Absorption Microspectroscopy of Bone Marrow Cells

By Sérgio de Carvalho

This paper presents some results of the absorption microspectroscopy of bone marrow cells in two main wavelengths: 4150 Å (violet region in the Soret band) and 2650 Å (UV). The first wavelength is indicative of heme structures in appreciable concentration in cells. As these are only achieved as Hb, the Soret band absorption microspectroscopy becomes specific for the cytochemistry of that substance. The 2650 Å length is known to be specific for nucleic acids.

Methods

The observations reported in this paper were done with a reflecting microscope according to the method described by Wilkins and the author.

Fig. 1. Human bone marrow, unfixed dry smear on quartz slide. Reflecting solid objective NA 0.90. Picture taken at 4150 Å, X1800. White cells and chromatin of red cells do not absorb. An erythroblast is in anaphase; chromosomes non-absorbing. Heme granules in the nucleus.

Results

The pictures show heme absorption in the cytoplasm as well as in the nuclei of the erythroblasts (fig. 1, 2b, 3a and 4). Comparing the pictures of the same

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Fig. 2.—Human bone marrow. Unfixed dry smear on quartz coverslip. Reflecting solid objective. NA 0.90. Same field photographed at 5500 (a), 4150 (b) and 2650 Å (c); respectively no absorption, heme absorption and nucleic acids absorption. These pictures show a complete absorption spectrum of a same cell throughout the continuum. The oval non-heme-absorbing cell (b) is a plasmaocyte as can be checked in the UV picture (c).

Fig. 3.—a same as fig. 2b; b same as fig. 2c. Another field. A major enlargement shows intimate details. Accurate observation of the pictures indicates that heme nuclear granules fall in between chromatin meshes in the same cell.

Fig. 4.—Human bone marrow. Suspension in 37 C. saline. Basophil erythroblast photographed in vivo with reflecting solid objective, NA 1.37 at 4150 Å (violet light). X2400. It clearly shows heme nuclear absorption.
Fig. 5.—Human bone marrow. Unfixed dry smear. A proerythroblast photographed with the technic indicated in pictures above. a at 4150, b at 2650 Å. In a, there is no heme absorption, in b heavy nucleic acid absorption.

Fig. 6.—Rat bone marrow. Unfixed dry smear. Picture taken with the technic indicated in pictures above, at 2650 Å (UV). Clearly shows nucleic acid absorption in the granules of neutrophilic cells.

Field taken at 4150 and 2650 Å we can see how heme absorption granules in the nuclei fall in between the meshes of the chromatin network (figs. 2, 3).

Following the maturation of the erythroblasts one can see that the proerythroblast is completely nonabsorbing in the Soret band, meaning that it contains no heme at all, while it contains much nucleic acid (fig. 5).

The maximum heme absorption in the nucleus corresponds to the basophilic erythroblasts (fig. 4 shows one of such cells), while it decreases in the polychromatolytic and orthochromatic ones as the nucleus becomes pyknotic (figs. 1 and 3a).

Ultraviolet pictures showed quite clearly nucleic acid absorption in the granules of the neutrophil leukocytes and their precursors (fig. 6).

DISCUSSION

The finding of heme absorption in the nuclei of the erythroblasts under the form of granules agrees with previous staining and other cytochemical methods by Villa, Riecker, Knoll, Ferrara, by the author, Curletto, and Hérmansky, as well as with fluorescence microscopy by Vannotti and Siegrist, electronic microscopy by De Robertis, and bulk analysis of the isolated nuclei by Stern, Allfrey, Mirsky and Saetren.

As to UV absorption in the neutrophils, it coincides with the observations of van den Berghe and Hoffman.
Using a microspectrographic technic, reflecting optics and narrow bands of the continuum (5500, 4150 and 2650 Å), the author has obtained pictures of human and rat bone marrow cells in unfixed dry smears as well as in suspensions in vivo; erythroblasts showed different absorption patterns. Violet light microspectrography, specific for heme absorption, allowed us to demonstrate the presence of intranuclear heme granules through various stages of maturation of these cells. This confirmed previous and simultaneous results arrived at by cytochemical and other methods.

The presence of nucleic acids has been demonstrated in neutrophil granulations.

REFERENCES

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