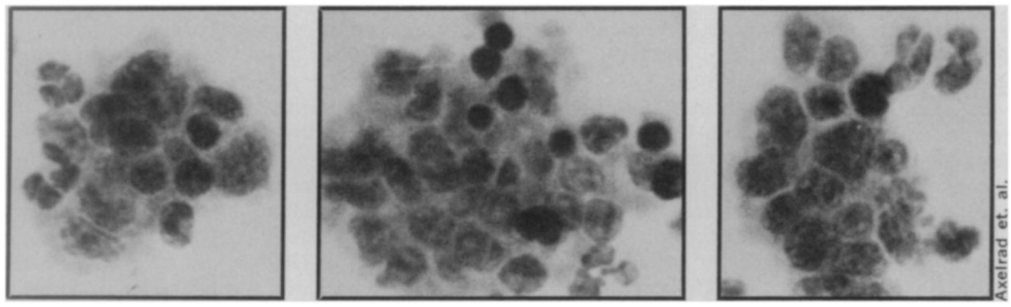
Out for Blood



Culturing blood cells outside marrow bones offers ways to study the hematic processes

by Joan Arehart-Treichel

If there is anything people get emotional about, it's the five liters of crimson fluid that courses through each of our bodies and replaces itself every 120 days. They faint at the sight of blood. They sweat blood. They act in cold blood. Their blood boils. If you want to pique the Irish, just call them "blood fools." Some Canadian and American hematology scientists, however, are hepped up about blood—for different reasons.

During the past three years they've learned how to grow bone marrow cells in culture so that they develop into red blood cells. They are using the technique to better understand red-blood-cell production and to see how this production differs in persons with various diseases. There is also the possibility that a few years from now the technique might also be used to make blood in the lab for blood transfusions. Scientists using the technique aren't especially optimistic about this possibility, though.

The story started more or less during the 1960's when E. A. McCulloch and J. E. Till of the Ontario Cancer Institute in Toronto developed a technique for measuring those cells in bone marrow that ultimately give rise to red blood cells, white blood cells and pieces of cells called platelets. The bone marrow cells are called pluripotent stem cells (or stem cells with many potentials). These cells are thought to give rise to committed stem cells that make red cells, white cells or platelets—or intermediary products thereof.

The technique involved taking a suspension of bone marrow cells and injecting the suspension into irradiated mice. The injected cells settled into the spleens of the animals, each cell giving rise to some million more cells. Each clump of a million cells or so could be seen on the surfaces of the animals' spleens after about ten days and indicated the presence of a pluripotent stem cell. In other words, if there were 10 clumps, one knew that 10 pluripotent stem cells were present.

Even now, there is no technique for measuring pluripotent stem cells in humans because people cannot be safely irradiated and injected with bone marrow. But there is reason to believe that pluripotent stem cells are at work in our bodies as well.

Then Arthur A. Axelrad, John Stephenson, David McLeod and Mona Shreeve of the University of Toronto built on the work of McCulloch and Till. By 1970 they had developed a technique for making red cells in culture. They could do it first with animal cells, then with human cells.

The technique called for putting a suspension of bone marrow cells in a test tube. (The cells were able to differentiate into either red cells or white cells; whether they were stem cells or some derivatives thereof was not clear.) A nutritive medium was added to the test tube, then plasma to clot and trap the bone marrow cells, then erythropoietin (EPO). EPO is a hormone made by the kidneys and known to regulate red-cell production. After staying in culture for several days, the bone marrow gave rise to erythroid colonies—colonies of cells with nuclei (see photographs above). Each of these cells in turn gave rise to a number of red cells.

"For the first time," Axelrad recalls, "it was possible to make colonies of red blood cells in culture in the presence of EPO."

Axelrad and his team have since improved their technique. They can now routinely harvest erythroid colonies containing up to a hundred cells each.

Axelrad and his team are also using their technique to probe normal and abnormal red-cell production. Their technique has also been adapted by several other research groups during the past year or two, notably by McCulloch, Arnold Tepperman of the Princess Margaret Hospital in Toronto and their co-workers; by Esmail Zanjani and his team at the Mount Sinai School of Medicine in New York City; by John Adamson and co-workers at the Veterans Administration Hospital in Seattle.

Regarding normal red-cell production, Adamson and his colleagues have found that if bone marrow is stimulated in culture to make red cells, that does not reduce the ability of the marrow to make white cells, and vice versa. Axelrad and his team have found that EPO does not control red-cell production by acting on stem cells, but on cells (or units) that are intermediary between the stem cells and the erythroid colonies.

As for abnormal red-cell production, McCulloch and his co-workers are probing a kind of leukemia that consists of abnormal red-cell production

Shreeve prepares red blood cell cultures under a tissue-culture hood.

Exploring effects of EPO on leeches.

(versus leukemias that consist of abnormal white-cell production). "We hope," McCulloch says, "that the technique will give us insight into the cellular mechanisms of leukemia, that is, the transition of relatively primitive cells to more differentiated cells, what controls that transition and where that transition breaks down."

But the disease that several of the teams are really tackling with the cell-culturing technique is polycythemia vera, a slowly progressive disease characterized by increased red-cell production. Although their results are preliminary and somewhat contradictory, they offer some tantalizing hints of what the disease is due to.

Axelrad and his team, which now includes J. F. Prchal, put bone marrow from 14 healthy persons in culture. The marrow would not make erythroid colonies and subsequently red blood cells without the presence of EPO. If EPO was added, colonies were produced, and the number of colonies and red cells produced depended on the concentration of EPO. Marrow taken from six polycythemia vera patients, however, could make erythroid colonies and red cells without EPO. And if EPO was put in their presence, erythroid colonies and red cells increased.

Tepperman and his colleagues have gotten similar but somewhat less consistent results. They have found that bone marrow from patients with the disease can make red cells in culture without EPO, but if EPO is added, red-cell production sometimes, but not always, increases.

Zanjani: After the EPO response.

How these results should be interpreted is open to some speculation. Says Tepperman, "They suggest that red-cell growth in this disease may be autonomous—something we have suspected for some time."

Says Axelrad, "I think it would be better to say that in polycythemia vera, there is a small population of erythrocytic colony-forming units that are either independent of EPO or have already been influenced by EPO in patients before being cultured and carry that influence over into culture."

Zanjani believes that even if red-cell growth in polycythemia vera can be autonomous, there is some role for EPO, and he wants to find out what it is. Several years ago he and his colleagues found a factor that they do not think is EPO, but that is able to increase red-cell production, probably by increasing the number of stem cells. They are now treating marrow from polycythemia vera patients with the factor and will then see whether such treatment alters the marrow's response to EPO. If it does, it may shed some light on the usual responses of polycythemia-vera marrow to EPO.

The Mount Sinai researchers are also exploring the effects of EPO on sheep fetuses, cancer cells, fish, worms and even leeches to see whether they can learn more about EPO's role in normal and abnormal red-cell production.

As far as the possibility of ever making blood in the test tube, these scientists are largely pessimistic. Says McCulloch, "I don't think it's in the books. We have a splendid way of making cells for transfusions by using people, and I don't see that counter methods will ever come within a million times of the efficiency required."

Adamson agrees. "I really don't see it as a feasible or practical way of going about things."

"No," says Tepperman, "there is no application to blood transfusions that I can foresee. You'd never be able to get the cells out or enough of them. It is a research tool to investigate with rather than a tool to produce red cells."

"I don't think it is beyond the realm of possibilities," Axelrad admits. "Even now our culture is lasting up to three weeks, and much will depend on whether the original cells can last in culture. But," he stresses, "we are already there with the thing that is really exciting—using this tool to probe human diseases." □