an atlas of Bone-marrow Pathology

by M. C. G. Israëls

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"*. . . hierfür ist eben ausschliesslich die Farbenanalyse zu verwerthen, durch dies es mit Leichtigkeit gelingt, die einzelnen Körnungen von einander zu trennen und sie und ihre Träger bis zu ihren Ursprüngen, den blutbereitenden Organen, zu verfolgen.*" (*It is here that analysis by staining is of peculiar value, by its use we can easily succeed in distinguishing one granule from another and in following them, and the cells that contain them, to their source in the blood-forming organs.*)

PAUL EHRLICH, *Z. f. klin. Med.*, 1879, I, 553.

"*Essential for any conception of the cell is that it is no static system. It is a material system and that to-day is to say an energy system. Our conceptions of it fail if not dynamic. It is a scene of energy-cycles, suites of oxidation and reduction, concatenated ferment actions. It is like a magic hive the walls of whose chambered spongework are shifting veils of ordered molecules and rend and renew as operations rise and cease.*"—C. S. SHERRINGTON, "Man on his Nature."

INTRODUCTION

THIS volume sets out to provide an authoritative, accurately illustrated account of the bone-marrow in health and disease for the guidance of physicians and pathologists. Other atlases have appeared before. The better ones were German texts published before 1941[10]; they are now difficult to obtain and unrepeatable. Blackfan and Diamond's excellently produced atlas[1] deals only with the blood in children and therefore does not attempt to deal with marrow. The Sandoz "Atlas of Hæmatology"[10] shows how much colour photomicrography has progressed, but also shows that the limitations, unavoidable when using an objective with a depth of focus less than the thickness of the cell, have not been overcome. Custer's excellent Atlas of photomicrographs[4] is mainly concerned with histological sections. The latest atlas by McDonald, Dodds and Cruickshank[7a] is a collection of first-class colour photomicrographs showing mostly typical blood and marrow fields and some individual cells, together with histological specimens showing changes in marrow, spleen, liver and lymph glands; there are also some phase-contrast photographs. The arrangement of the plates makes it more suitable for teaching than reference in the laboratory, but the reproduction of nuclear detail, though sometimes still blurred, is definitely better than in previous similar works.

The work on bone-marrow biopsy and pathology was originally started because, as a physician, I found the diagnosis of patients with blood diseases, especially those with macrocytic anæmias, unsatisfactory. Bone-marrow biopsy was not then undertaken by clinical pathologists, so the physician had to become his own pathologist, and this book is the essence of the experience so gained. Even today, when bone-marrow biopsy is a commonplace laboratory investigation, the physician in charge of patients with blood diseases, will be well-advised to study the bone-marrow smears and sections for himself and not be guided solely by the written reports. Bone-marrow puncture is important as an aid to diagnosis, and so to prognosis and treatment. But to use it for these purposes, morphology and functional pathology have had to be closely studied. Nomenclature has been kept relatively simple and well-established terms are used.

The book is divided into two sections. The first (Chapters 1–3) deals with technique and the identification of individual cells; the second (Chapters 4–7) deals with the changes in the marrow picture found in the different diseases, the interpretation of the various types of marrow picture, and provides tables for differential diagnosis. Throughout, the necessity is insisted upon of allowing for the variation inevitable in any biological investigation, especially when studying cells that are progressing along continuous lines of development. The first part is mainly of interest to the clinical pathologist; the second is chiefly concerned to show the physician what to expect in various diseases and how to interpret the

pathologist's findings. The illustrations are mostly from bone-marrow smears obtained by aspiration techniques: one plate (14) shows histological specimens obtained by the trephine method.

The Atlas has been designed in the hope that it will persuade more pathologists to undertake bone-marrow studies, and more physicians to ask for bone-marrow investigation in cases where it can help—and to ask in the early stages of the investigation before treatment has covered up valuable clues.

The accurate illustration of both individual cells and of typical changes in disease has been obtained only by close co-operation between author, artist, and publisher. Without Miss Davison to paint the original plates this work could not have been contemplated. Without the patience of Messrs. Heinemann in the face of my insistence on high-grade accuracy, the originals could not have been properly reproduced. To Miss Davison and Messrs. Heinemann, I am therefore very specially indebted. The photomicrographs on Plate 14 have been prepared by my colleagues Dr. E. J. Watson-Williams and Dr. John MacIver; to them, and to others who have sent me good material, I am very grateful.

M. C. G. I.

Manchester, 1966.

CONTENTS

CHAPTER		PAGE
	INTRODUCTION	xi
I.	NOMENCLATURE AND DEVELOPMENT OF THE BLOOD CELLS	1
II.	TECHNIQUE	7
III.	THE MORPHOLOGY OF THE MARROW CELLS	12
IV.	THE NORMAL BONE-MARROW PICTURE	24
V.	BONE-MARROW CHANGES IN DISEASE	27
VI.	BONE-MARROW IN INFANTS AND CHILDREN	40
VII.	DIFFERENTIAL DIAGNOSIS AND INDICATIONS FOR MARROW BIOPSY	42
	BIBLIOGRAPHY	49
	INDEX	82

LIST OF PLATES

PLATE		PAGE
1.	HÆMOCYTOBLASTS, PRO-ERYTHROBLASTS, GIANT ERYTHROBLASTS	55
2.	NORMOBLASTS	57
3.	MEGALOBLASTS	59
4.	GRANULOCYTES	61
5.	EOSINOPHILS AND MONOCYTES, RETICULUM CELLS	63
6.	LYMPHOCYTES AND PLASMA CELLS	65
7.	MISCELLANEOUS CELLS, MEGAKARYOCYTES	67
8.	NORMAL MARROW, INFANT AND ADULT; NORMOBLASTIC HYPERPLASIA	69
9.	MEGALOBLASTIC HYPERPLASIA	71
10.	IRON-DEFICIENCY ANÆMIA, BEFORE AND AFTER TREATMENT; POLYCYTHÆMIA VERA; ACUTE AGRANULOCYTOSIS	73
11.	LEUKÆMIAS, MYELOID AND LYMPHATIC	75
12.	MONOCYTIC LEUKÆMIA, INFECTIOUS MONONUCLEOSIS, MYELOMATOSIS, GAUCHER'S DISEASE	77
13.	TRANSITIONAL MEGALOBLASTS, ABNORMAL RETICULUM CELLS (RETICULOSIS AND LEUKÆMIA), TUMOUR CELLS	79
14.	SPECIMENS FROM GARDNER TREPHINE BIOPSY	81

ATLAS OF BONE-MARROW PATHOLOGY

CHAPTER I

NOMENCLATURE AND DEVELOPMENT OF THE BLOOD CELLS

A SCHEME of nomenclature for the cells of the blood and bone marrow has not yet been generally agreed on. Any descriptive account must therefore start with an explanation, as clearly defined as possible, of the nomenclature to be adopted and of the scheme of development of the blood cells that is followed. The history of hæmatology records many schemes of development and nomenclature; they will not be described here and those who are interested can find all the more important schemes admirably set out in Gradwohl's text-book.[5] The names given to cells representative of stages of development have been adopted for descriptive, classificatory, or purely historical reasons. "Polymorphonuclear" adequately describes the typical nucleus of these leucocytes. The suffix "blast" is used to denote a primitive, relatively undifferentiated cell, and the name "myeloblast" indicates the earliest differentiated stage of the granulocytic leucocytes which, for historical reasons, are still known as the "myeloid" series. The name "megaloblast" is an example of an historical name that has been attached to various stages of red blood-cell development, but originally was simply a large hæmoglobinated nucleated red cell. It must never be forgotten that the names do not represent absolutely distinct cells; they are convenient terms for distinguishable stages of cell development and these stages merge imperceptibly into those less or more mature. Sometimes the criterion that can be adopted is quite clear, like the appearance of granules in the cytoplasm or segmentation of the nucleus. But more often there is no such clear distinction and though the majority of cells will be placed by different observers in the same group, there are a minority that will be placed in different but adjacent groups by different observers. For instance, the promyelocyte has a large nucleus, basophilic cytoplasm, and red-staining, undifferentiated granules; the neutrophil myelocyte has a relatively smaller nucleus, less basophilic cytoplasm, and granules of a more orange shade: clearly cells will occur in the border line between these stages. Fortunately these difficulties do not significantly affect the over-all estimate of the bone-marrow picture—and it is this over-all estimate that is important in the clinical application of blood and bone-marrow changes in disease.

Most workers in medicine, using human material, have adopted a modified polyphyletic scheme in which it is agreed that, though most of the cells are derived from a common precursor, for the greater part of their life-history as seen in the

hæmopoietic organs, the main classes follow separate lines of development without interchange. The scheme followed here is set out in Fig. 1 which shows normal cell development. The nomenclature of the groups has been arranged so that differentiation between them has clinical significance: thus the "metamyelocyte" group comprises all forms between myelocytes and segmented polymorphonuclears including "band" forms and "staff" cells, since there is little to be gained—from a clinical point of view—in counting them separately. For the same reason the hæmohistioblast *vs.* hæmocytoblast controversy finds no place here. Deriving from a common hæmocytoblast, separate lines of maturation are shown for granulocytes from myeloblast to polymorphonuclear leucocyte, for erythroblasts from pro-erythroblast to erythrocyte, for lymphocytes and for monocytes. In conformity with modern views, the plasma cell series are shown with a line of their own[8, 59] maturing from an early form, the plasmablast, that derives separately from reticulo-endothelium; the evidence for this direct derivation is only tentative, but the scheme emphasises the fact that plasma cells are a separate class of cell and not modified lymphocytes. The megakaryocytes are represented deriving from a primitive "blast" form as shown by Barta,[16] Dameshek and Miller,[26] and others. Almost all the cells have been found from time to time in normal marrow; the exceptions are lymphoblasts, monoblasts, and plasmablasts and they are only seen in conditions where the particular series is hyperplastic, e.g. lymphatic leukæmia, monocytic leukæmia, and glandular fever, respectively. In addition to the cells shown in Fig. 1 there are purely pathological cells, like myeloma cells and pathological monocytes; these will be described under their respective sections in Chapter III.

The classification of erythroblasts has been a specially controversial issue centering mainly round the separation of the megaloblasts. Accounts of the issues involved in this problem have been given by the author,[41, 44] and by O. P. Jones,[50] and to them the reader, who wishes for historical detail, is referred. The present view is that megaloblasts are erythroblasts with a characteristic nuclear abnormality produced by the absence, relative or complete, of the growth factors vitamin B_{12} (cyanocobalamin) or folic acid, and the degree of abnormality to some extent varies with the degree of deficiency. This megaloblastic pattern can be seen in the erythroblasts in early embryonic life before the stage of hepatic erythropoiesis. The classification used here was proposed by the author in 1951[6] and is set out schematically in Fig. 2. It shows the megaloblasts breaking away from the normal line of development after the pro-erythroblast stage. The various subsequent stages of both normoblast and megaloblast development are divided by criteria depending on changes in nuclear structure and not on whether the cytoplasmic staining is basophilic, polychromatic, or orthochromatic. It has been shown elsewhere[43] and is again emphasized in Chapter III that such variations only indicate the stage of hæmoglobinization, and it is possible to see erythroblasts at the same stage of development with any of these cytoplasmic tints; this is shown in the hyperplastic normal group in Fig. 2.

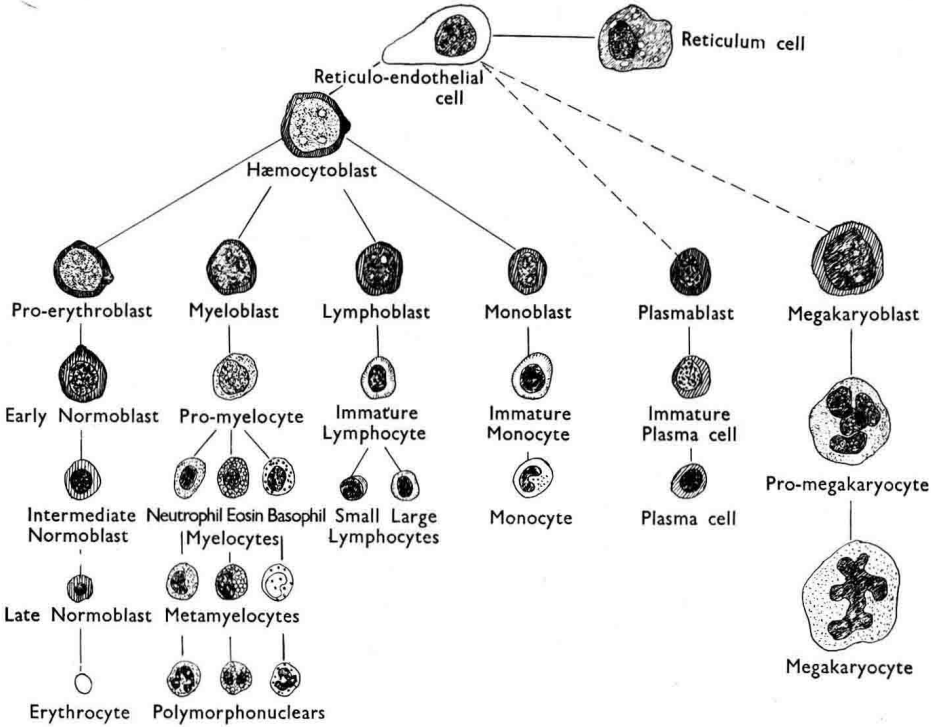


FIG. 1. Development Scheme for Normal Blood and Bone-marrow Cells.

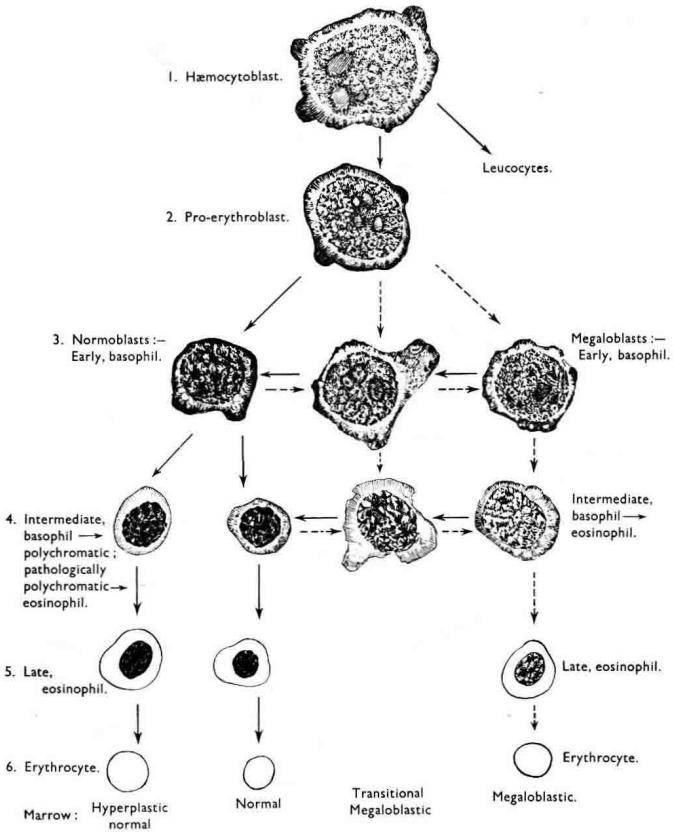


FIG. 2. Development of Normal and Pathological Erythroblasts.

In order to assist those used to other schemes, Table 1 sets out the nomenclature of the red-cell types adopted by representative workers in various countries compared, so far as is possible, with that used in this Atlas; comparison can be only approximate when the authors have used cytoplasmic tint-variations as criteria of maturity. For leucocytes there is more general agreement and no such table is necessary. The only comprehensive alternative is that proposed by Osgood and Ashworth,[8] but this has gained only limited acceptance. The nomenclature adopted throughout the present Atlas is briefly as follows; fuller descriptions of individual cell stages are given in Chapter III.

HÆMOCYTOBLAST: the large, basophilic precursor of erythroblasts, granulocytes, lymphocytes, and monocytes.

ERYTHROBLAST: a generic term for all differentiated nucleated red-cell types—pro-erythroblasts, normoblasts, and megaloblasts.

PRO-ERYTHROBLAST: the earliest differentiated erythroblast, the basophilic precursor of both normoblasts and megaloblasts.

NORMOBLAST: the normal stages of red-cell development from the pro-erythroblasts; divided into early, intermediate, and late stages according to the differentiation of the nucleus.

MEGALOBLASTS: a pathological group of nucleated red cells developing from the pro-erythroblast, possessing a distinctive nuclear pattern, and divided into early, intermediate, and late stages according to the differentiation of the nucleus.

MYELOBLAST: the basophilic, non-granular, most primitive member of the granulocyte series.

PROMYELOCYTE: the earliest granulated cell of the granulocyte series, the granules being not yet differentiated.

MYELOCYTE: the early differentiated granulocyte of bone-marrow, appearing in the three forms with neutrophil, eosinophil, or basophil, granules.

METAMYELOCYTE: intermediate forms between myelocyte and cells with lobed nuclei; the cytoplasm has the brownish tint of the mature granulocyte but the nucleus, though smaller than that of the myelocyte, is not segmented; "band forms" and "staff cells" are included in this group. The usual three differentiated forms are distinguished.

POLYMORPHONUCLEAR LEUCOCYTE: granulocytes with segmented nuclei and neutrophil, eosinophil, or basophil, granules.

LYMPHOBLAST: the basophilic precursor of the lymphocytes.

LYMPHOCYTES: divided into *immature* with an intermediate nuclear pattern, and small and large *mature* types.

MONOBLAST: the basophilic, non-lobed precursor of the monocytes.

MONOCYTES: divided into *immature* forms with unlobed nucleus, *mature* with typically folded and lobed nucleus, and *pathological* forms with extreme variation of nuclear shape.

TABLE 1

Nomenclature of Erythroblasts according to Different Authors

Present Author	Ferrata & Negreiros[21]	Ducie & White[15]	Leitner[7]	Gradwohl[5]	Blackfan & Diamond[2]
Hæmocytoblast	S.	S.	N.D.	N.D.	N.D.
Pro-erythroblast	N.D.	N.D.	N.D.	Mgbl.	N.D.
Normoblast, early	Pro-Ebl.	Pro-Nbl.	Pro-Nbl.	Mcbl.	Mgbl.
do., intermediate	Bas. Ebl.	Bas. / Poly. } Nbl.	Bas. Mcbl.	} Nbl.	Ebl.
do., late	Poly. Ebl.	Pyknotic } Nbl.	Poly. and Orth. Nbl.	} Nbl.	Nbl.
Megaloblast, early	Pro. Mgbl.	Pro. Mgbl.	Pro. Mgbl.	} N.D.	} N.D.
do., intermediate	Bas. Mgbl.	Bas. / Poly. } Mgbl.	Mgbl.		
do., late	Poly. Mgbl.	Pyknotic } Mgbl.	"Alter" Mgbl.		

Present Author	Wintrobe[13]	Sandoz Atlas[10]	Schulten[11]	Whitby & Britton[12]	Bessis[7]	Custer[4]
Hæmocytoblast	N.D.	N.D.	S.	S.	N.D.	N.D.
Por-erythroblast	N.D.	N.D.	S.	S.	N.D.	Pro-ebl.
Normoblast, early	Pro-Nbl.	Pro-Ebl.	Mcbl.	} S.	Pro-Nbl.	Early Ebl.
do., intermediate	Bas. Nbl.	Bas. / Poly. } Nbl.	Mcbl.		Bas. / Poly. } Nbl.	Intermed.
do., late	Poly. and Orth. Nbl.	Orth.	Nbl.		Acid.	Ebl. / Late Ebl.
Megaloblast, early	Pro-Mgbl.	Pro-Mgbl.	} Mgbl.	} S.	Pro-Mgbl.	Pro-Mgbl.
do., intermediate	Bas. / Poly. } Mgbl.	Bas. / Poly. } Mgbl.			Bas. / Poly. } Mgbl.	Bas. / Poly. } Mgbl.
do., late	Orth.	Orth.			Acid.	Acid

KEY. S. = Same name. N.D. = Not distinguished. Ebl. = Erythroblast.
Mgbl. = Megaloblast. Mcbl. = Macroblast. Poly. = Polychromatic.
Bas. = Basophilic. Orth. = Orthochromatic. Nbl. = Normoblast.

PLASMABLAST: a primitive cell with a nucleus showing characteristics of the plasma-cell series.

PLASMA CELLS: divided into *immature* large forms with large nucleus and the more typical *mature* forms with eccentric nucleus of small size; atypical forms appear in myelomatosis.

MEGAKARYOBLAST: primitive precursor of megakaryocytes having a characteristic unsegmented nucleus.

MEGAKARYOCYTES: divided into *immature* forms with segmented nucleus but non-granular cytoplasm, *mature* large forms with granular, platelet-forming cytoplasm, and the so-called "Lymphoid" form commonest in thrombocytopenic purpura.[26]

RETICULUM CELLS: a group of phagocytic cells with typically ill-defined structure. Primitive forms of reticulum cells are known with large well-patterned nuclei like other primitive cells, but with a characteristically coarse pattern.

RETICULO-ENDOTHELIAL CELLS: the theoretically multipotent cell of the bone-marrow sinuses; not normally seen in marrow smears.

CHAPTER II

TECHNIQUE

Aspiration Methods

VARIOUS, often quite elaborate, types of apparatus have been devised for obtaining marrow by aspiration. The Salah needle is recommended here: it is a fairly wide-bore (1·5 mm.) needle with a heavy top and an internal stylet (Fig. 3a): the length of the actual needle part is 4 cm. and a movable guard is provided. For carrying out a sternal puncture, the following apparatus is required:

Salah needle, dry-sterilized in hot-air oven at 160°C for 1 hour.

One c. cm. "tuberculin" syringe with well-fitting piston, thoroughly dried by washing through with acetone.

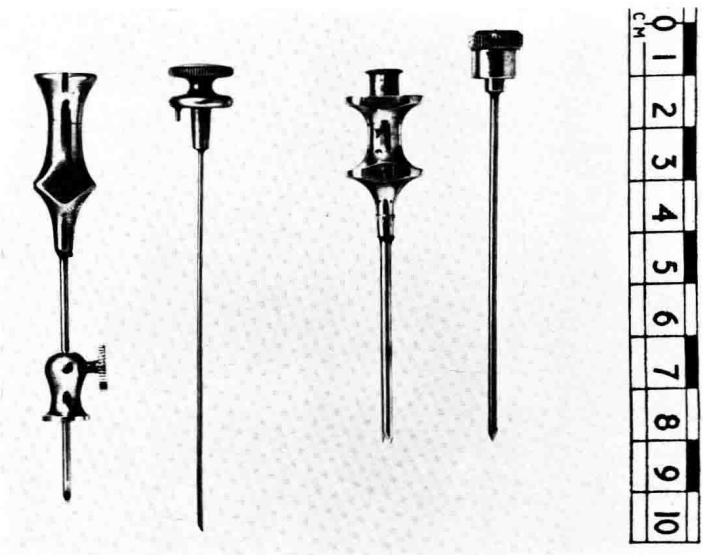
Clean slides for smears and coverslip for spreading.

Glass thimble containing a little dried pot. oxalate.

Local anæsthetic (2 per cent procaine), 2 c. cm. syringe, and needle.

The most useful site is the centre of the manubrium; the surface marking is halfway along the vertical line joining the centre of the suprasternal notch to the horizontal line joining the upper borders of the second costal cartilages. The skin is sterilized, and the area anæsthetized with a minimum (1 to 1·5 c. cm.) of local anæsthetic, care being taken to inject the periosteum over the sternum. The guard on the Salah needle is adjusted to give about 0·5 cm. entry into the sternum, allowance being made for the depth of the subcutaneous tissue over the bone—this can be estimated when injecting the anæsthetic. The needle is inserted vertically into the anæsthetized area as far as the bone surface, and then, with a steady twisting motion, pushed through the anterior sternal plate into the marrow cavity. In some patients the resistance of the bone suddenly gives way when the cavity is reached—and then there is no difficulty. But in many patients there is only a relative lessening of resistance to indicate entry, and in these cases a trial with the suction syringe should be made before pushing the needle any further in. When the marrow cavity is reached, the stylet is withdrawn and the dry syringe fitted into place. Gentle suction is applied and 0·2 c. cm. of marrow fluid—not more—is withdrawn; this causes a slight suction pain in most patients. The syringe is detached and the smears are made *at once*. By varying the size of the drop, different thicknesses of smear can be made, but they should all be relatively thin as thick smears are valueless. If it is desired to count the cells in the marrow sample, the rest of the fluid is ejected into the oxalated thimble. Marrow that has been mixed with anti-coagulant should *never* be used for making smears; the cytology is definitely rendered poorer. When these operations are concluded, the

needle is withdrawn and firm pressure exerted on the site for a minute or two and this usually stops all bleeding; finally, a dressing is applied. Sternal puncture can



(a) (b)

FIG. 3. Marrow Biopsy Apparatus.
(a) Salah marrow puncture needle.
(b) Gardner marrow trephine.

be done conveniently in the Out-patient's clinic; it is wise to make the patient rest for half an hour before leaving the clinic to be sure that bleeding has completely ceased.

The smears are allowed to dry in air and are then stained by the following method:

Jenner's stain (0·3 per cent in methyl alcohol), undiluted, for 3 minutes.

Dilute, on the slide, to about 50 per cent with buffer solution and stain for 1 minute.

Pour off, and without washing, add Giemsa stain (Gurr's R66), 10 drops to 5 c. cm. buffer solution, and stain for 5 minutes.

Wash off rapidly under the tap, blot, and dry in air.

(*Notes.* The buffer solution is of pH 6·4 and is made up as follows:

Pot. acid phosphate	2·7231 grams.
N/10 Na OH	64 c. cm.
Distilled water to	1,000 c. cm.

Check with brom-thymol-blue capillator (B.D.H.).

This buffer suits the stains named, but optimum pH may be slightly different for different batches of Giemsa stain and should be checked; if the result is too blue, the buffer is too alkaline, if the red cells are bright red but the leucocyte nuclei pale blue, the buffer is too acid.

The Giemsa stain is obtained from G. T. Gurr, 136 New King's Road, London, S.W.6.)

Sometimes very cellular marrow, as in chronic myeloid leukæmia, requires a stronger Giemsa solution, 20 drops to 5 c. cm. buffer solution; for this reason it is inadvisable to stain all the slides at once.

The smears need not be covered with a cover slip and the stain remains satisfactory for several years in temperate climates. If a cover slip is to be applied, one of the synthetic neutral materials like Cædax (Bayer) or Xam (Gurr) should be used and not Canada Balsam.

For a differential count, at least 500 cells should be counted; the best areas are usually near the edges at the thin end of the smear.

The above description is of the author's technique; many others have been proposed at different times. Needles with large, detachable handles, or the use of a small mallet to get the needle through the bone, are unnecessary and merely frighten the patient; one hand-power is enough in almost all cases. Some workers prefer to insert the needle into the cartilage between the manubrium and the gladiolus, and then push it upwards at an angle into the manubrial cavity. This is quite satisfactory but offers no special advantages. Occasionally no marrow is obtained from the manubrium; the gladiolus, at the level of the 3rd costal cartilage, should then be punctured.

For children, iliac crest puncture is preferable, and for this a handled instrument like that designed by R. C. Hill[36] is useful because it allows better directional control and can be fitted with needles of different sizes. The site used is the thickened area of the crest just behind the anterior superior iliac spine. The needle is pushed through the crest in a plane at right angles to the upper surface until a lessening of resistance is felt. Sometimes 0·2 ml. of marrow is easily aspirated, sometimes less, and it may be necessary to withdraw the needle and