INTRODUCTION

MORPHOLOGIC HEMATOLOGY

By Oliver P. Jones, Ph.D.

BECAUSE this issue is concerned primarily with morphologic aspects of hematology, a brief survey of past achievements in this field and an indication of recent trends should accompany the varied and interesting articles which follow. Ever since Ehrlich[1] developed the triacid stain in 1880, clinical hematologists have relied principally upon the study of dry smears to aid them in determining the activity of hematopoietic organs as reflected in the peripheral blood picture as well as in the organs themselves. Histologists have relied more on the use of tissue sections to determine the origin of blood cells and especially their relation to connective tissue. Most of the major contributions to morphologic hematology were made between 1880 and 1924. During this period myeloblasts, immature lymphocytes (lymphoblasts), pronormoblasts and promegaloblasts were recognized and accurately described. Wright[2] traced the origin of blood platelets from the megakaryocyte. Downey and Weidenreich[3] were the first to describe the heteroplastic origin of lymphocytes from the reticulum. Maximow[4] and others demonstrated that lymphocytes circulating in the peripheral blood do not represent cells in the terminal stage of development and that under certain conditions they may become transformed to other cell types. Knoll[5] was the first to report a case of leukemic reticulo-endotheliosis and describe cells more immature than the myeloblast in peripheral blood. Downey and McKinlay[6] published their classical paper on leukocytoid lymphyocytes in infectious mononucleosis.

Pappenheim, Ferrata, Naegeli, and Downey have repeatedly described minute differences in nuclear and cytoplasmic structure. Although these differences appear small, they are nonetheless constant for the conditions under which they are found. If this were not so, the dry smear method would not have withstood the acid test of practical application. For example, hematologists have repeatedly shown that the blood picture of infectious mononucleosis can be distinguished from that of acute leukemia with immature lymphocytes (lymphoblasts) present in varying numbers.[7] Leukemic reticulo-endothelioses have been separated from other leukemias and two types of monocytic leukemia (Naegeli and Schilling) are now recognized.[8] Blood and marrow pictures of anemias due to liver principle deficiency have been separated from macrocytic anemias due to causes other than such a deficiency through the recognition of a megaloblastic series of red cell development.[9] However, in order to make such distinctions the observer must have a disciplined eye and good preparations with which to work. The use of dry fixed smears is not necessarily limited to the field of descriptive or morphologic hematology but, with the proper selection of experimental material, may be utilized to good advantage in determining cell potentialities. This has been well exemplified in the recent

From the Department of Anatomy, School of Medicine, University of Buffalo.

studies by Kolouch[10] on inflammation, Berman[11] on tissue cultures, and Dougherty[12] on the cytogenesis of microglia.

The development of new cytological, cytochemical, and optical technics has enlarged the scope of morphologic hematology because blood is such an admirable tissue to which to apply these technics. We have been passing gradually from a purely descriptive to an interpretative hematology and are beginning to understand the nature of certain cellular structures. Or, as Dempsey and Wislocki[13] have pointed out, "Histochemical observations correlated with functional stages may serve to illuminate morphological problems." This does not mean that morphologic hematology will become subordinated; on the contrary, in some instances it will even necessitate a greater refinement of our observational powers.

Basophilic cytoplasm is an attribute of several cell types. The nature of this basophilia was suggested in the studies by Caspersson and Schultz,[14] who demonstrated by means of ultraviolet absorption spectroscopy the presence of nucleic acids in growing root tips. Since then, Brachet[15] and others have been able to abolish the basophilia of lymphocytes, primitive and definitive erythroblasts, and myeloblasts, following digestion in ribonuclease, and thereby identify the basophilic substance as ribonucleoprotein. On the other hand, Wislocki and Dempsey[16] have shown that mast cell granules contain neither ribonucleoprotein nor a mucopolysaccharide. However, it is interesting to note that the presence of heparin in mast cells, reported by Holmgren and Wilander,[17] has recently been confirmed by Paff and his associates[18] through studies of mastocytomas. Knowledge that cytoplasmic basophilia is due to ribonucleoprotein and that lymphocytes may shed off bits of cytoplasm (originally observed by Downey and Weidenreich)[5] formed the basis for Dougherty and White's[19] investigations on the production of gamma globulin and antibody formation from lymphocytes.

Practically all basophilic or lymphoid cells have pale or yellowish areas in their cytoplasm. This is particularly true of primitive erythroblasts from the embryonic rat yolk sac. In the past these have been referred to as hyaloplasm or paraplasm. After studying these light areas by means of various technics, Jones[20] concluded that they are negative images of underlying organoids—mitochondria for the most part. Cytochemical methods revealed that these light areas (mitochondria) are relatively rich in lipoid material and contain a carotenoid pigment, probably vitamin A. One of the most interesting findings was that cell organoids are preserved quite well in freshly dried smears of embryonic blood. This naturally makes such material particularly suitable for investigating the cytochemistry of a single cell.

It has been known for some time that neutrophilic granules have a lipoidal nature. Since Lison[21] first reported the superiority of sudan black over all other similar dyes for demonstrating total fat histochemically, several investigators have used it for studying myeloid granulation. Sheehan,[22] Discombe,[23] Baillif and Kimbrough,[24] and Wislocki and Dempsey[16] all agree that neutrophilic and eosinophilic granules are sudanophilic. The latter are peculiar in that they have a sudanophilic cortex and sudanophobic core. The combination of staining blood films with sudan black followed by May-Grünwald-Giemsa described by Baillif and Kimbrough[24] should

be useful for many hematological studies on the origin and relation of cells under pathological conditions. In our laboratory, Dr. Raymond Kibler[4,5] has found that Auer rods in acute leukemia are definitely sudanophilic, a property which distinguishes them from other forms of so-called azurophilic granulation including Kurloff bodies. Lison[26] has pointed out that the lipoidal nature of myeloid granules is an important factor in the observed localization of oxidase in the specific granules. Apparently the enzyme is located in the cytoplasm and the products of oxidation dissolve in the granules secondarily because of their fatty nature.

In studying the nucleus, it must be realized that several nucleic acids may stain with the usual basic dyes.[27] One of the methods long accepted as a standard for the identification of chromatin has been Pappenheim's methyl green pyronine. But, according to the rigid qualifications for a histochemical reaction which have been laid down by Lison and Baker,[26,28] this is a stain and not a reaction. It does not aid in distinguishing the type of nucleic acid present. On the other hand, the Feulgen reaction is specific for desoxyribonucleoprotein, which is present in chromatin and chromosomes. The recent study by La Cour[29] on mitotic nuclei in marrow from patients with pernicious anemia contains much of interest for hematologists in general. Observations of myelocytes during metaphase showed that they were almost all Feulgen-negative while megaloblasts were intensely Feulgen-positive. Since nucleic acids govern cell division, it would seem that the megaloblasts gain at the expense of the myelocytes and that both cell types compete for a common supply of nucleic acid. Of particular interest is the suggestion—which indeed warrants serious consideration—that the presence of a large neoplasm may produce a nucleic acid starvation of cells in other tissues. Hence it may be possible to detect malignancies by studying the nucleic acid starvation of myeloid cells and their accompanying abnormal mitoses.

Morphologic hematology has grown and matured since 1880, so that it may now take its proper place in the field of cytology. Instead of being senile, it is more alive than ever. In this respect, phase microscopy,[30] among other technics, has been responsible for enlarging its scope. With this new optical technic it is possible to study unstained living and dry fixed blood cells and to demonstrate structures usually hidden in routine preparations. By means of bright and dark phase microscopy, it was possible for Jones[31] to study unstained dry smears of embryonic blood and to localize filamentous mitochondria. When these same preparations were subsequently stained with Wright's stain, the negative images in the basophilic cytoplasm corresponded to the underlying mitochondria. Cunningham and Tompkins[32] pointed out that the methods of Ehrlich and Romanowsky are not capable of demonstrating certain specific data which the supravital technic reveals. This is only partially true in the case of primitive erythroblasts, for we now know that a fairly accurate estimate of the mitochondrial distribution may be obtained from a study of good dry smears alone.

REFERENCES

[1] Ehrlich, P.: Anämische Befunde De—und Regeneration rother Blutscheiben. Berliner klin. Wchnschr., *17:* 405, 1880.

[2] Wright, J. H.: The histogenesis of the blood platelets. J. Morph., *21:* 263, 1910.

[3] Downey, H., and Weidenreich, F.: Über die Bildung der Lymphocyten in Lymphdrüsen und Milz. Arch. f. mikr. Anat., *80:* 306, 1912.

[4] Maximow, A.: Über die Entwicklung der Blut—und Bindegewebszellen beim Säugitierembryo. Folia haemat., *4:* 611, 1907.

[5] Knoll, W.: Eine seltene Form der Anämie. Deutsches Arch. f. klin. Med., *136:* 237, 1921.

[6] Downey, H., and McKinlay, C. A.: Acute lymphadenosis compared with acute lymphatic leukemia. Arch. Int. Med., *32:* 82, 1923.

[7a] Downey, H.: The occurrence and significance of the "myeloblast" under normal and pathologic conditions. Arch. Int. Med., *33:* 301, 1924.

[7b] Heck, F. J.: Infectious mononucleosis. In Downey, H.: Handbook of Hematology. New York: Paul B. Hoeber, Inc., 1938, vol. 4, p. 2581.

[8] Downey, H.: Monocytic leucemia and leucemic reticulo-endotheliosis. In Downey, H.: Handbook of Hematology. New York: Paul B. Hoeber, Inc., 1938, vol. 2, p. 1273.

[9] Jones, O. P.: Morphologic, physiologic, chemical and biologic distinction of megaloblasts. Arch. Path., *35:* 752, 1943.

[10] Kolouch, F.: The lymphocyte in acute inflammation. Am. J. Path., *15:* 413, 1939.

[11] Berman, L.: Observations on dry films of cultures of lymphoid tissue. Arch. Path., *33:* 295, 1942.

[12] Dougherty, T. F.: Studies on the cytogenesis of microglia and their relation to cells of the reticulo-endothelial system. Am. J. Anat., *74:* 61, 1944.

[13] Dempsey, E. W., and Wislocki, G. B.: Histochemical contributions to physiology. Physiol. Rev., *26:* 1, 1946.

[14] Caspersson, T., and Schultz, J.: Pentose nucleotides in the cytoplasm of growing tissues. Nature, *143:* 602, 1939.

[15] Brachet, J.: La détection histochimique des acides pentosnucleiques. Compt. rend. Soc. de Biol., *133:* 88, 1940.

[16] Wislocki, G. B., and Dempsey, E. W.: Observations on the chemical cytology of normal blood and hematopoietic tissues. Anat. Rec., *96:* 249, 1946.

[17] Holmgren, H., and Wilander, O.: Beitrag zur Kenntnis der Chemie und Funktion der Ehrlichschen Mastzellen. Ztschr. f. mikr.-anat. Forsch., *42:* 242, 1937.

[18] Paff, G. H., Bloom, F., and Reilly, C.: The morphology and behavior of mast cells obtained from mastocytomas and cultivated in vitro. Anat. Rec., *97:* 460, 1947.

[19a] Dougherty, T. F., and White, A.: Functional alterations in lymphoid tissue induced by adrenal cortical secretion. Am. J. Anat., *77:* 81, 1945.

[19b] Dougherty, T. F., White, A., and Chase, J. H.: Relationship of antibody content of normal and malignant lymphocytes. Proc. Soc. Exper. Biol. & Med., *59:* 172, 1945.

[20] Jones, O. P.: Mitochondria and their relation to the so-called hyaloplasm. J. Lab. & Clin. Med. *32:* 700, 1947.

[21] Lison, L.: Sur de nouveaux colorants histologique specifiques des lipides. Compt. rend. Soc. de biol., *115:* 202, 1934.

[22] Sheehan, H. L.: The staining of leucocyte granules by Sudan black. B. J. Path., & Bact., *49:* 580, 1939.

[23] Discombe, G.: The nature of neutrophilic granulation. J. Path. & Bact., *58:* 572, 1946.

[24] Bailliff, R. N., and Kimbrough, C.: Studies on leucocyte granules after staining with Sudan black B and May-Grünwald-Giemsa. J. Lab. & Clin. Med., *32:* 155, 1947.

[25] Kibler, R. S.: On the nature of Auer rods. Anat. Rec., *97:* 393, 1947.

[26] Lison, L.: Histochemie Animale; méthodes et problèmes. Paris; Gauthier-Villars, 1936.

[27] White, M. J. D.: Nucleus, chromosomes and genes. In Bourne, G.: Cytology and Cell Physiology. Oxford University Press, 1942, p. 139.

[28] Baker, J. R.: Some aspects of cytological technique. In Bourne, G.: Cytology and Cell Physiology. Oxford University Press, 1942, p. 1.

[29] La Cour, L. F.: Mitosis and cell differentiation in the blood. Proc. Roy. Soc. Edinburgh, *62:* 73, 1943.

[30] Bennett, A. H., Jupnik, H., Osterberg, H., and Richards, V. W.: Phase microscopy. Tr. Am. Micro. Soc., *65:* 99, 1946.

[31] Jones, O. P.: A study of mitochondria in dry smears of embryonic blood. Anat. Rec., *97:* 347, 1947.

[32] Cunningham, R. S., and Tompkins, E. H.: The supravital method of studying blood cells. In Downey, H.: Handbook of Hematology. New York: Paul B. Hoeber, Inc., 1938, Vol. 1, p. 553.