"Monocytic" Cells of Normal Blood, Schilling and Naegeli Leukemia, and Leukemic Reticuloendotheliosis in Slide Chambers

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Cells of the monocytic series from both normal and leukemic bloods have been maintained and studied in special slide chambers in an effort to elucidate their interrelationships and transformations, and to determine the value of such studies in the diagnosis and characterization of monocytic and related leukemias.

The study of blood cells in the living state offered the possibility of aiding in cell identification. Supravital staining methods of Cowdry were utilized by such investigators as Sabin, Cunningham, Sabin and Doan, and Schwind and applied to studies of the white blood cells. Claims were even made that the blood cells could be more accurately distinguished than by use of the Romanowsky stains. Perhaps excessive stress on the importance of the vacuolar apparatus in the identification of cells, and the inability to visualize details of nuclear structure resulted in criticism and under-utilization of this method. Phase microscopy has permitted more satisfactory study of the living nucleus, and may be used with or without supravital staining. In simple petrolatum rimmed preparations, such physiologic characteristics as motility, cell spreading, and phagocytosis may be studied. In studies such as those of Dausset, however, weight of the coverslip has been a complicating factor in evaluating spontaneous cell flattening and spreading.

A slide chamber developed by Schrek and adapted to the study of motility and morphology of living cells by phase microscopy and cinemicrography, permits an extension of phase microscope studies. Counts of surviving cells are readily made. The chamber has the advantage of permitting study from day to day of the same cells as were obtained from the original blood specimen rather than their descendants. Cell transformations may be studied and conditions are good for detection of intermediate forms.

The duration of experiments is limited to a week or two, since change of medium is not practical in these chambers. Cell survival is enhanced by the use of relatively small numbers of cells in the chambers (1500 cells per cu. mm.). Survival of cells, as evidenced by their morphologic integrity and non-staining by eosin, has been of sufficient length for useful studies to be made. In the chambers, lymphocytes have a survival of 5–10 days, monocytes survive as macrophages 2–3 weeks, and immature blood cells survive 1–2 weeks. Mature granulocytes, on the other hand, are all dead in 2–3 days. This limits...
studies of granulocytes, but does clear the chambers for easier study of the surviving longer lived cells.

Optimal conditions are offered in the slide chamber not only for the study of the immediate cell morphology but also of the physiologic characteristics of motility, cell spreading, etc. Morphologic transformations of the cells and physiologic changes may be observed over a period of time. Observation of the cell for more than just a few hours permits the detection of changes in structure and function which may be useful in cell identification. "Blast" cells and other immature cells, for example, are characteristically rounded and non-motile during the first hours of incubation. After 24 hours' incubation at 37 C., many of them become elongated and motile. Visualization of structural detail is more satisfactory in the elongated, stretched-out state, and motility studies may be done. Mature granulocytes, on the other hand, show maximum flattening and spreading during the first hour or two of incubation and then quickly become motile and less flattened. Time lapse cinemicrography is readily adapted to the study of cells in the slide chamber. Motility is perhaps best studied in this fashion and has the advantage of providing a permanent record.

Schrek and Donnelly\textsuperscript{12} have studied normal, leukemic, and lymphosarcoma lymphocytes in the slide chambers and demonstrated morphologic and functional differences which were considered of diagnostic value.

The slide chamber also permits study of the response of cells to a variety of conditions. These may include external application of irradiation,\textsuperscript{13} changes in incubation temperature, or addition of reagents\textsuperscript{14} to the culture medium.

The present report is a study of normal and leukemic "monocytes" in slide chambers with emphasis on immediate phase morphology, cell flattening, motility, and—particularly—cell transformations after incubation at 37 C.

**MATERIALS AND METHODS**

Studies of normal monocytes were made on blood from technicians, physicians and patients who were first shown to be hematologically normal. Leukemic blood was obtained for study from patients at the Veterans Administration Hospital, Hines, Ill., and at the Cook County Hospital, Chicago, Ill. The diagnoses were established by the hematologic services at these institutions and were reviewed by us before use in the present study. Only those cases were selected which conformed closely to diagnostic criteria in the literature. Although these criteria are still admittedly controversial, they come reasonably close to being generally accepted. It will be evident in the results that these diagnoses proved useful as a starting point.

**Acute Monocytic Leukemia (Schilling Leukemia)**

Four cases were studied in the slide chambers. The diagnostic criteria conformed with those reviewed by Sinn and Dick\textsuperscript{15} and rested particularly on the following:

1. High total WBC counts, although sometimes not occurring until the terminal stage. Three cases had total WBC counts of 30–50,000 per cu. mm. The fourth ran a total WBC which never rose over 5,000 per cu. mm. Death in this case may have occurred prematurely as a result of therapy with 6-mercaptopurine. In all other respects this case conformed with the diagnostic criteria.

2. The finding of large numbers of grossly mature-looking monocytes in the peripheral blood, with some immature monocytes and blasts. On stained smears (fig. 1E) our
four cases all had 40–60 per cent of fairly mature monocytes, with up to 10 per cent immature monocytoid cells and a few blasts, 10–20 per cent lymphocytes, 10–20 per cent granulocytes with a few of these appearing as bands or metamyelocytes, and an occasional nucleated RBC.

3. The bone marrow aspirate showing increased numbers of blasts to almost total blastic infiltration, with an associated increase of reticulum cells and immature monocytic cells. Bone marrow aspirates in all our cases showed an infiltration with primitive reticulum cells (fig. 1F) differentiating along monocytic and histiocytic lines, and increased numbers of blast cells.

4. An acute clinical course with survival less than one year. All our cases were dead in less than six months from the onset of recognizable symptoms.

**Leukemic Reticuloendotheliosis**

Two cases of leukemic reticuloendotheliosis were studied in the slide chambers. The diagnoses were based on the criteria of Bouroncle et al.16 who noted that there was no idiopathic clinical sign or symptom, but that the hematologic picture was pathognomonic. They pointed out that the course of the disease might vary from acute to chronic, that splenomegaly might or might not be prominent, that anemia, thrombocytopenia or pancytopenia might be found, that bone marrow aspiration was usually difficult and that the picture might thus be readily confused with chronic progressive myelofibrosis. The diagnosis of leukemic reticuloendotheliosis rests then on the finding of numbers of reticulum cells in the peripheral blood and bone marrow with particular emphasis on the morphology of the living cells by phase microscopy.

*Case reports in brief.* 1. The patient (J. J.) was an 83 year old white male, former blacksmith, admitted to the Cook County Hospital on July 25, 1960, with a history of fatigue and weakness for several weeks. The pertinent physical findings included: marked pallor, ecchymotic areas on his body, absence of lymphadenopathy, liver palpable 2 cm., and spleen 4 cm. below the costal margin. The initial blood examination showed: hemoglobin 6.1 Gm. per 100 ml.; RBC 2.45 million per cu. mm.; WBC 2,900 per cu. mm. with 19 per cent neutrophilic polymorphs, 12 per cent band forms, 54 per cent lymphocytes, 1 per cent basophils, 1 per cent myelocytes, 5 per cent "blasts", 8 per cent monocytoid cells and reticulum cells (3 nucleated RBC were seen); platelets 83,000 per cu. mm. These findings varied little on repeated examinations throughout his course. The bone marrow was aspirated with difficulty. Megakaryocytes were present but reduced in number. The myeloid-erythroid ratio was 5:1. Erythropoiesis was normoblastic. There was a shift to the left of granulopoiesis. Reticulum cells were very prominent.

Despite several blood transfusions, the patient did poorly. A febrile course was attributed to a Clostridium septicemia. Death occurred on August 31, 1960.

2. A 64 year old white male (G. C.) admitted to Hines Veterans Administration Hospital on July 30, 1958, with a history of weakness and weight loss of six months' duration. The pertinent physical findings included liver palpable 4 cm. below the right costal margin and the splenic tip just palpable. There was no significant lymphadenopathy. Hemoglobin was 5.0 Gm. per 100 ml.; RBC count 2.0 million per cu. mm., total WBC 2,900 per cu. mm. with 2 per cent neutrophilic bands, 32 per cent lymphocytes, and 66 per cent reticulum cells. The bone marrow showed a marked reticulum cell proliferation. The patient was treated with 6-mercaptopurine without benefit and died on September 15, 1958.

On stained smears, reticulum cells were present in the peripheral blood (fig. 2E) in both cases. These were large cells, with spongy nuclear chromatin and cytoplasm which stained a pale blue. The bone marrow (fig. 2F) also showed an infiltration with reticulum cells.

**Acute Myelomonocytic Leukemia (Naegeli Leukemia)**

The diagnosis is based on the development of an acute "monocytic" stage during the course of granulocytic leukemia. Four cases of granulocytic leukemia in an acute "mono-
cytic" phase were studied in the slide chambers. In all, the monocytosis was a terminal stage, with survival for only a few weeks. They had in common high total white blood cell counts with large numbers of monocytoid cells in the peripheral blood and bone marrow associated with myeloblasts and immature granulocytes. Splenic enlargement was prominent.

Figure 3D illustrates the appearance on stained smears of the cells resembling monocytes grossly. Some of these, when well spread out (fig. 3E), were seen to have the spongy chromatin pattern of reticulum cells.

Methods for preparation of blood for slide chamber study, descriptions of the slide chamber and its use for inverted phase microscopy and time lapse cinemicrography have been detailed by Schrek.11 The culture medium used consisted of 50 per cent fresh human serum of the same blood type as the cells and 50 per cent Parker medium 199 (Difco). The slide chamber cultures were incubated at 37 C. immediately after preparation and were maintained continuously at this temperature. All microscopes were enclosed in plexiglass incubators.

RESULTS

Normal Monocytes in Slide Chambers

Morphology—0 day. The normal monocyte (fig. 1A) on initial examination after a half-hour incubation at 37 C. in the slide chamber has a large, pale, round, oval, or kidney-shaped nucleus with a fine, lacy, open chromatin pattern. The cytoplasm is in good quantity and is generally clearly demarcated into ecto- and endoplasm. A few small dark granules, presumably mitochondria, may be seen scattered in the endoplasm. A few vacuoles are sometimes present, but these have not been a prominent feature of the cells under the experimental conditions. The clear ectoplasm appears as a nongranular, ruffled, veil-like structure surrounding the cell and soon shows evidence of waving about in the fluid. High lights in the veil tend to give a spidery effect.

Cell flattening and spreading. A factor which has been very helpful in characterizing the white blood cells has been observation of differences in their tendency to flatten and spread out freely and spontaneously on the glass of the chambers. Both granulocytes and monocytes flatten and spread shortly after they settle to the bottom of the chamber. This is best observed after incubation at 37 C. for one-half hour. Flattening of monocytes in fresh serum may be so extreme that they become very thin and spread out on the glass to cover a large surface area. Careful focusing may be necessary to avoid missing them entirely. Lymphocytes and most immature or abnormal cells, on the other hand, fail to flatten and appear as round balls at zero time. It is sometimes difficult to distinguish granulocytes from monocytes in the early flattened state. The greater granularity and the multilobed nucleus help to identify the latter. The monocytes, and the macrophages derived from them, retain a degree of flattening throughout their chamber life. The granulocytes, on the other hand, soon become actively motile and in a few hours tend to give up their hold on the glass.

Motility of the monocyte in the slide chamber. The monocyte shows little tendency to move from place to place in the chamber. This contrasts markedly with the considerable motility of the granulocyte and lymphocyte as seen both at the microscope and with cinemicrography. The monocytes appear to be content to lie fixed to the glass with their veils waving about in the fluid medium. An occasional monocyte did show some ameboid movement.
Fig. 1.—Living cells in slide chamber incubated at 37 C., phase contrast, except (E) and (F) are Wright stained smears, all 1700 X. (A) Normal, 0-day monocyte, cell spreading and ectoplasm. (B) Normal, macrophage, 7-day, nucleus, and varied granules. (C) Normal, macrophage, 7-day, ectoplasm. (D) Normal, monocyte development to macrophage, 3-day, cell enlarged, onset of granules. (E) Acute monocytic leukemia, monocytes, peripheral blood, fixed, stained. (F) Acute monocytic leukemia, reticulum cell proliferation, bone marrow, fixed, stained. (G) Acute monocytic leukemia, 0-day monocyte, area increased by spontaneous spreading; resembles normal monocyte.

The Macrophage

The fully developed macrophage. After 7 days’ incubation, the macrophage is at the peak of its development. It is described as representing what we mean in this report by a normal macrophage. It will be shown below that certain deviations from the normal macrophage do occur with leukemic bloods.
At 7 days (fig. 1B) the macrophage is a large 40–80 μ in diameter when rounded and reaching up to 120 μ in length when extended. Both rounded and elongated, spindle-shaped forms are present. The voluminous cytoplasm is divided into an extensive granular endoplasm and a great ectoplasmic veil (fig. 1C). The endoplasm is filled with many granules, both light and dark, which vary greatly in size and shape. The most prominent, characteristic and numerous granules, however, are medium in size and are marked by their blackness and uniformity. Phagocytic and degenerative vacuoles are sometimes also seen.

The clear ectoplasmic veils resemble those of the monocyte. The ectoplasm encircles the cell, but becomes most apparent at the ends of the pseudopodial projections of the spindle-shaped cells. At the end of the typical long pseudopodial projection, there is usually a knob of endoplasm around which the ectoplasmic ruffle is clearly visible.

The nucleus is large, round to oval, and at this stage very sharply outlined. The well-defined nuclear membrane encloses a very light, clear nucleoplasm. This results in a marked contrast between the nucleus and the darker heavily granular cytoplasm. Usually one, sometimes two, clearly outlined nucleoli are seen.

The macrophage resembles the monocyte in its motility. There is a considerable waving of veils, but little locomotion from place to place in the chamber. Some ameboid movement in an occasional cell is seen.

The rounded forms have a propensity for sending out long pseudopodia and taking on a spindle shape. The projections of the spindle-shaped forms on casual observation give an impression of being fixed. Time-lapse photography, and sometimes prolonged observation at the microscope, demonstrate that they are often extended and withdrawn and that there are frequent changes in length and number of branches. This may give an impression of motility, yet the cells remain in one place for considerable periods of time.

**Transformation of monocyte into macrophage.** The transformation of monocytes into mature macrophages occurs gradually in the slide chambers over a period of several days and is usually completed in 5–6 days. The transformation involves a great increase in overall size of the cells, particularly of the cytoplasm. There is little increase in granularity during the first 2 days of incubation. About the 3rd day (fig. 1D) the number of endoplasmic granules begins to increase. In the next days of incubation there is a great progressive increase in the numbers of a variety of granules. By the 7th day the endoplasm is filled with granules. The clear ectoplasmic veils also increase considerably in amount. The nucleus too becomes larger, while the nucleoplasm becomes lighter and clearer, with usually 1, sometimes 2, prominent sharply defined nucleoli. Old macrophages tend to become less flattened and the nucleus may then become obscured by overlying granules.

**The Leukemic Monocyte**

A characteristic appearance, behavior and transformation of the normal monocyte in the slide chamber has been described above. Studies were made of leukemic cells of the histio-monocytic group seeking differences from the
normal monocyte which might be useful. Several cases of acute monocytic leukemia (Schilling leukemia), of myelomonocytic leukemia (Naegeli leukemia), and of leukemic reticuloendotheliosis were studied.

**Acute monocytic (Schilling) leukemia: Slide chamber studies.** In the slide chambers on initial examination after a half-hour incubation at 37°C., the monocytic cells from acute monocytic leukemia (fig. 1C) closely resembled normal monocytes. Some cells showed a slight increase in endoplasmic granularity. Spreading on the glass, motility and veil formation were much like the normal.

After 24 hours' incubation (fig. 2A), there regularly developed in the endoplasm of these cells a set of 10 to 30 coarse black granules. They were quite uniform in size and stood out in striking fashion against the background of the pale, thin, spread-out cells in which they lay. They resembled the heavy coarse black granules seen in fully developed macrophages from normal blood. After the 2nd day of incubation these granules were greatly increased in number. Heavy granules of this type were normally not seen so early. They started to appear in monocytes from normal blood about the 3rd day of incubation (fig. 1D) and became prominent on the 4th or 5th day.

Macrophage formation from acute leukemic monocytes after the second day of incubation proceeded very much as in the normal. The mature macrophages produced were for the most part similar to those developed from normal blood. Many, however, did not mature fully, were smaller than normal, and had fewer varieties of more uniform granules (fig. 2B).

The cell suspensions on initial examination contained a small number of cells which were recognized as blasts. During the first hours of incubation (fig. 2C) they were non-flattened, rounded, and about 10 to 12 μ in diameter. The nucleus was large with a clear nucleoplasm and prominent nucleoli. The cytoplasm was slight in amount, pale but darker than the nucleus. It contained a few granules, presumably mitochondria. No ectoplasm was demonstrable. These blast cells had no special characteristics to distinguish them from other blasts. After 24 hours incubation (fig. 2D), many of them became elongated and motile. Many of the motile blasts were seen to have slender, antenna-like cytoplasmic projections which, like the nucleus, appeared to be oriented anteriorly in the direction of the cells' movement.

Survival in the slide chambers, as with other immature cells, was prolonged and usually more than 2 weeks. An occasional mitotic figure was observed in the blast cells.

Intermediate forms between the blast and the monocyte were not evident on initial examination, nor was there evidence of maturation after further incubation.

**The "Reticulum" Cell**

The monocyte of acute monocytic leukemia (Schilling) in the slide chamber, as detailed above, had little to distinguish it from the normal monocyte. Cells with distinct characteristics, on the other hand, were found in slide chamber studies of the cells from patients with leukemic reticuloendotheliosis and in the acute "monocytic" phase of granulocytic leukemia (so-called Naegeli leu-
Fig. 2.—Living cells in slide chamber incubated at 37 C., phase contrast, except (E) and (F) are Wright stained smears, all 1700 X. (A) Acute monocytic leukemia, 1-day monocyte, early development of coarse granules. (B) Acute monocytic leukemia, 11-day macrophage, more uniform granules than in normal. (C) Acute monocytic leukemia, 0-day blast, mostly rounded, non-motile; this cell shows start of pseudopodia formation. (D) Acute monocytic leukemia, 1-day blast, elongated, motile, some ectoplasm at nuclear end which is oriented in direction of movement. (E) Leukemic reticuloendotheliosis, reticulum cells, peripheral blood, fixed, stained. (F) Leukemic reticuloendotheliosis, bone marrow, reticulum cell infiltration, stained smear.
kemia). These distinctive cells had a resemblance to monocytes, but differed from them in morphologic detail and with respect to spreading on glass, motility, and transformation to macrophages. The cells in the two diseases appeared more closely related to each other than to normal monocytes. These cells were labeled or defined as "reticulum" cells. They were like the reticulum cells of Bouroncle et al.16

**Leukemic reticuloendotheliosis: Slide chamber studies.** In the slide chamber after an initial half-hour incubation at 37 C., granulocytes, lymphocytes, and some "blast" cells were seen. The most frequent cell, the reticulum cell, bore some resemblance to the monocyte. Some reticulum cells were rounded, while others showed various degrees of flattening; but none showed the extreme flattening of the monocyte. They differed considerably from monocytes also in their structural detail.

The reticulum cells were about the same size as monocytes. Their nuclei were large, round to oval, and surrounded by a sharply defined nuclear membrane. The nuclear chromatin pattern was coarser than in the monocyte, with some clumping. There was a tendency for chromatin clumps to be attached to the nuclear membrane. Nucleoli were not prominent at this time, but could be made out in some of the cells. The cytoplasm was reduced in quantity compared to the monocyte. There was little or no ectoplasm as opposed to the extensive ectoplasmic veils of the monocyte. The cytoplasm contained many different sized granules which were much more numerous than in the normal monocyte. In contrast to the few, uniform-in-size, coarse, large, dark granules seen after 24 hours' incubation in the monocytes of acute leukemia, the granules in the reticulum cells were more numerous and varied considerably in size.

After 24 hours' incubation the cells were only slightly flattened and showed considerable ameboid motility. They moved about the chamber almost as actively as granulocytes. This motility contrasted greatly with the sluggish ameboid activity and veil waving of the monocyte.

Transformation to macrophages was slow and abnormal (fig. 3C). The enlarged, clear macrophage type nucleus developed, but ectoplasmic veils were often absent or much reduced. Some cells were star-shaped with several long, thin, granular pseudopodia (fig. 3B). These star-shaped cells did not develop from normal or acute leukemic monocytes. There was no tendency for clubbing of the ends of the pseudopodia and ectoplasmic ruffles about the pseudopodial tips were absent. Some star-shaped cells had very long, fine, filamentous fibrillar threads extending from the tips of their pseudopodia. A few normal macrophages did develop, but it was not certain whether they originated from a few normal monocytes in the original blood or from the reticulum cells.

**Acute myelomonocytic leukemia (Naegeli leukemia): Slide chamber findings.**

On initial examination after a half-hour incubation at 37 C., some flattened granulocytes, an occasional flattened normal monocyte, and a rare lymphocyte were seen. The balance of the cells were fairly evenly divided between "reticulum" cells and "blasts." The reticulum cells closely resembled the cells
Fig. 3.—Living cells in slide chamber incubated at 37 C., phase contrast, except (D) and (E) are Wright stained smears, all 1700 X. (A) Leukemic reticuloendotheliosis, 0-day reticulum cells. (B) Leukemic reticuloendotheliosis, 8-day star-shaped macrophage, granular endoplasmic pseudopodia, without ectoplasmic veil. (C) Leukemic reticuloendotheliosis, 14-day reticulum cell, minimal macrophage changes, no ectoplasm. (D) Naegeli leukemia, monocytoid cell, peripheral blood, stained smear. (E) Naegeli leukemia, reticulum cell, peripheral blood, stained smear. (F) Naegeli leukemia, 0-day reticulum cell, rounded, non-motile. (G) Naegeli leukemia, 0-day, blast, rounded, non-motile until 1-day incubation. (H) Naegeli leukemia, 1-day reticulum cell, motile. (I) Naegeli leukemia, 1-day reticulum cell, star-shaped form. (J) Naegeli leukemia, 1-day intermediate form, blast nucleus, reticulum cell cytoplasm.
seen in leukemic reticuloendotheliosis. Most of them (fig. 3F) were non-flattened and rounded, although an occasional cell spread out partially and some of these extended pseudopodia. The large round to oval nucleus had a coarse chromatin pattern with some chromatin clumping. Some chromatin clumps appeared to adhere to the sharply defined nuclear membrane. A nucleolus was seen only occasionally. The cytoplasm contained a large number of granules of varied size. Little or no ectoplasm could be seen. In the extended cells a small amount of ectoplasm was more readily detected.

The "blast" cells (fig. 3G) at this time were smaller than the "reticulum" cells, but they too were for the most part rounded and non-flattened. The nucleus was large with a pale clear nucleoplasm and contained one, and sometimes several, sharply defined nucleoli. A few dark granules were seen in the cytoplasm.

After 24 hours' incubation at 37°C, many of the reticulum cells (fig. 3H) had spread out partially; spindle-shaped, elongated, and star-shaped forms (fig. 3I) were present. On prolonged observation such cells were seen to retract their pseudopodia and round up. On cinemicrography, like the cells of leukemic reticuloendotheliosis, they displayed considerable ameboid motility. Granularity was unchanged.

On further incubation the reticulum cells were seen to develop in the direction of the macrophage. This development differed from the normal in that the macrophages formed were smaller than normal, developed more slowly, had fewer and less varied granules and differed in nuclear development. The nuclei were smaller and darker and the nucleoli less sharply defined. Star-shaped forms with little ectoplasm, similar to those seen in leukemic reticuloendotheliosis, were prominent. In one of the cases studied, the macrophages showed considerable ameboid motility.

Many of the blast cells at 24 hours also became elongated and motile. There were no basic structural changes noted in these cells and they retained a similar appearance throughout their chamber life, although they survived for about 2 weeks.

Cells intermediate between reticulum cells and blasts were observed initially and after incubation. Figure 3J shows such a cell after 24 hours' incubation at 37°C, with the pale nucleoplasm and the nucleolus of a blast, yet with the overall appearance of a reticulum cell.

**Discussion**

Slide chamber phase microscope studies, especially when used in conjunction with time-lapse photography, offer a means for observing detailed functional and morphologic changes in cells during periods of incubation of up to 2 to 3 weeks.

The indispensability of studies of living cells in the histiomonocytic leukemias is unquestionable. Even simple rimmed cover-slip preparations will provide much valuable data without recourse to the more complex technics and apparatus used in the present study. Other authors\(^{10,16}\) have stressed the importance of the study of the living cell as an adjunct to the study of stained
smears. Bouroncle et al.\textsuperscript{15} for example, noted that diagnoses of chronic or acute lymphatic leukemia based on stained smears had been made in some of their cases of leukemic reticuloendotheliosis before study of the living cells revealed that the predominant cells were reticulum cells.

\textit{Differential Cell Characteristics}

The slide chamber differential findings of the cells detailed in the results are reviewed in brief:

1. \textit{Normal monocytes}. Normal monocytes were characterized by their marked flattening and spreading in fresh compatible serum and by the sharply defined clear ectoplasmic veils encircling them. Their endoplasm contained a few vacuoles and mitochondria. Their pale, round to kidney-shaped nuclei had fine, lacy, open chromatin patterns. Monocytes were readily distinguished from lymphocytes, which had no demonstrable ectoplasm (except in small hyaline pseudopods), failed to flatten and spread on the glass, and were much more motile after incubation. Granulocytes did have some ectoplasm, although not as well developed as the monocytes, and did flatten and spread out on glass, but their heavier endoplasmic granularity, multilobed nuclei, and very active ameboid motility after incubation at 37 C. identified them.

2. \textit{Normal macrophages}. After incubation at 37 C., monocytes gradually developed over a period of 5–6 days into fully developed macrophages. This transformation involved a great increase in overall cell size, marked increase in cytoplasmic granularity (not pronounced until the 2nd or 3rd day of incubation), concomitant enlargement of the ectoplasmic veil encircling the cell, and the development of a prominent nucleolus in a pale, clear nucleus.

3. \textit{Monocytes and macrophages of acute monocytic leukemia}. The monocytes of acute monocytic leukemia resembled normal monocytes closely, although some cells showed an increase in mitochondria. Macrophage development was similar to that of normal monocytes except for the earlier development of heavy, coarse, dark cytoplasmic granules.

4. \textit{"Blasts"}. Some blast cells were found in all the cases of acute monocytic leukemia, acute myelomonocytic leukemia, and leukemic reticuloendotheliosis. These were small cells, rounded and non-motile at first, but becoming elongated and motile after several hours' incubation at 37 C. The nucleus was large with a prominent nucleolus, and the cytoplasm sparse with few mitochondria. The blasts from all cases were indistinguishable for practical purposes.

5. \textit{Reticulum cells}. The reticulum cells had a superficial resemblance to monocytes. They differed from monocytes in that cell flattening was slight or minimal, the nuclear chromatin pattern was coarser with some clumping, the ectoplasm was much less voluminous, the endoplasm had more numerous and varied mitochondria, and considerable ameboid activity was evident after incubation for several hours at 37 C. They were seen in both leukemic reticuloendotheliosis and myelomonocytic leukemia.

6. \textit{Abnormal macrophages}. The macrophages formed in both leukemic reticuloendotheliosis and acute myelomonocytic leukemia were similar, but dif-
fered from the normal. They developed slowly and abnormally and were smaller in size. Ectoplasmic veils were absent or much reduced. Star-shaped forms with several long granular pseudopodia without ectoplasmic ruffles at their tips were frequent. These cells did not develop from normal blood.

Observations on the Formation of Macrophages

The transformation of monocytes into macrophages in the slide chambers has been described in detail. The development of large phagocytic cells from the mononuclear cells of the blood was noted by the early tissue culturists. These large cells were given a variety of names, including: macrophages by Carrel, Lewis and Lewis; polyblasts by Maximow, and Phase II cells by Berman. Most observers have agreed that monocytes could transform into macrophages. Some investigators, such as Maximow, Bloom, Rebuck, and Klein thought that lymphocytes could also transform into polyblasts (macrophages) while others denied this.

In the slide chambers macrophages appeared to develop only from monocytes. Intermediate forms between monocytes and macrophages were regularly observed while intermediates between lymphocytes and macrophages were not seen. In experiments to be described in detail elsewhere, the impression that macrophages developed only from monocytes was reinforced. It was found that the number of macrophages formed in the slide chamber corresponded to the number of monocytes initially present. It was demonstrated, too, that lymphocytes, although not developing into macrophages, nevertheless had a potential for undergoing cell transformation in the slide chambers. Under the influence of phytohemagglutinin (Difco) they were observed to transform into blast-like cells.

Observations on Cell Relationships

The classification of the histiomonocytic leukemias has been the subject of much controversy over the years. As a point of departure in our slide chamber studies it was necessary to make use of the terminology which is in current clinical use. The slide chamber findings helped substantiate the diagnoses and served too as a basis for further speculation on the relationships of the cells involved.

The relatively normal appearance of the monocytic cells of acute monocytic leukemia has been difficult to reconcile with leukemia. In the slide chambers the similarity in appearance and development of normal and acute leukemic monocytes was evident. Blast cells, nevertheless, are usually found in the peripheral blood, as was true in all of our cases. The combined findings of high monocyte and total WBC counts, blast cells, acute clinical course with rapid death in the absence of any other evident cause, and reticuloendothelial proliferation have led to the acceptance of acute monocytic leukemia as an entity. Almost 40 years of improvement in diagnostic and therapeutic methods have helped to overcome Naegeli's contention that cases which were not a stage of granulocytic leukemia represented a reactive monocytosis.
Our experience has not included a case which could be labeled “chronic monocytic leukemia.” We suspect that in some instances this diagnosis may be synonymous with “leukemic reticuloendotheliosis.”

The “Reticulum” Cell

The cells which have been labeled “reticulum” cells were objects of great interest to us. On stained smears, as well as in the living state, they appeared to be related to the histiomonocytic series. In the slide chamber, on initial examination, they corresponded closely in appearance to the descriptions by Bouroncle et al. of the cells of leukemic reticuloendotheliosis. In the slide chambers the reticulum cells showed distinct differences in morphology, physiology and development from normal monocytes and from the cells of acute monocytic leukemia.

The similarity of the reticulum cells of leukemic reticuloendotheliosis and of myelomonocytic leukemia suggested that they were closely related, if not identical. It was noted by Downey that reticulum cells might be found in the blood smears of Naegeli leukemia. He showed illustrations of such cells which are similar to the reticulum cells in our stained smears. He thought that the finding of a few such cells indicated only a minor participation of the reticuloendothelial system and was probably of no importance. It is our impression, however, that the reticulum cells seen in Naegeli leukemia should not be ignored. In the living state, as seen in the slide chambers, these cells appear to be more numerous than is apparent on stained smears. Their true nature may not be evident unless they are studied while alive. We have stressed the finding of reticulum cells in myelomonocytic leukemia not only for its diagnostic value but also for its significance in better understanding the relationships of the cells involved.

The presence of “reticulum” cells in the blood of acute myelomonocytic leukemia offers a cellular differential from true monocytic leukemia. The finding of reticulum cells differentiates the cases which are first observed in an acute monocytic phase, with death occurring before evidence of granulocytic leukemia can become evident. We are confident of our ability to distinguish the two conditions on the basis of the slide chamber findings.

The “reticulum” cell seen in the blood in reticuloendotheliosis and myelomonocytic leukemia may be closely related to the hematopoietic reticulum cell. It, perhaps, corresponds to the “primitive cells” which Cunningham, Sabin and Doan placed between the hematopoietic reticulum cell and the blast cells. Intermediate forms between “reticulum” cells and blasts were often seen in the slide chambers. It is our interpretation that the reticulum cells produce blasts. Naegeli noted intermediate cells between myeloblasts and monocytoid cells in his preparations from myelomonocytic leukemia. He thought that the myeloblasts produced the monocytoid cells. Our findings are based on daily observation of the same group of living cells in slide chambers during incubation for 2 to 3 weeks. This would appear to provide more evidence than study of cells appearing at a given instant in a blood smear.

Further maturation of blast cells in the leukemias under discussion was not
seen, although we have occasionally noted some abnormal maturation of myeloblasts in granulocytic leukemia. It is not clear whether this maturation arrest of the blasts is due to an intrinsic defect of the cells, to deficiencies in the slide chamber environment, or to a combination of these factors.

We have speculated that during the granulocytic phase of Naegeli leukemia the cells found in the blood are the product of myeloblast proliferation and development. In the "monocytic" phase, on the other hand, there is a proliferation of pre-blast ("reticulum") cells which spill over into the blood. Some of these may represent cells which have undergone further abnormal maturation toward the monocyte, but blastic maturation is arrested. Variations in unknown factors in the disease process in Naegeli leukemia may at times cause an accentuation of myeloblast proliferation with production of granulocytic cells while at other times accelerating pre-myeloblast cell activity to produce the monocytoid cells. Such factors might also affect the type of cell actually released from the marrow.

Our views have been based on morphologic observation and interpretation. Biochemical and/or histochemical studies might be of aid in substantiating the concepts presented.

**Summary and Conclusions**

1. The study of living blood cells in slide chambers by phase microscopy and cinemicrography adds useful morphologic, developmental and physiologic data complementary to other methods for studying blood cells.
2. Normal monocytes were characterized by flattening in fresh serum with minimal ameboid motility, by ectoplasmic veils waving in the medium, and by transformation to fully developed macrophages in 5–7 days.
3. Cells from four patients with acute monocytic leukemia (Schilling type) showed only slight differences in morphology, development and function from normal monocytes in the slide chamber.
4. Monocytoid cells from four patients with myelomonocytic leukemia (Naegeli type), in the slide chamber, resembled those from two patients with leukemic reticuloendotheliosis in morphology, development and function, but differed from normal monocytes and cells of acute monocytic (Schilling) leukemia. The monocytoid cells were labeled "reticulum" cells with the implication that they were related to the primitive hematopoietic reticulum cell.
5. The "reticulum" cell in the slide chamber differed from the monocyte in having a coarse nuclear chromatin pattern with clumping of chromatins, larger numbers of varying sized cytoplasmic granules, less ectoplasm, development of characteristic star-shaped macrophage forms without ectoplasmic veils, and in exhibiting a considerable ameboid motility as opposed to the sluggish veil waving of the monocyte.
6. Speculation as to the significance of the "reticulum" cell has been presented with the suggestion that this cell precedes the blast cell in hematopoiesis. In Naegeli leukemia, unknown factors may cause alterations in the type of cell released from the marrow. There may also be shifts in emphasis between
myeloblast and pre-myeloblast proliferation which become reflected in the cells appearing in the peripheral blood.

**SUMMARIO IN INTERLINGUA**

1. Le studio de vive cellulas de sanguine in cameras-lamina per microscopia e cinemicrographia a contrasto de phase contribue utile datos morphologic, disveloppamental e physiologic que complementa le datos obtenite per altere methodos de studiar le cellulas del sanguine.

2. Monocytos normal esseva characterisate per applattamento in sero fresc, con un minimo de motilitate ameboide, per velos de ectoplasma undulante in le medio, e per le transformation ad in completely disveloppate macrophagos in le curso de 5 a 7 dies.

3. Cellulas ab quatro patientes con acute leucemia monocytic (typo de Schilling) monstrava in le camera-lamina solmente leve differentias ab mono-
cytos normal con respecto a lor morphologia, lor disveloppamento, e lor functionamento.

4. Cellulas monocytoide ab quatro patientes con leucemia myelomonocytic (typo de Naegeli) resimilava in le camera-lamina illos ab duo patientes con reticuloendotheliosis leucemic con respecto a lor morphologia, lor disvelop-
pamento, e lor function, sed illos differeva con respecto a ille tractos ab monocytos normal e etiam ab cellulas de sanguine a acute leucemia monocytic (typo de Schilling). Le cellulas monocytoide esseva designate como cellulas de “reticulo” con le implication que illos esseva affin al primitive cellulas hematopoietic del reticulo.

5. Le cellula de “reticulo” in le camera-lamina se monstrava differente ab le monocyto in tanto que illo esseva characterisate per un crude configuration de chromatina nuclear con agglutination del chromatina, plus grande numeros de granulos cytoplasmatic de varie dimensiones, minus ectoplasma, e le dis-
veloppamento de characteristic formas stellate de macrophago sin velos ecto-
plasmatic, e in tanto que illos exhibiva un considerabile grado de motilitate ameboide per contrasto con le lethargic undulation de velos exhibite per le monocyto.

6. Es presentate certe speculationes in re le signification del cellula de “reticulo”; es sugerite que iste cellula precede le blastocytos in le hematopoiese. In leucemia de Naegeli, factores incognoscite possibilemente causa alterationes in le typo de cellula liberate per le medulla. Alterationes in le proportiones del proliferation de myeloblastos e de illo de pre-myeloblastos etiam es possibilemente reflectite in le cellulas que appare in le sanguine peripheric.

**REFERENCES**

3. Cunningham, R. S., Sabin, F. R., and Schwind, J. L.: The supravital method...


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"Monocytic" Cells of Normal Blood, Schilling and Naegeli Leukemia, and Leukemic Reticuloendotheliosis in Slide Chambers

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