On the Presence of Hemoglobin in Erythroleukemia Cells

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The acute leukemia cell represents a growing malignant element arising in the hematopoietic tissue. In contrast to the normal blood cell development, differentiation is absent or defective, which gives the cell a general "blastlike" appearance, i.e., a basophilic cytoplasm and a big, irregularly rounded nucleus containing prominent nucleoli. Cytchemically, the acute leukemia cell is characterized by a high content of ribose nucleic acids in the cytoplasm, in common with the normal, immature hemocytoblast.1

Due to their poor differentiation, the different types of acute leukemia cells are often difficult to distinguish from each other cytologically. Sometimes, so-called peroxidase-positive granules indicate a granulocytoblastic origin.2,3 In the case of erythroblastemia, known both from human beings and animals, the deep cytoplasmic basophilia effectively hides an eventual hemoglobin color.

The present paper describes microspectrographic analyses of acute leukemia cells in an effort to determine whether leukemia cells of erythrogenic origin, as opposed to other leukemic cells, contain hemoglobin in detectable amounts. The different types of experimental virus-induced leukemias in fowls have been studied, since this material is cytogenetically well defined.

In the virus-induced acute leukemias of fowls, it can be shown that the tumor cells arise from specific types of ontogenetically determined blood stem cells in the bone marrow. In this laboratory, there are two distinct types of acute virus-induced leukemias under continuous passage: one "erythroleukemic," originally obtained from Dr. J. Engelbreth-Holm in Copenhagen and the other "myeloblastemic," furnished by Dr. J. Beard, Durham. Figures 1 and 2 represent early stages of leukemia induction in the two types and illustrate neoplastic changes in the erythropoietic and granulocytoblastic tissue, respectively.

Material and Methods

White Leghorn chickens (Edo) were used as experimental animals. The erythroleukemia was induced in 10-day-old animals by single intravenous injections of a large dose of cell-free cytoplasmic homogenate prepared from pooled leukemic spleens, as described earlier.4 This produces a uniform and very acute disease. The survival time of the chickens after the injection of such massive doses is about 11 days.5 The picture of the disease is characterized by the enlarged liver and spleen, a bone marrow completely filled with leukemia cells and a white blood cell count of about 100,000 per cu. mm., practically all immature blastlike elements. Anemia is slight or almost absent in these very acute cases. The erythroblastemic cell is round with well-defined borders, a markedly basophilic

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Fig. 1.—(left) Chicken bone marrow sinusoid, third day after the injection of erythro-leukemia virus. Intrasinusoidal, proliferating leukemia cells at top. Hematoxylin and eosin (X 500).

Fig. 2.—Chicken bone marrow, fourth day after the injection of myeloic leukemia virus. Extravascular, proliferating leukemia cells in the middle part of the figure. Hematoxylin and eosin (X 200).

cytoplasm and a round, usually regular nucleus containing one or two big nucleoli embedded in a finely dispersed chromatin.

The myeloic leukemia used has earlier been extensively described by Dr. J. W. Beard and collaborators. In our laboratory, using white Leghorn chickens, the maximal white cell count (up to 1 million per cu. mm.) and death of the animals is reached in about three weeks, after the virus has been inoculated as a filtered, cell-free plasma in three-day-old chickens. These cases also show the typical picture of enlarged liver and spleen together with a diffusely involved bone marrow. The myeloic leukemia cell is characterized by a slightly foamy, basophilic cytoplasm and a round or oval nucleus containing several nucleoli embedded in the irregularly dispersed chromatin. At times a clear cytoplasmic zone can be seen surrounding the nucleus.

All the chickens inoculated in the present experiment, 10 with each strain of virus, developed their diseases in a typical and uniform way in accord with our earlier experience. When the peripheral blood contained a high number of leukemia cells, very thin blood smears were prepared on standard microscope slides. The smears were air-dried and the leukemia cells were analyzed for their suspected hemoglobin content with the microspectrophotometer described below. For each analyzed cell, the coordinates on the object table of the microscope were noted. After each set of analyses, the slide was stained with May-Grunewald-Giemsa and the same cells were microphotographed.

In spite of the pathogenetically less well-known human erythroleukemia, it was also of interest to include some cells from a clinically typical case of this disease in the present investigation. Moreover, cells from a human case of acute, lymphatic leukemia were investigated, mainly for control purposes.

Case history of the human erythroleukemia: G.P., a 66-year-old man, suffered for some

*We are greatly indebted to Drs. N. C. Nordenson and G. Hammarsten, Södersjukhuset, Stockholm, and Dr. S. Franzén, Karolinska sjukhuset, Stockholm, for putting these cases at our disposal.
months from weakness and increasing pallor. On May 8, 1957, he had a profuse bleeding from his nose and was hospitalized at the Södersjukhuset in Stockholm. His liver and spleen were slightly enlarged. Laboratory studies revealed the hemoglobin to be 25% of normal value; red blood cell count 2.0 million per cu. mm. The white blood cells numbered 3,300 per cu. mm. with the following differential count: blast cells 24%, myelocytes 1%, metamyelocytes 0%, juvenile neutrophils 4%, segmented neutrophils 44.5%, eosinophils 1%, basophils 0%, lymphocytes 24.5% and monocytes 1%. All the blast cells were of the erythroblastic type. The large nuclei of these cells were irregular, round or oval with a fine chromatin network in which several big nucleoli were seen. The cytoplasms were deeply basophilic. The sternal punctate was rich in cells, and almost all of them were of an atypical erythroid type. The patient died on June 24, 1957 following splenectomy and liver biopsy. Besides arteriosclerosis and pulmonary edema the autopsy revealed atypical erythroblasts throughout the hematopoietic tissue, especially in the vertebra and spleen.

Case history of the acute lymphatic leukemia: E.K., a 48-year-old man, suffered 2 or 3 months from nasal pain and then developed diplopia. A biopsy was made from a small tumor observed in his nasal septum. Microscopical examination of the biopsy specimen revealed a picture of lymphosarcoma. In bone marrow smears, obtained on Dec. 4, 1957 by sternal puncture, there were numerous immature, atypical cells, some of which showed a slight differentiation towards lymphoblasts. Blood count: hemoglobin 86% of normal value; red blood cells 4.4 million per cu. mm., white blood cells 16,100 per cu. mm.; 30% of the white cells were lymphoblastlike elements.

Thin air-dried bone marrow smears were used for the microspectrography of the human leukemia cells. After each set of cells had been analyzed, the slides were stained with May-Grünwald-Giemsa and microphotographed.

The microspectrograph used in the analysis of cellular hemoglobin is schematically depicted in figure 3. To evaluate the analytic results presented later, a short description of the main working principles is necessary.

The light source (A) was a 30 watt tungsten filament-type lamp and gave a sufficient amount of light for the spectral range of the hemoglobin Soret-band.

The light from the lamp is focused on the entrance slit of the double prism Leiss monochromator (B). The monochromatic light emerging from the monochromator goes to a microscope stand (C) which is fitted with a Beck reflecting condensor and a Beck reflecting objective (N.A. 0.6). The image of the cell is located at a particular position in the field by means of an interchangeable cross-hair ocular. After a particular cell has been chosen, the image is diverted by a moveable mirror onto a set of vibrating mirrors (D). The mirror-chopper (E) oscillates at 50 cycles/sec. and is arranged in such a manner that the image of the cell falls on one mirror and a portion of the surrounding microscopic field falls on the other. A series of moveable shields allows us to blank out the cell image or the corresponding reference light or to let both the image and reference pass undisturbed. A pair of slits (F) situated between the chopper and the photomultiplier permits us to choose a small portion of the cell suitable for analysis. We usually choose areas of approximately 4 μ. The chopped light then falls on the photocathode of an RCA 1P28 photomultiplier (G).

The output of the photomultiplier consists of a train of square waves. One complete cycle consists of two parts; one represents the reference light and the other represents the light to be measured. The signal is amplified by a high-gain amplifier and detected by a chopper-type phase detector (H, I). The phase detector separates the reference signal from the measuring signal and along with the filter circuits changes both signals into direct current signals.

Since the spectral response of the photomultiplier and the spectral output of the lamp are not constant, the gain and sensitivity of the photomultiplier must be changed to compensate for this. This is accomplished by using the reference signal to control the gain and sensitivity (J).

*The instrument is based on constructions by L. Åkerman.*
The detected signal is recorded on a Leeds and Northrup Speedomax recorder (K, L). The reference signal is used as the compensating reference potential for the balancing potentiometer (15 K ohms) in the recorder. The trace recorded on the paper is thus displayed directly in transmission units, i.e., I I.

Since the leukemia cells were microspectrographed in air-dried smears, it is important to know the spectral properties of the cellular hemoglobin in the dry state. Figure 4 shows that the hemoglobin is partially oxidized by the air-drying and that also the specific extinction coefficient is somewhat lowered.

**RESULTS AND DISCUSSION**

Figure 5 shows the absorption spectra from the analyzed fowl leukemia cells together with the corresponding cell pictures after staining with May-Grünwald-Giemsa. For comparison, two red blood cells have been analyzed under identical conditions. It is evident that the erythroleukemia cells 1 to 5 display a low but distinct Soret-band absorption from areas in their cytoplasm. The bands are several times the noise level of the records and all in the same wavelength regions. The erythroleukemia cell No. 7 and all of the myeloleukemia cells did not show any specific absorption in this wavelength range.

Thus, in spite of the immature cytologic picture characterizing the typical, acute erythroleukemia of fowl, most of the cells investigated showed spectral properties corresponding to those of a porphyrin pigment. The wavelength of the absorption peaks in these spectra corresponded closely in all cases to that of hemoglobin. Other pigmented substances such as the peroxidases and cytochromes have peaks at nearby wavelengths, but the resolution of the spectrograph used is such that these differences in wavelength would
Fig. 4.—Absorption spectrum of the red cell hemoglobin in an air-dried blood smear compared with the spectra of met- and oxyhemoglobin. Absorption constant of the red cell hemoglobin at 4120 Å: 8.51 ± 1.73 \times 10^3. (Oxyhemoglobin in water solution at 4150 Å: 12.4 \times 10^3.)

be readily distinguishable from that of hemoglobin in cytologic preparations. This evidence is of course not conclusive, nor can anything be said about the type of hemoglobin which appears to be present; such questions are to be further investigated in this laboratory by means of microelectrophoretic methods.

These cells arise from stem cells which normally do not contain any detectable amounts of hemoglobin. A development of the circulating leukemia cells must have occurred, therefore, and by calculating a cellular content of hemoglobin, the degree of this development can be quantitatively estimated. In the case of the present experimental group of chickens the specific absorption relative to the noise level, suggests a roughly calculated concentration of hemoglobin between 2 and 5 µg per ml.

During normal erythropoiesis, microspectrographically significant concen-
Fig. 5.—Absorption spectra from areas in the cytoplasm and pictures of the analyzed cells after staining with May Grünwald-Giemsa. Cells 1 to 5 and 7 are from chicken erythroleukemia. Cells 8 to 10 are from chicken myeloid leukemia. Cells 6 and 11 represent mature chicken erythrocytes in the smear.
trations of hemoglobin are reached at a state when the cytoplasmic ribose-nucleic acids have decreased below 0.5 per cent. The hemoglobin-containing leukemia cells, on the contrary, possess a very high average concentration of ribose-nucleic acids, giving the cytoplasm its pronounced basophilia. Thus, if the concentration of hemoglobin is taken as a measure of the degree of differentiation, this differentiation is indeed abnormal.

The myeloleukemia cells arising from the intersinusoidal leukopoietic tissue have no specific absorption in the Soret band spectral region. Substances of the peroxidase types, present in normal myelocytes, are at concentrations too low to be detectable by their natural absorptions. Comparing the two types of leukemia cells, the formation of detectable amounts of specific substances does not show any correlation with the clinical malignancy; the erythroleukemia in fowl under our laboratory conditions is a more acute disease than the myeloleukemia. Instead, the cytochemical difference found reflects the different types of stem cells affected by the virus-like agents.

In figure 6, cells 12, 13 and 14 are atypical erythroid elements from the human erythroleukemia. Cells 16 and 17 represent the case of acute lymphatic leukemia. For comparison, an absorption spectrum of an erythrocyte in the smear has also been included in the figure. A surprisingly high Soret band absorption is shown by the erythroid cytoplasms, but since they look very non-homogeneous, the height of the Soret band might have been influenced by an unknown magnitude of multiple scattering. We do not find it advisable to calculate any concentration figure from the present measurements. No specific absorption is displayed by the lymphoid leukemia cells. These results on the human material are in general conformity with the findings on the fowl leukemias. However, with regard to the less well-known etiologic and pathogenetic events of the human leukoses, most of the points discussed above are not directly applicable. It must suffice to postulate that the erythroleukemia cells, in spite of their pronounced atypia, possess microspectrographically detectable concentrations of hemoglobin in contrast to the other acute leukemia cells.

**Summary**

The neoplastic cells in acute erythroleukemia of the fowl appear to contain microspectrographically detectable amounts of hemoglobin in their cytoplasms. The acute myeloleukemia cells show no measurable specific absorption at all in the Soret band spectral region. Similar findings were obtained on cells from one case of human erythroleukemia and one of acute lymphatic leukemia.

**Summario in Interlingua**

Le cellulas neoplastic in acute erythroleucemia de gallinas pare continer in lor cytoplasma microspectrographicamentemente detegibile quantitates de hemo-globina. Le cellulas de acute myeloleucemia monstra nulle mesurabile absorption specific del toto in le region spectral del banda de Soret. Simile resultatos esseva obtenite con cellulas ab un caso de erythroleucemia human e un altere de acute leucemia lymphatic.
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Fig. 6.—Absorption spectra from the cytoplasms and pictures of the analyzed cells after staining. Cells 12 to 14 are from the case of human erythroleukemia, and cells 16 and 17 from acute lymphatic leukemia. The dotted curve represents absorption spectrum from an erythrocyte.

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