

mice. In view of the similar radioactive iron uptake in comparison with normal mice, we doubt that there is a significant increase in the total surface area of the marrow sinus system in the polycythemic mouse, but we believe that the change is more likely to be a change in configuration. Indeed, in the absence of reduction in parenchymal mass, any enlargement of surface area could be viewed as an increased opportunity for cell migration. In addition, Dr. Boggs' alternative explanation does not explain the greater than twofold rise in reticulocyte count that occurs within 2 hr of bleeding the hypertransfused Ep-stimulated mice to normal hematocrits (Table 4). Our explanation of the phenomenon suggests a release of trapped reticulocytes by "unpacking" the marrow sinuses and is consistent with the experimental data.

As Dr. Boggs points out, reduced concentration of blood neutrophils might be expected to occur in polycythemic states if release of leukocytes is inhibited by polycythemia. We would anticipate the changes to be of less magnitude than reticulocyte changes because of leukocyte motility. However, the human polycythemic syndromes are not entirely comparable to the hypertransfused mouse. Polycythemia vera is associated with a significant increase in granulocyte production,<sup>2</sup> while erythrocytosis secondary to uncompensated hypoxia should be associated with increased levels of epinephrine and glucocorticoids, both of which produce increased leukocyte counts.<sup>3</sup> Perhaps polycythemia related to high oxygen affinity hemoglobins or compensated high altitude polycythemia would be more comparable to the hypertransfused mouse. Other than unreferenced statements in standard textbooks of hematology that indicate the leukocyte count is consistently normal in these states (in contrast to polycythemia vera), I have found no pertinent data. I expect this means that the counts are not elevated but that lower counts have not been excluded by systematic evaluation. We have not studied

leukocyte counts in hypertransfused mice, nor are we aware of such published data.

Regarding the technical question raised by Dr. Boggs, in Table 3 the number of reticulocytes in transit for the nontransfused controls should have read  $1.5 \pm 0.5$ , rather than  $0.5 \pm 0.5$ . The latter value was inadvertently mis-transcribed. The correct entry is more consistent with the overall message of the paper than the other as Dr. Boggs astutely pointed out.

Finally, the comments on accelerated release of neutrophils in response to erythropoietin preparations are of interest to us. Our studies would indicate that accelerated release occurs with Connaught step III preparations, as well as with step I and step II. However, even the step III preparation is a fairly crude preparation and, as Dr. Boggs suggests, endotoxin contamination could be responsible for the leukocytosis. Without endotoxin assay, however, one cannot assume this to be true, and other contaminants or even erythropoietin may be responsible. (Chamberlain et al: "Reduction in advential cell cover: An early direct effect of erythropoietin." *Blood Cells*—in press).

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#### *To the Editor:*

Post-implantation rodent and marsupial embryos can now be cultured in vitro for periods of 2-4 days. The nutrient medium most commonly used is homologous serum. This medium is usually prepared by withdrawing blood from the aorta of an anesthetized rat and allowing the blood to clot and stand overnight at 4°C.

The following morning the clot is broken, the blood centrifuged, and the serum decanted.

With serum prepared by this method New and Daniel<sup>1</sup> found that the paired heart primordia of "egg-cylinder" stage embryos usually failed to fuse, resulting in a double heart. However, Steele<sup>2</sup> showed that the method of prepara-

tion of the serum was critical for the development of egg-cylinders in culture. If the blood was centrifuged immediately after withdrawal, then embryos grown in this serum (or in plasma) had a single heart and general development was greatly improved. The growth of embryos explanted at a later stage (headfold) has also been improved by culture in immediately centrifuged (IC) serum rather than in delayed centrifuged (DC) serum.

The factor(s) responsible for different heart development in IC and DC sera have been investigated. It is now known that it is not the result of any difference in the sodium, potassium,<sup>2</sup> or calcium<sup>3</sup> ion concentration of the media, and complement concentration may also be unimportant, despite the fact that heat inactivation reduces the frequency of double heart formation in DC serum.<sup>3</sup> It was found that the harmful properties of DC serum appeared rapidly (within 30 min) during contact with a normal blood clot in which the blood cells were trapped in the fibrin coagulum, but

did not develop after 18 hr contact with separated blood cells and fibrin clot. I would be pleased to hear from hematologists who could suggest a possible explanation for this effect.

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#### To the Editor:

The prevention of leukemic meningitis in childhood acute leukemia by prophylactic cranial radiation with intrathecal methotrexate has been established.<sup>1</sup> Indications for prophylaxis against central nervous system (CNS) complications in adult acute leukemia have not been adequately studied. Wolk et al.<sup>2</sup> demonstrated a 27% incidence of leukemic meningitis based on autopsy or spinal fluid evaluation of adults with acute myelogenous leukemia following response to chemotherapy. This author felt that as the survival of adults with acute leukemia increased the incidence of leukemic meningitis would approach the frequency seen in childhood leukemia. Experimental evidence for this was suggested by Thomas.<sup>3</sup> He demonstrated that mice inoculated with L 1210 leukemia at autopsy demonstrated a marked increase in the frequency of arachnoidal leukemic infiltration if given subcutaneous methotrexate to prolong their lives by several days. The occurrence of leukemic meningitis has often been a late complication of acute leukemia.<sup>4</sup> The Southwest Cancer Chemotherapy Study Group found the median duration from onset of disease to the moment of leukemic meningitis to be approximately 9 mo.<sup>5</sup> The rarity of this syndrome in adults may therefore be due to their prior short survival.

Within the past 9 mo we have seen three adult patients with leukemic meningitis at Sinai Hospital of Detroit.

*Case 1.* M.L., a 48-yr-old white female, was diagnosed 2/26/74 as having acute myelogenous leukemia. Bone marrow demonstrated greater than 90% blasts with a peripheral leukocyte count of 46,800, consisting of 50% blasts. The patient was given induction therapy with vincristin, ARA-C, and prednisone, consisting of two courses, after which she developed a remission marrow. She was maintained on vincristin, ARA-C, and prednisone on a monthly basis. On 8/13/75 the patient complained of cephalgia and right orbital pain of 3 wk duration. Spinal fluid revealed 7,450 cells mostly mononuclear with some blasts and a protein of 545 mg/100 ml. Peripheral blood findings along with bone marrow aspirate and biopsy indicated a remission marrow. The patient was treated with intrathecal methotrexate 15 mg every 3 days with 2,000 rads of cranial radiation with clearing of the spinal fluid and loss of symptomatology.

*Case 2.* S.W., a 27-yr-old white female, was diagnosed as acute myelogenous leukemia 1/15/75 with a peripheral leukocyte count of 189,000 with 90% blasts. Bone marrow biopsy and aspirate revealed 95% blasts. She was treated with vincristin, ARA-C, and prednisone