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Kiyoshi Hiraki*

*Okayama University,

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Kiyoshi Hiraki

Abstract

By our method of bone marrow culture and peripheral leucocyte culture, the differentiation of leukemia from other diseases is simplified. By this method the acute form of leukemia can be differentiated from the chronic form, and the classification of leukemia by the leucocyte series becomes easy and exact. It is believed that this method is clinically quite useful.

STUDIES ON DIAGNOSIS OF LEUKEMIA BY TISSUE CULTURE

Kiyoshi HIRAKI.

Department of Internal Medicine, Okayama University Medical School
Okayama, Japan.

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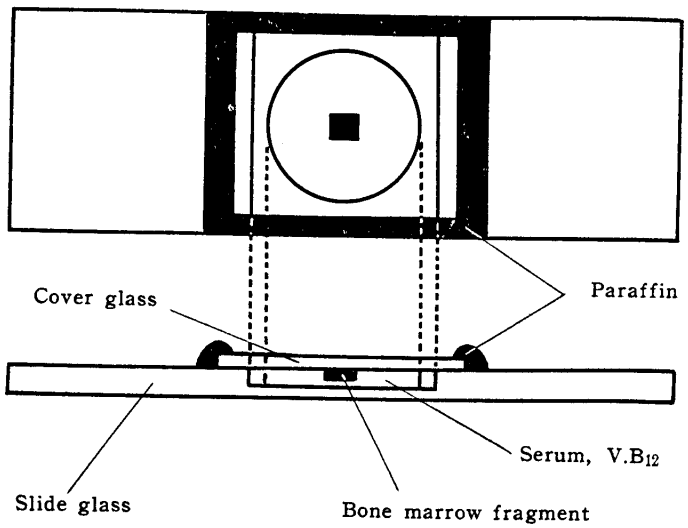
The diagnosis of leukemia is quite easy in the case of a typical chronic leukemia. In acute leukemia, especially an aleukemic case, the differentiation from other diseases such as aplastic anemia, agranulocytosis, or leukemoid reaction may be extremely difficult. Further, the classification of leukemia by cell type by conventional methods is not adequate, especially so in the case of acute leukemia in which there are many divergent views.

The anthor presents here a method of diagnosis of leukemia by tissue culture which is relatively feasible for clinical application and will give accurate results.

Method of Tissue Culture :

Schema of Tissue Culture Method
(Used in our clinic)

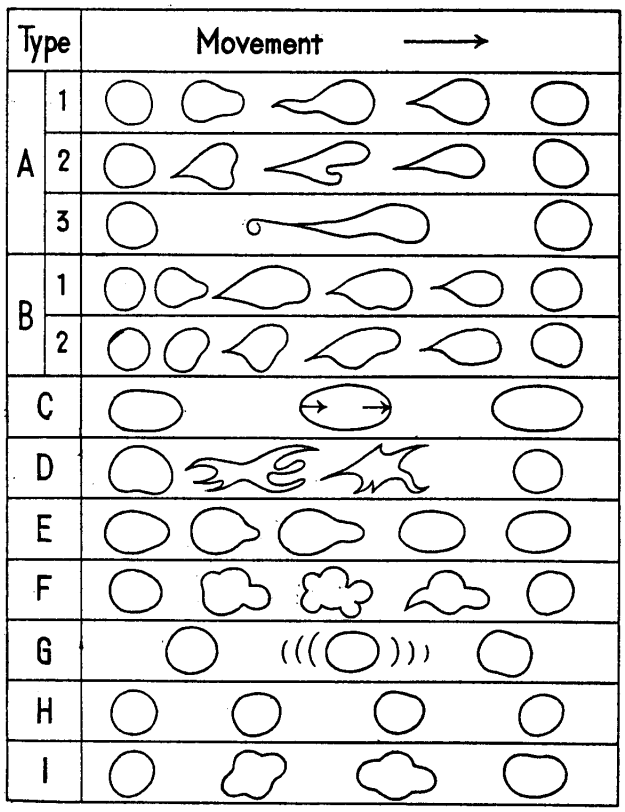


As shown in the above diagram tissue culture is conducted in a medium consisting of a drop each of normal human serum and vitamin B_{12} solution (containing 100γ in 1 c.c.) placed on a tissue culture plate of our own device. Six to 12 hours after explantation it is taken out of the incubator and observed. Generally

we use the aspirated bone marrow tissue and the fragments of the buffy coat of peripheral leucocytes obtained by using a silicon-coated test tube. Prior to the culture India ink and neutral red solutions are added to the medium in order to observe vital staining and phagocytosis of various cells. Acridine orange solution is added for the fluorescence-microscopic observations.

Findings on the Bone Marrow Tissue Culture of Normal Persons: On observing the growth zone in the bone marrow tissue culture of normal persons, young blood cells are seen quite densely packed in the central part near the explant, but in the intermediate to the peripheral zones the cells are scattered less densely; in these latter zones are found mainly leucocytes that are more mature and have more vigorous migratory capacity.

Classification of Type of Cellular Movement



We classify the different cells by observing motility, morphology of the nucleus and granules, etc; the classification of type of movement (A_1 in the left diagram to I), the characteristic behavior of various cells, and the findings of fluorescent microscopic observations.

In the course of culture, mature neutrophils appear early, then eosinophils, monocytes, and lymphocytes, in that order.

Next, observing the growth area under a fluorescence-microscope at a low magnification, the area is seen to be mainly diffusely red because the myeloid series contains red fluorescent granules. Among them are scattered yellow and green fluorescent points due to lymphocytes, monocytes, and various degenerated cells.

Findings on the Peripheral Leucocyte Culture of Normal Persons: As mature leucocytes appear in the growth zone close to the explant, they wander out to the periphery of the growth area with time. Cells in the central zone become extremely sparse and a space surrounding the explant appears, presenting a corona-like appearance. In the case of peripheral leucocyte culture,

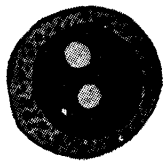
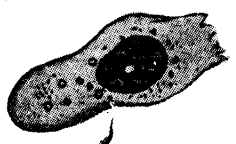
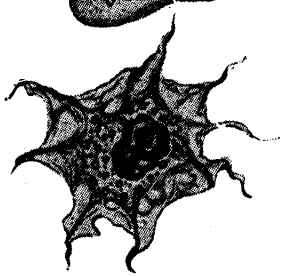
various cells appear in the same order as in the case of bone marrow culture.

Findings on Bone Marrow Tissue Culture in Leukemia: In acute leukemia,cells in the growth area are extremely dense and the boundary of the growth area is so distinctly defined as to make it readily distinguishable as acute leukemia. This characteristic pattern of the growth area is identical in acute myelogenous and acute lymphocytic leukemia.

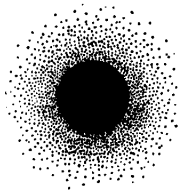
In chronic leukemia the well-defined boundary of the growth area with a high cell density, characteristic of acute leukemia, is surrounded by another less dense zone of mature cells, forming what we call a double-growth zone. This characteristic finding can be observed commonly in both chronic myelogenous and chronic lymphocytic leukemias.

Clinical symptoms of monocytic leukemia generally present symptoms intermediate between those of acute leukemia and chronic leukemias, and the growth pattern of bone marrow tissue in monocytic leukemia is somewhat similar to that in acute leukemia.

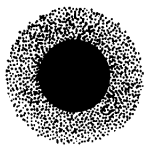
Characteristic Behaviors of VariousLeucocytes

Cells	Shape	Type of Movement
Myeloblast		H, I
Promyelocyte		H, I
Myelocyte		H, I
Metamyelocyte		B, E
Neutrophil		A, B
Eosinophil		A, B
Basophil		D, B
Lymphocyte		A, B, C
Monocyte		D

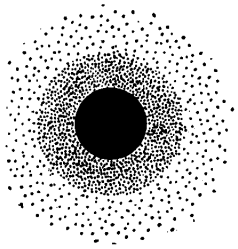
Bone Marrow Tissue Culture
(Pattern of tissue growth)



Normal Person



Acute Leukemia



Chronic Leukemia

The growth patterns of the bone marrow in acute and chronic leukemias as compared with that of the normal person are presented diagramnatically above (Figs. 1, 3, 4).

The growth patterns of normal bone marrow and of lenkemic marrow have been shown to be different. The author's contention for this difference will be explained here.

The leucocytes in leukemic bone marrow mainly consist of young leucocytes. The maturation of white cells is impeded. Young leucocytes of leukemic marrow and even mature leucocytes of leukemic marrow have a decreased migratory capacity. There is a differernce in degree of these factors between acute and chronic leukemias.

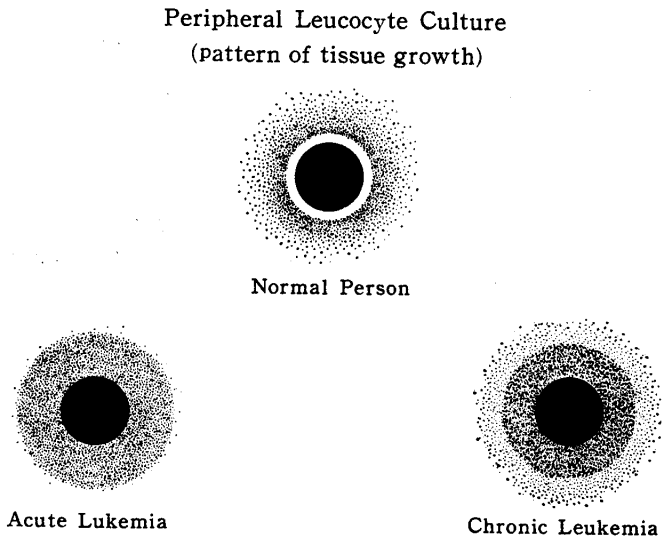
On the fluorescence-microscopic observations the growth area of leukemic bone marrow possesses these characteristics. It is noted that numerous cells belonging to the same series make their appearance, throwing off a peculiar fluorescence. In acute leukemia the growth area is green in color and distinctly defined with a clear-cut boundary. This is because blasts are green but do not possess reddish orange color granules. A few mature leucocytes appear in the growth area. In acute myelogenous leukemia the growth area therefore presents in addition to the green color a somewhat reddish tinge, while in acute lymphocytic leukemia a yellow tinge is notable. This phenomenon is especially marked in the periphery of the growth area.

In chronic leukemia the inner zone of the double growth zone reveals the reddish green color in myelogenous or the yellowish green color in lymphocytic leukemia. In the outer zone the fluorescence of mature leucocytes is especially predominant.

In monocytic leukemia there are many greenish orange promonocytes and mature monocytes, green monoblasts, a few neutrophils and yellow lymphocytes.

Monocytic leukemia presents multi-colored fluorescent effects, and the boundary of the growth area is relatively well defined.

Findings on the Peripheral Leucocyte Culture in Leukemia: As has previously been mentioned the corona-like formation can be seen when the fragments of the buffy coat of peripheral leucocytes from a normal person are cultured. In leukemia, as there already exist many young leucocytes in the original mass, the growth pattern is the same as in the bone-marrow tissue culture, as shown



diagrammatically above (Fig. 2). Likewise the fluorescence-microscopic findings are much the same as the bone marrow culture.

The Classification of Leukemias by the Leucocyte Series: By observing the behavior of the predominant living leucocytes appearing in the growth area, it is relatively easy to differentiate the specific type of leukemia as to cell type even in the case of acute leukemia which usually presents many conflicting problems.

Results obtained by tissue culture on 82 cases of leukemic patients admitted to our clinic during the past four years are shown in the right table. Although the number of cases is still too small to draw any definite conclusion, it has become apparent that monocytic leukemia is most numerous. Lymphocytic leukemia, thought to be rare in Japan, has been found relatively common in our experience. It is interesting to note

Classification of leukemic patients admitted in our clinic (Jan. 1954~April 1958)

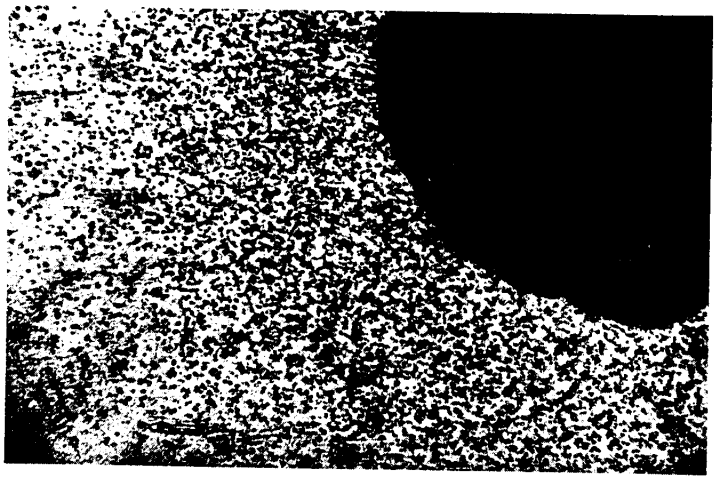
	Acute		Chronic	Total
Myelogenous L. (Neutrophilocytic)	19		10	29
Eosinophilocytic L.	0		0	0
Basophilocytic L.	2		0	2
Lymphocytic L.	16		1	17
Monocytic L.		32		32
Others	2		0	2
Total	39	32	11	82

that in Japan, unlike in Europe or in America, chronic lymphocytic leukemia is extremely rare. We have found in addition two cases of acute basophilocytic leukemia.

SUMMARY

By our method of bone marrow culture and peripheral leucocyte culture, the differentiation of leukemia from other diseases is simplified. By this method the acute form of leukemia can be differentiated from the chronic form, and the classification of leukemia by the leucocyte series becomes easy and exact. It is believed that this method is clinically quite useful.

Fig. 1

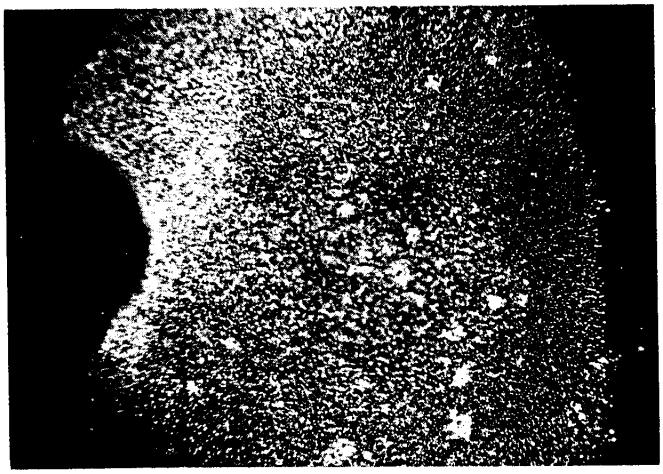


Normal person
Growth zone of bone marrow tissue culture

Fig. 2

Normal person
Growth zone of peripheral leucocyte culture

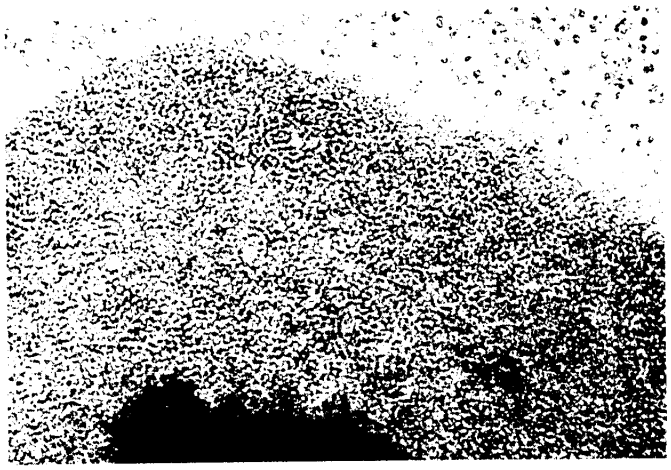
Fig. 3



Acute leukemia

Growth zone of bone marrow tissue culture

Fig. 4



Chronic leukemia

Growth zone of bone marrow tissue culture