rhenen and colleagues (6) and by Stavel (7). On the other hand, a joint exercise between Japanese hematologists and three members of the FAB group has shown a concordance of 82.5% (8).

The FAB group subsequently published proposals for improving the definitions of the two main subtypes of lymphoblastic leukemia, L1 and L2 (9), by introducing a scoring system that depends on the quantitative assessment. A similar approach was used in classifying the myelodysplastic syndromes (10).

In the last 3 years the group has worked on four areas of acute myeloid leukemia in which the original descriptions were notably imprecise (11): the distinction between lymphoblastic leukemia without maturation (M1) and lymphoblastic leukemia with maturation (M2); the distinction between M2 and myelomonocytic leukemia (M4); the distinction between M4 and monocytic leukemia (M5); and the definition of erythroleukemia (M6) and its distinction from refractory anemia or refractory anemia with an excess blast cells (RAEB).

Materials and Methods

An initial set of 40 slides from patients with acute myeloid leukemia or myelodysplastic syndrome were reviewed individually by all the authors and subsequently in a workshop of the group in Paris (March 1982). The problems concerning the reproducibility of the classification of acute myeloid leukemia were defined, and a tentative set of proposals was drafted. These proposals were tested in 1983 in a review of a second set of slides selected from 31 patients. At a second workshop in Paris in April 1983, the data generated from the individual diagnoses were analyzed. Problem cases were reviewed and points of difference among observers were defined.

The final recommendations are described here in the form of revised criteria for the diagnosis of acute myeloid leukemia with special reference to the four points outlined above. Lymphocytes, plasma cells, mast cells, and macrophages are not included in the differential counts aimed at classifying acute myeloid leukemia or myelodysplastic syndrome; however, qualitative or quantitative changes of these cells should be noted. Erythroblasts are defined for the purposes of this study as all nucleated erythroid precursors including proerythroblasts.

Results

Seventy-one cases that had been preselected because of their difficulty in classification according to the original FAB criteria were reviewed for the two workshops (52

From the University of Rochester Cancer Center, University of Rochester School of Medicine and Dentistry, Rochester, New York; Medical Research Council Leukaemia Unit, Royal Postgraduate Medical School, London, England; Institut de Recherches sur les Leucémies et les Maladies du Sang, Hopital Saint-Louis, Paris, France; Hematology Service, National Institutes of Health, Bethesda, Maryland; and Service Central d'Hematologie-Immunologie, Hopital Henri Mondor, Creteil, France.
cases of acute myeloid leukemia and 19 of myelodysplastic syndrome). The concordance among seven observers who used the original FAB criteria was as follows: All seven agreed on the diagnosis in 26 cases, six agreed in 17 cases, five agreed in 17 cases, and four agreed in 11 cases. Thus, major disagreement (concordance by only four members) occurred in 15% of cases. Analysis of the 28 cases with nonconcordance (agreement by five or less members) showed that the disagreements fell into three groups: differentiation in the subtype of acute myeloid leukemia (13 cases—7 between M2 and M4, 3 between M1 and M2, and 3 between M4 and M5); differentiation between subtypes of myelodysplastic syndromes (9 cases; these were almost always related to differences in the assessment of the proportion of blast cells); and differentiation between acute myeloid leukemia and myelodysplastic syndrome (these were exclusively in differentiation between refractory anemia with excess blasts cells in transformation [RAEB-T] and M2 or M6).

The overall degree of concordance between observers was higher after the new recommendations for acute myeloid leukemia subtypes were used. Of the 28 cases in which no agreement was established with the original FAB criteria, agreement (concordance by six or more members) was achieved in 15 cases. With the new criteria, five members agreed on the diagnosis in 9 cases and four agreed in 4 cases. These recommendations raised the overall concordance from 60% to 82% in these problem cases.

The recommendations that emerged from this analysis are presented below. No disagreements were seen in the diagnosis of M3 or M3 variant; thus, the previous proposals (12) have not been altered. Only minor modifications are suggested in the criteria for M1 and M5.

**Revised Classification of Acute Myeloid Leukemia and Distinction from Myelodysplastic Syndrome**

One difficulty in distinguishing M6 from myelodysplastic syndrome is that the variability in the percentage of erythroblasts in the bone marrow influences the assessment of the proportion of nonerythroid blast cells if the erythroblasts are included in the differential count. Thus, in any cases with more than 70% erythroblasts, a diagnosis of acute myeloid leukemia cannot be made on the original criterion (which specifies 30% as the minimal blast cell count for diagnosis). We now propose that in the initial assessment of a bone marrow specimen (based on a 500-cell count) with suspected acute myeloid leukemia or myelodysplastic syndrome, the first step should be to establish the percentage of erythroblasts (Figure 1). Cases with fewer than 50% erythroblasts are acute myeloid leukemia (M1 to M5) if 30% or more of all the nucleated bone marrow cells are blast cells in accordance with the original proposals (10). If the proportion of blasts is less than 30% of all nucleated cells, a diagnosis of myelodysplastic syndrome will be considered, again in accordance with previous proposals (10). However, if 30% or more of nucleated bone marrow cells are erythroblasts, acute myeloid leukemia (M6) or myelodysplastic syndrome is the diagnosis (see Criteria for M6). The differential diagnosis between M6 and myelodysplastic syndrome will now be made by assessing the percentage of blast cells within the nonerythroid cells, that is, by excluding only the erythroblasts from the court. The diagnosis of M6 will require 30% or more of the nonerythroid cells to be blast cells; when less than 30% of the nonerythroid cells are blast cells, the diagnosis is myelodysplastic syndrome (Figure 1). For further assessment of relevant cell types in M1 to M5, only nonerythroid cells should be considered in the differential count; that is, exclude from the differential count plasma cells, lymphocytes, mast cells, macrophages, and all nucleated erythroid precursors. A summary of the criteria for the diagnosis of acute myeloid leukemia subtypes (excluding M3) is given in Table 1.

**Criteria for M1**

The sum of blast cell types I and II, as defined previously (10), must be 90% or more of the nonerythroid cells, with at least 3% of these blast cells being peroxidase or Sudan black positive. The remaining 10% of cells will be maturing granulocytic cells from promyelocytes onwards, or monocyes.

**Criteria for M2**

The sum of blast cell types I and II is from 30% to 89% of the nonerythroid cells; monocytic cells are less than 20%; and the granulocytes from promyelocytes to mature polymorphonuclear cells are greater than 10%. Problems may arise in some cases that have early maturing cells with morphologic characteristics that do not conform to type I or II blast cells, normal promyelocytes, or the hypergranular promyelocyte characteristic of M3.

**Figure 1.** Suggested steps in the analysis of a bone marrow (BM) aspirate to reach a diagnosis of acute myeloid leukemia (AML) (M1 to M6) or myelodysplastic syndrome (MDS). BI = blast cells; ANC = all nucleated bone-marrow cells; NEC = nonerythroid cells, bone marrow cells excluding erythroblasts.
These cells have a fine nuclear chromatin; one or two nucleoli; and an abundant, often basophilic, cytoplasm with variable numbers of granules that sometimes coalesce. If these cells are less than 10% of the nonerythroid cells, the diagnosis remains M1. In some cases the proportion of these cells is greater than 10% and the diagnosis will become M2, even though few promyelocytes and later cells are present (Figure 2).

**CRITERIA FOR M4**

The diagnosis of M4 and its separation from M2 and M5 require assessment of both the peripheral blood and bone marrow films. In bone marrow, the blast cells are greater than 30% of the nonerythroid cells. The sum of myeloblasts, promyelocytes, myelocytes, and later granulocytes is 30% or more but less than 80% of the nonerythroid cells. More than 20% of the nonerythroid cells are cells of the monocytic lineage at different stages of maturation; usually they are promonocytes and monocytes. When the mononuclear cells exceed 80%, the diagnosis is M5 (monocytic leukemia).

When the bone marrow findings are as above and the peripheral blood monocyte count (monoblasts, promonocytes, monocytes) is $5 \times 10^9/L$ or more, the diagnosis is M4. If the monocyte count is less than $5 \times 10^9/L$, a diagnosis of M4 can still be made if the bone marrow findings are as described above and the presence of a significant monocytic component is confirmed by ancillary laboratory tests, such as serum lysozyme estimations or cytochemical methods that incorporate either a double esterase reaction, specific chloroacetate esterase and nonspecific alpha-naphthyl acetate esterase (13), or other esterase stains that identify monocytes, such as naphthol-ASD acetate, with and without incubation with sodium fluoride. The diagnosis of M4 is established if more than 20% of the bone marrow precursors are monocytes, as shown by cytochemical reactions, or if the lysozyme concentrations exceed three times the normal values in serum ($11.5 \pm 4 \mu g/mL$) or urine ($2.5 \mu g/mL$) (14) or the reference values in individual laboratories. If the bone marrow resembles that of cases of M2, a diagnosis of M4 is still possible if the peripheral blood monocyte count is $5 \times 10^9/L$ or more and one of the above tests provides evidence for an increased monocytic component in the bone marrow.

**M4 WITH EOSINOPHILIA**

In a small percentage of cases of M4, eosinophils are present in the bone marrow; they usually amount to 5% or more of nonerythroid cells. These eosinophils are abnormal, and some have, in addition to the characteristic specific eosinophilic granules, large basophilic (immature) granules and may have a single unsegmented nucleus (Figure 3A). In contrast to normal eosinophils, these cells have cytochemical reactions for chloroacetate esterase and periodic acid Schiff that are distinctly positive (15) (Figure 3B). We proposed that such cases be designated as M4 with eosinophilia. Although cells with these features can rarely be identified in cases of M2, overall the cases with M2 and bone marrow eosinophilia lack the abnormal eosinophil granules seen in M4 with eosinophilia (4).

**CRITERIA FOR M5**

The diagnosis of M5 is based on the appearance of bone marrow. The definition remains unchanged from that in the first FAB classification (1). One criterion suffices: 80% or more of all the nonerythroid cells in the bone marrow are monoblasts, promonocytes, or monocytes. The definitions of the two subtypes M5a and M5b are modified as follows: For M5a, 80% or more of all the monocytic cells are monoblasts; for M5b, less than 80% of all the monocytic cells are monoblasts, the remainder being predominantly promonocytes and monocytes.

**CRITERIA FOR M6**

We have previously described quantitative and qualitative abnormalities of the erythroblasts as well as various aspects of unusual erythropoiesis (1). To avoid confusing M6 with other megaloblastic and dyserythropoietic states, we specified that more than 30% of all the bone marrow cells were myeloblasts or promyelocytes (1). Using this criterion we have since recognized that in some cases of M6, in which a high proportion of the erythroblasts are bizarre cells, the erythroid component was so large that it was impossible to diagnose M6 from the original FAB definition because the blast cells accounted...
for less than 30% of all nucleated cells of the bone marrow. We now revise our criteria for the diagnosis of M6 by requiring that 30% or more of the remaining nonerythroid cells are type I or type II blast cells. The proposed criteria for the diagnosis of M6 are thus different from those used for the other subtypes of acute myeloid leukemia (M1 to M5) (Figure 1). Subtype M6 can now be diagnosed when less than 30% of all nucleated cells of the bone marrow are blast cells; some of these cases would previously have been classified as refractory anemia with excess blast cells (RAEB) or with excess blast cells in transformation (RAEB-T) with a major erythroid component (10). When 50% or more of all nucleated cells are erythroblasts, the diagnosis becomes myelodysplastic syndrome if less than 30% of the nonerythroid cells are blast cells (Figure 1).

Example 1: A bone marrow specimen shows 65% erythroblasts, 21% maturing granulocytes, and 14% type I and II blast cells. The first criterion for M6 is met because more than 50% of all nucleated cells are erythroblasts. The second criterion for M6 is met because 40% of the nonerythroid cells are blast cells.

![Figure 2A. M2 with translocation 8:21. The blasts are large, the Golgi apparatus is prominent, and large Auer rods or large granules are seen in the blast cells. The size of the blast cells and the presence of these Auer rods are characteristic of M2 with the cytogenetic transformation (original magnification, x 1200).](https://example.com/fig2a.jpg)

![Figure 2B. M2. This clearly shows the importance of the maturing granulocytic elements in making the diagnosis of M2. In addition to the promyelocytes, there are granular blast cells with a high nuclear:cytoplasmic ratio, a centrally placed nucleus, and no obvious Golgi region.](https://example.com/fig2b.jpg)

![Figure 3A. M4 with eosinophilia. Bone marrow showing many of the abnormal eosinophilic precursors with anomalous immature granules. These granules are characterized by their large size and their violaceous red.](https://example.com/fig3a.jpg)

![Figure 3B. The cytochemical reaction of eosinophilia in M4 with eosinophilia. The chloroacetate esterase stains two very large cells that are strongly positive (red). They are neutrophil precursors that show normal positivity. The other weakly positive cells are the eosinophilic precursors. Normally, the eosinophilic precursors and the eosinophils are not stained by this cytochemical reaction.](https://example.com/fig3b.jpg)

Example 2: A bone marrow specimen shows 30% erythroblasts, 45% granulocytic cells, and 15% type I and II blast cells. The first criterion for M6 is not met, and this case cannot be classified as M6; the diagnosis is refractory anemia with an excess of blast cells. Note that in myelodysplastic syndrome, the percentage of blast cells is calculated as a percentage of all nucleated cells.

Discussion

The classification proposed by the FAB group has been adopted by many workers as a basis for making biological and clinical observations about acute leukemia. The close association seen between several of the acute myeloid leukemia subtypes and specific chromosome abnormalities suggest that the original descriptions of the subtypes have some biological significance. Thus, a subgroup of M2 is associated with t(8;21); both M3 and M3 variant are associated with t(15;17); and M5 is associated with 11q- (2, 4, 16-20). However, the reproducibility of the original proposals (1) was not optimal, and the difficulty in making the differential diagnosis from myelodysplastic syndrome became recognized (10). The group has there-
fore reviewed critically the four areas in which the criteria used to separate some acute myeloid leukemia subtypes were imprecise: the boundaries between M1 and M2, between M2 and M4, between M4 and M5, and between M6 and myelodysplastic syndrome, particularly refractory anemia with excess blast cells. In formulating the new recommendations we have tried to preserve, as far as possible, the original definitions while increasing their precision.

A major departure in the assessment of the percentage of the various cell types has been introduced. For the diagnosis of erythroleukemia (M6) when less than 30% of all nucleated cells of the bone marrow are blast cells, we suggest that erythroblasts be excluded when assessing the proportion of blast cells; only the nonerythroid cells are counted. Similarly, for the evaluation of the blast cells as well as the granulocyte and monocyte components in M1 to M5, it is recommended that erythroblasts be excluded from the differential count. This change should bring uniformity in diagnosing M6 and make its separation from myelodysplastic syndrome easier. The rationale for the change is that reduced granulocytic differentiation and a higher relative proportion of blast cells are compatible with a diagnosis of M6 rather than refractory anemia with excess blast cells. The need for a more accurate separation between myelodysplastic syndrome and acute myeloid leukemia is emphasized by the development of chemotherapy programs for patients with myelodysplastic syndrome.

The new definition of M1 requires less than 10% of cells to be differentiated granulocytic or monocytic cells, the predominant cells being blast cells of types I or II (10) (that is, cells with few or no azurophil granules). A type of blast cell with more abundant cytoplasm and more granules that is characteristically seen in M2 with t(8;21) (17) is considered to have maturation features for the purpose of the classification, although we recognize that it is not morphologically a promyelocyte.

Subtype M4 may be found in three situations. In the revised recommendations, the bone marrow and peripheral blood findings are considered separately. First, in the presence of significant monocytosis (5 × 10^9/L or more), the diagnosis is M4 if 20% or more of the cells show monocytic differentiation. Second, when the monocyt count in the peripheral blood is less than 5 × 10^9/L, M4 can be diagnosed only when 20% or more of the marrow cells show monocytic differentiation and, in addition, the serum lysozyme concentration is raised or the cytochemical reactions confirm the increased monocytic component in the bone marrow. Third, if the bone marrow appearance resembles that of M2 and the peripheral blood shows monocytosis, the diagnosis is still M4 if a raised serum lysozyme concentration indicates an increased monocytic component or if appropriate cytochemical tests show the presence of more monocytic differentiation in the bone marrow than is suggested by the Romanovsky preparation. The requirement for an additional test in cases with M2-like bone marrow and monocytosis is a departure from the original definition of M4. The revised classification of M4 also recognizes a variant with an increased proportion of bone marrow eosinophils, M4 with eosinophilia, with distinct staining and cytochemical properties. Recently, this form of M4 has been shown to be strongly correlated with specific abnormalities of chromosome 16; del(16)(q22) and inv(16)(p13q22), both with the common breakpoint at 16q22 (4, 21, 22).

The original description of monocytic leukemia (M5) has not been altered, but improved definitions for the immature (M5a) and mature (M5b) forms have been introduced. The distinction may be important, because M5a is more frequently associated with a recently described chromosome abnormality 11q→ with a breakpoint at 11q23 (4, 18).

Since the first FAB classification, rare cases of acute leukemia, perhaps 2% in adults and less than 1% in children, have been recognized that do not fit exactly in any of the categories previously described for acute lymphocytic leukemia, acute myeloid leukemia, or myelodysplastic syndrome. When tests for immunologic markers are done, including those for the detection of antigens specific for lymphoblasts (for example, the common acute lymphocytic leukemia antigen [CALLA] or SmIg) and the enzyme terminal transferase concentration is measured, almost all cases of childhood acute lymphocytic leukemia can be classified morphologically as L1, L2, or L3. In adults and children, other forms of leukemia may be seen. Acute leukemia with a megakaryoblastic component (23) requires special methods for showing that the blast cells belong to the megakaryocytic lineage: for example, the platelet-peroxidase reaction on electron microscopic examination of specific monoclonal antibodies to platelet glycoproteins (24-28). Rare cases of myeloblastic leukemia have been described that require the ultrastructural demonstration of peroxidase to confirm their granulocytic nature. Although these cases may be a minority of those of M1, some reports indicate that the most sensitive light microscope methods for the demonstration of peroxidase were not always used and often the Sudan black B reaction was omitted. Other types of acute leukemia that are not classified according to the lymphoid and myeloid system are basophilic leukemia and hypoplastic acute myeloid leukemia (29, 30). Mixed acute leukemias (for example, proliferation of blast cells with lymphoid and myeloid characteristics) have now been recognized by means of immunologic and ultrastructural methods (31, 32).

Finally, the secondary leukemias that often evolve through a myelodysplastic syndrome phase have atypical features and often cannot be easily classified according to the FAB proposals. The group plans to work on these problems in the near future. Despite these unresolved issues, it is hoped that the simple morphologic and cytochemical methods now proposed will improve the reproducibility of the classification of acute myeloid leukemia and myelodysplastic syndrome and that these proposals will be helpful.

> Requests for reprints should be addressed to Harvey R. Grahmick, M.D.; Hematology Service, CPD, Clinical Center, Building 10, Room 2C390, National Institutes of Health, Bethesda, MD 20205.
PROCEDURE OF BONE MARROW ASPIRATION AND BIOPSY FROM THE POSTERIOR SUPERIOR ILIAC SPINE

1. This technique must be demonstrated and initially supervised by an operator experienced in performing the procedure.

2. POSITION the patient in the lateral decubitus posture with the top knee drawn toward the chest.

3. LOCATE the most prominent point of the posterior superior iliac crest and mark with ink. Put on gloves.

4. PREPARE the skin with antiseptic, paint a wide area at least twice, as for a surgical field. Remember, this procedure is invasive, done in an area of inevitable fecal contamination and the patient often has impaired immunity. Drape the area.

5. ANESTHETIZE the area using 2-5 ccs. of 2% Xylocaine with Epinephrine. Good results are obtained if first the subcutaneous tissue is infiltrated with about 1 cc. of local anesthetic using a #25 needle, then the needle is withdrawn and an intracutaneous wheal made. Disconnect the syringe from the needle, leaving the needle in the skin as a marker of the initial injection. Then, using 1½-#21 needle (introduced through the skin at the point marked with the #25 needle), infiltrate the periosteum, injecting at multiple points so as to anesthetize an area about 1.5 - 2 cms. in diameter.
6. Incise the skin about 3 mm using a #11 blade.

7. **ASPIRATION**: Hold the needle with the shank firmly between the thumb and index finger (the thumb positioned along the axis of the needle), maintain the trochar in place with the middle finger. Advance the needle with pressure and an alternating clockwise-counterclockwise motion. Entrance into the marrow cavity is generally detected by decreased resistance. Remove the trochar and aspirate the sample with a 20 cc. syringe using a rapidly applied negative pressure. Do not maintain suction too long—the sample will be diluted with blood. Remember, only 1 cc. will be a sufficient sample for most purposes. Delay in emptying the syringe will result in a clotted sample.

8. **BIOPSY** using the Jamshidi needle: (Operator must be experienced before attempting in children).
   a) Hold the needle with the proximal end in the palm and the index finger against the shaft.
b) With the trochar locked in place, introduce the needle through the incision, bring it in contact with the P.S.I. spine and point it toward the anterior superior iliac spine.

c) Advance the needle using firm pressure and an alternating clockwise-counterclockwise motion until it is just firmly fixed into cortical bone.

d) Remove the trochar, support the needle with your other hand.

e) Advance the needle as in (c) toward the anterior superior iliac spine approximately 2 cms.

f) Pull the needle back 2 mm and direct the tip at a slightly different angle, advance the needle 2 mm. to cut the core.

g) Rotate the needle with quick twists four or five times clockwise and counterclockwise. Slowly remove the needle with rotating motions. On occasion gentle suction with a syringe will help maintain the core in the needle.
h) Remove the core with the probe introduced through the distal cutting end.

i) Apply firm pressure until no bleeding. Apply a dressing and have the patient lie on the biopsy site to maintain hemostatic pressure. The patient should not shower or bath for 24 hours.

9. COMMON MISTAKES.
   a) Discarding a small sample. There may be enough material in the needle tip alone to provide information.

   b) Too much or too little local anesthetic - maximum dosage of Xylocaine is 5 mg/kg.

   c) Inadequate sedation in children or anxious adults.

   d) Inadequate periosteal anesthesia. This is the major cause of patient discomfort.

   e) Maldirection of Jamshidi needle or introducing needle to a needlessly dangerous depth.

   f) Contamination of field or instruments by operator or technician.
Two types of preparations are made from the marrow specimen — direct films and squash preparations of marrow granules. The first are made at the bedside immediately after the specimen is aspirated. The slides are laid out ready, and the tip of the syringe is quickly touched to each in turn, and films are quickly spread on these slides. The remainder of the marrow is placed in the small test tube containing EDTA, mixed to prevent coagulation and brought back to the lab where the squash preparations are made.

Technique for squash preparations:

The bone marrow is poured from the tube into a small watch glass. Upon close examination small clear granules should be seen. These granules are picked up with fine tipped forceps and placed on slides. A second slide is placed over one of these at a right angle and gentle pressure applied to spread out the granules. The spreader slide is then slid smoothly along to the end spreading the granule out.
SQUASH PREPARATIONS OF BONE MARROW GRANULES

1. ARE THERE GRANULES?

The easiest and quickest way of telling at the bedside if granules have been aspirated is to squirt a small amount of marrow from the syringe onto a slide and tilt slide. You can usually see the granules this way. Immediately put remaining specimen in 10% EDTA and mix well. Make slides quickly. Try to make slides fairly thin by picking up a small amount on the tip of the slide and spreading with a lower angle than normally used for making peripheral blood films.

2. HOW TO MAKE A GOOD SQUASH

For best results make squash preparations as soon as you return to the lab. Don’t use 10% EDTA that looks cloudy.

3. WHAT TO DO WITH A CLOTTED SPECIMEN

Swirl the EDTA specimen on a watch glass with tweezers. Granules will gradually keep coming out of the clot while you swirl. You can make squashes with each individual granule, but quite often these will have fibrin in them.

4. WHAT ARE YOU LOOKING FOR IN A SQUASH?

a) CELULARITY

Learn what normal cellularity looks like first. This takes practice. Look at as many marrows as you can from the file and see what the range of normal cellularity looks like. After that, judging whether cellularity is increased or decreased is easier.

b) MEGAKARYOCYTES

Normally one can see 2-10 megakaryocytes per low power field

c) ABNORMAL CELLS

Either individual cells or cells in clumps. Clumped cells such as metastatic ca are often more easily recognized on the spread films.

d) INFILTRATION OF CELLS

Cells that are naturally occurring in the bone marrow may be abnormal if in excess of certain numbers, i.e. lymphocytes, plasma cells, etc.

e) IRON STORES

Iron stores are assessed by performing an iron stain on squashes of bone marrow granules. Every patient should have an iron stain.

5. HOW MANY SQUASHES SHOULD YOU MAKE?

This will vary from case to case, depending on what is suspected. A good practice is to make from 15 to 20, if no biopsy obtained. Otherwise 10 squashes will do.

(Rev Apr/90)
LEUKOCYTES, ERYTHROCYTES, THROMBOCYTES

Cell Morphology—general

All blood cells originate from undifferentiated mesenchymal cells. From these stem cells, clones of cells differentiate and ultimately appear in the circulating blood as red cells, platelets, and various types of white cells. The earliest cells of each cell line have similar morphological characteristics and cannot be differentiated by appearance alone. They are given specific names such as “myeloblast,” “lymphoblast,” or “rubriblast” depending on the tissue in which they are found, the cells with which they are associated, and the definitive cell that they are destined to produce (Tables I and II). As the embryonic cells change from their primitive forms to mature cell types, they undergo changes in nuclear and cytoplasmic characteristics common to all cells (Fig. 1).

Maturation sequence. Immature cells as a class are large and become progressively smaller as they mature (Fig. 1 A). The nuclei of young cells of the maturation sequence are large and relatively large in relation to the cytoplasm. As the cells age, the absolute and relative size of the nucleus decreases (Fig. 1 B). In cells of the erythrocytic series, the small and degenerated nuclei in the older cells are extruded.

The cytoplasm of primitive cells is predominantly blue and contains large amounts of ribonucleic acid (RNA), which has an affinity for the basic or blue dye (methylene blue). As the cytoplasmic structures and secretory products are manufactured, the color of the cytoplasm becomes more red and less blue (Fig. 1 A). The nuclear chromatin strands of immature cells contain deoxyribonucleic acid (DNA), which has an affinity for the acidophilic (eosinophilic) red dye. As the nucleus ages, it is more intensely stained, and the color changes from light red to dark blue (Fig. 1 B).

The most reliable criterion for the age of a cell is not its size nor its color but the structure of the nuclear chromatin. In undifferentiated cells or blasts the nuclear chromatin strands are distinctly visible. No part of the chromatin is darker or more compact than other portions. In some cells, the pattern is linear, whereas in others, the superimposed, tortuous and twisted chromatin threads appear as red granules or short rods. If injured in the process of aspiration or spreading on a slide, the delicate and lace-like chromatin strands become thick andropy but remain intact and distinct. When the nucleus degenerates, the bonds of the helical structure of the elongated DNA molecules are broken, and the chromatin strands widen and become more coarse and clumped. In the terminal degenerative and senile stages, the nucleus is small, round, dark, and structureless (Fig. 1 C).

One of the signs of immaturity in blood cells is the presence of nucleoli in the nucleus. These small islands of cytoplasmic material, manufactured within the nucleus, are signs of metabolic activity and growth. Nucleoli are best seen in very thin smears and may be indistinct and

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TABLE I NOMENCLATURE

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</tr>
<tr>
<td>Thrombocyte</td>
<td>Band</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>Segmented</td>
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<tr>
<td>Monocyte</td>
<td>Basophil</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>Plasmocyte</td>
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TABLE II BLOOD CELLS
observed in thick or darkly stained smears. Nucleoli vary in size, number, and shape, but their diameter is usually within the 2 to 4 micra range, the shape round or oval, and the number 1 to 4. They have a fairly homogeneous structure and a color similar to that of the cytoplasm. In methylene blue and eosin stains, the color is predominantly blue. Nucleoli are not bounded by membranes, but the expanding intranuclear masses tend to compress the surrounding chromatin strands to give the appearance of a dark boundary (Fig. 1 D, Fig. 8 A, B, C).

The combined changes in size and color of the cytoplasm and the size, color, and structure of the nucleus are visualized in Fig. 1 D.

The cytoplasmic shape of cells is influenced by the mechanical trauma to which the cells are subjected, the pressure of surrounding cells, and their amoeboid activity. Primitive cells tend to be fixed by their cytoplasmic extensions in the ground substance. When torn away from their attachments, as in the process of bone-marrow aspiration, the margins are disrupted, and the edges have a frayed and jagged appearance. Mature and free cells in the circulating blood stream have smooth margins. Actively amoeboid cells, such as mature granulocytes and lymphocytes, tend to round up when exposed to the air and when spread on a glass surface. Slowly amoeboid cells such as large mononuclears or histiocytes may have pseudopods (Fig. 6 H, I, Fig. 13).

Fixed-tissue and early cells which move slowly have round, oval, or slightly indented nuclei. Actively motile and mature cells, such as monocytes and granulocytes, have indented, kidney shaped, lobulated, or segmented nuclei (Fig. 4, Fig. 6). The nuclei of mature lymphocytes are usually round, but may be slightly indented. The nuclei of red cells and plasma cells in all stages of maturation are round.

Immature cells that are metabolically active reveal in their cytoplasm a relatively light zone adjacent to the nucleus. This area, known as the Golgi area, contains smooth endoplasmic reticulum and centrioles. In this juxtanuclear area, colorless (achromatic) mitochondria tend to aggregate. The light zone near the nucleus is best exemplified in plasmocytes but is visible in immature blood cells of all types.

Granules are not present in stem cells (blasts). In the series of cells which characteristically develop granules, the primary granules are dark and predominantly blue. The specific and secondary granules that later develop in the more mature cells stain less intensely and are more red and less blue, as exemplified by eosinophils and neutrophils (Fig. 4). Manufactured products within cells, such as globulin in plasmocytes or hemoglobin in red cells, are indicative of maturation.

Phagocytosis of particulate matter is a manifestation of functional activity characteristic of differentiated cells, but the absence of phagocytosis in a given cell at a given time has no value in determining the maturity of a cell. The cytoplasmic, granular, and nuclear characteristics are usually well synchronized in normal cells, but in pathological conditions, the maturation sequences of the various cell structures may be out of step with each other as in the nucleated red cells of pernicious anemia which may have advanced hemoglobin synthesis and immature nuclear characteristics or in iron deficiency in which the hemoglobin is inadequately formed in cells with pyknotic nuclei.
Reproductive sequence. In addition to the changes in cell morphology as a manifestation of differentiation and maturation, there are morphological changes that are manifestations of reproduction and multiplication. A few primitive cells in hematopoietic tissues remain undifferentiated and undergo mitotic division that produces likewise undifferentiated cells. Other cells, after the first mitosis, differentiate to a degree before they divide again. When these slightly more mature cells undergo mitosis, the daughter cells maintain the cytoplasmic characteristics of the parent cells from which they derive. The majority of cells enter the mitotic cycle in the intermediate stages of maturation, as for example, in the promyelocyte or myelocyte stages of the granulocytes or the prorubricyte or rubricyte stages of the nucleated red cells. After one or several mitotic cycles, the nucleus degenerates and loses its ability to divide.

After the mitotic division of the nucleus, the cleavage of the cytoplasm, and the formation of two cells from the parent cell, the nuclear membrane reforms around the chromosomes. The cell and its nucleus progressively enlarge. Nucleoli appear in the nucleus. During the last few hours of the division cycle, the nuclear membrane and the nucleoli disappear, and the chromatin condenses into dark, compact masses. Each chromatin thread divides, spindles extend outward from the centrosomes, and the chromosomes migrate to the opposite poles; then, lines of cleavage appear in the cytoplasm, and two new cells are formed. At the time of mitosis, the cytoplasm, like the nucleus, is in a state of unrest and assumes a granular or bubbly appearance.

Cells that have long growth periods between divisions are large, whereas rapidly dividing cells are small. Each cell approximately doubles in size and doubles its component of genetic material before it divides. The size of a given cell during the reproductive cycle is dependent on the stage that cell happens to be in at the time the smear is made.

The cytoplasmic shape of cells throughout interphase is variable. Cells observed during the time of mitosis often have very irregular shapes and blunt and frayed cytoplasmic protrusions caused by the violence of cytoplasmic movement during the act of tearing apart and separating.

The nuclei of cells during interphase are round. During prophase and in the later stages of mitosis (metaphase, anaphase, and telophase) the nuclear shape is irregular.

The color of the cytoplasm of undifferentiated and blast cells participating in the division cycle is blue, but in the more differentiated cells, such as hemoglobin-containing nucleated red cells or eosinophils undergoing mitosis, the color of the cytoplasm and the structures within the cytoplasm are dependent on the stage of development of the individual cell at the time it entered the reproductive cycle. Whether cells become more differentiated while they are in the premitotic phase is a moot point.

The nuclei of cells may undergo one or more mitotic divisions without the corresponding division of the cytoplasm, producing giant cells with multiple nuclei as exemplified by megakaryocytes and by double nuclei in plasmocytes and in nucleated red cells. In other cells, the chromosomes may replicate without disruption of the nuclear membrane and without division of the nucleus (endomitosis). Other abnormalities of nuclear division include: nuclei with too few or too many chromosomes; multiple nuclei within a single cell which differ in size, color, and structure; uneven number of nuclei; and clefts or cleavage lines.
Neutrophils, Eosinophils and Basophils

Granular leukocytes (granulocytes) develop in the bone marrow from undifferentiated cells called myeloblasts. Maturation of the myelocytic series of cells is characterized by the development of dark and blue-staining primary granules which are later replaced by secondary granules that differ in their affinity for various dyes. Cells that have granules with an affinity for blue or basic dye are called basophils; those that are stained reddish-orange with the acid dye eosin are called eosinophils; and the cells with granules which do not stain intensely with either dye are called neutrophils. As the cells acquire mobility, the nuclei of the neutrophilic, eosinophilic, and basophilic systems of granular cells undergo progressive changes from round to multilobular forms designated respectively as myelocytes, metamyelocytes, band, and segmented forms (Fig. 2, Fig. 4).

Myeloblast. Myeloblasts vary in diameter from 15 to 20 micra. There is a moderate amount of bluish nongranular cytoplasm, which stains unevenly and is lighter next to the nucleus than at the periphery. Cytoplasmic tags are often demonstrable. The nucleus is round and stains predominantly red. The interlaced chromatins strands are delicate, well defined and evenly stained. Two or more nuclei are usually demonstrable (Fig. 2, Fig. 4 A).

Promyelocyte (Progranulocyte). A cell ceases to be a myeloblast and becomes a promyelocyte when it develops distinct granules (Fig. 2, Fig. 4 B). The earliest granules are dark-blue or reddish-blue. Most of the granules are round, but some may be elongated, curved, and irregular in shape. Granules may be visible above and below the relatively lightly stained and purple-red nuclei.

The nuclei are round or oval and relatively large in relation to the cytoplasm (Fig. 4 B). The chromatin structure is slightly coarser, and the strands of chromat in are blue and not as red as in the myeloblast. Nucleoli may be visible but are unusually indistinct. The cytoplasm is blue with a relatively light zone adjacent to the nucleus. The cytoplasmic margins are smooth, and the cell does not indent nor is it compressed by neighboring cells. Since some of the early granulocytes are still capable of reproduction, the size may be quite variable, depending on the stage of a given cell in the mitotic cycle.

A promyelocyte becomes a myelocyte when the granules differentiate to such a degree that one can identify the granules as basophilic, eosinophilic, or neutrophilic.

Neutrophilic myelocyte. The first sign of neutrophilic differentiation or “dawn of neutrophilia” takes place in the form of a small (Fig. 4 G, Fig. 7 A), relatively light island of ill-defined reddish granules, which develops adjacent to the nucleus. In older myelocytes, the dark granules become less prominent, and the neutrophilic granules predominate. Neutrophilic myelocytes are usually smaller than progranulocytes and have relatively larger amounts of cytoplasm. The nuclei are round, oval, or flattened on one side. The chromatin strands are unevenly stained and thickened. Nucleoli are indistinct (Fig. 2, Fig. 4 G, Fig. 7 A, G).

Neutrophilic metamyelocyte (Juvenile). Neutrophilic metamyelocytes have a slightly indented nucleus and small, pinkish-blue granules. As a class, they are slightly smaller than myelocytes and have a relatively smaller nucleus and less well-defined chromatin structures (Fig. 2, Fig. 4 H, Fig. 7 D). Neutrophilic metamyelocytes are rarely seen in normal peripheral blood, but are often found in conditions in which there is myelocytic hyperplasia.

Neutrophilic band (N. non-segmented, N. non-filamented, N. staff or stab). As the neutrophilic metamyelocytes mature, the nucleus indentation becomes more marked until a stage is reached in which the indentation is greater than half the width of the hypothetical round nucleus. The opposite edges of the nucleus become approximately parallel for an appreciable distance giving a horseshoe appearance. Neutrophilic bands are slightly smaller than metamyelocytes. The nucleus shows degenerative changes, and there is usually a dark pyknotic mass at each pole where the lobe is destined to be. The granules of band neutrophils are small, evenly distributed, and stain various shades of pink and blue (Fig. 2, Fig. 4 I).

Neutrophilic band forms constitute from 1% to 6% of the leukocytes in the peripheral blood of healthy individuals. An increase in non-filamented forms and other immature neutrophils is known as a “shift to the left” and is an indication of an abnormal response.

Neutrophilic segmented (N. filamented, N. polymorphonuclear, PMN, polymorphonuclear neutrophilic granulocyte). These cells differ from neutrophilic bands in that the nucleus is now separated into definite lobes with a very narrow filament or strand connecting the lobes. The mature neutrophils are approximately twice the size of erythrocytes. The cytoplasm in an ideal stain is light pink, and the small, numerous, and evenly distributed granules have a light-pink to bluish-purple color (Fig. 2, Fig. 3, Fig. 4 J).

Segmented neutrophils in the peripheral blood of older children and adults range from 50% to 70% with an average of 60%. On the average, 5% of the neutrophils have one lobe, 35% two lobes, 41% three lobes, 17% four lobes, 2% five or more lobes. In pernicious anemia and related B12, and folic-acid deficiencies, there is an increase in hyperlobulated (six or more lobes) neutrophils (Fig. 31 G).

The transition between the various stages of granulocytes is gradual. Many cells are borderline and difficult to distinguish from each other. The major difficulty is that of differentiating between band and segmenting forms and deciding whether the margins of the isthmus between two lobes are parallel and whether the connecting link is wide enough to be interpreted as a “band” or narrow enough to be identified as a “filament.” A “band” is defined as a connecting strip or isthmus with parallel sides and wide enough to reveal two distinct margins with nuclear chromatin material visible between the margins. A “filament” is defined as a thread-like connection between two lobes so narrow that there is no visible chromatin between the two sides. In its most characteristic form, the shape of a “band” (non-segmented, non-filamented) nucleus is that of a bent stick, a horseshoe, or a curved link of sausage. Lobes of nuclei often touch or are superimposed so it is impossible to see connecting links. If the margin of a given lobe can be traced as a definite and continuing line from one side across the isthmus to the other side, it is assumed that a filament is present even though it is not visible. In differentiating between segmented (filamented) and band (non-segmented, non-filamented) nuclei, evaluation should not be restricted to any single morphological characteristic but to combined features including parallel sides and width of the connecting link, visibility of chromatin at the narrowest portion, and superimposition of lobes. In case of doubt, the rule is to place the questionable cell in the more-mature or segmented category most likely to be correct.
**Eosinophil** (Acidophil). Eosinophils are characterized by relatively large, spherical granules which have a particular affinity for the acid eosin stain. The earliest recognizable eosinophils have a few dark and bluish primary granules intermingled with the secondary and specific red granules. As the eosinophils pass through their various developmental stages, the bluish granules, characteristic of the promyelocyte and the early myelocyte stages, disappear (Fig. 2, Fig. 4 K-N). Because the percentage of eosinophils is usually low in bone marrow and in peripheral blood smears, no useful clinical purpose is served by routinely separating the eosinophils into their various myelocyte, metamyelocyte, band, and segmented categories. On the other hand, in situations in which the eosinophils are greatly increased, an analysis of the incidence of the various stages is indicated.

The eosinophils as seen in normal peripheral blood smears are about the size of neutrophils, and usually have a band or two-lobed nucleus. The granules are spherical, are uniform in size, are usually evenly distributed, fill the cell, and rarely overlap the nucleus (Fig. 2, Fig. 3, Fig. 5 K-N). In unstained smears, the granules take a bright reddish-orange stain with brownish tints. On focusing up and down, one can bring out highlights on individual granules or reveal them as little circles. Often this spherical shape will enable the identification of the cell when the stain is unsatisfactory. (Eosinophils can be identified readily in moist preparations without the use of stains, because the granules are distinct, round, relatively large, and of a brownish color.) Often it is difficult to distinguish eosinophils from neutrophils when the granules of the neutrophil are prominent and they stain darkly. In case of doubt, the questionable cell should be called a neutrophil. By moving to the thin part of the field (with bright illumination), and paying attention to the size, uniformity, and shape of the granules rather than to the color alone, one can often make distinctions that otherwise would be guesswork.

Eosinophils constitute from 1% to 6% of the leukocytes in smears of peripheral blood of normal individuals. There is a relative and absolute increase in eosinophils in association with antigen-antibody reactions.

**Basophil** (Basophilic granulocyte). Basophils have round, indented, band, or lobulated nuclei (Fig. 2, Fig. 3, Fig. 4) and may be classified according to the shape of their nuclei as basophilic myelocytes, metamyelocytes, bands, and segmented forms, but the cells are so few in peripheral blood and bone marrow that there is no clinical advantage in placing the cells in separate categories. The granules of the basophil are dark, and are usually visible above and below the relatively light nucleus as well as lateral to the nucleus (Fig. 4 C-F). The granules are unevenly distributed and vary in number, size, shape, and color. Often the granules are adjacent to, or are surrounded by, a relatively unstained area.

Basophilic myelocytes may be indistinguishable from promyelocytes, for both have granules, an absence of eosinophilic or neutrophilic granules, and a round nucleus. As a rule, basophilic myelocytes are smaller, have less cytoplasm, and have a more mature nucleus than promyelocytes. In case of doubt, it is recommended that the cell be called a promyelocyte rather than a basophilic myelocyte.

Neutrophilic granules may be darkly stained or have prominent so-called “toxic granules” which superficially resemble basophilic granules. When in doubt, interpret the cell as a neutrophil.

**Lymphocytes**

In the early stages of embryonic development, progenitor cells of the lymphocytic series, like the parent cells of all other types of blood cells, are derived from morphologically undifferentiated mesenchymal stem cells in the yolk sac. Primitive cells, destined to become lymphocytes, enter the blood stream and are seeded first in the thymus, liver, and spleen. In later fetal and post-natal life they are seeded in the bone marrow, lymph nodes, and islands of lymphocytic cells in the gastrointestinal tract, lungs, and all other organs, with the exception of the brain and spinal cord. In older children and in adults, lymphocytes in the blood originate in the lymph nodes and lymphocytic aggregations in various organs.

Lymphocytes in older children and adults constitute from 20% to 40% of the leukocytes of the peripheral blood cells and from 5% to 15% of the nucleated cells in the bone marrow. During the first few years of life while children are developing immunity to infectious agents and other foreign environmental factors, lymphocytes in smears of blood are in the 30% to 70% range and in the bone marrow in the 10% to 30% range.

Lymphocytes vary in size (Fig. 5). The diameter of small lymphocytes is usually in the 7 to 10 micra range, and the diameter of the nuclei is approximately that of normal red cells in the same microscopic field. Large lymphocytes are comparable in size to granulocytes or large mononuclears. There are intermediate sizes. In general, the larger forms are less mature cells, but size is not a reliable criterion for the age of a lymphocyte, for these cells are easily flattened, and the number of large and intermediate cells is significantly greater in thin than in the thick portions of the same blood smear.

In small lymphocytes, the nucleus is relatively large. Usually there is a narrow rim of cytoplasm. In some lymphocytes, the cytoplasm is barely visible. One explanation for the paucity of cytoplasm is that these cells are continually shedding and losing portions of their cytoplasm in the process of fulfilling their feeder (trophocyte) function and supplying nutrient materials and antibodies.

In fresh and warm moist preparations and in cultures, lymphocytes are actively motile cells with irregular and hand-mirror shapes. The exposure to air, chilling, contact with glass surfaces and drying during the process of making smears causes them to round up rapidly and to assume spherical shapes (Fig. 3 H, Fig. 5).

Lymphocytes are often compressed and indented by adjacent erythrocytes to corrugated and holly-leaf shapes (Fig. 3 B, Fig. 5 J-L). One of the shapes seldom seen in any other type of cell is the spindle form with an oval nucleus, tapering ends, and filaments extending outward from both ends (Fig. 5 F). The lines of force created by the spreader slide in the process of making the smear may produce, in some fields, multiple oval or spindle-shaped lymphocytes with their long axes parallel.

Under high magnification and critical illumination of thin preparations of rapidly dried smears of fresh blood or bone marrow, lymphocytes with a few pointed cytoplasmic protrusions or numerous microvilli may be observed (Fig. 5 E).

Deep marginal indentations, spindle cells with oval nuclei and filaments at both ends, elongated cells in parallel arrangements, and cells with flagellated (hair-like)
cytoplasmic projections are to be considered in the identification of cells as lymphocytes and in differentiating these cells from other nongranular cells with round nuclei.

The cytoplasm of lymphocytes stains blue, varying from light sky blue to dark blue shades. In thin smears and with the aid of a good microscope, one can observe in some of the lymphocytes multiple, tiny, colorless globules. In pathological states such as infectious mononucleosis (Fig. 37), amaurotic familial idiocy (Tay-Sachs disease), and the Sézary syndrome (Fig. 45), cytoplasmic vacuoles may be demonstrable.

The majority of lymphocytes do not have true granules, but in normal blood there may be, particularly in the larger cells, a few well-defined granules (lysosomes) which are unevenly distributed (Fig. 3 B, Fig. 5 I-L). These granules are roughly spherical, vary in size; and often have a clear zone or halo around them. They are called “azurophilic granules” because they stained with the originally used azure stains. With the Wright stain and other methylene blue- eosin dyes now used, the lymphocyte granules take a predominantly red color. Granules in mature lymphocytes may be conspicuous in association with chronic infectious states and in virus diseases but are rarely observed in lymphocytic leukemia.

The nuclei of lymphocytes are usually round but may be slightly indented (Fig. 5 C, F, G, K). Filamented forms are not observed. The nuclear chromatin of lymphocytes is lumpy or cumbled, and there is a tendency for the nuclear chromatin at the periphery to be stained intensely (peripheral condensation of chromatin). The light coming up from below and striking the darkly stained nucleus is reflected and highlights the surrounding nongranular cytoplasm, giving a perinuclear halo or “silver-lining” effect.

Nucleoli are demonstrable in small lymphocytes when examined in thin sections by electron microscopy but are not visible when examined by light microscopy. The explanation is that lymphocytes round up when smears are made. The chromatin strands of the thick and spherical nuclei obscure the nucleoli. The fact that nucleoli may be present is evidence that some of the cells which appear to be old and degenerate are metabolically active and capable of mitosis and transformation into other cell types.

Smears of blood from patients with infectious mononucleosis (Fig. 37), infectious hepatitis, and numerous other viral diseases reveal atypical lymphocytes. These include blast-like cells with finely-meshed chromatin and distinct nucleoli; plasma-like lymphocytes with eccentric nuclei, dark blue cytoplasm, and prominent juxtanuclear chromophobic areas; and monocytoid cells with indented or lobulated nuclei, blunt pseudopods, and variable granularity. Other atypical forms include lymphocytes with nuclear clefts, nuclear and cytoplasmic vacuoles, unevenly stained bluish cytoplasm, prominent purple granules, and deep indentations by surrounding cells. Lymphocytes revealing the above morphological characteristics are not specific for viral diseases and should not be called "virocytes," for similar atypical cells may be found in numerous non-viral conditions such as fungal and protozoal infections, auto-immune states, allergic reactions, and after transfusions and tissue grafts.

Cells, morphologically identified as small lymphocytes, when stimulated by antigenic agents are capable of transforming into large cells with blue cytoplasm and immature nuclear characteristics. The morphologically undifferentiated cells replicate and ultimately differentiate into typical antibody synthesizing plasmocytes. The transition in the sequential stages of development from lymphocytes to stem cells to plasmocytes leads to the production of a spectrum of reactive cells which display an infinite number of morphological characteristics. There is no single or several words that can possibly encompass all of the intermediate cells. It is recommended that questionable cells be reported as "other lymphocytes" and described. Eponyms, terms implying activation, reaction or disease, and terms implying presumed function and destiny are to be avoided.

Lymphocytes that have been in contact with antigens and have responded by participating in immune reactions acquire the capability of retaining a memory of this immunological experience. They also possess the capability of transferring "immunological memory" to successive generations of daughter cells. Descendants of previously challenged lymphocytes, when again confronted by antigens of the same type at a later date, respond more rapidly and more effectively to the later challenge than at the time of the original contact.

Extensive immunological investigations have revealed evidence of two distinct types of lymphocytes designated as "T" lymphocytes and as "B" lymphocytes. "T" lymphocytes are thymus-dependent and are concerned with the production of cellular (cell or tissue-mediated) immunity.

"B" lymphocytes are independent of the thymus and are related to the lymphocytic cells which in humans are the equivalent of the thymus cells of other species. The exact anatomical location of the bursa-equivalent cells in man is not known. "B" lymphocytes and plasmocytes derived from "B" lymphocytes synthesize and secrete immunoglobulins or antibodies of the humoral or circulating type. There are no morphological features demonstrable in Wright stained smears that make it possible to determine the immunological competence of a given lymphocyte by its appearance.

The survival time of individual lymphocytes is variable. Some live for a few days, degenerate, and are phagocytized. Other lymphocytes survive for hundreds of days and perhaps longer. Lymphocytes are capable of passing through endothelial cells of the capillaries and post-capillary venules (emperipolysis), between lirrillar cells, and through basement membranes into interstitial tissues. From tissue spaces they enter efferent lymphatic channels and finally get back into the blood stream by way of the thoracic duct which empties into cervical veins. In addition to recirculating in blood and lymph, lymphocytes also have the ability to enter the rapidly regenerating and continually sloughing epithelial cells of the intestine and thus aid in the defense of the body against pathogens in the lumen of the gut.

Prolymphocyte and Lymphoblast. Lymphoblasts, prolymphocytes, and small lymphocytes have similar morphological characteristics in that each has a relatively large, round or slightly indented nucleus, and blue cytoplasm (Fig. 2, Fig. 8 A, D, G). Differentiation is based principally on differences in nuclear structure. In lymphoblasts (Fig. 8 A), the chromatin strands are thin, evenly stained, and purplish red. One or several nucleoli usually are demonstrable. In mature lymphocytes, the nucleus stains darkly, is predominantly blue, and the chromatin is clumped. Nucleoli are not demonstrable. In prolymphocytes, the nucleoli are indistinct and the chromatin color and structure intermediate. These differences are subtle. It is often a matter of opinion as to the category in which individual lymphocytic cells should be placed. In case of doubt, whether a given cell is a lymphoblast, prolymphocyte, or lymphocyte, identify it as a lymphocyte.
**Monocytes** / *large Mononuclears*

Monocytes as a class are larger than neutrophils in thin portions of the smear where the cells are flattened out, but in the thicker portions where the cells are more spherical, there is less difference in size. In mature monocytes, there is a relatively large amount of cytoplasm in relation to the nucleus (Fig. 6).

The shape of monocytes is variable. Many are round or oval. Others reveal blunt pseudopods which are manifestations of their slow motility (Fig. 6 E, H, I). These amoeboid and aggressive cells continue to move while the blood film is drying and become fixed before there is time to retract their cytoplasmic extensions. The pseudopods vary in size and in number. The outer portion of the outstretched cytoplasm (ectoplasm) often has a transparent or hyaline appearance as contrasted with the granular inner cytoplasm (endoplasm) (Fig. 6 I).

The cytoplasm of monocytes in the Wright stained smear is dull gray-blue as contrasted with the color of the cytoplasm of the neutrophils in adjacent fields which is less intensely stained and is pink.

The granules of monocytes are usually fine, lightly stained, numerous, and evenly distributed, giving to the cells a ground-glass appearance (Fig. 6 A). In other cells, there may be, in addition to the small granules, varying numbers of prominent granules (Fig. 6 C). Vacuoles are often prominent in the cytoplasm (Fig. 6 I). Phagocytized erythrocytes, leukocytes, nuclei, cell fragments, pigment, bacteria, and fungi may be demonstrable in digestive vacuoles.

The nuclei of the monocytes are usually round or kidney-shaped, but may be deeply indented or have two or more lobes separated by narrow filaments (Fig. 6 G). One of the most distinctive and diagnostic features of the monocyte is the presence of superimposed lobes, giving the nucleus the appearance of brain-like convolutions (Fig. 6 B, C, F). Another feature of the nucleus of value in identification is the tendency for the nuclear chromatin to be loose with light spaces in between the chromatin strands, giving a coarse, linear pattern in contrast to the lymphocyte with its clumped chromatin.

In smears of peripheral blood from healthy individuals, the percentage of monocytes is usually within the 2% to 10% range with the average value approximately 5%. In smears of bone marrow from persons in good health, monocytes usually constitute less than 2%.

Monocytes are derived from stem cells in the bone marrow. As these cells grow, they are transformed into macrophages too large to pass readily through capillaries. Extremely large mononuclear phagocytes are seldom seen in blood smears but are demonstrable in body fluids other than blood. Some of the macrophages become anchored in connective tissues where they are entrapped by reticular and collagen fibers. The smaller monocytes, the larger wandering macrophages, and the semi-fixed or fixed phagocytic histiocytes (tissue macrophages) are thought to be capable of reversible transformation from one to the other.

Phagocytic cells of varying size and mobility remove from the circulating blood injured and dead cells and cell frag-
Plasmocytes

Plasmocytes constitute approximately 1% of the nucleated cells of the normal bone marrow but are not seen in the peripheral blood smears of healthy adults. They may be present in the circulating blood of young children and in the blood of patients with viral infections including exanthemas, herpes, viral hepatitis, and infectious mononucleosis. Plasmocytes are also observed in the bone marrow with serum sickness, allergic states, chronic bacterial and fungal infections, toxoplasmosis, and multiple myeloma. In plasmocytic leukemia, in occasional auto-immune states, and in situations in which the immune system is suddenly challenged by antigenic materials, the plasmocytes may be markedly increased.

 Mature plasma cells, when found in blood smears, vary in size from 15 to 25 micra. They are usually round or oval with smooth or slightly irregular margins. The cytoplasm is nongranular and stains a dark blue. In addition the cytoplasm has a brilliant translucency. This rich and velvety quality, variously described as cornflower, larkspur, or pigeon blue, is thought to be due to numerous relatively unstriated mitochondria and to lightly stained or reddish secretory products that allow the light to be transmitted through the cytoplasm containing numerous dark-blue (basophilic) ribosomes. The cytoplasm adjacent to the nucleus is relatively pale (perinuclear clear zone). Fibrillar structures that take a blue stain may be demonstrable (Fig. 8 C, F, II. In many plasmocytes, there are one or several vacuoles. There is no evidence of phagocytosis of visible particles.

The nuclei of mature plasmocytes are relatively small, are oval or round, and are eccentric. The nuclear chromatin is coarse and lumpy.

In tissues fixed in formaldehyde or other fixatives and perhaps poorly dehydrated in the process of staining, there may be produced in the nuclei artifacts characterized by the tendency of the chromatin to clump and to adhere to the nuclear membrane, giving the vague visual impression of the spokes of a wheel. In stained blood and marrow smears, aggregates of chromatin are demonstrable in some mature cells, but “cart-wheels” are figments of the imagination. The use of the phrase “cart-wheel nucleus” is a cliche copied from older textbooks of pathology, and it should be discarded.

Most plasmocytes in bone marrow are fixed or semi-fixed cells which are torn in the process of aspiration and appear in marrow smears with irregular spiculate margins (Fig. 42 C). Plasmocytes in the marrow are often seen in groups clustered around large nongranular or finely granular tissue cells. It is thought that the contact of plasmocytes with large histiocyte cells is a manifestation of the immune response in which antigenic material, processed by macrophages, is transferred to the plasma cells which in turn will manufacture immune globulins. Proliferating plasma cells in association with malignancy of the plasmocytic system are not grouped around large mesenchymal cells.

The immune globulins, manufactured by plasma cells, produce many striking morphological variants. The proteinaceous material is usually in the form of round, red globules called “Russell bodies” or “eosinophilic globules.” The globules do not always take the red stain but may be colorless or reveal pastel colors of pink, blue, or green. The globules may fill the cytoplasm giving the appearance of a bunch of grapes (grape, berry, or morula cells). The secretory bodies are usually perfectly round (Fig. 43, Fig. 44).

In some cells, the spherules are so numerous and so tightly packed that they assume hexagonal or honey-comb shapes. In other cells, the fiery red color has a diffuse distribution producing cells called “flame cells” or “flaming plasmocytes” (Fig. 42 E). The red staining material may appear as granules, as pools at the margins, or as extrusions through the cell membrane (Fig. 42, Fig. 43). After the escape of secretory products, the residual cytoplasmic stroma appears tattered and torn. The proteinaceous material within the cytoplasm may crystallize and produce elongated and pointed structures which may be colorless or stain various shades of red to purple (Fig. 42). In rare instances, there may be secretory globules of varying size and number in the nuclei (Fig. 44).

Transitional cells between lymphocytes and plasmocytes are sometimes encountered. These plasma-like lymphocytes or lymphocyte-like plasmocytes in the older hematological literature were called “Türk’s irritation cells.” Their presence is an indication of antigenic contact and an active immunological response. They are found in small numbers in smears of blood from healthy young children and in blood from patients with virus and allergic diseases and with chronic infections.

Plasmo blasts and Proplasmocytes, like the mature plasmocytes, have a dark-blue, nongranular cytoplasm, prominent juxtanuclear clear zones, and eccentric round nuclei (Fig. 2, Fig. 8 C, F). The major difference is in the structure of the nuclear chromatin in which the plasmo blasts is reticular, in the mature forms lumpy, and in the proplasmocytes intermediate. The nuclei of primitive plasmocytes are relatively red and light as compared with the dark-purple stains of the definitive cells.

Nucleoli are demonstrable in typical plasmo blasts and are absent or ill-defined in proplasmocytes. It is rare to find cells of the plasmocytic system in mitosis or to identify primitive cells in any conditions other than multiple myeloma and plasma-cell leukemia.
Erythrocytes

Rubriblast (Proerythroblast, pronormoblast, megaloblast [Sabn]). The earliest cells of the erythrocytic sequence are similar to other undifferentiated cells or “blasts.” Nucleoli are usually visible, and the chromatin strands are linear and distinct. In the very earliest forms, the cytoplasm stains a light blue, but in later and more frequently occurring forms, there is a superimposed reddish tint which imparts to the cytoplasm a peculiar dark and royal-blue color which is quite similar to that seen in certain plasmocytes (Fig. 1 D, Fig. 2, Fig. 9).

Prorubricyte (Basophilic erythroblast, basophilic normoblast, early erythroblast). This cell is differentiated from the rubriblast by the coarsening of the chromatin pattern and ill-defined or absent nucleoli. The cytoplasm contains varying amounts of hemoglobin which has a reddish tinge but the predominant color is blue (Fig. 1 D, Fig. 2, Fig. 9).

Rubricyte (Polychromatophilic erythroblast or normoblast, intermediate erythroblast or normoblast). Rubricytes are smaller than prorubricytes, have relatively more cytoplasm, and take varying mixtures of red and blue stains. The nuclear chromatin is thickened and irregularly condensed, and nucleoli are no longer visible (Fig. 1 D, Fig. 2, Fig. 9).

Metarubricyte (Normoblast, orthochromatid erythroblast or normoblast, late erythroblast or normoblast). The metarubricyte has a predominantly red cytoplasm and minimal amounts of residual blue. The nucleus is relatively small and has a nonlinear clumped chromatin structure or a solid blue-black degenerated nucleus (Fig. 1 D, Fig. 2, Fig. 9). Nucleated red cells with fragmented or partially extruded nuclei and naked black nuclei (Fig. 20 G) in bone-marrow smears are classified as metarubricytes.

Diffusely Basophilic Erythrocyte (Polychromatophilic erythrocyte). These cells have lost their nucleus and still maintain some of their bluish color (Fig. 1 D, Fig. 2, Fig. 9). As a class, they are larger than the more mature and senile cells. These cells, when stained with new methylene blue or other supravital dyes before they are fixed, reveal granulofilamentous structures (polysomes and endoplasmic reticulum).

Erythrocyte or Red Cell (Normocyte, discocyte). Normal erythrocytes are biconcave discs, 6 to 8 micra in diameter and 1.5 to 2.5 micra thick, which appear in stained smears as circular objects with distinct and smooth margins. The intensity of the stain in the central portion where the cell is thinnest is less than at the thicker marginal area (Fig. 1 D, Fig. 2, Fig. 9). In very thin coverslip preparations and at the extreme ends of smears, the red cells are flattened out like pancakes and do not reveal their true biconcave shape. Very thin areas of smears where the erythrocytes are of uniform thickness are favorable for the visualization and identity of malarial parasites and other cytoplasmic objects but are unfavorable areas for the evaluation of the decreased concentrations of hemoglobin (hypochromia).

Erythrocyte variants. The immature cells of the erythrocytic series of cells, as well as the mature cells, are subject to marked morphologic variations in size, shape, and hemoglobin content (Figs. 20, 25, 26, 27, 28).

In pernicious anemia and related conditions due to a deficiency of B₁₂ and/or folic acid, cells have long growth periods between divisions and contain an increased amount of hemoglobin. The nuclear chromatin structure in these dysplastic cells is often atypical, and there is asynchronism between the nucleus and cytoplasm (Fig. 20 A-E). The abnormal cells characteristic of pernicious anemia are called rubriblasts, prorubricytes, rubricytes, and metarubricytes with the qualifying phrase “pernicious anemia type,” or with appropriate descriptions (Fig. 9, left column).

In blood loss or iron deficiency states, the mature and immature erythrocytes are smaller than normal, are irregular in shape, have less hemoglobin, and take a more basic stain than do comparable cells of the normal series. The nucleated red cells of patients with hypochromic microcytic anemia are classified as rubriblasts, prorubricytes, rubricytes, and metarubricytes with the modifying adjective phrase “of the iron deficiency type” or with a description of the observed morphological abnormalities (Fig. 9, right column).

Megakaryocytes and Thrombocytes

C ells of the Megakaryocytic System are peculiar in that the nucleus undergoes multiple mitotic divisions without cytoplasmic separation, thus producing giant polyploid cells (Fig. 2, Fig. 10, Fig. 11). All the nuclei in a given cell undergo mitosis at the same time (Fig. 11) producing 2, 4, 8, and in rare instances 16 or 32 nuclei. The multiple nuclei usually remain attached to each other and are often superimposed giving a lobular appearance. The dividing nuclei maintain the distinct linear chromat pattern of young cells while the cytoplasm undergoes maturation changes characterized by the development of granules and membranes, culminating in platelet differentiation and liberation.

Well-defined platelet masses usually appear at the margins of megakaryocytes in the 4 to 8 nucleate stages of development, but in some cells platelets form in cells with single or double nuclei. When the nuclei and the cytoplasm are out of step with each other, it is recommended that the identity of the individual cell be established by the characteristics of the cytoplasm rather than by the chromatin structure or the number of nuclei. This is a departure from the rule that the structure of the nucleus is the most reliable criterion for identification.

Intact megakaryocytes, fragments of megakaryocytes, and naked nuclei are occasionally demonstrable in smears of peripheral blood from patients with myeloproliferative diseases such as chronic myelocytic and megakaryocytic
leukemia and in leukemic myelofibrosis. They are seldom observed in peripheral blood smears of normal individuals. In bone marrow smears and in sections of marrow tissue from normal individuals, the megakaryocytes constitute 1 to 4 per 1,000 nucleated cells. Most of the megakaryocytic cells are in the third and fourth stages of maturation.

**Megakaryoblast.** Megakaryoblasts are large, irregularly shaped cells with a single nucleus or with several round or oval nuclei and with a blue, nongranular cytoplasm. There may be blunt pseudopods which stain various shades of blue and which may contain multiple chromophobic globules (Fig. 10 A, Fig. 11 A). Spongy ectoplasm of this type is often demonstrable in sarcoma and in other malignant cells but is not present or is inconspicuous in primitive cells of the erythrocytic or leukocytic series of blood cells. The nuclear chromatin strands in megakaryoblasts are distinct. Nucleoli usually are demonstrable (Fig. 10 A).

**Promegakaryocyte.** Promegakaryocytes differ from megakaryoblasts in that there are bluish granules in the cytoplasm adjacent to the nucleus. The nucleus in this second stage of maturation has usually divided one or more times, and the cell has increased in size. Often there are bluish cytoplasmic extensions with rounded contours which may have a homogeneous or a bubbly appearance (Fig. 2, Fig. 10 B, Fig. 11).

One of the variants of the promegakaryocyte is a cell with one or more nuclei with granular cytoplasm adjacent to the nucleus encircled by a collar of vacuolated cytoplasm and by a third and distinct marginal zone characterized by dark-blue and rounded cytoplasmic protrusions which stain unevenly and often contain small colorless globules (Fig. 11 C).

**Megakaryocyte** (Megakaryocyte without thrombocytes). Megakaryocytic cells in the third stage of maturation are large cells with relatively large amounts of cytoplasm, round shapes, even margins, and multiple nuclei. The chromatin pattern of the nuclei is linear and coarse with distinct spaces between the chromatin strands. The cytoplasm contains numerous small, rather uniformly distributed granules which have a reddish-blue hue (Fig. 2, Fig. 10 C). Light staining areas may be demonstrable.

**Metamegakaryocyte** (Megakaryocyte with thrombocytes). Megakaryocytic cells in the fourth stage of maturation are characterized by the aggregation of granular cytoplasmic material into masses which are separated from each other by relatively clear spaces (demarcation membranes or vesicles). These units of granular cytoplasm tend to aggregate near the periphery of the cell (Fig. 2, Fig. 10 D).

Megakaryocytes in the more advanced stages of maturation are slowly amoeboid. They extend portions of their cytoplasm through the basement membranes and between the endothelial cells of the sinusoids of the bone marrow. From these cytoplasmic protrusions, the differentiated and membrane-bound platelets separate and are swept into the flowing blood stream. Other megakaryocytes escape into the vascular channels of the marrow and are transported by veins to the lungs where they lodge in the terminal pulmonary arterioles and alveolar capillaries. From these sites, they continue to differentiate and to liberate portions of their cytoplasm in the form of platelets (Fig. 2, Fig. 10 E). The naked nuclei disintegrate or are phagocytized.

**Thrombocyte** (Platelet). Thrombocytes are fragments of cytoplasm of megakaryocytes. In spreads of blood from normal individuals, the diameter of individual platelets varies from 1 to 4 micra, but in various diseases, the size may range from barely visible structures to masses larger than red cells or leukocytes (Fig. 35, Fig. 50-53). As a rule, thrombocytes have multiple pointed filaments or tentacle-like protrusions (Fig. 10 F). Round, oval, spindle, and discordant shapes with smooth margins are also observed. The cytoplasm stains a light blue and contains variable numbers of small, blue granules which tend to aggregate in the center (granulomere, chromomere) as contrasted with the marginal zone which is nongranular (hyalomere).

Platelets tend to adhere to each other (Fig. 3). Individual platelets and clumps of platelets are most numerous at the distal (feather) ends of blood smears. In thin portions where the erythrocytes and leukocytes are well separated, the number per oil-immersion field varies from 5 to 25. The number of platelets in the average oil immersion field multiplied by 20,000 gives the approximate number per cubic millimeter. No report of a blood smear is complete unless the platelet number is stated and morphological abnormalities are described.

**TABLE III** **MORPHOLOGICAL FEATURES OF CELLS OF THE MEGAKARYOCYTIC SERIES**

<table>
<thead>
<tr>
<th>STATE OF MATURATION</th>
<th>CYTOPLASMIC GRANULES</th>
<th>THROMBOCYTES</th>
<th>CYTOPLASMIC TAGS</th>
<th>NUCLEAR CHARACTERISTICS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Megakaryoblast</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
<td>Single, Fine chromatin structure Nucleoli</td>
</tr>
<tr>
<td>Promegakaryocyte</td>
<td>Few</td>
<td>Absent</td>
<td>Present</td>
<td>Double</td>
</tr>
<tr>
<td>Megakaryocyte</td>
<td>Numerous</td>
<td>Absent</td>
<td>Usually absent</td>
<td>Two or more nuclei</td>
</tr>
<tr>
<td>Metamegakaryocyte</td>
<td>Aggregated</td>
<td>Present</td>
<td>Absent</td>
<td>Four or more nuclei</td>
</tr>
</tbody>
</table>
FIXED TISSUE CELLS

IN ADDITION to the free blood cells of the peripheral blood and the precursors in the bone marrow, there are various types of fixed tissue cells. These cells are relatively immobile and are attached to other cells or imbedded by their cytoplasmic extensions in the ground substance of the marrow and entrapped within the network of reticular and collagen fibers. They are aspirated with difficulty and are best seen in tissue sections.

**Stem Cells** (Undifferentiated Mesenchymal Cells, Hemohistioblasts). These cells are usually large in relation to the differentiated blood cells in the same microscopic fields. The shape is irregular and there may be marginal extensions (Fig. 12 A, Fig. 13 B). Nuclei are round or oval. The nuclear margins are distinct. The nucleus takes a light and predominantly red stain. Chromatin strands vary in texture but are well defined and uniformly stained. One or more nucleoli are usually demonstrable. The light-bluish non-granular cytoplasm has a homogeneous or delicate reticular structure. (Fig. 14 A).

Primitive cells of the bone marrow and other hematopoietic organs are multipotent cells capable of producing the various types of fixed tissue cells as well as free blood cells. It is not possible on the basis of morphological characteristics to differentiate totipotent stem cells (hemohistioblasts) from "hemocytoblasts" that are committed to the production of the various types of circulating blood cells.

Between the most primitive nongranular stem cells and fully developed tissue granulocytes, fibrocytes, and fat, bone, cartilage, and endothelial cells there are intermediate (pro-) maturation forms.

There is no agreement about the best terms to use for transitional cells before they have developed their mature characteristics. It is recommended that questionable immature cells with nonspecific granules and other cytoplasmic structures be tallied as "unclassified" and described. Such cells should not be called "reticuloendothelial cells" or "reticulum cells." Interpretation of early and intermediate forms should be made on the basis of size, shape, color, and structure in the stained smear as well as their association with other cells; their appearance and number on tissue sections; their affinity for special stains; and the total clinical picture including combined laboratory tests.

**Tissue Basophils** (Mast Cells, Heparinocytes). Tissue basophils are fixed tissue cells that are traumatized in the process of aspiration and therefore often have jagged margins. Many of the cells have spindle shapes and oval nuclei. The size varies. As a rule, the diameter of the more round tissue basophils is from 2 to 4 times that of red cells in the same field. The nucleus is relatively small, round, or oval. The cytoplasm is filled with intensely-stained violet-blue granules. The granules are uniformly round and are approximately the same size (0.1 to 0.3 micron). The granules frequently overlie the margins of the relatively pale nucleus or may partially or completely obscure the nucleus (Fig. 15 A).

Tissue basophils are widely scattered in various organs including the bone marrow. They usually are not encountered while performing a differential count of a few hundred cells but may be relatively increased and conspicuous in conditions associated with pancytopenia and myelosclerosis. In conditions in which they are readily demonstrable in the marrow smears, there is often an associated hemorrhagic tendency.

Tissue basophils and blood basophils are closely related in their chemical characteristics and in their functions. Their difference is mainly one of motility.

**Tissue Eosinophils.** In smears of bone marrow, one occasionally sees large cells with elongate and tapering cytoplasmic extensions and containing typical red granules of the type seen in the eosinophils of the circulating blood. The nucleus in such cells, instead of being indented or lobulated, resembles that of the other fixed tissue cells, being round or oval and having a well-defined reticular chromatin pattern and often nucleoli (Fig. 15 B).

It is thought that tissue eosinophils are fixed tissue variants of the more motile eosinophils of the circulating blood.

**Tissue Neutrophils** (Ferrata Cells). One of the cell types, which is encountered in small numbers (less than 1%) in practically every smear of normal bone marrow, is a cell which resembles the undifferentiated mesenchymal cell except for the fact that it contains varying numbers of neutrophilic granules. Tissue neutrophils are large with ample cytoplasm. In rare instances, one may find a round or oval variant with smooth contours, but as a rule, the shape is bizarre with a combination of blunt pseudopods and multipointed and nebulous cytoplasmic streamers. These cells are readily indented by adjacent cells or are squeezed in between them. Often there are long and tenuous cytoplasmic extensions which seem to wrap around other cells. These cells are not phagocytic and seldom have vacuoles in their cytoplasm. The cytoplasm stains light blue and has a fine lattice-like structure (Fig. 13 C, Fig. 14 B, C). Granules vary in number. The granules stain varying shades of red to blue, but the majority take a brilliant red or reddish-purple stain. Many of the granules tend to be arranged in chains. The bead-like aggregates extend into the cytoplasmic projections where they tend to be parallel to each other and to the cytoplasmic margins (Fig. 15 C).

The large round or oval nucleus has a coarse chromatin structure with a distinct linear pattern. Nucleoli are usually conspicuous (Fig. 15 C).

Tissue neutrophils (Ferrata cells) are increased in bone-marrow smears in conditions in which there is proliferation of neutrophilic cells. These cells may be prominent in bone marrow smears in myelocytic and mono-myelocytic leukemia (Naegeli type of monoblastic leukemia), in leukemic myelosis, in pernicious anemia, and in conditions in which there is injury to cells associated with maturation arrest and a neutropenic state due to chemicals and cytotoxic agents. Tissue neutrophils may be demonstrable in the peripheral blood of patients with myelocytic or monomyelocytic leukemia.

**Histocytes** (Macrophages). The etymological and nonspecific meaning of the word "histocyte" is "web (tissue) cell," but by long usage and general acceptance the word has evolved to mean a large fixed or slowly motile cell of the
tissue spaces that is actively phagocytic or is potentially capable of phagocytizing particulate matter including intact cells, fragments of cells, bacteria, fungi, crystals and other objects.

Vacuoles are often demonstrable. The background color of the cytoplasm is light blue and is usually unevenly stained. Fibrillar structures may be visible (Fig. 12 C, Fig. 15 A, Fig. 16).

Phagocytic histiocytes vary in size but are usually large. The cytoplasm is often outstretched (Fig. 16). The more motile cells reveal blunt pseudopods. The more fixed cells that are torn away from their moorings in the process of aspiration have shaggy margins and multiple pointed and tapering cytoplasmic projections (Fig. 13 A).

The nuclei of phagocytic histiocytes are relatively small in relation to the cytoplasm. The nuclei are round, oval, or slightly indented and have a linear chromatin pattern. Nucleoli are often demonstrable.

Phagocytic histiocytes of the fixed tissue spaces and the phagocytic mononuclear cells (monocytes) of the circulating blood are closely related and are thought to be variants of the same type of cell. These cells tend to be aggressive, to compress or indent other cells, to shove them aside, to go under or over them, or to eat them. They are not as readily indented by other cells as are lymphocytes and tissue neutrophils.

It is morphologically impossible to differentiate potentially phagocytic cells from stem cells when they are isolated and do not contain granules or visible phagocytic objects in their cytoplasm, but it is possible to identify undifferentiated cells as histiocytes when they appear in the center of a cluster of closely attached nucleated red cells or cells of the lymphocytic and plasmacytic series.

Malignancies involving mesenchymal cells that are morphologically undifferentiated and those that are potentially or actively phagocytic constitute a large, complex, and overlapping spectrum of diseases which have been given an infinite number of names. Variants which are characterized by the demonstrable ability of some of the cells to phagocytize are monocytic leukemia, histiocytic-monocytic leukemia, and histiocytic medullary reticulosis (malignant reticuloendotheliosis). Another entity, characterized by cells which have long finger, web, and veil-like cytoplasmic extensions and feeble phagocytic activity, is designated as "histiocytic leukemia" ("hairy cell leukemia") (Fig. 41).

**Fat Cells.** Fat cells are seldom seen in thin smears of bone marrow, for they are ruptured in the process of aspiration. On spreading on a slide, the contained globules of fat tend to escape and leave the stroma and cell membranes as unidentifiable debris. In thicker portions of the marrow smears, individual fat cells or groups of fat cells can be seen, surrounded by other marrow cells.

Mature fat cells are large round cells, comparable in size to megakaryocytes and osteoclasts (50 to 80 micra in diameter). The small, round or oval nuclei are located eccentrically, presumably pushed to one side by the pressure of globules of fat in the cytoplasm. The chromatin structure in many of the nuclei is definite and linear. Often there is a globular body in the nucleus thought to be fatty material in the process of manufacture (Fig. 17).

The globules of fat in the cytoplasm are of varying size, are chromophobic, or stain a light blue or pink. The fat globules have smooth margins. The globules compress each other, producing irregular shapes. The lipid masses are separated in compartments by cytoplasmic material which appears as delicate blue lines (Fig. 17). The fixed character of the cells is revealed by multiple fibrils which extend outward from the cell margins (Fig. 17) and interfere with the fibers of fibrocytes and endothelial cells. The lipid material in fat cells has an affinity for various Sudan dyes.

Mesenchymal cells which manufacture fat are to be differentiated from secretory plasma cells with large agglomerates of proteinaceous material in their cytoplasm. The secretory droplets in the grape or morula variants of plasma cells are spherical rather than irregular in shape and appear as perfect superimposed circles as if drawn by a small compass (Fig. 44). Cells producing fat are also to be differentiated from cells which phagocytize fat, the so-called "lipid-laden" histiocytes or "lipophages." In phagocytic histiocytes, the lipid particles tend to be small, giving to the cytoplasm a foamy or bubbly appearance.

**Osteoblasts.** An osteoblast is a large cell with ample cytoplasm and relatively small, round, and eccentrically placed nucleus (Fig. 18). These cells may be traumatized in the process of aspiration and smearing and often have irregular shapes and cytoplasmic streamers. The cells may have comet or tadpole shapes. The nucleus may be partially extruded or may rest outside the cell, like a small, round head on a round body. The nuclear chromatin strands and the nuclear margins are well defined. Usually there is a distinct nucleolus which takes a predominantly blue color in contrast to purple-red stain of the chromosomes.

The basic color of the cytoplasm is blue. Wavy fibrils are often visible. Throughout the cytoplasm there are small spherical bodies which are colorless and give to the cytoplasm a bubbly appearance. Within the cytoplasm there is a prominent round or oval zone which takes a lighter stain than the rest of the cytoplasm. This area may be adjacent to the nucleus but is usually away from the nucleus (Fig. 18).

Osteoblasts morphologically resemble plasma cells, for both have irregular shapes, pointed cytoplasmic protrusions, blue cytoplasm, eccentric nucleus, spherical bodies within the cytoplasm, chromophobic areas, cytoplasmic fibrils, and vacuoles.

Osteoblasts as a class are larger than plasma cells. The relatively unstained zone of the plasmaocyte is adjacent to the nucleus and partially surrounds the nucleus as a collar, whereas the clear zone of the osteoblast is often distinctly separate from the nuclear margin and when adjacent to the nucleus does not surround or enclose the nucleus. The protein secretions of plasma cells impart a reddish background color which is not demonstrable in osteoblasts.

Osteoblasts in marrow smears often appear in groups or aggregates which may be misinterpreted as malignant cells. The margins of cells in a malignant cluster are indistinct, and one cannot tell where one cell begins and the other ends. Malignant cells are crowded and distorted. The size of the cells and the color and structure of the nuclei tend to be quite variable, whereas in osteoblasts the cells are more orderly and uniform. Light staining areas in the cytoplasm away from the nuclei are characteristic of osteoblasts and are seldom demonstrable in malignant cells.
**Osteoclasts.** The osteoclast is a very large, irregularly shaped, and elongated cell with multiple round or oval nuclei which are approximately the same size. The number of nuclei is quite variable. The nuclei are separate and are distributed haphazardly within the cytoplasm (Fig. 19). It is thought that the large number of separated nuclei within a given cell is due to the fusion of the cytoplasm of multiple osteoclasts into a single large cell (osteoclast). The nuclear chromatin is usually linear and nucleoli are often visible. The abundant blue cytoplasm has a finely granular or ground-glass appearance. In some cells there may be distinct granules. In thin smears, it is sometimes possible to demonstrate a ruffled cytoplasmic fringe consisting of diaphenous veils, finger-like cytoplasmic protrusions, and sacular invaginations.

Osteoclasts and megakaryocytes are sometimes difficult to differentiate, for both may be very large with a granular cytoplasm, irregular shapes, and multiple nuclei. The nuclei of megakaryocytes are connected by strands or are superimposed, whereas the nuclei of osteoclasts are usually separated and have no visible connections with each other. The number of nuclei in megakaryocytes is even, whereas the number of nuclei in osteoclasts may be uneven.

Osteoclasts are usually demonstrable in areas where bone is in the process of demineralization and absorption. It is thought that osteoclasts synthesize and secrete enzymes that aid in dissolution of osteoid tissue and calcific bone.

**Fibrocytes** (Fibroblasts). Fibrocytes are connective tissue cells present in blood-forming organs as well as in all other parts of the body. These cells are responsible for the synthesis and secretion of polypeptides (trophocollagen) that, after release from the cytoplasm, aggregate (polymerize) into long fibrils. These fibrils form bundles of varying size with varied physical and staining characteristics. They are identified as reticular, collagen, and elastic fibers.

In tissue sections and in cultures, mature fibrocytes are elongated and spindle-shaped cells with oval nuclei, nongranular or finely granular cytoplasm, and multiple branching cytoplasmic protrusions. These cells are not capable of phagocytizing large particles. Fibrocytes are so tightly bound by their intertwined cytoplasmic extensions, by connective tissue ground substance, and by fibers that they are aspirated with difficulty. They are seldom seen or at least not identified as fibrocytes in smears or imprints of hematopoietic organs.

Although fibroblasts usually are not thought of as blood cells, they are essential constituents of blood-forming organs. After injury to marrow cells due to any cause, there is a proliferation of fibrocytes producing fibrosis (scar tissue). Malignancies involving fibrous tissue elements are known as fibrosarcomas. When the malignant process involves fibrocytes as well as other types of bone marrow cells, the condition is known as "leukemic myelosis" (leukemic myelofibrosis, agenogenic myelocytic metaplasia). In Hodgkin's disease, there is a malignant proliferation of fibrocytes as well as undifferentiated mesenchymal cells and lymphocytes.

**Endothelial Cells.** Occasionally one sees in marrow smears fragments of small intact vascular channels, the lumens of which are bounded by elongated nongranular cells with oval nuclei. Spindle or oval cells may be scraped from the lining of blood vessels or from the heart chambers by the point of a needle in the process of collecting blood. These cells are identified as endothelial cells by their organoid arrangement. Individual endothelial cells are not identifiable.

**Osteocytes and Chondrocytes.** These cells are not present in bone-marrow smears although they are normal constituents of bones and cartilage.
Proposals for the Classification of the Acute Leukaemias

FRENCH–AMERICAN–BRITISH (FAB) CO–OPERATIVE GROUP

J. M. BENNETT, D. CATOYSKY, M. THERÈSE DANIEL, G. FLANDRIN,
D. A. G. GALTON, H. R. GRAINICK and C. SULTAN

*University of Rochester Cancer Centre, University of Rochester School of Medicine and Dentistry,
Rochester, New York, U.S.A., †M.R.C. Leukaemia Unit, Royal Postgraduate Medical School,
London, ‡Institut de Recherches sur les Leucémies et les Maladies du Sang, Hopital Saint-Louis,
Paris, France, §Hematology Service, National Institutes of Health, Bethesda, Maryland, U.S.A.,
¶Service Central d’Hématologie–Immunologie, Hopital Henri Mondor, Creteil, France

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SUMMARY. A uniform system of classification and nomenclature of the acute leukaemias, at present lacking, should permit more accurate recording of the distribution of cases entered into clinical trials, and could provide a reference standard when newly developed cell-surface markers believed to characterize specific cell types are applied to cases of acute leukaemia. Proposals based on conventional morphological and cytochemical methods are offered following the study of peripheral blood and bone-marrow films from some 200 cases of acute leukaemia by a group of seven French, American and British haematologists. The slides were examined first independently, and then by the group working together. Two groups of acute leukaemia, ‘lymphoblastic’ and myeloid are further subdivided into three and six groups. Dysmyelopoietic syndromes that may be confused with acute myeloid leukaemia are also considered. Photomicrographs of each of the named conditions are presented.

Chemotherapy has become increasingly effective in the treatment of acute leukaemia, particularly in childhood. However, the range of morphological variation in both lymphoblastic and myeloblastic leukaemia is very great and in recent years attempts have been made to define subgroups and to see whether there are any correlations between the subgroups and clinical and laboratory findings, response to treatment and prognosis. Several different classifications have been proposed, but the same terms have been used in different ways, and different terms have been applied to the same conditions. A group of seven French, American and British haematologists have met to discuss the problem, with the aims of making proposals on nomenclature and classification that might serve as a basis from which a generally acceptable system could be worked out, and of defining features of each named entity as objectively and unambiguously as possible.

At the present time there is no general agreement on nomenclature or classification, or even on the methods for distinguishing myeloid from lymphoblastic leukaemias. It is gener-
ally accepted that this latter distinction is important, because, since the first cytotoxic drugs were tested, it has been clear that the response of lymphoblastic leukaemia was better than that of the acute myeloid leukaemias. It is equally possible that the results of treatment improve, certain subtypes of both main groups may prove to be more responsive than others.

Other advantages of an agreed nomenclature and classification are: (1) the results of different therapeutic trials could be compared with one another with more confidence; (2) discrepancies in the results quoted by different workers when applying new techniques for the characterization of leukaemic cells would be reduced.

Before meeting in Paris from 14 to 19 October 1974, Romanowsky-stained films of peripheral blood and bone marrow from 150 cases of acute leukaemia and disorders that might be confused with acute leukaemia were circulated among the participants for diagnosis. At the Paris meeting the original diagnoses and those made by each participant were discussed, and in cases of disagreement the slides were re-examined. The points of agreement were identified and provided the basis on which the present proposals were made. After 8 months of correspondence and a further working meeting in London from 8 to 10 July 1975, the group reached a general agreement on all outstanding issues, and achieved a uniformity of 85% in applying the provisional classification to a fresh set of slides deliberately chosen to test previously controversial categories. As a result of this high degree of agreement we decided to present this classification as a basis for discussion.

Our first task was to try to establish objective criteria for the separation of the broad group of myeloid from the non-myeloid ('lymphoblastic') leukaemias and we believe we have been successful in this. We then attempted to provide similar criteria for defining the morphological borderlines between already well-recognized categories (and some less well recognized) in each of the two major groups. In the myeloid leukaemias our subdivisions are largely conventional, but difficulty arose in the case of the non-myeloid leukaemias, because the variation observed is not easily characterized objectively. This group includes the subgroup of childhood leukaemias widely known as lymphoblastic and a much more heterogeneous subgroup of undifferentiated leukaemias that cannot be characterized by any conventional methods. The cells in a minority of cases in both subgroups have been shown to possess surface markers specific for normal lymphocytes, but because the lymphoblastic nature has not been established in a majority of cases we have thought it better to include 'quotes' in describing the whole group as 'lymphoblastic'.

In attempting to define boundaries between overlapping groups, in examining any one case, major attention was given to the predominant cell type and providing some indication of the range of variability within the population. The latter was often sufficient to preclude attempts to provide measurements to indicate cell size, nuclear-cytoplasmic ratio, granularity, degree of cytoplasmic basophilia, and similar features.

The classification is based entirely on the morphological appearances of the bone marrow and peripheral blood in Romanowsky-stained films, supplemented in appropriate circumstances by certain cytochemical reactions. Blood films, though essential, especially in the myeloid leukaemias, were not considered by themselves as sufficient for diagnosis. Other methods now under investigation, but not yet of established diagnostic value, are also referred to. In the course of our work we were impressed with the importance of technical excellence in the preparation and staining of blood and marrow films, and of choosing areas.
Classification of Acute Leukaemias

for examination in which the cells are not distorted in shape or poorly stained by overcrowding. It was also found that great caution was required before diagnosing leukaemia when the marrow sample was hypocellular.

We now classify all our cases of leukaemia according to the scheme presented. The following descriptions are applicable only to material from patients who have received no cytotoxic drug treatment: they are of little or no value when applied to material from patients in relapse. In general, they apply to cases in which the bone-marrow films contain fragments and trails that are hypercellular or at least normocellular.

RESULTS AND COMMENTS

I. 'LYMPHOBLASTIC' LEUKAEMIAS

We describe three types (L1, L2 and L3), defined according to (a) the occurrence of individual cytological features, and (b) the degree of heterogeneity in the distribution among the leukaemic-cell population of some or all of these features. The features considered are: cell size, nuclear chromatin, nuclear shape, nucleoli, amount and basophilia of cytoplasm. For each of the features considered, up to 10% of the cells may depart from that characteristic of the type. A summary of the characteristics of each type is given in Table I.

<table>
<thead>
<tr>
<th>Cytological features</th>
<th>L1</th>
<th>L2</th>
<th>L3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size</td>
<td>Small cells predominate</td>
<td>Large, heterogeneous in size</td>
<td>Large and homogeneous</td>
</tr>
<tr>
<td>Nuclear chromatin</td>
<td>Homogeneous in any one case</td>
<td>Variable—heterogeneous in any one case</td>
<td>Finely stippled and homogeneous</td>
</tr>
<tr>
<td>Nuclear shape</td>
<td>Regular, occasional clefting or indentation</td>
<td>Irregular, clefting and indentation common</td>
<td>Regular—oval to round</td>
</tr>
<tr>
<td>Nucleoli</td>
<td>Not visible, or small and inconspicuous</td>
<td>One or more present, often large</td>
<td>Prominent; one or more vesicular</td>
</tr>
<tr>
<td>Amount of cytoplasm</td>
<td>Scanty</td>
<td>Variable; often moderately abundant</td>
<td>Moderately abundant</td>
</tr>
<tr>
<td>Basophilia of cytoplasm</td>
<td>Slight or moderate, rarely intense</td>
<td>Variable; deep in some</td>
<td>Very deep</td>
</tr>
<tr>
<td>Cytoplasmic vacuolation</td>
<td>Variable</td>
<td>Variable</td>
<td>Often prominent</td>
</tr>
</tbody>
</table>

Features of L1 (Fig 1). Predominantly small cells, up to twice the diameter of the small lymphocyte. Although characteristically the cells vary little in size, heterogeneity is compatible with L1 if all the other features are present. The nuclear chromatin is variable from case to case but homogeneous in any one case. It is usually finely dispersed but may appear clumped in the smaller cells. The nuclear shape is regular, but the presence of at least some degree of clefting, indentation, or folding is compatible with L1. Nucleoli are often not visible; the nucleolus when present is usually small and not prominent. The cytoplasm is usually scanty (high nuclear:cytoplasmic ratio). Deep cytoplasmic basophilia is uncommon. Characteristically, L1 is homogeneous in respect of all the features listed.
Features of L2 (Fig 2). Usually the majority of cells are more than twice as large as small lymphocytes. In many cases there is marked heterogeneity in cell size. The nuclear chromatin varies from finely dispersed to coarsely condensed, and is characteristically heterogeneous in any one case. Nuclear clefiting, indentation and folding are characteristic and gross irregularities in shape are common. Nucleoli are nearly always present; they are variable in size and number often large. The amount of cytoplasm in the cells of any one case is variable and often abundant. Cytoplasmic basophilia is also variable and may be marked in some cases. Characteristically, L2 is heterogeneous in respect of all the features listed and there is consequently more variation between different cases than in L1 as well as in any one case.

Features of L3 (Burkitt type) (Fig 3). The cells are large and characteristically homogeneous. They have a dense but finely stippled nuclear chromatin. The nucleus is oval to round and regular. One or more prominent vesicular nucleoli are present in the majority of cells. The cytoplasm is moderately abundant and completely surrounds the nucleus. Intense cytoplasmic basophilia is present in every cell. L3 is characteristically homogeneous in respect of every feature listed, both from case to case, and in an individual case. A high mitotic index (about 5%) is characteristic. Prominent cytoplasmic vacuolation is often present in a majority of the cells; vacuolation may also be present in L1 and L2, but is usually less prominent.

General Remarks on 'Lymphoblastic' Leukaemia

L1 represents the type of acute leukaemia common in childhood. L2, less common in children, requires differentiation from myeloblastic leukaemia without maturation (M1) and is sometimes designated 'undifferentiated leukaemia'. A minority of cases of lymphoblastic leukaemia are difficult to classify as either L1 or L2 on morphological characteristics alone. The myeloperoxidase reaction or Sudan Black B staining are essential in the investigation of all cases of suspected lymphoblastic leukaemia. Scattered azurophilic granules in primitive cells in Romanowky-stained films are present in rare cases of L3 but they are invariably myeloperoxidase-negative. This is the main difference between L2 and M1 (see below).

Because of the heterogeneity of L2 it may not always be possible to distinguish between positive leukaemic blasts and residual normal myeloid cells; the presence of up to 5% of myeloperoxidase-positive blast cells is still considered compatible with L2. This is arbitrary, but is suggested as a safe limit. Rarely L2 may be confused with poorly differentiated monocytic leukaemia (M5) but the fluoride-sensitive esterase reactions will permit the distinction to be made.

B-lymphocyte markers have been found in most cases of L3 studied, and T-lymphocyte markers in about 25% of cases of lymphoblastic leukaemia (other than L3). Therefore, at the present time lymphocyte markers cannot be demonstrated in the majority of cases of lymphoblastic leukaemia. There are no morphological features characteristic of the minority in which T-lymphocyte markers are found. Although 'lymphoblastic' is not a satisfactory term for the whole group, 'undifferentiated' is perhaps even less appropriate.

II. MYELOID LEUKAEMIAS

We describe six main types (M1, M2, M3, M4, M5 and M6)* defined according to (a) the

* The categories M1 to M6 are substantially the same, though not identical with those of Galton & Dick (1975).
Classification of Acute Leukaemias

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Direction of differentiation along one or more cell lines, and (b) the degree of maturation of the cells. Thus, M1, M2 and M3 show predominantly granulocytic differentiation and differ from one another in the extent and nature of granulocytic maturation; M4 shows both granulocytic and monocytic differentiation, M5 predominantly monocytic differentiation and M6 predominantly erythroblastic differentiation.

Myeloblastic leukaemia without maturation (M1) (Fig 4). The cells in the bone-marrow show some evidence of granulocytic differentiation as shown by either: (a) blasts in Romanowskystained films which are non-granular and usually containing one or more distinct nucleoli; 3% or more of the blasts are myeloperoxidase positive (Fig 5), or (b) varying proportion of blasts containing at least a few azurophilic granules, Auer rods or both. Further maturation is not seen.

Myeloblastic leukaemia with maturation (M2) (Fig 6). The defining characteristic which distinguishes M2 from M1 is that maturation at or beyond the promyelocyte stage is present. The promyelocyte is the largest cell in the normal granulocyte series and contains numerous azurophilic granules. More than 50% of the bone-marrow cells are myeloblasts and promyelocytes. The leukaemic cells are often nucleolated and have varying amounts of cytoplasm, usually with many azurophilic granules; cells containing Auer rods, almost always single, are common. In rare cases almost all the myelocytes, metamyelocytes, and mature granulocytes are eosinophils. In M2, in contrast to M1, myelocytes, metamyelocytes, and mature granulocytes (often demonstrating abnormal features) may be found in varying proportions.

Bilobed or reniform myeloblasts may sometimes be mistaken for monocytes. However, they have the small nucleoli and fine chromatin pattern of myeloblasts, and their less abundant cytoplasm lacks the ground-glass grey appearance characteristic of monocytes. The use of cytochemical reactions specific for monocytes will be helpful in these cases (see later). The polymorphs may also show abnormalities in varying proportions and combinations: they may have few granules or appear to have none; their nuclei may resemble those of the Pelger-Huet anomaly, or the cells lack myeloperoxidase activity. Cases with erythroid hyperplasia (but less than 50% erythroblasts) without the morphological abnormalities of erythroleukaemia, are included in this group. In some cases the combined percentage of myeloblasts and promyelocytes in the bone marrow may help to distinguish M2 from one of the dysmyelopoietic syndromes (see later).

Hypergranular promyelocytic leukaemia (M3) (Fig 7). Characteristic features of this disorder are: (a) The great majority of cells are abnormal promyelocytes, with a characteristic pattern of heavy granulation. (b) The nucleus varies greatly in size and shape and is often reniform or bilobed. (c) The cytoplasm of most of the cells is completely occupied by closely packed or even coalescent large granules staining bright pink, red or purple by Romanowsky dyes. In some cells the cytoplasm is filled with fine dust-like granules. Characteristic cells containing bundles of Auer rods ('faggots') randomly distributed in the cytoplasm are almost invariably present in the bone marrow and sometimes in the peripheral blood. The cytoplasm of the cells which contain faggots is often clear and pale-staining but may also contain azurophilic granules. (d) A variable but often high proportion of the hypergranular promyelocytes and the faggot cells are disrupted, with granules and Auer rods lying free, over and among other cells. It is necessary to distinguish M2 cases in which the percentage of promyelocytes is high.
from those of M3. In M2 the cytoplasmic granulation is usually less heavy and does not obscure the basophilic cytoplasm as is usual in M3; however, in M2 occasional cells in which the cytoplasm is completely filled with coarse granules may be seen.

Myelomonocytic leukaemia (M4) (Figs 8 and 9). Both granulocytic and monocytic differentiation are present in varying proportions in the bone marrow and peripheral blood. M4 resembles M2 in all respects except that the proportion of promonocytes and monocytes exceeds 20% of the nucleated cells in the bone marrow, the peripheral blood or both. However, promonocytes and promyelocytes may not always be readily distinguishable in Romanowsky-stained preparations unless the cytochemical reactions specific for monocytes are carried out (see later). In some cases the distinction between M2 and M4 depends on the high count of promonocytes, monocytes or both in the peripheral blood in M4 (almost always above 5 x 10⁹/l). In these cases the marrow appearance alone may be that of M2. When the percentage of promonocytes and monocytes in the marrow is high the appearance approaches that of M5 (well differentiated), but the percentage of myeloblasts and promyelocytes always exceeds 20%.

Monocytic leukaemia (M5). The diagnosis, although based on a Romanowsky-stained film of blood and bone-marrow, requires confirmation of cytochemical methods (see later). Two subtypes occur: (a) Poorly differentiated (monoblastic) (Fig 10) is characterized by large blasts in the bone-marrow and sometimes in the peripheral blood. The blasts have delicate lacy chromatin, and one, occasionally up to three, large prominent vesicular nucleoli. The cytoplasm is voluminous, often shows one or more pseudopods, is basophilic and may contain rare azurophilic granules. The pseudopodia or buds may be more translucent than the rest of the cytoplasm and this gives the appearance of a double membrane. A low percentage of promonocytes may be present. (b) Differentiated (Fig 11): monoblasts, promonocytes and monocytes are found, but the proportion of monocytes in the peripheral blood is higher than in the bone marrow, in which the predominant cell is the promonocyte. This cell is similar to the monoblast but has a large nucleus with a cerebriform appearance; nucleoli may be present, but the cytoplasm is less basophilic, has a greyish ground-glass appearance and fine azurophilic granules are often scattered throughout.

In both types there is almost total replacement of the marrow by leukaemic cells; however, a minor granulocytic component may be present, rarely more than 10%, exceptionally up to 20%. A few cells may contain Auer rods in both types of M5. The diagnosis (from M2 or M4) must then be confirmed by the fluoride-inhibited esterase reactions (Figs 12 and 13).

Erythroleukaemia (M6) (Fig 14). The erythropoietic component usually exceeds 50% of all the nucleated cells in the bone marrow, and the erythroblasts show, in varying degree, bizarre morphological features, especially multiple lobation of the nucleus, with variation in size of the lobes, multiple nuclei, presence of one or more nuclear fragments, giant forms, and megaloblastic features. If the proportion of erythroblasts with bizarre dyserythropoiesis exceeds 10% and the other abnormalities (see below) of M6 are present, then an erythropoietic component of 30%, may suffice. Erythroblasts are often present in the peripheral blood. The granulopoietic cells show an increased proportion of myeloblasts and promyelocytes, and Auer rods may be seen. The percentage of myeloblasts and promyelocytes accompanying these dyserythropoietic changes is variable, but, when it is less than 30%
of all the nucleated cells, an alternative diagnosis such as dysmyelopoietic syndrome should be considered (see below). Abnormal megakaryocytes (mononuclear forms or micromegakaryoblasts) may be present. M6 almost always progresses to M1, M2, or M4.

We recognize that megaloblastic anaemia, congenital dyserythropoietic anaemias, and other conditions in which certain cytotoxic drugs (especially cytosine arabinoside and methotrexate) have been administered, may be confused with M6.

III. DYSMYELOPOIETIC SYNDROMES (DMS)

In contrast to the foregoing sections in which the need for cytotoxic-drug therapy is widely accepted, there is a range of conditions for which an immediate recommendation to start therapy cannot be made or may not be indicated. These are disorders associated with bone-marrow hypercellularity in which confusion with acute myeloid leukaemia is possible. Conditions such as aplastic anaemia or PNH which may terminate in frank leukaemia will not be considered.

Two broad types of dysmyelopoietic syndrome can be recognized and both are more common over the age of 50 years. The conditions are refractory anaemia with excess of blasts (RAEB) and chronic myelomonocytic leukaemia (CMML). The presence of a prominent monocytic component in the bone marrow and peripheral blood distinguishes CMML from RAEB. In CMML the granulocytic component of the bone marrow is more prominent than in RAEB, while in RAEB the erythroid component of the bone marrow is more prominent than in CMML. Both types require frequent follow-up of peripheral blood counts and repeated bone-marrow examination to check for their possible progression towards acute myeloid leukaemia.

Refractory anaemia with excess of blasts (RAEB) (Fig 15) includes conditions variously described as 'preleukaemia', 'smouldering leukaemia', 'subacute leukaemia', and 'atypical leukaemia'. Erythropoietic hyperplasia and dyserythropoietic changes in the bone marrow, with or without the presence of ring sideroblasts, are common especially in the initial stages, whether or not anaemia is severe. Neutropenia is common, and the absolute monocyte count is below $1 \times 10^9/l$. Blasts are not always found in the peripheral blood; the mature granulocytes may appear hypogranular or agranular in Romanowsky-stained films, or have Pelger–Hüet type nuclei. The bone marrow is hypercellular, and promegakaryocytes and blasts together account for 10–30% of all the nucleated cells. Abnormal granulocytic maturation is present in varying degree. It is suggested that a search for Auer rods should be made because their presence may prove to correlate with prognosis although this is not yet established. Progressive increase in the percentage of myeloblasts and promegakaryocytes towards 50% probably indicates the development of myeloid leukaemia. Megakaryocytes are variable in number, and mononuclear forms and micromegakaryocytes may be present (Fig 15, arrow).

Chronic myelomonocytic leukaemia (CMML) is the only leukaemic condition in which the clinical term chronic has been retained in an otherwise strictly morphological classification. In the peripheral blood, the monocyte count is at some stage of the disease higher than $1 \times 10^9/l$, and the monocytes often appear atypical. The absolute neutrophil count is variable. Blasts and promonocytes are rarely present, and the neutrophils may show the same abnormalities as in RAEB. The bone marrow is hypercellular as in RAEB and usually shows an
excess of myeloblasts and promyelocytes (up to 30%). There is also an increase in promonocytes and monocytes but, as in M4, this may not always be apparent unless the cytochemical reactions specific for the monocytic series are carried out (see below). Serum lysozyme concentrations are raised in CMML but not in RAEB.

**SPECIAL INVESTIGATIONS**

**Cytochemical tests of diagnostic value.** Two cytochemical methods, the myeloperoxidase and non-specific esterase reactions (the latter before and after exposure to NaF with naphthol AS- or ASD-acetate as substrates) are recommended for routine use in the investigation of every new case of acute leukaemia. The myeloperoxidase reaction, or alternatively Sudan Black B staining, is positive in all the myeloid leukaemias. Its special importance is in making the distinction between myeloblastic leukaemia (M1) (Fig 5) and 'lymphoblastic' leukaemia (especially L2). The naphthol AS- or ASD-acetate esterase reaction (NASDA) is positive in the majority of monocytic, granulocytic, and myelomonocytic leukaemias, but in monoblasts, promonocytes, and monocytes the reaction is often stronger (Fig 12) and is completely or almost completely inhibited by sodium fluoride (Fig 13). In granulocytes the positivity of the reaction is unaffected by exposure to fluoride.

**Cytochemical tests recommended for future assessment.** The PAS reaction for glycogen is negative in L3 but gives variable results in L1, L2 and in the myeloid leukaemias.

The acid-phosphatase reaction gives strong positivity localized to the Golgi region in more than 80% of the blast cells in some cases of lymphoblastic leukaemia. This pattern has been observed in a high percentage of cases in which T markers were demonstrated. The acid-phosphatase reaction described is relevant only in the context of lymphoblastic leukaemia, because the enzyme is also present in myeloid cells.

**Lysozyme estimations.** The serum lysozyme concentration is elevated in conditions with a prominent monocytic component. Concentrations above four times the upper limit of the normal range are almost always confined to M4, M5 and CMML. The cytotbacterial method indicates the production of lysozyme by individual cells, and the majority of cells of the monocytic series react positively.

**Tests for lymphocyte markers.** Tests for B and T lymphocyte markers are now increasingly being applied to the investigation of leukaemias and may become of value in classification as information accumulates.

**REFERENCE**

Proposals for the classification of the myelodysplastic syndromes


Received 12 November 1981: accepted for publication 18 December 1981

SUMMARY. New diagnostic criteria for the diagnosis of the various myelodysplastic syndromes (MDS) are proposed, and a detailed description is given of the features that may help define MDS. Five MDS are described: (1) refractory anaemia (RA), (2) RA with ring sideroblasts, (3) RA with excess of blasts (RAEB), (4) chronic myelomonocytic leukaemia (CMML), and (5) RAEB in transformation. One of the main distinguishing features of these conditions is the proportion of blast cells in the peripheral blood (PB) and/or bone marrow (BM). The morphological features of the blast cells that are of diagnostic importance have been redefined. In RA, with or without ringed sideroblasts, there are fewer than 1% of blasts in the PB and fewer than 5% in the BM; RAEB is defined as having between 5% and 20% of blasts in the BM and fewer than 5% in the PB; RAEB in transformation (a newly defined category) will be considered when any one of the following features is present: (i) more than 5% of blasts in the PB, (ii) 20–30% in the BM, and (iii) the presence of Auer rods in granulocyte precursors in BM or PB. In accordance with these newly defined criteria, it is now proposed that over 30% of bone marrow blasts will suffice for the diagnosis of acute myeloid leukaemia (AML) in any of its forms (M1–M6). The proposed descriptions of the MDS should facilitate the interpretation of data emerging from cytogenetic and bone marrow culture studies and the search for features of possible prognostic significance. Recognition of the new category, RAEB in transformation, may throw light on the pathogenesis of AML.

Correspondence: Dr D. Catovsky, MRC Leukaemia Unit, Royal Postgraduate Medical School, Ducane Road, London W12 OHS.

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In 1976 the FAB co-operative group published proposals for the morphological classification of the acute leukaemias which have been adopted widely (Bennett et al, 1976). A distinction was made between the common types of acute leukaemia, usually presenting with symptoms of recent onset, and requiring immediate treatment, and the range of less acute disorders usually presenting in persons above 50 years of age with symptoms of variable duration that were described collectively as the dysmyelopoietic or myelodysplastic syndromes (MDS); for these patients, urgent treatment was not indicated. Two broad types of MDS were described, namely 'refractory anaemia with excess of blasts' (RAEB), and 'chronic myelomonocytic leukaemia' (CMML) (Bennett et al, 1976; Gralnick et al, 1977). For both conditions it was recognized that the distinction from acute leukaemia was sometimes difficult to make, and that in a proportion of cases of MDS, the disease underwent transformation to a more acute phase often indistinguishable from overt acute myeloid leukaemia. This change was associated with an increase in the proportion of blast cells and sometimes of promyelocytes in the bone marrow (BM) and often in the peripheral blood (PB). It was suggested that a diagnosis of MDS could still be retained if the sum of blast cells and promyelocytes in the BM was as high as 30%, and that proportions between 30% and 50% probably indicated the development of acute leukaemia which was defined by myeloblasts and promyelocytes together accounting for 50% or more of the nucleated cells in the BM.

In the past 5 years it has become clear that the range of morphological appearances in the PB and BM consistent with a diagnosis of MDS is very wide, that there is great variation in the risk of transformation to a blastic phase, and that the risk appears to be correlated with the morphological features. The FAB group therefore decided to examine more cases of MDS to see whether morphological features could be specified that would permit the recognition of different subtypes.

The group met in Paris (April 1980) and agreed, after reviewing 50 cases, on the range of morphological features that would be recorded in its subsequent work. At a meeting in London (May 1981), sets of slides from 30 cases were examined, of which half had been deliberately selected because their diagnosis had proved difficult. In this report we describe the morphological features examined in each case, and the way in which these features were used to define five subtypes of MDS (see below for definitions). In particular the features of a new type designated as 'RAEB in transformation' are specified. We have also included two groups of refractory anaemia, one without ringed sideroblasts, and refractory anaemia with ringed sideroblasts, because in both groups morphological abnormalities in the granulocytic and megakaryocytic series identical with those present in other subtypes of MDS may occasionally be found in varying degree. Some cases of CMML with high neutrophil and monocyte counts often have minimal dysplastic changes in the erythroid and megakaryocytic lineages and may have little in common with MDS showing trilineage dyspoiesis. However, the group examined enough cases with typical RAEB but monocyte counts above $1 \times 10^9/l$ to permit the conclusion that CMML is closer to MDS than to other chronic myeloproliferative disorders.

Although the majority of patients with MDS are elderly, an increasing number of younger patients are now being seen: they are mostly patients who have received prolonged treatment with radiotherapy, cytotoxic drugs, particularly alkylating agents, or both forms of treatment.
Myelodysplastic Syndromes

(Foucar et al., 1979; Kitahara et al., 1980). Although there are some differences, MDS following these forms of treatment is essentially similar to idiopathic MDS, and four of the 30 cases examined were examples of it.

Finally, during the course of our work it was decided to revise the definition and limits of MDS and AML, in terms of the blast-cell percentage and other features as described below.

Features used to define the myelodysplastic syndromes

Dyspoiesis. Anaemia, leucopenia (neutropenia) and thrombocytopenia are common findings in the MDS. These cytopenias occur despite a normocellular or hypercellular BM. The qualitative and quantitative abnormalities of these cell lines are considered to result from ineffective haematopoiesis. The morphological abnormalities of the erythroid, granulocytic and megakaryocytic series can be seen in well-prepared PB and BM films stained with Romanovsky dyes (e.g. May-Grünwald-Giemsa). We have listed the qualitative changes which can be readily identified (Table 1). In some patients the mere presence of these abnormalities is adequate for their inclusion in the MDS. In others, some quantitative assessment of the qualitative defect may be necessary. Systematic accumulation of these data and correlation with other disease features in a large series of patients might demonstrate their diagnostic value and prognostic significance.

Dyserythropoiesis. Qualitative and quantitative abnormalities of the erythroid precursors in the BM and aberrations of red cell morphology in the PB are very common in MDS. Qualitative abnormalities in the BM include the presence of ringed sideroblasts, multinuclearity, nuclear fragments of various sizes, abnormal nuclear shape (e.g. single, double or triple indentations resulting in two or more nuclear lobes of equal or unequal size or an irregular outline) and abnormal cytoplasmic features (Fig 1) (e.g. irregularities in the density of staining which, when extreme, shows as clear unstained areas with ill-defined edges occupying a variable proportion of the cytoplasm, and punctate basophilia). PB abnormalities are primarily seen as changes in the RBC morphology and rarely by the presence of nucleated RBC, except in patients totally dependent on transfusion. The presence of RBC abnormalities in the PB is often an indication to look for dyserythropoiesis in the BM. We believe that these abnormalities are primarily a result of disordered erythropoiesis. An almost universal quantitative abnormality of the erythroid series is anaemia which is usually regenerative (ineffective erythropoiesis). Other quantitative abnormalities of the BM which may reflect extreme defects in RBC production are: the presence of more than 15% of ring sideroblasts, and the proportion of erythroid precursors being either excessive (e.g. greater than 60%) or decreased (e.g. less than 5%) of the nucleated BM cells.

Dysgranulopoiesis. Morphological abnormalities in granulocytes may be seen in the PB and the BM. In the PB the neutrophils may appear agranular or hypogranular on May-Grünwald-Giemsa stain (Fig 2A). In some cases the cytoplasmic abnormalities are represented by the persistence of basophilia in mature cells. Abnormalities in the nuclear segmentation may be seen as hyposegmentation (Pelger-Huët-like anomaly) or hypersegmentation with bizarre shapes.

In the BM, abnormal staining of the primary (azurophilic) granules is one of the major
### Table 1. Suggested features to record when analysing a case of MDS

<table>
<thead>
<tr>
<th>Name, age, sex, full blood count</th>
<th>Peripheral blood</th>
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<tbody>
<tr>
<td><strong>Bone marrow</strong></td>
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<tr>
<td>(1) Blast cells (types I and II)</td>
<td></td>
</tr>
<tr>
<td>&lt; 5%, 5–10%, 10–20%</td>
<td>0, 1–5%, 5–10%,</td>
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<tr>
<td>20–30%, &gt; 30%</td>
<td>10–20%, &gt; 20%</td>
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<tr>
<td>Auer rods</td>
<td>Auer rods</td>
</tr>
<tr>
<td>(2) Dyserthropoiesis</td>
<td>RBC abnormalities</td>
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<tr>
<td>Ringed sideroblasts ≥ 15%</td>
<td>Circulating NRBC</td>
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<tr>
<td>Multinuclearity</td>
<td></td>
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<tr>
<td>Nuclear fragments</td>
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<td>Other nuclear abnormalities</td>
<td></td>
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<tr>
<td>Cytoplasmic abnormalities</td>
<td></td>
</tr>
<tr>
<td>Erythroblasts &lt; 5%, &gt; 60%</td>
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<tr>
<td>(3) Dysmegakaryocytopenia*</td>
<td>Large platelets</td>
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<tr>
<td>Micromegakaryocytes</td>
<td></td>
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<tr>
<td>Large mononuclear forms</td>
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<td>Multiple small nuclei</td>
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<tr>
<td>Reduced numbers</td>
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<tr>
<td>(4) Dysgranulopoiesis</td>
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<tr>
<td>Nuclear abnormalities</td>
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<td>Hypoplastic cells</td>
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<td>(5) Monocytes</td>
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<td>Monocytes and promonocytes &gt; 1 x 10⁹/l</td>
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<tr>
<td>(6) Cellularity</td>
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* Examine at least 10 megakaryocytes.

Abnormalities found, in the promyelocytes and myelocytes. In some cases the cells seem to be devoid of granules and in others the primary granules appear larger than normal. In the BM, the secondary granules may be absent or reduced in the myelocytes and later forms including mature granulocytes (Fig 2B and D). In addition, irregular distribution of the cytoplasmic basophilia may be seen, with a dense rim of basophilia in the periphery of the cells, and a lack of it in the perinuclear area. The same nuclear abnormalities of the mature granulocytes seen in the PB are present in the BM.

**Dysmegakaryocytopenia.** Qualitative abnormalities are frequently found and sometimes a reduction in the number of megakaryocytes, a quantitative defect, is also present. Qualitative
abnormalities found, in the promyelocytes and myelocytes. In some cases the cells seem to be devoid of granules and in others the primary granules appear larger than normal. In the BM, the secondary granules may be absent or reduced in the myelocytes and later forms including mature granulocytes (Fig. 2B and D). In addition, irregular distribution of the cytoplasmic basophilia may be seen, with a dense rim of basophilia in the periphery of the cells, and a lack of it in the perinuclear area. The same nuclear abnormalities of the mature granulocytes seen in the PB are present in the BM.

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</tr>
<tr>
<td>Cytoplasmic abnormalities</td>
</tr>
<tr>
<td>Erythroid blast cell &lt; 5%, &gt; 60%</td>
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<tr>
<td>(3) <strong>Dysmegakaryocytopoiesis</strong></td>
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<td>Micromegakaryocytes</td>
</tr>
<tr>
<td>Large monocellular forms</td>
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<tr>
<td>Multiple small nuclei</td>
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<tr>
<td>Reduced numbers</td>
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<tr>
<td>(4) <strong>Dysgranulopoiesis</strong></td>
</tr>
<tr>
<td>Nuclear abnormalities</td>
</tr>
<tr>
<td>Hypogranular cells</td>
</tr>
<tr>
<td>(5) <strong>Monocytes</strong></td>
</tr>
<tr>
<td>Monocytes and promonocytes &gt; 1 × 10⁹/l</td>
</tr>
<tr>
<td>(6) <strong>Cellularity</strong></td>
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<td>RA with excess of blasts (RAEB)</td>
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<td>RAEB 'in transformation'</td>
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<tr>
<td>Chronic myelomonocytic leukaemia (CMML)</td>
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* Examine at least 10 megakaryocytes.
abnormalities include micromegakaryocytes, large mononuclear megakaryocytes (Fig 3A) and megakaryocytes with multiple small separated nuclei (Fig 2c and Fig 3B, C and D). Occasionally megakaryocytes with giant and/or abnormal granules are seen. Varying proportions of these abnormal forms and of normal megakaryocytes are found. Rarely, micromegakaryocytes can be recognized in the peripheral blood; more commonly giant platelets are seen. The significance of the abnormalities in the megakaryocytic line in relation to the stem cell affected in the MDS is at present unknown.

Description of the blast cells seen in MDS

In our experience the inclusion of the typical promyelocyte with myeloblasts in the enumeration of blast cells (e.g. in RAEB) has not increased our ability to distinguish different forms of MDS or predict a leukaemic evolution. We believe that the revised definition of the blast cells proposed here could be more helpful in defining the various forms of MDS and may be clinically more useful.

In our revised blast-cell category we include certain blast cells with cytoplasmic granules but we exclude normal appearing promyelocytes (see below). Likewise, recognizable promonocytes, proerythroblasts and megakaryoblasts are not included in the blast category but are noted.

We recognize two types of blasts in MDS which differ primarily in their cytoplasmic characteristics.

Type I. These blasts vary from cells indistinguishable from myeloblasts to cells of various size which may be unclassifiable (Fig 2C and D). Cytoplasmic granules are always absent. These cells usually have prominent nucleoli and an uncondensed chromatin pattern. The nuclear/cytoplasmic ratio of the smaller blasts tends to be higher (i.e. 0.8) than in the larger ones.

Type II. These blasts have a few primary (azurophilic) granules. Otherwise they resemble type I blasts but the nuclear/cytoplasmic ratio tends to be lower and the nucleus remains in a central position.

When the following characteristics are present the cells are no longer considered to be type II blasts and are classified as promyelocytes: (i) eccentric nucleus, (ii) a developed Golgi apparatus (seen as a clear zone in the vicinity of the nucleus), (iii) more dense and/or clumped chromat in pattern, (iv) numerous granules, and (v) low N/C ratio. Deficiency of primary granules or their failure to take up the stain may result in a hypogranular or agranular promyelocyte which is recognized by the remaining four features.

Conditions included in MDS: definitions

The five types of MDS are: (1) refractory anaemia (RA), (2) RA with ring sideroblasts, (3) RA with excess of blasts (RAEB), (4) chronic myelomonocytic leukaemia (CMML), and (5) RAEB in transformation. The features that characterize these forms of MDS can be defined as follows:

(1) Refractory anaemia (RA) is usually found in patients over the age of 50 years with
anaemia as the main presenting symptom. The peripheral blood shows reticulocytopenia, variable dyserythropoiesis and infrequently dysgranulopoiesis. Blast cells are not seen in the peripheral blood; when present they do not exceed 1%. The bone marrow is normo- or hypercellular with erythroid hyperplasia and/or dyserythropoiesis. The granulocytic and megakaryocytic series almost always appear normal and there are always fewer than 5% of blast cells. Rarely, patients with neutropenia and/or thrombocytopenia but no anaemia can be included in this category. The group recognizes that although the term RA may not be satisfactory, it is widely used and generally well understood, and therefore it would be better to retain it.

(2) RA with ring sideroblasts (acquired idiopathic sideroblastic anaemia). The main difference from the above is the presence of ringed sideroblasts accounting for more than 15% of all nucleated cells in the bone marrow. Ringed sideroblasts have several siderotic granules on the nuclear membrane arranged in a collar around the nucleus (Dacie & Lewis, 1975). Deficient haemoglobinization in some of the red cell precursors leads to a dimorphic picture in peripheral blood films.

(3) Refractory anaemia with excess of blasts (RAEB). The age incidence is similar to that of RA. There is always some degree of cytopenia affecting two or more of the bone marrow series. The peripheral blood shows conspicuous abnormalities in all three cell lines. Dysgranulopoiesis, in contrast to RA, is a common feature. There may be a small proportion of circulating blast cells (<5%). The bone marrow is hypercellular and shows varying degrees of either granulocytic or erythroid hyperplasia. There is always evidence of dysgranulopoiesis, dyserythropoiesis and/or dysmegakaryopoiesis; ringed sideroblasts may be seen. The percentage of blasts (type I and II) in the bone marrow, by definition, is equal to or greater than 5% up to 20%. There is almost always evidence of maturation in the granulocytic series to promyelocytes and beyond.

(4) Chronic myelomonocytic leukaemia (CMML). The defining feature is the presence of an absolute monocytosis (over 1 x 10^9/l). Often this is associated with an increase in mature granulocytes with or without evidence of dysgranulopoiesis (e.g. hypogranular and/or Pelger forms). The percentage of blasts in the peripheral blood is less than 5%. The bone marrow resembles that of RAEB but may show a significant increase in monocyte precursors (promonocytes) often with fewer than 5% of blasts. In some patients with moderate monocytosis and bone marrow features identical to those of RAEB, the percentage of blasts may be higher than 5%, up to 20%.

(5) RAEB in transformation. This type includes cases of cytopenia in patients of any age, often with symptoms of brief duration, which do not strictly fit into either of the above categories or in any of the AML types (M1–M6). The haematological features are similar to those of RAEB but include any of the following: (i) 5% or more of blasts in the peripheral blood, (ii) more than 20% and up to 30% of blasts (types I and II) in the bone marrow, (iii) presence of unequivocal Auer rods in granulocyte precursors.

Modification of the definition of AML

The recognition of the new category of RAEB in transformation made it necessary to modify
slightly the minimum requirement of bone marrow blasts compatible with a diagnosis of AML. The original FAB minimum required the sum of blasts and promyelocytes to be at least 50%, and the percentage of blasts did not have to be specified. In the present revision more than 30% of blasts of types I and II (see above), with or without Auer rods, will suffice for the diagnosis of any of the M1–M6 types. The limit of 30% had already been applied in M6. The maturation features for defining the various types of AML remain unchanged.

Special features of secondary MDS
AML induced by radiotherapy and/or chemotherapeutic agents often evolves through a MDS phase which may be indistinguishable from the conditions described above, particularly RAEB. Some additional features may be observed in the bone marrow: (i) hypocellularity, (ii) increased fibrosis with hypercellular marrow, and (iii) frequent demonstration of numerous ringed sideroblasts. As a result of (i) and (ii) aspirates may be difficult to obtain. There may also be a higher proportion of blasts in the peripheral blood than would be expected from the percentage in the bone marrow. Abnormal and immature megakaryocyte precursors are often seen in peripheral blood and bone marrow films.

Conclusions and implications for future work
It has been apparent in the last 5 years that the two broad categories of MDS described in the first proposals of our group (Bennett et al, 1976), RAEB and CMML, did not cover the whole range of this heterogeneous group of conditions. In addition there were no well-defined features of established prognostic significance or which indicated progression to AML. Features intermediate between RAEB and AML (M2) have led to diagnostic difficulty in some cases. We hope that the definitions for each variant within the haematological spectrum of MDS that we now present will make it possible to assess the prognostic significance of some features, like the proportion of bone marrow or peripheral blood blast cells, presence of Auer rods, degrees of dyserythropoiesis, etc. The group felt that the systematic recording of the features outlined in Table I may help in the diagnosis of MDS and may, in future series, help determine their diagnostic and/or prognostic value. In addition, the application of the new definitions in the related disciplines of cytogenetics (Second International Workshop on Chromosomes in Leukemia, 1980) and bone marrow culture (Spitzer et al, 1979) may lead to the recognition of correlations which might help in patient management. The proposed criteria for MDS, including the revised minimum percentage of blasts (over 30%) used for the definition of AML, should be applicable to all cases presenting with MDS and should greatly reduce the number of unclassifiable cases. The term RAEB in transformation has been introduced to define a group of patients who in the experience of the group appear to have a shorter course of their MDS and a higher risk of evolving more rapidly into AML than either RA or RAEB cases. The FAB group recognizes that the diagnosis of some difficult cases may not always be possible by morphology alone and that just as the characterization of leukemic cells has been greatly improved by new techniques in recent years, it also expects similar advances in the MDS.
The importance of MDS relates to three features: (1) the development of AML in a proportion of cases, (2) the presence of marked features of MDS in some de novo AML, which may indicate a subclinical MDS phase, and suggests the possibility of an aetiology different from that of the more common de novo AML without MDS features, and (3) the frequency of a preleukaemic phase with florid features of MDS in the development of secondary AML.

REFERENCES


