Morphology and Cytochemistry of the Granule-Vacuole Body of Leukemic Cells

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A RARE and hitherto undescribed vacuole-like structure has been found within the cytoplasm of certain leukemic cells from seven patients with acute leukemia. For descriptive purposes this cytoplasmic structure shall be designated as the granule-vacuole body. It was the purpose of this investigation to study the morphology and cytochemistry of this structure and compare it briefly with the cytoplasmic granulation and Auer bodies of leukemic cells.[1, 2]

Materials and Methods

Approximately 500 cases of acute and chronic leukemia reviewed over an eight year period from the Hematology Service, University Hospital, Ohio State University, have revealed seven cases in which granule-vacuole bodies were recognized. These included three cases of granulocytic leukemia and four cases of monocytic leukemia; Auer bodies were found associated in four of these cases.

Fresh moist films prepared by touching a clean coverslip to a drop of blood or bone marrow and inverting it upon an unstained or supravitally (neutral red and Janus green) prepared slide were examined with the phase contrast and bright field microscopes.[3] In addition, Wright's stained films were routinely examined.

Air dried films were fixed with formalin vapor for three minutes and subjected to a number of cytochemical procedures: A comparison of ribonuclease digested and undigested control films stained with Wright's stain was made to detect the presence of pentosenucleoprotein.[4] Desoxypentosenucleoprotein was demonstrated by means of the nucleal reaction.[5] Metachromasia following exposure to an aqueous (0.2%) solution of toluidine blue served as an indication of sulfate esters of mucopolysaccharides or pentosenucleoprotein.[6] The periodic acid Schiff reaction was employed to demonstrate mucopolysaccharides, saliva-labile glycogen, cerebrosides and possibly unsaturated lipids.[5] Lipids were demonstrated following exposure to a 70% alcoholic solution of Sudan black B.[1] Neutral fat was stained with a saturated aqueous solution of Nile blue sulfate.[7] Acetal lipids were shown by the plasmal reaction.[8] Acid and alkaline phosphatase activity was suggested by modifications of the Gomori procedures.[9] Peroxidase activity was indicated by the method of Sato and Sekiya.[10] The M. nadi test was utilized for the possible demonstration of cytochrome oxidase or lipoid material.[5]

Observations

Granule-vacuole bodies exhibit their most characteristic appearance in unstained moist and supravital films. They appear as round, moderately refractile yellow globules (0.5–4.0 u) and contain one or more small granules (figs. 1–3). Between one and six granule-vacuole bodies may be present in the cytoplasm of the leukemic cell. Most commonly these bodies are found in monoblasts and young monocytes of acute monocytic leukemia and in late B and C myelocytes and mature neutrophils in granulocytic leukemia. Although the frequency of these bodies in acute leukemia is markedly less than the frequency of Auer bodies, these two aberrant structures have often been associated in the cells of

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TABLE 1.—*Frequency of Granule-Vacuole Bodies and Auer Bodies Found in the Blood and Bone Marrow of Seven Patients with Acute Granulocytic and Monocytic Leukemia*

Type of Leukemia	Per cent granule-vacuole bodies in leukemic cells		Per cent Auer bodies in leukemic cells	
	Blood	Bone Marrow	Blood	Bone Marrow
Granulocytic				
1	0.0	19.2	0.0	8.0
2	*	20.0	*	0.0
3	0.0	1.0	0.0	0.0
Monocytic				
1	0.0	68.0	0.0	0.2
2	0.0	6.4	0.0	0.0
3	1.8	*	0.2	*
4	1.6	*	0.4	*

Percentages calculated from a count of 500 leukemic cells.
* No films available for examination.

the same patient (table 1). However, granule-vacuole bodies and Auer bodies are rarely seen together in the same leukemic cell.

The granule-vacuole bodies are not the result of in vitro alteration since they are present upon immediate examination of unstained moist films (fig. 3). Granule-vacuole bodies do not seem to impair motility or mitotic activity in the leukemic cells containing these structures. Frequently, these bodies are extruded from the cytoplasm of the mature neutrophil into the plasma without apparent alteration in the activity of the cell or in the morphology of the bodies (fig. 2).

The granule-vacuole bodies arise from certain cytoplasmic granules which undergo morphologic and cytochemical changes with the elaboration of a vacuole surrounding this granule. These aberrant granules are stained a deep orange-red (acidic) with neutral red. Smaller vacuoles may be stained a light orange-red color, but this property disappears as the vacuole enlarges.

Marked variations in the stainability and solubility of the granule-vacuole bodies are revealed following exposure to Wright's stain. In most instances the vacuolar portion of the inclusion is dissolved by the action of the alcohol present in the dye solution while the granular inclusion, being less alcohol-labile, exhibits a strong azurophilic and basophilic reaction (fig. 6). The smaller vacuoles are also less alcohol-labile than the larger vacuoles and stain pink to deep red with the Romanowsky dye.

The cytochemistry of the vacuolar portion of the granule-vacuole bodies differs in some respects from that of the granular portion. The vacuole appears to consist of a rather fluid matrix consisting of a strongly PAS-positive material (fig. 4), most probably a mucopolysaccharide, and exhibits a strong oxidase and peroxidase reaction. The vacuole has a sudanophilic membrane or cortex demonstrable with Sudan black after prior fixation with osmic acid vapor (fig. 5); neutral fat is not shown with Nile blue sulfate. Pentosenucleoprotein, desoxypentosenucleoprotein, acetal lipids, glycogen, acid and alkaline phosphatase are not found in the vacuoles. The granular portion of this inclusion is sudanophilic, PAS-positive, plasmal-positive and exhibits a strong oxidase and peroxidase activity as well as a slight acid and alkaline phosphatase reaction.

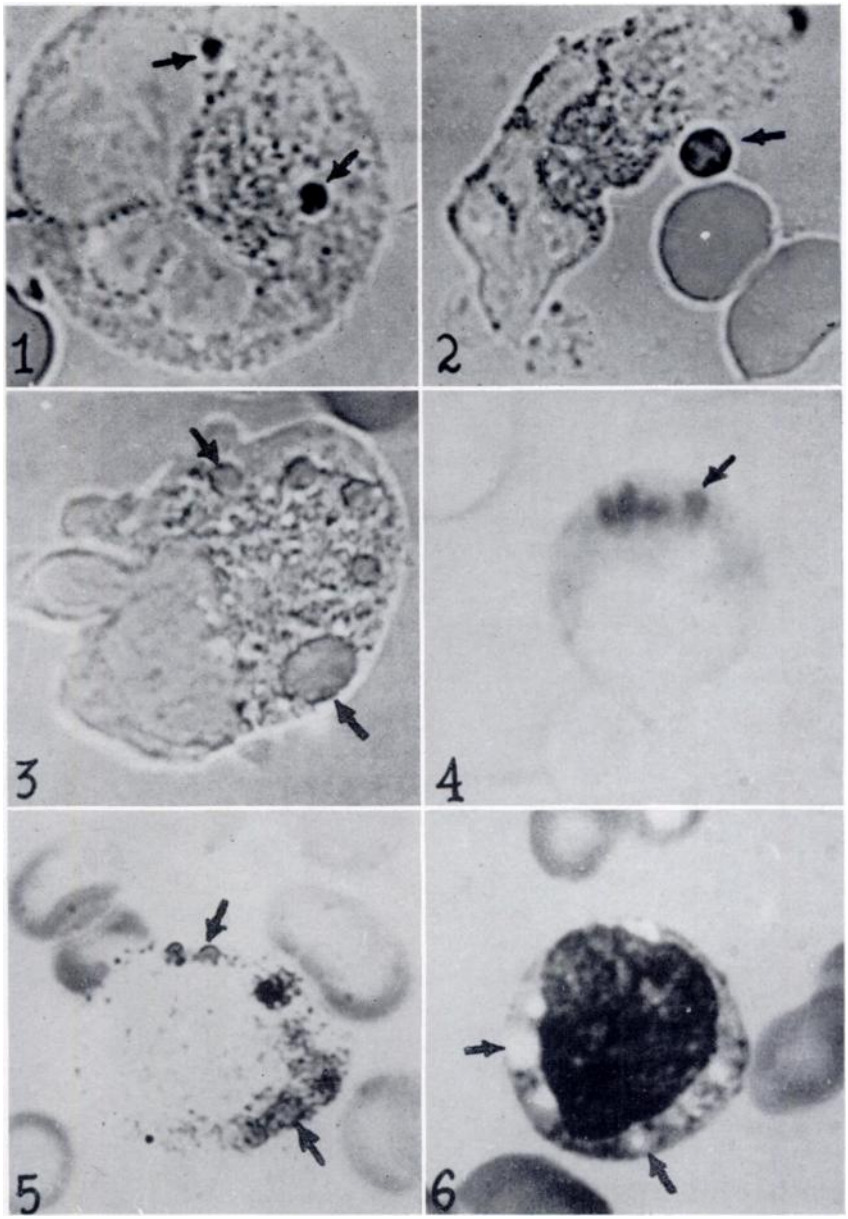

FIG. 1.—A myelocyte "C" containing two moderate sized granule-vacuole inclusions Supravital. × 2700.

FIG. 2.—An actively motile neutrophil which has just extruded a granule-vacuole inclusion into the plasma. Supravital. × 2700.

FIG. 3.—A myelocyte "C" with four moderate sized and one large vacuolar inclusion. No granules could be observed within the vacuoles. Unstained moist preparation. × 2700.

FIG. 4.—A late myelocyte "B" containing five deeply staining vacuole inclusions. The cytoplasm stains a lighter color than the inclusions. Periodic acid-Schiff. × 2700.

FIG. 5.—A myelocyte "B" containing several vacuole inclusions. Two of the inclusions appear to have an outer capsule which stains with the Sudan black B. Sudan black B with no counterstain. × 2700.

FIG. 6.—A young monocyte exhibiting several inclusions. A small amount of the vacuolar material still remains in each inclusion. A darker staining granule may also be observed at the edge of two of the inclusions. Wright's stain. × 2700.

TABLE 2.—*Summary of the Chemical Composition of Several Cytoplasmic Organelles and/or Inclusions*

Reaction	Young Cytoplasmic Granules	Auer Body	Granule-Vacuole Body		Kurloff Body
			Granule	Vacuole	
Neutral red	acidic to more neutral	acidic to more neutral	acidic	unstained	network ppt. by dye—acidic, remainder unstained
Birefringence	—	—	—	—	network ppt. by dye—positive
Azurophilia	+++ -++++	+++ -++++	+++	—	++++
Metachromasia	+	+	+	—	++++
Mucopolysaccharide	++-+++	++	++	+++	++++
Glycogen	--++	—	—	—	—
Neutral fat	—	—	—	—	—
Phospholipid	++-+++	+++	+++	++	—
Acetal phospholipid	+	+	+	—	—
Pentosenucleoprotein	+	++	++	—	?
Desoxypentosenucleoprotein	—	—	—	—	—
Cytochrome oxidase (?)	+++ -++++	++++	++++	++++	—
Peroxidase	++++	++++	++++	+++	—
Acid phosphatase	--+	—	--+	—	—
Alkaline phosphatase	--+	—	--+	—	—
General solubility	soluble protein and phospholipid	soluble protein and phospholipid	soluble protein and phospholipid	highly soluble phospholipid membrane	relatively insoluble

Table 2 represents a summary of the cytochemical composition of the granule-vacuole bodies and attempts to compare the intensities of the reactive substances with that of the cytoplasmic granules and Auer bodies of leukemic cells and with the intracytoplasmic globular Kurloff bodies seen in the lymphocytes of normal guinea pigs.[2]

DISCUSSION

The cytochemical reactions indicate a close correlation between the granule-vacuole inclusions, cytoplasmic granules and Auer bodies. The inclusions differ cytochemically from cytoplasmic granules and Auer bodies principally in the amount of each constituent as determined by the relative intensity of the reaction. The PAS, oxidase and peroxidase reactions produce a more intense color in the inclusions than in either the cytoplasmic granules or Auer bodies. The lipid membrane of the vacuole inclusion is extremely alcohol-labile in con-

trast with the partial solubility of the granules and Auer bodies. The cytochemical similarity of Auer bodies, cytoplasmic granules and the granule-vacuole bodies suggests that the granule-vacuole inclusions arise as the result of degeneration or malformation of certain cytoplasmic granules due to an alteration in the metabolism of certain leukemic cells. It is also possible that the vacuole surrounding the granule may result from a cytoplasmic reaction to the presence of these aberrant granules.

Summary

A rare and hitherto undescribed vacuole-like structure, the granule-vacuole body, found in the cytoplasm of certain cells of granulocytic and monocytic leukemia has been described morphologically and cytochemically. This inclusion has been compared with the cytoplasmic granules and Auer bodies occurring in leukemic cells.

Summario in Interlingua

Es presentate un description morphologic e cytochimic de rar e usque nunc non ancora describite structuras vacuoloide, designate como corpores granulovacuolari, que esseva trovate in le cytoplasma de certe cellulas in leucemia granulocytic e monocytic. Iste inclusiones es comparate con le granulos cytoplasmic e le corpores de Auer que occurre in cellulas leucemic.

REFERENCES

[1] Ackerman, G. A.: Microscopic and histochemical studies on the Auer bodies in leukemic cells. Blood *5:* 847–863, 1950.

[2] ——: Cytochemistry and morphology of the cells of the hemopoietic system. Dissertation, The Ohio State University, 1954.

[3] —— and Bellios, N. C.: A study of the living cells of the blood and bone marrow in supravital films with the phase contrast microscope. II. Blood and bone marrow from various hematologic dyscrasias. Blood *10:* 1183–1203, 1955.

[4] White, J. C.: The cytoplasmic basophilia of bone marrow cells, J. Path. & Bact. *59:* 223–234, 1947.

[5] Pearse, A. G. E.: Histochemistry, Theoretical and Applied. Boston, Little, Brown & Co., 1953.

[6] Rheingold, J. J., and Wislocki, G. B.: Histochemical methods applied to hematology. Blood *3:* 641–655, 1948.

[7] Cowdry, E. V.: Laboratory Technique in Biology and Medicine. Baltimore, Williams & Wilkins Co., 1952.

[8] Hayes, E. R., A rigorous re-definition of the plasmal reaction. Stain Technol. *24:* 19–23, 1949.

[9] Rabinovitch, M., and Andreucci, D.: A histochemical study of "acid" and "alkaline" phosphatase distribution in normal human bone marrow. Blood *4:* 580–594, 1949.

[10] Wintrobe, M. M.: Clinical Hematology, Philadelphia, Lea & Febiger, 1951.