Long-Term Mixed Cultures of Human Hemic Cells, with Granulocytic, Lymphocytic, Plasmocytic and Erythrocytic Series Represented

By John H. Brooke and Edwin E. Osgood

Stabilized strains of rapidly multiplying cells established from peripheral blood or bone marrow of patients with various hemic disorders, and representing cells of each hemic series, have been the aim of our laboratory for several years. The present communication reports new cell strains (table 1) containing cells derived from monocytic, granulocytic, lymphocytic, plasmocytic and erythrocytic series. Growth of cells from lymphocytic, plasmocytic and erythrocytic series was obtained by adding these cells to a previously well established cell culture such as Oregon J111. The observations that cells of these series grew well in the immediate vicinity of cells of monocytic or granulocytic series, and that in the DeBruyn mouse lymphoma cell strain, which has been cultured for many years, lymphoid cells grew initially only in the presence of a "fibroblast-like" cell, the work of Bichel, and results obtained by the feeder layer technic of Puck—all these observations led to the hypothesis that perhaps human lymphocytic, erythrocytic and plasmocytic series might be more easily grown if they were added to a cell strain well established in culture. This has proved to be a correct assumption, and several of the subsequently described cell strains were originated in this manner.

Methods

The methods now recommended for maintenance of cell strains isolated from human blood or bone marrow are as follows.

Medium.—The medium used for maintaining these cell strains is essentially that of Eagle. The 100 × concentrates of amino acid mixture, vitamin mixture and glutamine are added to a simplified Osgood balanced salt solution of the following composition: NaCl, 7.50 Gm.; KCl, 0.375 Gm.; Na₂HPO₄, 0.125 Gm.; KH₂PO₄, 0.03 Gm.; MgSO₄, 0.075 Gm.; CH₃COONa, 0.5 Gm.; dextrose 2.0 Gm.; and distilled water to make 1 liter. To this
TABLE 1.—Long-Term Cultures of Human Hemic Cells Now Available*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Date Isolated</th>
<th>Source</th>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Blood Type</th>
<th>Cell Series Predominant</th>
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<tr>
<td>J96</td>
<td>4/7/54</td>
<td>B</td>
<td>OSK112</td>
<td>26</td>
<td>M</td>
<td>ALML A</td>
<td>A Rh+</td>
<td>Monocytic</td>
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<td>B</td>
<td>OSK222</td>
<td>25</td>
<td>F</td>
<td>ALML A</td>
<td>A Rh--</td>
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<td>1957</td>
<td>J96</td>
<td>J128</td>
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<td>9/2/55</td>
<td>B</td>
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<td>F</td>
<td>CLGL O</td>
<td>O Rh+</td>
<td>Granulocytic</td>
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<tr>
<td>M166,J111</td>
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<td>OSK1063</td>
<td>65</td>
<td>M</td>
<td>CLLL B</td>
<td>Rh+</td>
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<td>3/15/57</td>
<td>B</td>
<td>MSH244304</td>
<td>1</td>
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<td>3/29/57</td>
<td>B</td>
<td>MCH241428</td>
<td>9</td>
<td>F</td>
<td>CLGL O</td>
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<td>J181,J111</td>
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<td>11/15/57</td>
<td>B</td>
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<td>12/6/57</td>
<td>M</td>
<td>MCH84010</td>
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<td>M</td>
<td>ALBL A</td>
<td>O Rh+</td>
<td>Basophilic</td>
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</tbody>
</table>

*Seeds cultures from these strains are available from R. W. Brown, Carver Foundation, Box 188, Tuskegee Institute, Ala.

18 = blood, M = marrow.

*Clones isolated by Theodore Puck's method at University of Colorado Medical Center, Denver. We are indebted to Mrs. St. Vincent of the Pediatrics Dept. for J111 and to Dr. Puck of the Biophysics Dept. for J96.

was added 10 per cent pooled human serum (Courtland Laboratories) and 100 units penicillin for each ml. Before use, the pH is adjusted to 7.2-7.3 with sterile 5 per cent CO2 in air, and the CaCl2 is added from a 100X stock sterilized separately, CaCl2, 25 Gm., and water to make 1 liter; 24 ml. rectangular borosilicate glass bottles are used for the bulk culture flasks, and white gum rubber stoppers are used for closure. Ten ml. of medium adequately covers one flat side of the bottles used.

Medium change.—The medium is routinely changed two times a week in the case of dense cultures. Sparse cultures will require less frequent change, and very dense populations require additional medium changes when the pH approaches 6.8.

Subculture.—Subcultures are made only from bottles with a very dense population (15 to 30 million cells) on a glass surface of 60 sq.cm., and large initial inocula (2 to 5 million cells) are used to set up new cultures.

To subculture a dense bottle, the supernatant medium is first discarded and replaced with 10 ml. of fresh medium; to this is added 1 ml. of a 1.5 per cent solution of disodium EDTA in distilled water and the pH raised to 7.3-7.4 with one to three small drops of 5 per cent Na2CO3 solution. After 10 to 15 minutes, the cells have been released from the glass, and the bottle is agitated to break up cell clumps. To this cell suspension, 30 ml. of additional medium is added and mixed. A 1 ml. aliquot is removed for counts and smears and the remaining 40 ml. of cell suspension is distributed, 10 ml. into each of 4 new sterile culture bottles. After the 1:4 dilution of the EDTA the cells reattach to the glass in two to three hours, and the supernatant medium may be decanted and replaced with fresh medium to eliminate the EDTA. This last step may be done the following day.

Here it should be emphasized that it has been found to be mandatory to use large initial inoculum and to maintain a high population density when dealing with the mixed cell strains; otherwise the desired cell type may be lost. This is particularly true of the erythrocytic (J173; J184B) and lymphocytic series (M166; J169), which require a high gradient factor. The number of cells per cu.mm. should be sufficient to give at least a gradient factor of 2 to 5 with the volume of medium and the size and shape of the container used. The gradient factor is defined as the product of the
depth in cm. times the cell count per cu.mm. expressed in thousandths. Much higher gradient factors are necessary for initial isolation of the lymphocytic and erythrocytic series.

**ESSENTIAL CLINICAL DATA**

Note that all cultures were made from blood except 184B and that in every instance in which only the mixed cultures were successful, unsuccessful efforts were made to culture the patients' cells alone at the same time and in the same media.

**Oregon J128.—OSK26,** from whose blood culture J128 was derived, was first seen in the Division of Experimental Medicine on April 22, 1955. This 59 year old white farmer's wife first noticed enlargement of her abdomen on December 15, 1953. She was first diagnosed as having chronic leukemic granulocytic leukemia in June, 1954. Between June and August of 1954 she had had 11 deep x-ray treatments to the spleen. On August 3, 1954, her leukocyte count was 102,000, with 8.7 Gm. of hemoglobin. She had received no therapy since August of 1954, and since 1953 had lost 71 pounds (from 215 pounds to 144 pounds).

Physical examination on April 22, 1955 revealed no petechial bleeding, 1+ cervical and axillary lymph nodes and an enormous spleen extending into the right iliac fossa. The liver measured 12 cm. below the right costal margin. She was started on titrated, regularly spaced Pu therapy on that day. Culture J118 was started from her blood on this date and grew well but was lost 106 days later as a result of a laboratory accident. On April 22, 1955 her leukocyte count was 355,000/cu.mm., with a typical differential for chronic granulocytic leukemia, and a hemoglobin of 6.8 Gm. On September 2, 1955, when culture J128 was started, her leukocyte count was 76,800, with 5.5 Gm./100 ml. of hemoglobin. The differential count on the day of the culture was 11 per cent myeloblasts, 4 per cent progranulocytes, 3 per cent myelocytes, 6 per cent metamyelocytes, 22 per cent band cells, 19 per cent basophils, 2 per cent eosinophils, 3 per cent lymphocytes, 2 per cent monocytes, 2 per cent nucleated erythrocytes and the balance disintegrated cells. Her leukocyte alkaline phosphatase score, by the method of Kaplow,* was 50, somewhat higher than that usually seen in cases of chronic granulocytic leukemia. She responded well to titrated, regularly spaced Pu therapy, and was able to return to heavy farm work in addition to her housework. The spleen had receded to 10 cm. below the costal margin by May 23, 1956, but on this date the blood picture of the acute terminal phase of chronic granulocytic leukemia was first noted. From this time on she had a rapid downhill course with splenic infarction, terminal bronchopneumonia and death on June 10, 1956, caused by the acute terminal phase of chronic granulocytic leukemia, confirmed by necropsy.

**Oregon M166, J111.—OSK1063,** a 65 year old married male, from whose blood culture M166, J111 was started, was first seen in the Division of Experimental Medicine on February 12, 1957. He had been referred from Idaho for treatment of chronic leukemic lymphocytic leukemia. In October, 1950, he had a leukocyte count of 29,000 with 65 per cent lymphocytes. Repeated counts since then had totaled 65,000 or over, with lymphocytes from 82 to 96 per cent. He had no specific therapy, although he had noted progressive fatigue. A basal cell carcinoma of the nose was first noticed in 1954 and was still present. He had had a transurethral prostatectomy in 1952 for benign prostatic hyperplasia. He had no complaints other than fatigue and nocturia one or two times.

Physical examination revealed a weight of 190 pounds with no weight loss, blood pressure 170/100, and a spleen extending 12 cm. below the left costal margin to within 2 cm. of the midline. The liver and lymph nodes were not significantly enlarged. There was no bleeding.

His initial leukocyte count on the day of culture (February 12, 1957) was 136,000, with 51 per cent lymphocytes and 46 per cent disintegrated lymphocytes, 1 per cent segmented neutrophils and 1 per cent monocytes. The bone marrow examination on this
date showed almost complete replacement of the marrow by cells of the lymphocytic
series. He was started on titrated regularly spaced P₇ therapy after the culture was
taken, and has shown an excellent response. He is still living and doing all his usual
activities as of April 1, 1958. On March 5, 1958 his leukocyte count was 16,000 and
hemoglobin 15.8 Gm., and the spleen was not palpable.

Oregon J169,J128.—M5H244304, from whose blood culture J169,J128 was derived, is
a female infant born September 23, 1955 who was first admitted to the Doernbecher
Unit on March 14, 1957 because of ecchymoses on the forehead and tibia dating from
March 10, and 4+ lymphadenopathy of cervical, posterior occipital, axillary and inguinal
nodes, her liver palpable 4 cm. below the right costal margin and her spleen 5 cm.
below the left costal margin. The hemoglobin on admission was 8.4 Gm. and leukocyte
count 352,500, with 60 per cent lymphoblasts and prolymphocytes. X-rays of the skull,
chest and long bones revealed no abnormalities. She was seen only in consultation by
members of the staff of the Division of Experimental Medicine, and culture J169,J128
was started the day after admission. Bone marrow aspiration on March 15, the day of
culture, showed only lymphoblasts and prolymphocytes with no megakaryocytes found.
She was treated with Meticorten, which produced a good clinical response, and dis-
charged from the Doernbecher Unit on April 11, 1957, to be followed by her local
physician. She was readmitted on June 20, 1957 with extensive hematomas and a leu-
kocyte count that had risen to 129,000 from its low point of 2,000 on March 25 and
was treated with 6-mercaptopurine. She expired on June 29, 1957. Necropsy revealed
acute lymphocytic leukemia with petechial and purpuric hemorrhages involving skin,
lungs, heart, renal pelves and mucosa of the bowel; enlargement of lymph nodes (inguinal,
cervical, abdominal and mesenteric); spleen weight of 60 Gm. and liver weight of 550
Gm. There were acute gastric ulcers with mucosal hemorrhages and leukemic infiltration
of kidneys, heart, lungs, gastrointestinal tract, liver and ovaries.

Oregon J173,J111.—M5H241428, born December 6, 1947, from whose blood culture
J173,J111 was derived, was admitted to the Doernbecher Hospital Unit on November 14,
1956 with a history of multiple staphylococcal abscesses since February of 1956 and a
gradually enlarging abdomen since the middle of September, at which time an enlarged
spleen was first noticed. Penicillin and other antibiotic therapy had failed to control
the furunculosis. Significant initial laboratory findings were: urinalysis 1.014; protein,
slight trace; 1+ pus; occasional red cells; hemoglobin, 6.5 Gm.; leukocytes 14,500 with
20 per cent segmented neutrophils, 1 per cent eosinophils, 15 per cent band cells, 33
per cent lymphocytes, 1 per cent monocytes and 5 per cent disintegrated cells, 15 per
cent myelocytes, 4 per cent metamyelocytes, 12 per cent promyelocytes and 4 per cent
myeloblasts. There were 11 rubricytes seen per 100 leukocytes and 85 metarubricytes
per 100 leukocytes. The serum bilirubin direct was 0.2 mg. per 100 ml., and the indirect,
1.4 mg. per 100 ml., total 1.6 mg. per 100 ml. On December 10, 1956, the direct serum
bilirubin was 0.2 mg. per cent, indirect 1.0 mg. per cent, and total was 1.2 mg. per cent.
Urinary urobilinogen was 75 mg. for 24 hour volume. A red cell fragility test done on
December 5, 1956 was interpreted as normal. Skeletal survey on November 14 showed an
elongated, well circumscribed, radiolucent defect in the left proximal tibia which was
interpreted as a probable benign cortical defect.

The patient's skin lesions cleared with local therapy, and she was first seen in con-
sultation by members of the staff of the Division of Experimental Medicine on Novem-
ber 23, at which time the laboratory study in the Division of Experimental Medicine re-
vealed the following: hemoglobin, 7.3 Gm.; erythrocytes, 3.70 million; total nucleated
cells in the blood 32,700/cu.mm.; promyelocytes, 7.0 per cent; early basophilic myelocytes
3.0 per cent; early myelocytes, 1.0 per cent; myelocytes, 6.0 per cent; basophilic myelo-
cytes, 2.0 per cent; metamyelocytes, 7.0 per cent; band neutrophils, 10.0 per cent; seg-
mented neutrophils, 4.0 per cent; lymphocytes, 12.0 per cent; disintegrated cells, 7.0
per cent; rubricytes, 13.0 per cent; metarubricytes, 28.0 per cent. In addition to the
cells reported in the differential count, many bizarre rubriblasts and prorubricytes were
found in the blood. The diagnoses of leukemoid reaction secondary to disseminated staphylococcal infection, myeloproliferative disorder, erythroleukemia and chronic granulocytic leukemia were all entertained at various times during the course of her illness. Splenic puncture on December 14, 1956 yielded only a blood clot. Curette marrow biopsy on December 19 showed active bone marrow with no evidence of fibrosis and a marked hyperplasia of the erythrocytic, granulocytic and thrombocytic series.

In all, she had five admissions to the Doernbecher Hospital Unit of the University of Oregon Medical School Hospitals and Clinics, November 14, 1956 to February 10, 1957, April 10 to April 21, April 27 to May 4, May 10 to May 13, and May 25 to June 5, 1957. The culture of J173, J111 was started on May 29, at which time her total nucleated cell count in the blood was 82,500 with 60 per cent of the cells nucleated erythrocytes in all stages of development, 8 per cent myeloblasts, 4 per cent progranulocytes, 9 per cent myelocytes, 14 per cent lymphocytes, and 8 per cent disintegrated cells and the remainder more mature cells of the granulocytic series. Both the progranulocytes and proerythroblasts showed large nucleoli, and the diagnosis at this time was either chronic leukemic granulocytic leukemia or erythroleukemia or both. The progranulocytes and proerythroblasts were markedly enlarged. She had been started on Myleran therapy on January 5, 1957, and had been on 1 mg. daily since her first discharge on February 10, at which time the liver was 12 cm. below the right costal margin and the spleen 17 cm. below the left costal margin. During this last admission, Myleran was increased to 2 mg. daily, and the otitis media which had developed again showed coagulase-positive hemolytic Staphylococcus aureus on culture and responded well to antibiotics. She was discharged on June 5, and expired at home after a progressive downhill course on August 3, 1957. Permission for necropsy was not obtained.

The most probable final diagnosis was chronic leukemoid granulocytic leukemia, but an associated erythroleukemia cannot be excluded, as on all occasions on which her blood was examined during these five hospital admissions, great numbers of immature cells of the erythrocytic series were present in her blood. On each of the admissions, the spleen and liver remained huge with relatively little lymphadenopathy, and recurrent staphylococcal infections led to fever varying from the normal range to 104°F. On these admissions, she had normocytic anemia with a hemoglobin varying from 6 to 9 Gm.

Oregon J181, J111.—OSK1228, from whose blood culture J181, J111 was isolated, was a 79 year old male who was first seen in consultation at Portland Sanitarium on October 17, 1957, complaining of pain in the back, loss of weight, weakness and a recent laparotomy. He had lost weight—from 155 pounds to 135 pounds since August of 1957—but had felt well until August 24, when on getting out of bed he suddenly noted severe back pain at the level of D-8, which has persisted to date. He was admitted to Portland Sanitarium on October 5, 1957 under the care of a local physician when x-rays of the spine revealed collapse of the 8th dorsal vertebra and partial collapse of the 10th dorsal vertebra, with rarified areas in the pubic bone, which were interpreted as due to metatstatic osteolytic lesions. Barium studies of the gastrointestinal tract revealed markedly thickened mucosal folds, which were interpreted as a possible primary malignancy of the stomach. On October 15, 1957, a laparotomy was done which failed to reveal a major lesion in the stomach, but biopsy from the stomach wall showed infiltration by cells of the plasmocytic series, so a sternal marrow biopsy was done on that date; it revealed almost total replacement of the marrow by plasmoblasts and proplasmocytes, which were very immature and had large nucleoli.

Physical examination revealed an alert, elderly, emaciated male with a recent laparotomy wound on the anterior abdominal wall. He was afebrile with normal blood pressure and a marked acute dorsal kyphosis with tenderness over the 8th and 10th dorsal vertebrae on percussion. There was no enlargement of lymph nodes or spleen. There was slight spasticity in the right lower extremity and a questionable positive Babinski. Pain was produced by turning or weight bearing, chiefly localized over the 8th and 10th dorsal vertebrae. He had had no bleeding tendencies.

Laboratory studies revealed a BUN of 25 mg.; blood calcium of 9.8; phosphorus,
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4.6; alkaline phosphatase of 3 units; total serum protein of 6.8 Gm., 3.5 Gm. of which was globulin; sedimentation rate was 37/95. Hemoglobin was 10.5 Gm.; hematocrit 36 per cent; and the leukocyte count 5,900. Seven per cent of the cells in the blood were plasmoblasts and proplasmocytes. The urine showed no Bence Jones protein and no other protein. Electrophoretic studies of the blood serum indicated 55.2 per cent albumin plus alpha globulin, 14.8 per cent alpha-2 globulin, 25.2 per cent beta globulin and 4.8 per cent gamma globulin. The plasma showed the presence of a cryofibrinogen in considerable quantity present on every examination. On chilling the plasma, a heavy precipitate would form, which on washing in the cold did not dissolve but would redissolve on warming and was then quantitatively precipitated by adding thrombin, even at incubator temperature.

A diagnosis of acute subleukemic plasmocytic leukemia which had started as plasmocytic myeloma was made. On October 21, 1957, the date the culture was started, his blood showed 11,000 nucleated cells with a differential count of 5 per cent plasmoblasts and proplasmocytes, 1 per cent nucleated erythrocytes, 52 per cent segmented neutrophils, 22 per cent band neutrophils, 14 per cent lymphocytes, 2 per cent monocytes, and the balance disintegrated cells.

His subsequent course was progressively downhill. Because he was a food faddist, he refused to eat any protein foods and would regurgitate a stomach tube despite all efforts of special nurses to keep it in place. He developed orthopnea, and hence it was difficult to apply traction or a cast, and eventually, notwithstanding antibiotic therapy, he developed a terminal hypostatic bronchopneumonia and expired on November 7, 1957.

A complete necropsy confirmed the diagnosis. Significant findings included infiltration of the sternum, ribs and vertebrae with proplasmocytes and plasmoblasts and local extradural hemorrhage at the level of D-9 and D-10. There was only moderate infiltration of the liver, spleen and lymph nodes with proplasmocytes and plasmoblasts. At the level of the 9th and 10th dorsal vertebrae, a mass of plasmocytic and plasmoblastic tissue extended from the vertebrae so as to occupy most of the upper right ilio-psoas muscle, and sections of the involved vertebrae revealed very little remaining bone tissue. The spleen weighed 120 Gm. and the liver 1450 Gm.

Oregon J182, J111, J—VAH116158, a 43 year old male lumber mill worker, from whose blood culture J182, J111, J was started, was first admitted to the Portland Veterans' Hospital on October 11, 1957, complaining of weakness, vertigo, pallor, purpura, palpitation and a blind spot in the left visual field; these symptoms had all appeared between August 1, and September 30, 1957.

Blood counts in another hospital had been normal in June of 1957 when he was admitted for a myelogram and herniated disc operation. Physical examination revealed pallor, weakness, a small hemorrhage in the fundus below the left optic disc, blood pressure 100/45, pulse 108, generalized petechial rash and lymphadenopathy, with no significant enlargement of liver or spleen.

Laboratory studies revealed a hemoglobin of 4.9 Gm., total nucleated cell count in blood 8,600 on day of admission and 16,000 on November 15, 1957, the day of culture, with a differential count of 2 per cent rubriblasts, 6 per cent prorubricytes, 27 per cent rubricytes, 24 per cent metaruibcrites, 21 per cent segmented neutrophils, 2 per cent band neutrophils, 6 per cent lymphocytes and 12 per cent disintegrated cells; no clot retraction, reticulocytes 0.8 per cent, prothrombin 100 per cent, Coomb's test, direct and indirect, negative, Schilling cobalt-60-B12 absorption test 11 per cent, with intrinsic factor 19 per cent, and no free HCl in gastric contents. The marrow showed predominantly immature cells of the erythrocytic series, but many cells were so bizarre as to be unidentifiable with certainty. X-ray studies of bones, chest, and the gastrointestinal tract showed no significant abnormalities.

Vitamin B12, folic acid, prednisone and repeated fresh blood transfusions failed to control the anemia and bleeding tendency, and he expired from major gastrointestinal hemorrhage on November 17, 1957.

Necropsy confirmed the final clinical diagnosis of acute "leukemic" erythrocytic sarcoma with myelophthisic, thrombocytopenic bleeding.
LONG-TERM MIXED CULTURES OF HUMAN HEMIC CELLS

Oregon J184B.—MCH84010, a white married male, born on August 29, 1906, entered Multnomah County Hospital the last time on November 20, 1957, referred for diagnosis of the cause of pancytopenia and splenomegaly. Culture 184B was started from his marrow. He had been seen in the University of Oregon Clinics and Hospitals previously on several occasions between January 12, 1938 and February 28, 1952. On all of these occasions there was nothing in the history, physical or laboratory studies related to the complaints on this last admission. Repeated blood studies were entirely within normal limits. From 1948 to 1953 his intake of alcohol had been excessive. In 1954 he had an x-ray of the back because of a previous back injury, and an enlarged spleen was seen. He had had repeated attacks of angioneurotic edema related to exposure to cedar dust or to work in a bakery. An enlarged spleen had been palpated by a physician in January of 1957, and he had developed diarrhea and exertional and nocturnal dyspnea at about this time. He had been admitted to another Portland hospital on November 6, 1957 where on the next day the first recorded abnormal blood count was reported, with 9.9 Gm./100 ml. hemoglobin, 3.0 million erythrocytes, 3,800 leukocytes and 36,000 thrombocytes per cu.mm. No abnormal cells were reported in the blood, and the diagnosis based on two marrow studies from this hospital was pancytopenia of undetermined etiology associated with marked splenomegaly and possible Laennec's cirrhosis.

Physical examination revealed dependent edema, weight 206 pounds, blood pressure 132/80, pallor, generalized purpura, splenomegaly and hepatomegaly. The spleen was palpated 5 cm. below the costal margin, and the liver at the corresponding position, with a rounded edge. His initial blood count showed 8.7 Gm. hemoglobin, with 2.2 million erythrocytes, and 3,250 leukocytes, 42 per cent segmented neutrophils, 6 per cent eosinophils, 1 per cent neutrophil band cells, 50 per cent lymphocytes and 1 per cent basophils. Very similar counts were recorded almost daily during his admission, the last on December 24, 1957 being 5.5 Gm. hemoglobin, 1.52 million erythrocytes, 550 leukocytes; at no time were nucleated erythrocytes or abnormal leukocytes seen in his blood. Liver function tests revealed a 4+ cephalin flocculation, a 23 per cent retention of Bromsulphalein, and a prothrombin activity of 20 to 34 per cent, and alkaline phosphatase of 13 King-Armstrong units. Repeated marrow aspirations consistently showed a marked hyperplasia of the erythroidic series, with many cells containing large nucleoli and many bizarre mitoses, and large cells in clumps that were strongly suggestive of malignant tumor cells; bone survey, gastrointestinal x-rays and chest films failed, however, to reveal any evidence of a primary tumor.

The first culture started from his marrow, J184, was not successful, but the second culture from his marrow, 184B, grew rapidly from the very start and continues to show both cells resembling cuboidal epithelial tumor cells and cells of the erythroidic series.

The differential cell count on the aspirated marrow of December 6, 1957, from which the culture was started, showed 0.5 per cent progranulocytes, 3.0 per cent neutrophilic myelocytes, 1.0 per cent neutrophilic metamyelocytes, 18.0 per cent neutrophilic band cells, 3.5 per cent neutrophilic segmented cells, 14.0 per cent lymphocytes, 7.0 per cent promyelocytes, 0.5 per cent rubricytes, 11.0 per cent metarubricytes, 6.5 per cent disintegrated cells (granulocytes) and 34.5 per cent disintegrated cells (type unknown). Most of the early cells of the erythroidic series showed large nucleoli, and there were many bizarre mitoses in cells of the erythroidic series.

The final diagnosis was erythrocytic sarcoma or erythroleukemia with probable metastatic malignant epithelial tumor metastasizing to the marrow, primary site undetermined, and Laennec's cirrhosis. A complete necropsy was compatible with these diagnoses, but no primary malignancy could be found. The spleen weighed 1400 Gm. and the liver 1600 Gm.

Oregon J185J111d.—OSK1261, from whose blood culture J185J111d was derived, was a 52 year old white male who was seen only once on December 3, 1957, the day the culture was started. His blood and marrow smears had been submitted by his local physician in Victoria, B. C., for diagnosis. His only complaints were weakness and easy fatigue beginning in July of 1954, at which time during a routine insurance examination an enlarged spleen was found. In September, 1954, the first blood count was done, and showed
a striking eosinophilia. In 1955 he had a leukocyte count of 24,000 with 60 per cent eosinophils. On November 14, 1957, a leukocyte count of 54,000 with 84 per cent eosinophils was recorded, and the marrow on this date showed 51 per cent eosinophils. Studies for parasitic disease, chest x-rays and liver function tests were all repeatedly negative. He had been in the Mediterranean region in the 1940's and had what was thought to be malaria, but it had never been proved. He had no fever since the first recognition of the splenomegaly and high eosinophil count. He had infectious hepatitis in Sicily before his discharge from the Army in 1943.

Physical examination revealed a well developed, well nourished, white male who did not appear ill. Relevant findings were: one + enlargement of the cervical, axillary and inguinal lymph nodes, a spleen palpable 8 cm. below the costal margin, and a liver palpable 2 cm. below the right costal margin. There was no evidence or history of bleeding.

The total nucleated cell count in the blood was 80,000 with 54 per cent segmented eosinophils, 13.2 per cent band eosinophils, 1 per cent eosinophil metamyelocytes, 0.2 per cent eosinophil myelocytes, 0.2 per cent early eosinophil myelocytes with nucleoli, and 14 per cent disintegrated cells, most of which were eosinophils. A total of 82 per cent of all cells in the 80,000 leukocyte count belonged to the eosinophilic series. In addition, there were 2.8 per cent segmented neutrophils, 0.4 per cent basophils, 0.2 per cent basophil myelocytes, 12.2 per cent lymphocytes, 0.6 per cent monocytes, and 4.8 per cent metamyelocytes. Stool studies for parasites were negative, and the smears revealed no evidence of malaria or other parasitic diseases. Hemoglobin was 12.2 Gm. per 100 ml., and the erythrocyte count was 3.3 million. The marrow showed a total nucleated cell count of 179,000, predominantly cells of the eosinophilic series including all stages, with the more mature stages predominant.

In view of the long history of splenomegaly, lymphadenopathy and hepatomegaly, with repeated negative studies for parasites and pulmonary or gastrointestinal disease, a diagnosis of chronic eosinophilic leukemia was made, with the reservations that this is known to be a dangerous diagnosis, and that high eosinophilias usually turn out to be due to other causes. At last report (February, 1958) this patient was living and had responded to the titrated regularly spaced total-body irradiation that was recommended as therapy; the leukocyte count had decreased to 25,000, and the spleen was much smaller, but the liver was somewhat larger.

Oregon J189,J111, from whose blood culture J189,J111 was derived, was a 84 year old male born June 14, 1893, a carpenter, married with four children. He was first seen in the Division of Experimental Medicine August 13, 1957, complaining of weakness, fatigue, swollen ankles, pallor and intermittent fever. He had been diagnosed by his local physician as having leukemia, on April 3, 1957, on the basis of blood counts and bone marrow aspiration. He had had four transfusions on April 15, June 1, July 1 and August 1 of 1957, but no specific therapy for the leukemia.

Physical examination revealed no bleeding or ecchymoses, one small lymph node in the right supraclavicular region, one + lymph nodes in the axilla, liver and spleen palpable at the costal margin.

Examination of blood and marrow on August 13, 1957, revealed the typical picture of acute subleukemic basophilic leukemia, with hemoglobin of 7.8 Gm./100 ml., RBC 2.8 million, leukocytes 6,000, predominantly early basophil myelocytes with large nucleoli and water-soluble metachromatic basophil granules. The neutrophils and eosinophils showed no evidence of involvement in the leukemic process. The initial thrombocyte count was 43,000. The marrow also revealed a preponderance of early basophil myelocytes with large nucleoli, and a few basophil myelocytes with metamyelocytes. All other marrow cells had essentially normal morphology. He was treated with prednisone and was able to be up and about and active, though requiring many transfusions and developing a progressively increasing amount of thrombocytopenic bleeding. His leukocyte counts prior to culture oscillated between 6,000 and 28,000. On December 11, 1957 he developed a thrombophlebitis of both ankles and stomatitis and on December 18, the
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day the culture was started, his leukocyte count was 36,000, hemoglobin 8.4 Gm. The leukocyte alkaline phosphatase by the method of Kaplow was negative, and the differential count continued to show predominantly early basophil myelocytes with progranulocytes and blast cells. He was admitted to Providence Hospital that same day, and from then on had a progressively downhill course, and died on December 28. At necropsy, the spleen weighed 850 Gm., the liver, 2,060 Gm. and the anatomic diagnosis was (1) acute granulocytic leukemia, (a) generalized lymphadenopathy, (b) splenomegaly, (c) hepatomegaly, (d) generalized petechial hemorrhages, (e) leukemic infiltration of lungs; (2) bilateral pulmonary congestion and edema, marked.

CHARACTERISTICS OF THE VARIOUS CELL SERIES IN CULTURE

Monocytic series.—The cultures Oregon J96 and Oregon J111 derived from blood of patients with acute monocytic leukemia have been previously described. These are obviously mixed populations of several cell types. Cells of the monocytic series predominate, but granulocytic and erythrocytic series cells are also present. Cells of the monocytic series are very pleomorphic with great variation in size, nuclear and nucleolar number, and morphology. They tend to form loose irregular colonies in early growth and later dense epithelioid sheets. They will, now, after more than four years in culture, tolerate a very wide range in gradient factor. See the figures in references 1 and 2 for illustrations of monocytic pleomorphism, and figures 7 and 9 in reference 11 for the appearance of monocytic colonies.

The cloned strains* were derived from J96 and J111. J96, is the monocytic component of J96, and J111, is a chance isolation of the component in J11 derived from the granulocytic series. We interpret these cells as belonging to the granulocytic series because the leukocyte alkaline phosphatase by the method of Kaplow* is positive for nearly all cells in this strain.

Granulocytic series.—The granulocytic series is represented in several cultures. Oregon J128, started from the blood of a patient with chronic granulocytic leukemia, has a large number of cells derived from the granulocytic series mixed with cells of the monocytic series. The cells of the granulocytic series do not seem to survive beyond the progranulocytic stage and tend to form round, sharply demarcated colonies several cells thick, in contrast to the spread-out, elongated cells of a monocytic colony. Oregon J185,J111, is a mixed culture derived by adding blood of a patient with chronic eosinophilic leukemia to the clone J111,1. It contains numerous immature cells of the granulocytic series, but the cells do not now survive to the stage of developing typical mature eosinophil granules. Oregon J189,J111, was derived by adding blood from a patient with acute basophilic leukemia to J111,1, and continues to show numerous cells with basophil granules, which are easily demonstrated by toluidine blue staining; the cells rarely survive beyond the myelocyte stage in this culture. Curiously, all cultures of granulocytic cells tend to lose their peroxidase reaction very early, but have retained the alkaline phosphatase reaction even though it was initially weak.

Lymphocytic series.—The cells of the lymphocytic series are best repre-

*These clones were established in the laboratory of Dr. T. Puck, Department of Biophysics, University of Colorado Medical Center, Denver, Colo.
presented in two mixed cultures, Oregon M166,J111 and Oregon J169,J128. M166,J111 has numerous cells growing in chains with lymphocytic morphology if the culture is maintained at the proper population density. When the population density is low, cells of the monocytic series will predominate until enough growth has occurred to restore a high population density. When the high population density necessary for granulocytic and lymphocytic series growth has been restored, these cells will again become numerous. In dense areas of growth of the lymphocytic series in this culture the resemblance to the cords of a lymph node medulla is striking (see figures 4 and 8 in reference 11 and figure 9 in reference 12). In culture J169,J128 the cells of the lymphocytic series tend to form colonies resembling the follicles of a lymph node (see figures 3 and 4 in reference 7 for comparison of the morphology of colonies from acute and chronic lymphocytic leukemia).

Plasmocytic series.—Cells derived from the plasmocytic series are best represented in culture Oregon J181,J111, a mixed culture that has been selectively grown for eight months with a low to medium population density which seems to be favorable for plasmocytic proliferation. These cells, although larger than average cells of the plasmocytic series and showing considerable chromosome duplication, retain characteristic nuclear morphology of the plasmoblast and proplasmocyte and the dense opaque cytoplasm with many mitochondria. They tend to grow in sheets one cell thick (see figure 11 in reference 7) and produce red staining inclusions resembling Russell bodies.

Erythrocytic series.—The cells of the erythrocytic series are most numerous in two cultures, Oregon J173,J111, a mixed culture, and Oregon J184B, the only one of these cultures that was started from bone marrow. J184B contains cells resembling a cuboidal epithelial carcinoma. In both of these cultures, cells of the erythrocytic series have a greatly shortened survival time; and relatively few cells reach the non-nucleated stage. However, when maintained at a high population density for 10 to 15 days, the production of cells of the erythrocytic series increases and a few non-nucleated erythrocytes may be found. Hemoglobin production is reduced relative to the normal, but some hemoglobin production is demonstrable with the Lepehne stain. It must be remembered, however, that no one as yet has devised a medium in which normal erythrocytes will remain unhemolyzed at 37 C. for any considerable portion of their normal life span. Cells of the erythrocytic series tend to grow in the middle of dense clusters of other cell types and to form colonies with a single immature nucleated cell at one end and a double chain or cord of progressively more mature cells as distance from the single cell increases, a finding which suggests that there is one division prior to production of the differentiating line, as illustrated in figure 13 of reference 7, but that these first two cells produced retain the ability to divide repeatedly without, themselves, maturing. For these cells we use the term “alpha cells,” while for the cells which will differentiate and die, “n cells.”

Suggested Studies with These Cell Lines

A number of diversified studies for which these cell strains would be suitable are mentioned below. The isolation of many cloned strains by the
methods of Puck,\textsuperscript{5} Goldstein,\textsuperscript{13} or Moskowitz\textsuperscript{14} with resultant separation of each hemic cell series in individual cultures would greatly facilitate some of these studies. The cloning of the lymphocytic and erythrocytic series may be difficult because of the high gradient factor required by these series for growth. The metabolism of each cell type may be studied and nutritional requirements determined, as has been done by Eagle and associates for several cell strains.\textsuperscript{6} The histochemical specificity should be studied, and screening with a battery of histochemical tests would probably define additional differences not now recognized in these cells. Hemoglobin synthesis could perhaps be studied using one of the strains containing cells of the erythrocytic series, but at present very little hemoglobin is produced in the media we have used. The growth pattern\textsuperscript{15} and colony form has proved valuable in identifying cells of the lymphocytic, monocytic, granulocytic and erythrocytic series in culture. Additional studies on these patterns in culture may give additional information on how these cells are produced and their net rate of motility. The number of colonies produced will give the number of alpha cells, capable of division, and the initial rate of labeling with tritiated thymidine will give the division rate, whereas the difference between this rate and the net increase in cell number will give the number of n cells, which differentiate and die.\textsuperscript{11,12} The time from initial labeling to the appearance of the label in the dead cells should give the life span. The numerous chromosomal aberrations in these cell strains seem to result chiefly from mitotic bridges with resultant accessory chromosomes and endomitotic divisions with resultant polyploidy. It is possible that these cells may be useful in certain phases of radiation biology; preliminary experiments indicate an increased radiation resistance possibly associated with increased degrees of polyploidy. It is probable that these cell strains may prove useful in the screening of chemotherapeutic agents. Culture strain Oregon J96 has been useful in virus screening and has been reported to have a wide virus spectrum.\textsuperscript{16} Probably others of these cell strains may be useful for viral studies. Quantitative studies could be carried out on the response of various types of hemic cells to particulate material of various kinds and the chemotactic response and phagocytic ability of each cell type determined.\textsuperscript{12} The inverted slide cap method\textsuperscript{11,17} is especially well adapted to such studies. The plasmocytic culture Oregon J181, J11L, should be especially well suited to antibody production studies in vitro. Our observations to date on the ecology of cells\textsuperscript{2,18} indicate that the number and type of cells present greatly influence the multiplication rate of other cells, and this is one of the many ecologic factors which deserve extended study. To date, we have been unable to grow cells of the lymphocytic or erythrocytic series in the absence of a relatively dense population of cells of the monocytic or granulocytic series. A good illustration of the importance of such ecologic factors in vivo is the distribution of cell growth after intravenous marrow transplantation. Observation of these culture strains since isolation has revealed many examples of evolutionary change; the cells have become adapted to much lower gradient factors than were necessary originally; the mean doubling time for some cells has changed from 60 days to 24 hours; the mean life span of the n cell has become greatly shortened; the peroxidase reaction of the granu-
locytic series has been lost; and even in a cloned strain great variation in multiplication rate of different colonies has been observed. A great number of enzyme systems and nutrient requirements remain to be studied. Our unifying concept of leukemias, lymphomas, and cancers suggests that one of the most important studies to be done would be to see if the missing factor necessary for a normal life span for these cells could be found with resultant production of the normal inhibitor of logarithmic multiplication. The essential feature of this concept is that cancer results from any genetic change in the alpha cell leading to shortening of the life span of the n cell with resultant deficiency of the specific unstable inhibitor produced by the n cell as a normal homeostatic feedback mechanism for controlling growth. This shortening of the life span of the differentiating cell gives the appearance of maturation arrest or dedifferentiation without the necessity of there being any reversal or arrest of the process of differentiation.

**Summary**

New culture strains containing human hemic cells of monocytic, lymphocytic, granulocytic, plasmocytic and erythrocytic series are described with suggestions as to maintenance of the desired cell series in culture.

The addition of cells from lymphocytic, plasmocytic and erythrocytic series to a previously well established culture has made possible long-term culture of these cell types, since this procedure aids in maintaining the necessary high gradient factor for their growth and multiplication.

Although the life span of these cells has been considerably shortened in culture and immature forms predominate, the cells show recognizable characteristics of the cell series of origin. A high population density must be maintained in cultivation of the mixed cultures or the desired cell type may be lost.

**Summario in Interlingua**

Es descrebite nove racias cultural continente cellulas de sanguine human del series monocytic, lymphocytic, granulocytic, plasmocytic, e erythrocytic. Es facite suggestiones relative al mantenentia del desirate serie cellular.

Le addition de cellulas ab series lymphocytic, plasmocytic, e erythrocytic a previemente ben establite culturas ha rendite possibile le cultivation a longe vista de iste typos de cellula, proque iste manovra contribue al mantenentia del alte gradiente que es necessari pro le crescentia e multiplication cellular.

Ben que le longevitate de iste cellulas ha esite reducite considerabilemente in le cultura e ben que formas immatur predomina, le cellulas exhibi recognoscibile caracteristicas del serie de lor origine. Un alte densitate de popula- tion cellular debe esser mantenite in le cultivation de culturas mixte. Si non, on curre le risco que le desirate typo cellular es perdite.

**References**

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Long-Term Mixed Cultures of Human Hemic Cells, with Granulocytic, Lymphocytic, Plasmocytic and Erythrocytic Series Represented

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