Pathology of the Bone Marrow

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Gustav Fischer Verlag
Stuttgart · New York

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With 230 Figures and 140 Tables

Gustav Fischer Verlag · Stuttgart · New York
1984

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Gustav Fischer Verlag GmbH & CoKG, Wollgrasweg 49, D-7000 Stuttgart 70
(Register HR Nr. A 7591/HR B 4976). Stuttgarter Volksbank 45 290 (BLZ 600 901 00), Deutsche Bank 80 20 000 (BLZ 600 700 70), Postscheck Stuttgart 135 56-709 BLZ 600 100 70).

For USA and Canada:
Verlag Chemie International Inc., 303 N.W. 12th Avenue, Deerfield Beach, Florida 33441

CIP-Kurztitelaufnahme der Deutschen Bibliothek
Pathology of the bone marrow / ed. by Karl Lennert and Klaus Hübner.
Stuttgart ; New York : Fischer, 1984.
ISBN 3-437-10935-9 (Stuttgart)
ISBN 0-89574-195-4 (New York)
NE: Lennert, Karl [Hrsg.]

Satz und Druck: Calwer Druckzentrum GmbH, Calw
Einband: Großbuchbinderei H. Koch, Tübingen
Printed in Germany

ISBN 3-437-10935-9
ISBN 0-89574-195-4 NY

Preface

In the 19th century and up into the 1920's the pathomorphology of the bone marrow was the domain of pathologists and was generally confined to postmortem diagnoses. After the sternal puncture technique had been introduced at the end of the 1920's, bone marrow cytology became a new clinical instrument for haematological diagnoses. Histological diagnoses on bone marrow *biopsies* did not come into practice until the 1950's. New techniques for embedding bone marrow specimens in plastic (BURKHARDT, BLOCK) and refined methods of paraffin embedding (DUHAMEL, RYWLIN, SCHLEICHER), including those suitable for histochemical stainings (SCHAEFER), were the basis of many investigations. Several monographs and numerous other publications helped to spread the use of those techniques. Recently, such histological studies have revived discussion of the pathology and classification of chronic myeloproliferative diseases (GEORGII, BURKHARDT). The pathology of bone marrow transplantation and malignant non-Hodgkin's lymphomas also had to be re-evaluated.

There has been a gigantic increase in knowledge in recent years. Hence it appeared to be worthwhile to choose the pathology of the bone marrow as the main theme of the 67th Meeting of the German Society of Pathology (May 1983) and, so to say, make an inventory. Most of the papers presented at the meeting have been translated into English for this monograph. We thank all the authors not only for their valuable contributions to the topic but also for the trouble they took to translate their papers from German into English. Mrs. MARTHA SOEHRING deserves our gratitude for language editing of the manuscripts and Dr. G. H. GRIESSER for preparing the subject index. Finally, we thank the publishers, especially Mr. B. VON BREITENBUCH, for the prompt printing and excellent appearance of this book.

Kiel and Frankfurt, August 1984 K. LENNERT · K. HÜBNER

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Technical Aspects

Introduction

K. LENNERT[1]

In the twenties and thirties no pathologist seriously dealt with bone marrow. Thus clinical hematology took command by introducing the sternal puncture technique and developing it to ever greater diagnostic perfection. The zenith of this development was a monograph on human bone marrow by ROHR (1939, 3rd Edition 1960).

After World War II we had to start all over again, i. e., resume where MAXIMOW had left off, as WIENBECK (1938) had done just before the War. The only way to do this was to compare bone marrow smears with sections. Indispensable prerequisites for such comparisons were optimum fixation (Zenker-formalin method of MAXIMOW) and equivalent stains for sections and smears (Giemsa or analogous methods). With these techniques it was also possible to make exact cytologic diagnoses on autopsy material.

There was only one factor left to heed, viz.: the cells in smears change during the preparation procedure and subsequent drying. The spherical form of a cell and especially its nucleus becomes a flat disc, in which the nuclear chromatin is condensed and the nucleoli are often much less prominent (Figs. 1 and 2).

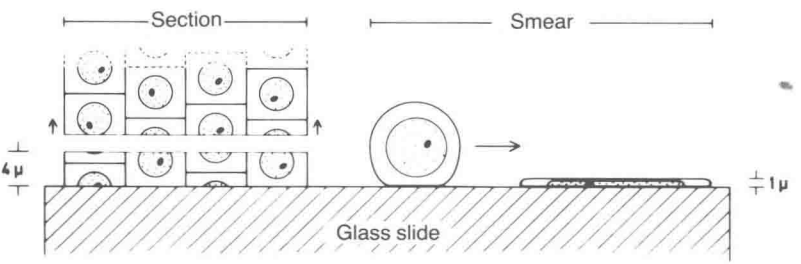

Fig. 1: Fundamental difference in demonstration of cells between section and smear. (From LENNERT, 1972)

It was thus possible to recognize the cells of smears in sections and to make cytologically founded diagnoses on bone marrow sections (LENNERT, 1952). The article published on this subject was hardly noted by clinicians. Consequently, the general impression, at least in Germany, was that the pathologic-anatomic evaluation of bone marrow did not become possible until a new technique for embedding in plastics was developed (BURKHARDT, 1956).

Now a variety of methods are available for examining bone marrow (Table 1). The advantages and disadvantages of most of these methods will be evident from the following chapters. A few new techniques that promise to be useful in diagnosis and research will also be presented.

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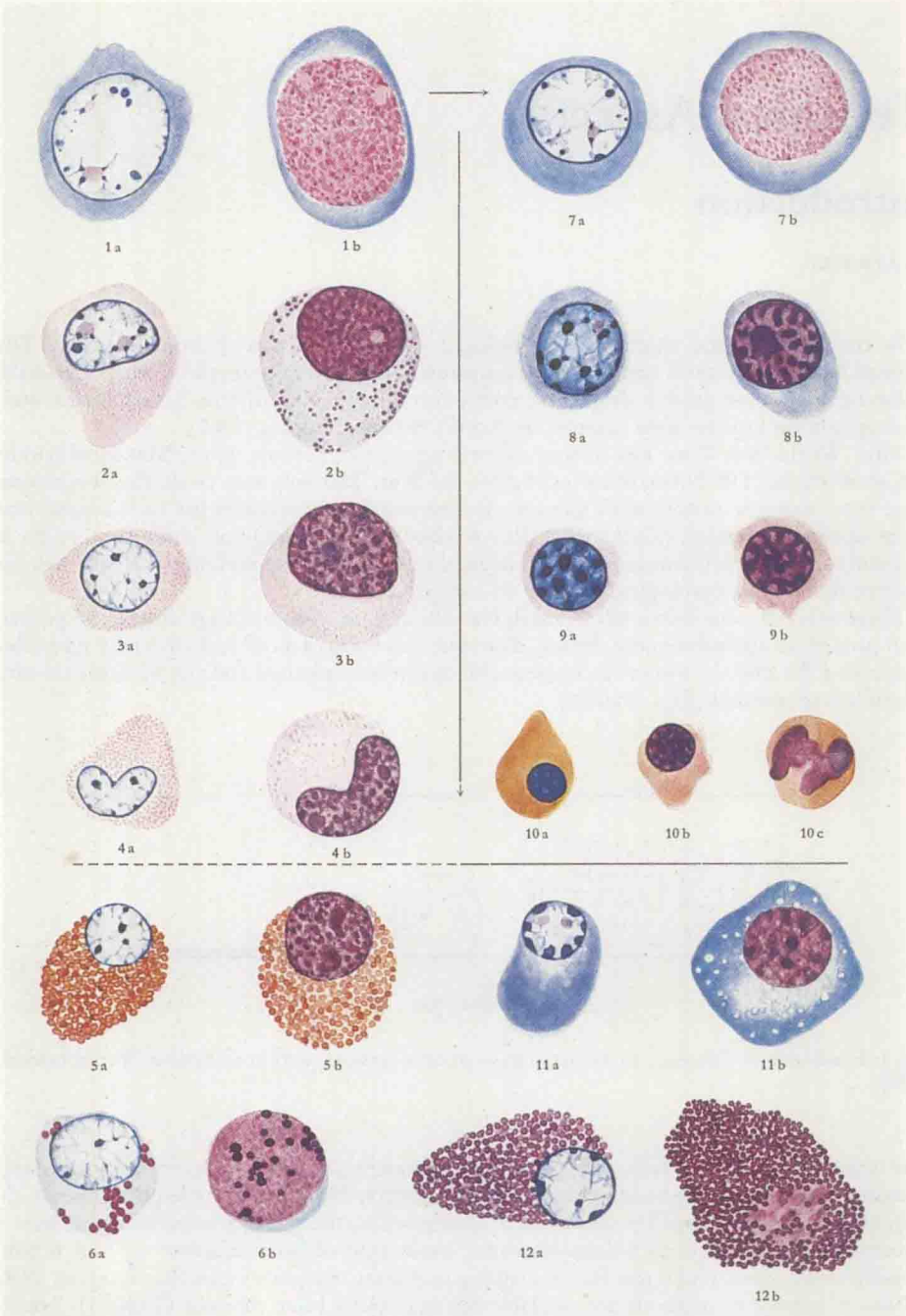

Fig. 2: Comparison of the most important bone marrow cells in sections (*a*, left cell of each pair) and smears (*b*, right cell of each pair). (From LENNERT, 1952)
1, proerythroblasts. 2, promyelocytes. 3, neutrophil myelocytes. 4, neutrophil metamyelocytes. 5, eosinophil myelocytes. 6, basophil myelocytes. 7, macroblasts. 8, basophil erythroblasts. 9, polychromatic erythroblasts. 10, orthochromatic erythroblasts (normoblasts). 11, plasma cells. 12, mast cells.

Table 1: The most important methods for examining bone marrow.

I. Sternal puncture

1. Smears ± cytochemistry
2. Embedding of small marrow specimens in paraffin

II. Trephine biopsy of iliac crest

1. Embedding in paraffin ± histochemistry after decalcification
2. Embedding in plastic without decalcification
3. Re-embedding of paraffin-embedded tissue in plastic after decalcification, when necessary
4. Frozen sections for histochemistry and immunohistology

References

BURKHARDT, R.: Die Myelotomie; eine neue Methode zur kombinierten cytologisch-histologischen Knochenmarksbiopsie. Blut *2*, 267 (1956). – LENNERT, K.: Zur Praxis der pathologisch-anatomischen Knochenmarksuntersuchung. Frankf. Z. Path. *63*, 267 (1952). – LENNERT, K.: Wertigkeit von Schnitt und Ausstrich. Verh. dtsch. Ges. inn. Med. *78*, 196 (1972). — ROHR, K.: Das menschliche Knochenmark. Anatomie, Physiologie und Pathologie, nach Ergebnissen der intravitalen Markpunktion. 3., vollst. umgearb. Aufl. Thieme, Stuttgart, 1960. – WIENBECK, J.: Die Granulopoese des kindlichen Knochenmarkes und ihre Reaktion auf Infektionen. Beitr. path. Anat. *101*, 268 (1938).

Sternal Puncture

F. Heckner[1]

The high value of microscopic investigation of bone marrow for the diagnosis of haematopoietic diseases and a number of further aspects can be considered to be a matter of fact. While the aspiration of bone marrow and the subsequent cytological diagnosis lies almost exclusively in the hand of the clinical haematologist, the pathologist takes an increasing part in the haematological diagnosis owing to the introduction of the technique of bone marrow trepanation. I will be able to demonstrate by comparing investigations in 300 relevant cases that there are not only domains of cytology by aspiration but also main applications of histological diagnosis on biopsies. These two methods do not compete; they rather supplement each other; any other point of view would be unreasonable.

Without doubt the clinical haematologist will apply the aspiration of bone marrow more frequently than the technique of biopsy leading to histological processing. The reason for this is a practical one, considering the lower time consumption and smaller technical effort; on the other hand, there is a large group of clinical cases (e.g. anaemia) for which the cytology by aspiration is entirely sufficient. However, as soon as improper marrow material is extracted or even a punctio sicca is obtained, a biopsy has to be performed applying the usual methods, which are neglected here. Besides, a larger number of indications requires a priori a combined cyto-histological diagnosis.

The examples given in Table 1 give some criteria for the value of both techniques of investigation. While *anaemias* occurring most frequently can be classified by the cytology of aspiration in connection with blood smear, *aplastic anaemia* requires additional histological proof. In cases of *panmyelopathic* diseases with high cellularity, the cytology is superior if *preleukaemic states* with an insignificantly developed myeloblastic clone are concerned. Here histologically false judgements can be made as well as in cases of some *acute leukaemias*, which can be characterized more quickly and better by cytochemistry on smear prepara-

Table 1: Microscopic examination of bone marrow. Value of cytological and histological methods (based on evalutation of 300 cases).

Diagnosis	Cytology	Histology
Anaemia (in general)	+++	Rarely
Aplastic anaemia	+	++
Panmyelopathy	+	+
Preleukaemia	++	(+)/+ (E?)*
Acute leukaemia	+++	Rarely (E?)
Chronic myeloid leukaemia	+	+
Polycythaemia vera	+	++
Megakaryocytic myelosis, myelofibrosis	+/−	+++
Non-Hodgkin's lymphoma		
Chronic lymphocytic leukaemia	+	+
Hairy cell leukaemia	(+)	++
Other types	+/++	++
Plasmacytoma	+++	+ (E)
Hodgkin's disease	−/(+)	+
Metastasis of carcinoma	+	++
Cytochemistry		
Fe	+	+
Other reactions	+++	Rarely

* E = source of error

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tions. Of the *myeloproliferative disorders, polycythaemia, megakaryocytic myelosis* and *especially myelofibrosis* are advantageously examined by histological techniques. For *non-Hodgkin's lymphomas* the cytology can give very good results if the bone marrow has been slightly involved; but a histological diagnosis is always indispensable, especially for *hairy cell leukaemia* which is often recognized in the haematological laboratory merely from a few large hairy cells in the leucocyte concentrate. Surprisingly we find a positive result for the objectification of a *plasmacytoma* by means of the histological method in only 60 % of the cytologically clear cases. The reverse is found for *Hodgkin's disease* in which an infiltration of bone marrow can rarely be recognized. Also *metastases of cancer* in the bone marrow are often recognized histologically; in addition the *micro-structure of the bone and the architecture* of the marrow can be judged only by histological investigations.

Thus changes in the single cell or minute accumulations of pathological cells are mainly and best recognized by cytodiagnosis; interrelations in the micro-structure and alterations leading to punctio sicca, such as fibrosis, aplasia, or closely interlaced proliferations, can be discovered and therefore interpreted correctly only by biopsy, followed by histology. Lucky those clinical haematologists who have qualified pathologists as steady diagnostic consultants!

How to Fix, Decalcify and Stain Paraffin Embedded Bone Marrow Biopsies

H. E. Schaefer*, [1]

The question arising is not of whether, but of how to prepare bone marrow biopsies for paraffin embedding. The paraffin technique has become basically discredited on account of a rather poor cytologic quality. In fact, paraffin sections of decalcified bone marrow stained with Giemsa's solution sometimes appear necrotic due to a loss of basophilic structures. This artificial fading of nuclei, basophilic granules, and cytoplasmic RNA is produced by the hydrolytic action of the acid pH common to most rapid decalcifiers. In combination with simple formalin fixation, acid decalcifying media are particularly detrimental for the general preservation of tissue.

In order to prevent this artifact, bone marrow biopsies should be decalcified at neutral pH by organic chelating agents as recommended by Sreebny and Nikiforuk (1951). For this purpose we prefer a solution of disodium salt of ethylene-diamine-tetraacetic acid (EDTA) buffered by tris-(hydroxymethyl)aminomethane (TRIS) to pH 7.0. This non-acid pH has the additional advantage of a higher solubility of EDTA (Table 2).

Even in the case of decalcification by a neutral chelating agent, formalin-fixed bone marrow subsequently embedded in paraffin tends to produce typical shrinking artifacts seriously interfering with the cytologic analysis of hematopoietic cells. Moreover, under those conditions glycogen will be poorly preserved.

Table 1: Fixation of bone marrow biopsies.

Fixative
2 ml of 25 % glutardialdehyde
3 ml formalin (37 % formaldehyde) and
1.58 g anhydrous calcium acetate are mixed and dissolved in distilled water to obtain a total volume of 100 ml

Procedure
Fresh bone marrow biopsies are placed overnight in this fixative. Biopsy cylinders exceeding a diameter of about 3 mm should be split lengthwise using a sharp microtome blade after one hour of primary fixation in order to facilitate further impregnation of tissue by the fixative

Caution
The final aldehyde concentration of this fixative is very low and may drop due to autooxydation or polymerization. Therefore the solution should be stored at 0 °C for only a few weeks

Table 2: Decalcification of bone marrow biopsies.

Decalcifying medium
10 g of ethylene-diamine-tetra-acetate disodium salt (Merck: Triplex III®) and
3.3 g Tris-(hydroxymethyl)-aminomethane (TRIS, THAM)
are dissolved in distilled water to obtain a final volume of 100 ml. The resulting pH ranges from 7.0–7.2.

Procedure
Fixed biopsy samples are placed in this medium for at least two days (depending on thickness and bone content) at room temperature. To accelerate decalcification a gentle shaking movement of the container is advisable.
Exposure to ultrasound is able to shorten the duration of decalcification to a few hours.

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* With kind support from the Deutsche Forschungsgemeinschaft.

Table 3: Chloroacetate esterase.

Incubation medium
a) 100 ml PBS (pH 7.2) are mixed with
b) 0.5 ml of a solution of hexazotized pararosaniline to obtain pH 6.5–6.8 and
c) 1 ml of substrate solution is finally added.

ad a): Prior to use PBS (phosphate-buffered saline) is obtained by diluting 5 ml of a 20fold concentrated stock solution with distilled water to a total volume of 100 ml. The PBS stock solution can be stored at room temperature without any danger of algal or fungal pollution. It is composed of 29.25 g $Na_2HPO_4 \cdot 2H_2O$, 4.90 g KH_2PO_4, 160.00 g NaCl dissolved in distilled water to give a total volume of 1000 ml.

ad b): A solution of hexazotized pararosaniline is prepared as follows. 50 mg pararosaniline · HCl are completely dissolved in 3 ml 1 N HCl, the vial is cooled from outside with pieces of melting ice and the solution is mixed drop by drop with 0.5 ml 1 M sodium nitrite (0.0345 g $NaNO_2$ dissolved in 0.5 ml H_2O). Effective hexazotation is indicated by a color shift from brown to faint yellow. The resulting solution is ready for use after reacting time of about 5 min or can be stored in small portions deep frozen (–30 °C) for weeks.

ad c): 3.58 mg naphthol-AS-D-chloroacetate are dissolved in a mixture of 0.1 ml Triton X-100® and 0.9 ml dimethyl-sulfoxide. Can be stored for weeks deep frozen (–30 °C).

Procedure
Deparaffinize sections in xylene and hydrate to water through graded alcohols. Place sections in the incubation medium for 1 h at room temperature. Wash in tap water (10 min) and once in distilled water. Nuclei are counterstained with Mayer's hemalum (10 min). Wash in tap water (10 min) and mount in glycerine-gelatine.

Result
Chloroacetate esterase-containing structures (the neutrophilic granulopoietic series, tissue mast cells, occasionally myeloma cells) stain brick red.

To circumvent this latter type of artifact, we have designed empirically a fixative mixture composed of an aqueous solution of 10^{-1} M calcium acetate, glutaraldehyde and formaldehyde at low concentrations. Bone marrow fixed in this medium, decalcified in a neutral EDTA solution and embedded in paraffin under conventional conditions exhibits hardly any shrinkage (Table 1). Sections stained with Giemsa's solution show a distribution of acidophilic and basophilic structures identical with the aspect of bone marrow smears. For example, megaloblasts disclose a prominent ROMANOWSKY (1891) phenomenon comparable to polychromous stained films of bone marrow.

In contrast, most types of resin embedding elicit a shift towards a more or less pronounced affinity for basic dyes due to oxidative reactions exerted by the process of polymerization. In thin polychromous stained paraffin sections the fine granulation of mature megakaryocytes is discernible and even the labile granulation of basophilic granulocytes remains intact and stains metachromatically with Giemsa or toluidine blue. PAS-reactive glycogen is well preserved in the endosteal cells for instance. To obtain complete fixation of glycogen the same calcium acetate-glutaraldehyde-formaldehyde solution may be applied on specimens of parenchymatous organs as well. Because of the relatively slow penetration velocity specific to all fixatives containing glutaraldehyde, tissue samples should not exceed a thickness of very few millimeters. Also the fixation of mucopolysaccharides – e. g. mucins present in metastatic adenocarcinomas – is superior to simple formalin fixation. Lectin-binding sites can be analyzed in metastases of breast carcinomas by the usual cytochemical techniques (KLEIN et al., 1979). Because of a relatively strong background autofluorescence common to all glutaraldehyde-fixed tissues, lectins as well as antibodies are at best labeled by horseradish peroxidase or alkaline phosphatase. Using the PAP technique we have demonstrated intracytoplasmic immunoglobulins in plasma cells or myeloma cells successfully. The neutrophilic series of granulopoiesis (and tissue mast cells) can be identified by the chloroacetate esterase staining reaction (GOMORI, 1953; MOLONEY et al., 1960). This enzyme is resistant to paraffin embedding (LEDER, 1964). The hitherto described techniques have the

Table 4: Tartrate-resistant acid phosphatase.

Incubation medium

a) 100 ml 0.1 M sodium acetate buffer (pH 5.6) are added to
b) 1.0 ml of a solution of hexazotized pararosaniline to obtain a final pH of 5.2 and dissolve in this mixture as substrate
c) 25 mg naphthol-AS-BI-phosphate sodium salt (final concentration: 5×10^{-4} M) and as inhibitor add
d) 150 mg L(+)tartaric acid added to 1.8 ml 1 N NaOH.

ad a): Acetate buffer (pH 5.6) is prepared as follows. 4.8 ml 0.2 M acetic acid (12.0 g glacial acetic mixed with distilled water to a total volume of 1000 ml) are added to 45.2 ml 0.2 M sodium acetate (27.2 g sodium acetate · $3H_2O$ dissolved in distilled water to a total volume of 1000 ml) and are filled up with distilled water to a total volume of 100 ml.
ad b): cf. Table 3 b.

Procedure

Deparaffinize sections in xylene and hydrate to water through graded alcohols. Place sections in the incubation medium for 2.5 h at 37 °C. (Incubation for a longer time – up to 12 h – is advisable only at room temperature.) Wash in tap water (10 min) and once in distilled water. Counterstain with Mayer's hemalum. Wash in tap water (10 min) and cover with glycerine-gelatine.

Result

Osteoclasts stain intensely red. A fainter brown-red staining appears occasionally in phagocytic reticulum cells. The cytoplasm of leukemic hairy cells (if present) discloses a faint reddish tint best seen if counterstaining of nuclei is omitted.

drawback of sporadically false negative results due to the extremely low solubility of the substrate naphthol-AS-D-chloroacetate in water. Therefore we have designed a method with the main advantage of completely dissolving a definite amount of substrate in the aqueous incubation medium by way of a primary solution of substrate in a mixture of dimethyl-sulphoxide and the detergent Triton X-100. This medium remains clear and must not be filtered.

Another enzyme-cytochemical test useful for application on paraffin sections of decalcified bone marrow biopsies is the staining reaction for tartrate-resistant acid phosphatase. This group of acid phosphatase isoenzymes characterizes the neoplastic cells of hairy cell leukemia (Yam et al., 1971), Gaucher cells and the non-storing blood monocytes in Gaucher's disease (Schaefer et al., 1977), all types of macrophages storing ceroid or lipofuscin-like material, sea blue histiocytes and pseudo-Gaucher cells included (Schaefer, 1981), in a more granular type of distribution neoplastic lymphoid cells in prolymphocytic leukemia (Buscard et al., 1976), and in an intermediary type of leukemia ranging between the three categories of hairy cell leukemia, chronic lymphocytic leukemia and lymphoplasmacytoid immunocytoma (Fohlmeister et al., 1981). Of most practical interest is the observation of a high activity of tartrate-resistant acid phosphatase in osteoclasts and mononuclear osteoclastic precursor cells. This enzyme is rather resistant to neutral chelating decalcification and paraffin embedding. To diagnose various types of osteoclastic osteopathies as well as to evaluate quantitatively or morphometrically the density and distribution of osteoclasts, we routinely stain paraffin sections for tartrate-resistant acid phosphatase. Cases of myeloma tending to marked hypercalcemia and accelerated osteolysis due to an activation of osteoclasts are to be identified in this way. An anomalous pattern of intracellular distribution of this osteoclastic enzyme has been reported as diagnostic for Paget's disease (Schaefer, 1980). Even in hairy cell leukemia tartrate-resistant acid phosphatase can be detected in paraffin sections successfully. In hairy cells this enzyme activity is lower as compared with osteoclasts. To visualize this fainter staining intensity, counterstaining of nuclei should be omitted.

Needless to say that a battery of other staining reactions are to be applied on paraffin sections of bone marrow decalcified with neutral chelating agents. Our routine program includes Perls' iron method (Perls, 1867) and silver impregnation of argyrophilic fibers.

References

Buscard, N. A., D. Catovsky, A. Okos, J. M. Goldman, and D. A. G. Galton: Prolymphocytic leukemia: Cell studies and treatment by leucapheresis. Haematol. and Blood Transfusion. Vol. 18, p. 237, Springer-Verlag Berlin, 1976. – Fohlmeister, I., H. E. Schaefer, B. Mödder, K. P. Hellriegel und R. Fischer: Chronische lymphoproliferative Erkrankung unter dem Bild einer Haarzell-Leukämie. Blut *42*, 367–377 (1981). – *Gomori*, G.: Chloroacyl esters as histochemical substrates. J. Histochem. Cytochem. *1*, 469–470 (1953). – Klein, P. J., R. A. Newman, R. Müller, G. Uhlenbruck, P. Citoler, H. E. Schaefer, K. J. Lennartz, and R. Fischer: The presence and significance of the Thomsen-Friedenreich antigen in mammary gland. II. Its topochemistry in normal, hyperplastic and carcinoma tissue of the breast. J. Cancer Res. clin. Oncol. *93*, 209–214 (1979). – Leder, L. D.: Über die selektive fermentcytochemische Darstellung neutrophiler myeloischer Zellen und Gewebsmastzellen am Paraffinschnitt. Klin. Wschr. *42*, 553 (1964). – Moloney, W. C., K. McPherson, and L. Fliegelman: Esterase activity in leucocytes demonstrated by the use of naphthol-AS-D-chloroacetate substrate. J. Histochem. Cytochem. *8*, 200–207 (1960). – Perls, M.: Nachweis von Eisenoxyd in gewissen Pigmenten. Virchows Arch. pathol. Anat. *39*, 42–48 (1867). – Romanowsky, D. L.: On the question of parasitology and malaria. Dissertation No. 38 (in Russian), Imperial Medical Military Academy, St. Petersburg, 1891. – Schaefer, H. E., K. P. Hellriegel und R. Fischer: Vorkommen Tartrat-resistenter saurer Phosphatase in verschiedenen Zelltypen des lymphoretikulären und hämatopoietischen Systems. Blut *34*, 393–397 (1977). – Schaefer, H. E.: Ein atypisches Verteilungsmuster der tartratresistenten sauren Phosphatase in Osteoklasten bei Ostitis deformans Paget – zur pathogenetischen und diagnostischen Bedeutung. Verhandl. dtsch. Ges. Path. *64*, 569 (1980). – Schaefer, H. E.: The role of macrophages in storage diseases. In: Disorders of the Monocyte Macrophage System. Pathophysiological and Clinical Aspects. Edts.: F. Schmalzl, D. Huhn, and H. E. Schaefer. Haematology and Blood Transfusion. Vol. 27, pp. 121–130, Springer, Berlin 1981. – Sreebny, L. M., and G. Nikiforuk: Demineralization of hard tissue by organic chelating agents. Science *113*, 560 (1951). – Yam, L. T., C. T. Li, and K. W. Lam: Tartrate-resistant acid phosphatase isoenzyme in the reticulum cells of leukemic reticuloendotheliosis. New Engl. J. Med. *284*, 357–360 (1971).

Histology of Plastic Embedded Biopsies

R. Burkhardt[1]

Summary

A method of embedding undecalcified iliac crest biopsies in plastic is described. Serial sections of 0.1–30 microns may be obtained. These are virtually free of artifacts, such as shrinkage and swelling, when observed on light microscopy. For routine histology sections of 3 microns are recommended. For optimum results a fixative consisting of methanol, formalin and buffer is used. The embedding medium consists of methylmethacrylate, a softener (Plastoid N) and a catalyst, benzoyl peroxide. After removal of the plastic the sections may be stained by most stains commonly employed in routine histopathology, such as Giemsa, Gomori, Ladewig, PAS, and Berlin Blue. The whole procedure takes four and a half days. This method has been used in our laboratory for the past 18 years with consistently reproducible results. Examples illustrating the advantages of the method for the demonstration of cytologic, interstitial and stromal details are given.

An embedding technique for semithin cutting of undecalcified bone was developed in our laboratory (Burkhardt, 1966 a and b) modified from the method of Boellaard and Hirsch (1959). The combination of the methylic ester of methacrylic acid with polyethyleneglycol as softener, as recommended by these authors, showed some flaws: unequal penetration, artifacts of polymerization especially with fatty tissues, insufficient elasticity when cut, and hygroscopy. We tested a number of fixatives, combinations of different softeners and either methyl-ethyl- or butyl-methacrylate, and numerous catalysts to find the most suitable mixture and the optimal physical conditions for impregnation and polymerization (TeVelde et al., 1977; Bayerlein, 1978). The resulting method has proved itself during the past eighteen years and about 32,000 biopsies. It yields regular results with samples of bone and bone marrow of different sizes and different composition of mineralized tissue, fat and water.

Table 1 shows a protocol of the procedure that may be carried out twice a week for processing about 70 samples. It produces about 700 sections of 3 microns that are stained according to 5 different techniques. The mixtures used for fixation and embedding are indicated in the abbreviations in the table. The following are the intrinsic working principles: uniform fixation in neutral milieu, complete dehydration, and careful soaking with the freshly prepared embedding mixture. To this end the monomeric methyl-methacrylic acid has to be destabilized with aluminum-oxide and mixed with the other ingredients, benzoylperoxide and nonylphenolpolyglycol ester-acetate, before use. The polymerization is quickly started in a hot water bath, and the tissues are transferred to a cooler bath before the polymerization heat rises to the point of creating bubbles. The hardened blocks are as transparent as glass and are not hygroscopic, even when extremely fatty tissue is enclosed. For cutting serial sections a heavy-duty microtome is needed; the fixation and dehydration times can be shortened by automatic fluid exchange. The most tedious and time-consuming part of the preparation of the slides is to avoid folds while mounting the sections on the glass slides that have to be coated with chromic-aluminum-gelatin. The staining procedures may be performed as usual, with only slight modifications that are required by the reduced thickness of the sections. This technique avoids the decalcification of the osseous tissue with its subsequent artifacts, and allows for a cutting range between 0.1 and 30 microns; the optimum for our routine is 3 microns.

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