Acid Phosphatase Activity in Auer Bodies

By ARTHUR F. GOLDBERG

AUER BODIES and leukocyte granules appear to be closely related structures, on the basis of similarities found in electron microscopic[1,2] and histochemical studies.[3,4] One of the enzymes which can be demonstrated in leukocyte granules both by histochemical[5,6,7] and biochemical methods[8,9] is acid phosphatase. Because we considered Auer bodies to be abnormal granules, this study was undertaken to see if acid phosphatase activity would also be found in this organelle just as it had been demonstrated in leukocyte granules. The presence of an enzyme common to both structures could be further favorable evidence of the similarities between them.

MATERIAL AND METHODS

Peripheral blood smears stained with Romanowsky-type dyes were obtained from three patients who had Auer bodies present in some of their peripheral leukocytes. Two patients had acute myeloblastic leukemia and one myelo-monocytic leukemia. Slides were stained for acid phosphatase activity with a slightly modified Gomori method.[10] Alkaline phosphatase activity was demonstrated by Kaplow's technic.[11] Other slides were stained with Jenner-Giemsa at pH 6.8.

Gomori's method was employed on smears of peripheral blood, air dried and fixed for 16 to 24 hours at 4 C. in 10 per cent neutral formalin-acetate (0.1 M sodium acetate = 900 ml., 40 per cent formalin = 100 ml. adjusted to pH 7.0). Slides were washed with distilled water and placed into a Coplin jar containing the incubation medium, for 6 hours at 37 C. The incubation medium was made in the following manner: *Solution A:* 0.2 M sodium acetate buffer at pH 5.0 (300 ml. of 0.6 per cent glacial acetic acid was added to 700 ml. of solution containing 27 Gm. sodium acetate· 3 H_2O in 1000 ml. distilled water). *Solution B:* 0.05 M acetate buffer at pH 5.0 (0.6 Gm. of lead nitrate was added to 125 ml. of solution A and made up to 500 ml. with distilled water). *Incubation medium:* 5 ml. of freshly prepared 3 per cent sodium glycerophosphate* (40 per cent α, 60 per cent β) was added to solution B. A fine precipitate forms which must be filtered.

Control slides were incubated in a medium omitting the glycerophosphate. After incubation, slides were washed with distilled water 4 times, placed for 1 minute in dilute acetic acid (0.1 per cent) to eliminate nonspecific bound lead, and then washed again. The final reaction product, lead sulfide was subsequently visualized after treatment in 1 per cent aqueous ammonium sulfide for 3 minutes. Smears were washed in distilled water, counterstained in dilute Harris' hematoxylin (1:20) for 5 minutes and washed again. They were then either mounted in Glycerogel or dehydrated through alcohols and xylene and mounted in Permount. No artifactual nuclear staining was seen either in the

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This study was supportel in part by PHS Research Grant CA-07178-02 from the National Cancer Institute.

Submitted Aug. 29, 1963; accepted for publication Feb. 7, 1964.

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control or test uncounter-stained preparations even after 9 hours incubation. Other control smears incubated for 10 minutes at 97 C. showed no phosphatase activity. Initially prolonged fixation times were used and thus a long incubation time was required to produce satisfactory activity. If shorter fixation times were employed, a much shorter incubation demonstrated similar enzyme sites.

Results and Discussion

With Gomori's method, acid phosphatase activity was detected in Auer bodies (fig. 1 a-d). Intermediate forms between fully developed Auer rods and typical spherical granules were similarly demonstrated (fig. 1d). Marked variations in the shape of Auer bodies and the presence of transitional forms between Auer bodies and granules have been recognized using other technics.[1-3,12] Gomori's method permits the fine localization of acid phosphatase activity clearly delineating the shape of the Auer bodies similar to that seen with other methods under the light microscope as well as in the electron microscope.

Ackerman,[3] also using the lead salt method, could not demonstrate this enzyme in Auer bodies. In another early study, however, Harada[4] showed with Gomori's method that some Auer bodies stained positively for acid phosphatase activity.

It is felt that the Auer body may be a grossly abnormal, elongated leukocyte granule, although it may develop from the fusion of smaller ones. Normally, immature myeloid cells contain very few elongated granules.[13] Most of the granules present are spherical. The predominant ones in mature polymorphonuclear leukocytes are elongated. The presence of an exaggerated structure such as the Auer body in immature cells may represent another manifestation of the asynchronous maturation and development of specific granules in leukemic cells. In preliminary observations with electron cytochemistry of normal leukocytes, acid phosphatase activity has been demonstrated in both the elongated and spherical granules of polymorphonuclear leukocytes.[14]

Acid phosphatase along with a series of other acid hydrolases, has been found in a newly described subcellular particle called the lysosome.[15,16] Leukocyte granules are considered to be such structures.[5,8] The presence of an acid hydrolase associated with lysosomes, as well as other reported morphologic and cytochemical similarities between Auer bodies and leukocytic granules, supports the concept that Auer bodies may be abnormally shaped granules. The Auer body could thus be an abnormal lysosome in a neoplastic cell and would probably also contain the other acid hydrolases found in leukocyte granules.

Using an azo-dye method, no alkaline phosphatase activity was found in younger myeloid cells. None was present in Auer bodies. Similar results were found by Ackerman[3] using Gomori's method for alkaline phosphatase. Harada,[4] however, described Auer bodies as exhibiting a positive reaction for alkaline phosphatase. We consider that these structures were probably falsely stained due to diffusion artifacts.[17] One may conjecture that while acid phosphatase is present in Auer bodies, the absence of alkaline phosphatase may be indirect evidence that this enzyme resides in a different organelle from the granule containing acid phosphatase in mature leukocytes.

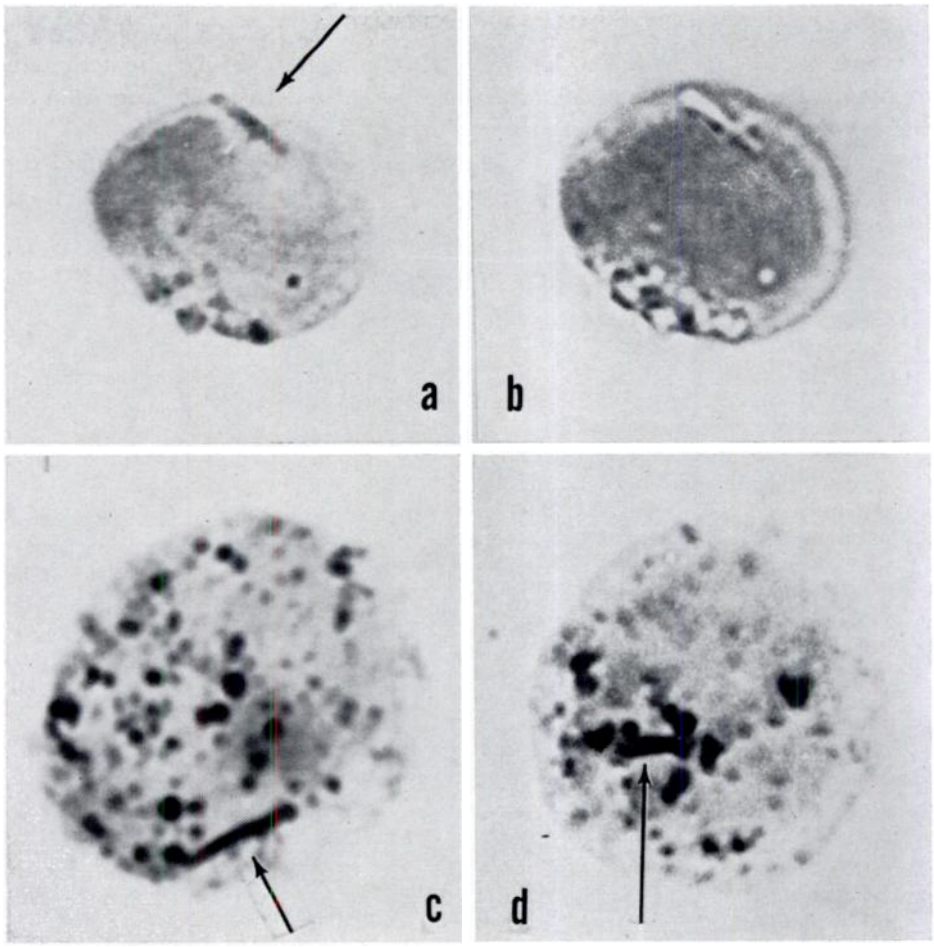

Fig. 1.—Peripheral leukemic cells stained for acid phosphatase activity by the lead salt method of Gomori (original magnification 1000 X, enlarged to 10,000 X). (a) Acid phosphatase activity in Auer body (arrow) of leukemic myeloblast. A smaller Auer body overlies the larger one. Because of the three dimensional nature of the preparation, it, as well as the nucleus and some granules, lie in another cellular plane. At opposite pole, activity in granules is present. (b) Same cell as in (a) viewed in modified dark field with microscope condenser lowered. Auer body and granules appear as discrete crystalline-like structures. (c) Leukemic pomyelo-monocyte with acid phosphatase activity distributed diffusely in granules and Auer body (arrow). (d) Leukemic promyelo-monocyte with small Auer body (arrow). Two other Auer bodies situated at both its poles are more vertically oriented. Activity is also present in variably sized granules.

Summary

Employing the Gomori lead salt method (pH 5.0), acid phosphatase activity was demonstrated in Auer bodies. The presence of this enzyme may be considered further evidence of the similarity between leukocyte granules, known to contain acid phosphatase, and this structure. No alkaline phosphatase activity was exhibited in these bodies. The significance of these findings is discussed.

SUMMARIO IN INTERLINGUA

Con le uso del methodo de Gomori a sal de plumbo (pH 5,0), le activitate de phosphatase acide esseva demonstrate in corpores de Auer. Le presentia de iste enzyma pote esser considerate como un evidentia additional pro le similitude inter le granulos leucocytic e le corpores de Auer. Nulle activitate de phosphatase alcalin esseva trovate in le corpores de Auer. Le signification de iste constatationes es discutite.

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