in optical astronomy, as interest in theoretical astrophysics, radio astronomy, and space astronomy grows, the proposed program is still based upon a straightforward projection. In view of the important contributions being made to the development of astronomy by observers with U.S. optical telescopes, to provide less than is recommended here would, in the opinion of the panel, mean a loss of momentum, and would constitute a retrenchment.

There is no single index in radio astronomy that represents the ground-based observing potential in the United States, particularly since modern antenna systems consist of both arrays and paraboloids. The growth of the major U.S. facilities is barely a decade old, and no long-term trends or growth rates can be said to have been established. The panel proposes a set of facilities demanded by the nature of the problems now faced in this field. These facilities will create capabilities for observational research commensurate with these demands, and quite beyond any yet provided. The manpower, the techniques, and the engineering competence for realization of this goal are all in sight.

Note

1. The full report, *Ground-Based Astronomy, A Ten-Year Program*, may be obtained from the Printing and Publishing Office, National Academy of Sciences, Washington, D.C., 20418, $4. The report was prepared under the sponsorship of the Committee on Science and Public Policy of the National Academy of Sciences and supported by the National Science Foundation.

Tissue Culture Studies of the Human Lymphocyte

Experiments under controlled conditions provide new information on this cell's function and potentiality.

Jay H. Robbins

The lymphocyte has long been the subject of study and debate (*1–3*). This white blood cell is prominently involved in certain inflammatory, immunological, and malignant processes (*4, 5*). However, despite its presence in both normal and diseased states, the specific functions and potentialities of this cell have, until recently, remained largely unknown.

In stained smears of human peripheral blood the lymphocyte is readily distinguishable from the other white blood cells because of its dark, rounded nucleus surrounded by what is usually a nongranular cytoplasm. Most of these peripheral-blood lymphocytes are less than 10 microns in diameter and are classified as "small" lymphocytes (*6; 7*, p. 53). Some larger lymphocytes (10 to 18 microns) may also be present. The presence of nucleoli in lymphocytes of human peripheral blood has been demonstrated with the electron microscope (*8*), but they are not usually seen in stained dry smears with the light microscope (*7*). However, large lymphocytes with prominently visible nucleoli are plentiful in lymphoid tissue such as the lymph nodes and spleen (*7*, p. 89; *9; 10*). There they divide and apparently give rise to the peripheral-blood lymphocytes, which later gain access to practically all the organs and tissues of the body.

A long-standing and still largely unresolved question concerns the potentialities of mammalian lymphocytes and the control of their differentiation (*2*). Some small lymphocytes are capable of undertaking an immunological response and apparently can be transformed in animals into large immature-appearing cells (*11, 12*). It is possible, also, that lymphocytes may develop in vivo into antibody-producing cells (*13–16*), and there is considerable evidence that some peripheral-blood lymphocytes can develop into macrophages (*3; 7*, p. 76; *10; 14; 17; 18*). The small lymphocyte has been considered a potential stem cell for all the formed elements of the blood (*1, 2, 16, 19*).

A notable advance in our knowledge was made when the small lymphocyte of human peripheral blood was shown to be transformed in vitro into a large, morphologically primitive, "blast-like" cell capable of undergoing mitosis (*18, 20–23*). This transformation, which may be referred to as "blastogenesis" (*24*), has been widely studied within the last 2 years. This article describes blastogenesis and some stimuli which induce it. Information is presented, obtained in studies of this phenomenon, concerning the differentiation and potentialities of the lymphocyte of human peripheral blood. Figure 1 summarizes both the established and the suggested transformations of this cell. Many of the findings obtained from the study of blastogenesis have direct and important medical applications. However, in this article I mention the pathological and clinical aspects only insofar as they seem to provide fundamental information about the lymphocyte and blastogenesis.

Effect of Phytohemagglutinin on the Small Lymphocyte

In general, when leukocytes of normal, human peripheral blood are cultured in vitro, few, if any, of the cells enlarge and undergo mitosis. Nowell (*25*) attempted to obtain dividing cells for chromosome analysis and reported that there were more blastoid and dividing cells in his cultures of normal, human peripheral blood than had usually been obtained in cultures reported by previous investigators. Investigating this interesting finding, he found that a substance known as phytohemagglutinin (PHA), an extract of the red kidney bean, was re-

The author is a research associate in the Section on Neurochemistry, Clinical Neuropharmacology Research Center, National Institute of Mental Health, Saint Elizabeths Hospital, Washington, D.C.

sponsible for this effect. Phytohemagglutinin had previously been used (*26, 27*) to agglutinate the erythrocytes, thereby facilitating their separation from the leukocyte-rich plasma which was to be cultured. This blastogenic effect was evidently not due simply to the removal of the agglutinated erythrocytes, for PHA was found to induce blastogenesis even when the erythrocytes remained in cultures of whole blood (*25*).

Evidence has since been presented which identifies the small lymphocyte as the cell undergoing blastogenesis in the presence of PHA (*18, 20–23*). In several of these experiments the initial inoculum consisted almost entirely of small lymphocytes. In the presence of PHA these small lymphocytes decreased in number and, simultaneously, many blastoid cells appeared, all prior to the onset of visible cell division. Numerical considerations clearly required the conclusion that transformation of many of the small lymphocytes initially present was responsible for the origin of many of the developing blastoid cells. It is not known whether the larger lymphocytes also undergo blastogenesis. It seems that the monocyte does not undergo blastogenesis in the presence of PHA; it probably either remains unchanged or becomes a macrophage (*28*). This view is supported by the findings that none of the blastoid cells give the cytochemical reactions characteristic of a monocyte (*29*).

On the third day of culture (*30*), in the absence of PHA the small lymphocytes are essentially unchanged. In cultures containing PHA, on the other hand, more than 70 percent of the small lymphocytes may be transformed into blastoid cells—that is, into large cells which have deeply basophilic, nongranular cytoplasm surrounding a large rounded nucleus which contains homogeneously staining chromatin and prominent nucleoli. In the cytoplasm there is often a lightly stained area bordering one side of the nucleus. In addition, there are often several small, unstained, cytoplasmic vacuoles. The diameter of the largest cells may be three times that of a small lymphocyte. Smaller blastoid cells, intermediate in size between these very large cells and the small lymphocyte, are also present. Many, but not all, of the blastoid cells are found aggregated in clusters. The single cells, as well as the free borders of cells in clusters, are actively ameboid (*31, 32*). Some blastoid cells can be seen in mitotic division after the 48th hour of culture.

The cytoplasm of the blastoid cells stains intensely with the dye pyronin (*23, 32, 33*). This pyroninophilia indicates the presence of ribonucleic acid, for the staining does not occur after exposure of the cells to the enzyme ribonuclease (*23*). Microdensitometric measurements of Feulgen-stained preparations indicate that the deoxyribonucleic acid doubles in amount as the nucleus enlarges during blastogenesis, in preparation for visible mitotic division (*32*).

Like the lymphocytes from which they arise and unlike the monocytes and granulocytic leukocytes, the blastoid cells do not stain in the peroxidase reaction (*29*). The blastoid cells show no evidence of stainable lipid or glycoprotein (*29*); the latter substance sometimes stains in certain γ-globulin-producing cells (*34–36*) and possibly results from globulin complexed to polysaccharide. Other histochemical studies (*29*) show that the developing blastoid cells have active dehydrogenase systems and can have much stainable glycogen. By the third day of culture, however, most of the large, developed blastoid cells have no detectable glycogen (*29*). Glucose is utilized during blastogenesis, and lactic acid is produced (*32*). These facts suggest that glycolysis may be the main energy source for blastogenesis. This suggestion is further supported by the observation that blastogenesis can occur in the complete absence of oxygen (*25*).

Electron microscopy has contributed further information about the structure of the blastoid cells. On the third day of culture the cytoplasm of the characteristic blastoid cell contains a well-formed Golgi body, sparse endoplasmic reticulum, and many free ribosomes (*23, 37–39*). These features are often found in cells capable of division (*15*). Some of the bodies in the cytoplasm that appear opaque when viewed with the electron microscope are believed to be the vacuoles seen with the light microscope (*23, 37, 39*). On the basis of their electron microscopic findings some investigators have speculated that these bodies may contain globulins (*39*); others have suggested that they are composed of lipid (*23, 37*). As noted above, the histochemical studies have so far failed to suggest the presence of either of these substances.

Radioactive nucleic acid precursors have been used to study the synthesis of nucleic acid during blastogenesis (*20, 31, 32, 40–42*). The results of these studies are in accord with the histochemical and morphological results presented above and support the conclusion that both ribonucleic and deoxyribonucleic acids are being actively synthesized by the cells in culture. Synthesis of ribonucleic acid begins during the first 24 hours of culture; that of deoxyribonucleic acid, soon thereafter.

Cultures from Patients with Chronic Lymphocytic Leukemia

Of great interest is the finding that very few or no blastoid cells develop in cultures from some patients with chronic lymphocytic leukemia (*28, 30, 43, 44*), a disease in which there is usually an overabundance of small lymphocytes in the peripheral blood and lymphoid tissue (*5*). This impairment in blastogenesis is greater in cultures from patients with high peripheral-blood lymphocyte counts than in cultures from patients with low counts, even though the concentration of cells in all the cultures is made equal (*44*), and it persists when the patients' cells are grown in culture fluid containing plasma from normal individuals (*28, 30*). It is unlikely that the impairment is due to a substance in the leukemic culture fluid (which contained the patient's plasma), for lymphocytes from normal persons undergo blastogenesis normally in the PHA-containing leukemic culture fluid. This result indicates also that the blastogenic factor of PHA is not significantly used up or inactivated in the leukemic cultures (*45*).

These results suggest that most of the lymphocytes from some patients with chronic lymphocytic leukemia may be inherently unable to respond to the blastogenic stimulus of PHA and thus differ from the majority of lymphocytes from normal persons. Even if this is so, it cannot be concluded that these leukemic lymphocytes are "abnormal" in the sense that such cells are not present in a normal person's blood, for, as already noted, in cultures from normal persons there are always some lymphocytes which do not undergo blastogenesis even in the presence of high concentrations of PHA.

To summarize, most small lymphocytes of normal, human peripheral

blood, in response to the blastogenic action of PHA, engage actively in certain metabolic processes characterized by the synthesis of ribo- and deoxyribonucleic acids. Consonant with these metabolic activities are the described changes in morphology, as revealed by the light and electron microscopes. One outstanding result of these changes is the consequent mitotic division of many of the resulting blastoid cells. Other lymphocytes—that is, a small percentage of the lymphocytes in a normal person's blood and most found in the blood of some patients with chronic lymphocytic leukemia—do not appear to respond to the blastogenic action of PHA in these short-term tissue cultures.

Chemistry and Properties of Phytohemagglutinin

The term *phytohemagglutinin* has been applied to the hemagglutinating extract obtained by Rigas and Osgood (*26*) from the red kidney bean, *Phaseolus vulgaris*. In low concentration PHA agglutinates only the erythrocytes (*26*); in high concentration it agglutinates leukocytes also (*46–49*). Other preparations with blastogenic activity have been obtained from *P. vulgaris* (*41, 50–55*). In some of these preparations there was blastogenic activity but no erythrocyte-agglutinating activity (*41, 52*). The possible independence of these two activities had been originally suggested in reports that certain batches of PHA had erythrocyte-agglutinating, but no mitogenic, activity (*40, 56, 57*). Furthermore, it was known that when PHA was kept in solution for several weeks its mitogenic activity decreased at a higher rate than its erythrocyte-agglutinating activity (*46, 52*).

Several investigators have successfully separated the blastogenic activity of PHA from the erythrocyte-agglutinating activity by adsorbing the latter from PHA onto erythrocytes (*30, 48, 49, 54, 57*) or erythrocyte stroma (*53, 58*). Leukoagglutinating activity remains with the blastogenic activity after this treatment (*48, 49, 58*). However, both these latter activities can be removed by exposing the PHA to leukocytes (*48, 49*), from which the activities can then be recovered by elution (*49*). It is probable that the blastogenic factor is proteinaceous, for it is nondialyzable (*46, 51, 52*), it is destroyed at 100°C (*25, 41*), and its activity is always found in the presence of protein (*41, 53, 54*). Of interest is the fact that the available PHA preparations are found to contain several proteins when they are analyzed electrophoretically (*41, 49, 50, 53, 57, 59*). The purification and characterization of the blastogenic factor, or factors, of PHA will be of great interest.

Other Blastogenic Factors

The discovery that PHA could induce blastogenesis in the lymphocyte has stimulated a search for other factors which might have similar activity. Within the last 2 years several such factors have been described. Simply for the purposes of discussion, these can be classified into three groups: (i) tuberculin-purified protein derivative (PPD) and some other antigenic substances; (ii) homologous leukocytes; and (iii) antiserum to leukocytes.

1) *Tuberculin-purified protein derivative and other antigens.* The first known blastogenic factor other than PHA was tuberculin-purified protein derivative. Its blastogenic activity was discovered independently by Pearmain, Lycette, and Fitzgerald (*60*) and by Schrek (*61*). Purified protein derivative produces blastogenesis and mitosis in leukocyte cultures (*60*) from the peripheral blood of donors who have been infected with tuberculosis or immunized with the bacillus of Calmette and Guerin, which is antigenically similar to PPD. Most of these findings have since been confirmed by other investigators (*62, 63*). It appears highly probable that the lymphocyte, rather than the monocyte, is the cell transformed by tuberculin, for the blastoid cells which develop resemble morphologically (*60–63*) and cytochemically (*63*) those obtained with PHA. However, it is not known whether it is the small or the large lymphocyte (or both) which undergoes this transformation.

Soon after the action of tuberculin was reported, Elves, Roath, and Israels (*64*) presented evidence that poliovirus vaccine and tetanus toxoid produced blastogenesis in leukocytes from specifically sensitized donors. Subsequently, several substances (*65*) which can be antigenic for man in vivo have been reported (*48, 64, 66–69*) to induce blastogenesis in cultures of peripheral-blood leukocytes from donors believed to have been sensitized to these substances through natural infection, prophylactic immunization, or other means. It has been generally assumed, but not proved, that the lymphocyte is the cell transformed by these antigens.

An interesting feature of the blastogenic response to tuberculin and to several (*66*), but not all (*66, 67*), of these antigenic substances is the fact that only a limited and small percentage of cells may become blastoid in the presence of any one substance [for example, usually less than 5 percent after the third or fourth day of culture with tuberculin (*63, 66*)]. More blastoid cells are formed in a culture challenged with two such antigenic substances than in one challenged with either alone (*66*). Some substances have been reported to produce a high percentage of blastoid cells [for example, 65 percent with heat-inactivated smallpox vaccine lymph (*67*) and 71 percent with staphylococcal filtrate (*66*)]. It remains to be elucidated whether such variations are due to differences in technique, differences in cell sensitivity, or differences among the blastogenic substances and their mode or modes of action.

Elves and his co-workers (*70*) have reported that a fluorescein-labeled antiserum to human globulin (prepared in rabbits) was bound to ethanol-fixed blastoid cells that were induced by either antigen or PHA. However, insufficient evidence was presented to establish the specificity of binding. Even if the binding was specific, it was not shown that the binding was to globulin *synthesized* by the cells in vitro rather than to globulin obtained by the cells from the plasma in the culture fluid. Nonspecific binding is known to occur (*71*), as is the adsorption of serum proteins to mammalian cells in vitro (*72, 73*). In the same report (*70*) these workers presented some evidence to show that the blastoid cells induced by one antigen bound the fluoresceinated form of that antigen. Their conclusions were, essentially, as follows: (i) γ-globulin is produced by blastoid cells induced either by PHA or by a specific antigen; and (ii) those cells induced by a specific antigen produce a specific antibody directed against the inducing antigen. Their interesting findings await confirmation.

Hirschhorn and his co-workers (*48*) have reported that the blastoid cells

incorporated radioactive amino acid into γ-globulin and that the blastoid cells from normal persons (but not from a patient with agammaglobulinemia) fluoresced when "incubated" with fluoresceinated antiserum to human γ-globulin. However, no adequate experimental evidence was presented to prove that the uptake of the fluoresceinated antiserum to human γ-globulin was specific or due to newly synthesized γ-globulin rather than to γ-globulin which may be present on normal human and other mammalian leukocytes and which can be washed completely from intact cells only after several washes (*74*), if at all (*72*). A detailed report of Hirschhorn's important studies, particularly a report concerning the techniques used, is eagerly awaited.

2) *Homologous leukocytes.* Soon after it had been established that lymphocytes of human peripheral blood undergo blastogenesis, Bain, Vas, and Lowenstein (*75*) recognized that blastogenesis occurred when the peripheral blood leukocytes of unrelated individuals were cultured together in vitro. These workers were the first to report adequately, and to recognize the significance of, the occurrence in such cultures of a reaction which was similar to, though less intense than, the reaction caused by PHA. The degree of blastogenesis appeared to be related to the degree of expected genetic compatibility between the leukocyte donors, for blastogenesis was never observed in mixed leukocyte cultures from pairs of monozygotic twins and was sometimes absent when the donors were closely related—for example, dizygotic twins. Since the blastoid cells produced in these homologous cell cultures resemble those induced by PHA, they probably arise from lymphocytes. However, experiments to prove definitively what cell type is transformed have not yet been performed. Many of these findings have been confirmed by others (*48, 76, 77*), who showed, in addition, that lymphocyte extracts (*76*) and certain cell fractions (*77*) retain the ability to induce blastogenesis in an unrelated individual's peripheral blood leukocytes. Attempts to apply the findings and technique of Bain, Vas, and Lowenstein to the clinical aspects of human organ transplantation are in progress (*48, 78*).

3) *Antiserum to leukocytes.* On the assumption that PHA might be inducing blastogenesis by attaching to some structure of the leukocytes, Grasbeck, Nordman, and de la Chapelle (*79, 80*) attempted to prepare a substance likely to make such an attachment. They succeeded by preparing, in rabbits, antiserums to human leukocytes. Their antiserums, which possessed leukoagglutinating activity due, presumably, to antileukocyte antibodies, induced formation of blastoid cells. While specific studies were not performed to determine the cell of origin of these blastoid cells, it seems quite possible that the lymphocyte was the cell transformed, since the antiserums produced blastoid cells and had mitogenic activity similar to that of PHA (*79*).

Relationship of Blastoid Cells to Other Hematologic Cells

What relationship, if any, do the blastoid cells induced in vitro have to other hematologic cells and to the known and suggested transformation of lymphocytes? (See Fig. 1.)

The blastoid cells differ greatly in structure from macrophages (*28*). The typical phagocytosis of macrophages (*7*, p. 78) does not appear to be a function of the blastoid cells, for the

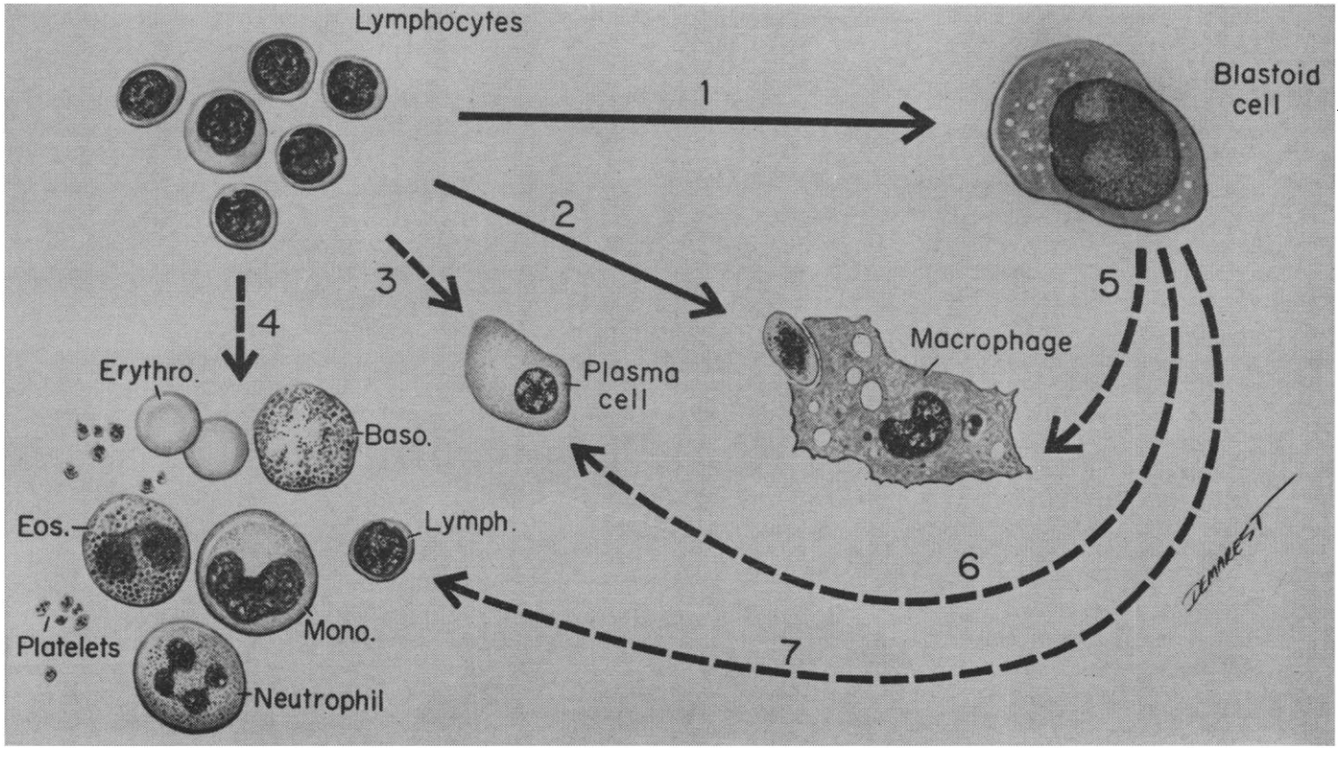

Fig. 1. Diagrammatic representation of the established transformations (solid arrows) and suggested transformations (broken arrows) of human lymphocytes: 1, blastogenesis known to occur in vitro; 2, development into a macrophage; 3 and 4, transformations inferred from in vivo studies of lymphocytes in other mammals; 5–7, possible developmental pathways of the blastoid cells (see text). *Erythro.*, erythrocyte; *Baso.*, basophil; *Eos.*, eosinophil; *Mono.*, monocyte; *Lymph.*, lymphocyte.

latter do not phagocytize cell debris (*37, 81*) or bacteria (*37*).

At least some of the specific antibody of mammals, including human γ-globulin, is produced in cells of the plasma cell series (*13, 15, 35, 82–84*). There is very little similarity between the morphology of the typical blastoid cell and that of the mature plasma cell. While the cytoplasm of both is markedly pyroninophilic, mature plasma cells have large amounts of well-developed endoplasmic reticulum (*15, 36, 85, 86*), whereas the blastoid cells have very little (*23, 37–39*). The staining of mature and immature human plasma cells with fluorescein-labeled antiserum to human globulin usually involves the entire cytoplasm (*84*). In the report of Elves *et al.* (*70*) no detailed description of the staining of the blastoid cells is presented. In Hirschhorn's studies (*48*) the fluorescence of the blastoid cells was mainly "ring-like." Neither group of investigators has presented evidence that their staining was specific for human γ-globulin. There has been no demonstration that soluble antibodies, capable of combining specifically with any known antigen or blastogenic factor, are produced in, and released from, the blastoid cells. Clearly, there is insufficient evidence at this time to warrant the conclusion that the blastoid cells are, or can develop in vitro, into plasma cells.

Similarly, there is no convincing evidence that the blastoid cells can give rise in vitro to any of the cells found in normal peripheral blood (Fig. 1, pathway 7). The blastoid cell does not have the structure or the cytochemical properties characteristic of the erythrocyte, monocyte, or granulocyte. Since certain blast cells of normal human bone marrow have been shown to undergo differentiation in vitro to a more mature cell under certain conditions (*87*), attempts, such as that with erythropoietin (*88*), to obtain further differentiation of the blastoid cells induced from peripheral-blood lymphocytes would be of interest (*89*).

The question arises, Do the blastoid cells or their progeny revert in vitro to small lymphocytes? Some investigators have suggested that this reversion might occur (*21, 31, 32, 43, 90*), but no detailed convincing evidence has been presented. This question may be resolvable, for the progeny of radioactively-labeled blastoid cells can be traced by means of autoradiography.

The PHA-induced blastoid cells have some morphological resemblance to normal primitive cells (for example, reticulum cells, lymphoblasts, plasmablasts) (*18, 21, 33, 39, 57, 81*) and cells sometimes present in diseases such as acute leukemia (*37*) and glandular fever (*39*). However, such a morphological resemblance does not necessarily mean that these cells have the same origin, function, or potentialities as the blastoid cells formed in vitro.

Of more significance, however, are the reactions in vivo in which a large pyroninophilic cell with immature morphology is formed in response to factors which are blastogenic in vitro. Thus, in the homograft and graft-versus-host reactions in mammals a large pyroninophilic cell develops (*11, 86, 91*) which apparently can be derived from the small lymphocyte (*11*). This cell closely resembles the blastoid cell formed in vitro (*18, 23, 37–39, 62, 75*). A morphologically similar cell is formed in lymphoid tissue stimulated with antigens (*13, 75, 82, 92*). The extent of the similarity between the blastoid cells formed in vitro and those formed in these immunological responses in vivo remains to be elucidated.

Mode of Action of the Blastogenic Factors

It has been suggested that the blastogenic factor of PHA produces blastogenesis as a result of its attachment to some structure of the lymphocyte (*25, 48, 49, 79, 80*), possibly the cell's membrane (*93*) or nucleus (*94*). The antiserum to human leukocytes, prepared in rabbits, presumably contains antibodies that combine with the groups on or within the lymphocytes which had induced formation of the antibodies. It is possible that PPD induces blastogenesis by attaching specifically to the sensitized lymphocytes or to an antibody attached to the lymphocytes. The existence of antibodies attached to cells is well documented (*72*), and there is evidence that peripheral-blood leukocytes from persons sensitized to tuberculin-purified protein derivative may contain "cell-fixed" antibodies to PPD (*95, 96*) and may possibly accumulate PPD in vitro (*97*).

Some investigators have suggested that the mode of action of PHA (*48, 63, 79, 80*) might differ qualitatively from that of the specific antigens such as tuberculin in the induction of blastogenesis. This contention is based essentially on the fact that PHA can induce blastogenesis rapidly in the majority of lymphocytes of all healthy people, whereas tuberculin induces blastogenesis slowly (*60, 62, 63, 80*) in but a small percentage (*63, 66, 80*) of lymphocytes only from persons who have previously been sensitized to tuberculin. However, an apparent difference in the *time* required for blastogenesis induced by two different substances does not necessarily mean that they are acting by different mechanisms; it may signify only a difference in the intensity of the blastogenic stimulus. The difference in the *number* of cells responding to different factors may reflect primarily a difference in the number of the cells to which the blastogenic factor can become attached.

If a blastogenic factor attaches to some structure of the cells which undergo blastogenesis, the question arises, Is blastogenesis initiated in some manner as the result of that attachment, without further processing of the factor by the cell, or does the attachment serve as a means for making the factor available, in an effectively high concentration, for reactions in other locations of the cell? If the latter is the case, then the means by which blastogenesis is induced by PHA, tuberculin, and antibody to leukocytes may be the same, the different results they produce being explained primarily on the basis of differences in the extent of their attachment to the lymphocyte and the means by which such an attachment was made possible.

No electrophoretically pure, proteinaceous blastogenic factor has yet been used in these in vitro studies; even tuberculin-purified protein derivative contains several substances (*95*). Therefore, the fact that some component in such a mixture is found attached to, or incorporated within, the lymphocytes which undergo blastogenesis does not prove that component to be the blastogenic factor. The mode of action of some of these blastogenic factors might involve an indirect, rather than a direct, effect on the lymphocytes which become blastoid. Thus, an interaction of a blastogenic factor with some plasma component or with a few sensitized cells might result in the formation or release of a substance which

acts on lymphocytes, causing them to undergo blastogenesis. Systems have been reported in which specific antigens may cause the in vitro release from sensitized cells of substances which can stimulate deoxyribonucleic acid synthesis (*98*) and can transfer delayed hypersensitivity (*99*). The participation of such substances must be ruled out before the cells which are to undergo blastogenesis in vitro can validly be considered "sensitized" or "immunologically committed" cells.

The induction of blastogenesis by PHA occurs in most of the lymphocytes from all the healthy humans who have been tested. It is known, also, that blastogenesis and mitosis occur in the presence of PHA in cultures of peripheral-blood leukocytes from several species of animals (*68, 100*). In view of this widespread response to PHA, it is important to study those cell cultures in which the lymphocytes fail to react normally, such as cultures of cells from patients with chronic lymphocytic leukemia. Other possible sources of lymphocytes showing impaired blastogenesis have been suggested. These sources, such as fetal blood from the human umbilical cord and peripheral blood of patients with agammaglobulinemia or sarcoidosis, are from humans who have certain impairments of immunological mechanisms. Lymphocytes from cord blood (*30*) and from the few hypogammaglobulinemic (*30*) and agammaglobulinemic (*48*) patients studied formed a normal number of blastoid cells in response to PHA. However, there are, as yet, insufficient data to determine conclusively whether or not blastogenesis induced by PHA and specific antigens is impaired in cultures of cells from patients with sarcoidosis (*101*) and from patients with different types of agammaglobulinemia.

Conclusion

Tissue culture studies show convincingly that the lymphocyte of human peripheral blood can be transformed into an immature-appearing, blastoid cell capable of mitotic division. Many "activating stimuli" are now known to induce this phenomenon of blastogenesis, and some insight into their possible mechanism of action has been obtained. The blastoid cells may be able to develop into other blood cells, but there is as yet no convincing evidence for this from the tissue culture studies. Although interesting functions have been attributed to these cells, no function other than that of mitotic division has yet been adequately demonstrated. The blastoid cells have some morphological resemblance to many blood cells found in healthy and in diseased individuals, and they appear to be very similar to cells formed in vivo in certain immunological reactions. It may reasonably be expected that the study of blastogenesis under controlled conditions in vitro will provide further understanding of the cellular and molecular basis of cell differentiation, antigen-cell interaction, and other immunological phenomena.

References and Notes

1. A. A. Maximow, in *Special Cytology*, E. V. Cowdry, Ed. (Hafner, New York, 1963), vol. 2, p. 601; W. Bloom, in *Handbook of Hematology*, H. Downey, Ed. (Hoeber, New York, 1938), vol. 1, p. 373.
2. J. M. Yoffey, *Quantitative Cellular Haematology* (Thomas, Springfield, Ill., 1960).
3. O. A. Trowell, *Intern. Rev. Cytol.* **7**, 235 (1958).
4. W. A. D. Anderson, *Pathology* (Mosby, St. Louis, ed. 4, 1961); *The Lymphocyte and Lymphocytic Tissue*, J. W. Rebuck, Ed. (Hoeber, New York, 1960); S. Raffel, *Immunity* (Appleton-Century-Crofts, New York, ed. 2, 1961).
5. M. M. Wintrobe, *Clinical Hematology* (Lea and Febiger, Philadelphia, ed. 5, 1961); W. Dameshek and F. Gunz, *The Leukemias* (Grune and Stratton, New York, 1958).
6. G. A. Daland, *A Color Atlas of Morphologic Hematology*, T. H. Ham, Ed. (Harvard Univ. Press, Cambridge, 1959), p. 19; C. C. Sturgis, *Hematology* (Thomas, Springfield, Ill., ed. 2, 1955), p. 730.
7. A. A. Maximow and W. Bloom, *A Textbook of Histology* (Saunders, Philadelphia, ed. 7, 1957).
8. F. N. Low and J. A. Freeman, *Electron Microscopic Atlas of Normal and Leukemic Human Blood* (McGraw-Hill, New York, 1958), p. 64; J. F. Rinehart, *Am. J. Clin. Pathol.* **25**, 605 (1955); F. N. Low, in *The Lymphocyte and Lymphocytic Tissue*, J. W. Rebuck, Ed. (Hoeber, New York, 1960), p. 54.
9. W. M. Copenhaver and D. D. Johnson, *Bailey's Textbook of Histology* (Williams and Wilkins, Baltimore, ed. 14, 1958), pp. 253–254.
10. J. W. Rebuck, H. I. Coffman, G. B. Bluhm, C. L. Barth, *Ann. N.Y. Acad. Sci.* **113**, 595 (1964).
11. K. A. Porter and E. H. Cooper, *Lancet* **1962-II**, 317 (1962); J. L. Gowans, D. D. McGregor, D. M. Cowen, C. E. Ford, *Nature* **196**, 651 (1962); J. L. Gowans, *Ann. N.Y. Acad. Sci.* **99**, 432 (1962); J. L. Gowans, D. D. McGregor, D. M. Cowen, in Ciba Foundation Study Group No. 16, *The Immunologically Competent Cell: Its Nature and Origin* (Little, Brown, Boston, 1963).
12. W. H. Hildemann, W. D. Linscott, M. J. Morlino, in *Ciba Foundation Symposium on Transplantation* (Little, Brown, Boston, 1962), pp. 236–263; D. L. Vredevoe and W. H. Hildemann, *Science* **141**, 1272 (1963); for further data, references, and discussion see Ciba Foundation Study Group No. 16, *The Immunologically Competent Cell: Its Nature and Origin* (Little, Brown, Boston, 1963).
13. J. C. Roberts, Jr., in *The Lymphocyte and Lymphocytic Tissue*, J. W. Rebuck, Ed. (Hoeber, New York, 1960), p. 82.
14. M. Holub, *Ann. N.Y. Acad. Sci.* **99**, 477 (1962).
15. W. Bernhard and N. Granboulan, in *Ciba Foundation Symposium on Cellular Aspects of Immunity* (Little, Brown, Boston, 1960), p. 92.
16. J. M. Yoffey, *Lancet* **1962-I**, 206 (1962).
17. W. Bloom, *Proc. Soc. Exptl. Biol. Med.* **24**, 567 (1927); A. Maximow, *ibid.*, p. 570; J. W. Rebuck, R. W. Monto, E. A. Monaghan, J. M. Riddle, *Ann. N.Y. Acad. Sci.* **73**, 8 (1958); J. C. Sieracki and J. W. Rebuck, in *The Lymphocyte and Lymphocytic Tissue*, J. W. Rebuck, Ed. (Hoeber, New York, 1960), p. 71.
18. L. Berman and C. S. Stulberg, *Lab. Invest.* **11**, 1322 (1962).
19. J. M. Yoffey, D. B. Thomas, D. J. Moffatt, I. H. Sutherland, C. Rose, in Ciba Foundation Study Group No. 10, *Biological Activity of the Leucocyte* (Little, Brown, Boston, 1961), p. 45. See G. Cudkowicz, M. Bennett, G. M. Shearer, *Science* **144**, 866 (1964), for further data and references concerning the possible pluripotency of the bone marrow's "small, round, mononucleated cells" which "are lymphocytic in appearance."
20. A. A. MacKinney, Jr., F. Stohlman, Jr., G. Brecher, *Blood* **19**, 349 (1962); O. R. McIntyre and F. G. Ebaugh, *ibid.*, p. 443.
21. K. Carstairs, *Lancet* **1962-I**, 829 (1962).
22. W. H. Marshall and K. B. Roberts, *ibid.* **1963-I**, 773 (1963); M. W. Elves and J. F. Wilkinson, *Exptl. Cell Res.* **30**, 200 (1963).
23. W. H. Marshall and K. B. Roberts, *Quart. J. Exptl. Physiol.* **48**, 146 (1963).
24. In this article the term *blastogenesis* is defined as the acquisition by a cell of a morphology which resembles that of the immature blood cells known as "blasts." Substances causing blastogenesis are referred to as "blastogenic," and the resulting cells, as "blastoid." No relationship to functional criteria or to similar terms in biology is intended.
25. P. C. Nowell, *Cancer Res.* **20**, 462 (1960).
26. D. A. Rigas and E. E. Osgood, *J. Biol. Chem.* **212**, 607 (1955).
27. E. E. Osgood and M. L. Krippaehne, *Exptl. Cell Res.* **9**, 116 (1955); D. A. Hungerford, A. J. Donnelly, P. C. Nowell, S. Beck, *Am. J. Human Genet.* **11**, 215 (1959).
28. R. Schrek and Y. Rabinowitz, *Proc. Soc. Exptl. Biol. Med.* **113**, 191 (1963).
29. D. Quaglino, F. G. J. Hayhoe, R. J. Flemans, *Nature* **196**, 338 (1962).
30. J. H. Robbins, *Experientia* **20**, 164 (1964).
31. E. H. Cooper, *Brit. J. Haematol.* **8**, 304 (1962).
32. ———, P. Barkhan, A. J. Hale, *ibid.* **9**, 101 (1963).
33. G. Astaldi, E. Strosselli, S. Sauli, *Haematol. (Pavia)* **47**, suppl., 1 (1962).
34. A. G. E. Pearse, *J. Clin. Pathol.* **2**, 81 (1949); R. G. White, *Brit. J. Exptl. Pathol.* **35**, 365 (1954).
35. J. P. Thiery, in *Ciba Foundation Symposium on Cellular Aspects of Immunity* (Little, Brown, Boston, 1960), p. 59.
36. M. C. Bessis, *Lab. Invest.* **10**, 1040 (1961).
37. Y. Tanaka, L. B. Epstein, G. Brecher, F. Stohlman, Jr., *Blood* **22**, 614 (1963).
38. D. R. Inman and E. H. Cooper, *J. Cell Biol.* **19**, 441 (1963).
39. M. W. Elves, J. Gough, J. A. Chapman, M. C. G. Israels, *Lancet* **1964-I**, 306 (1964).
40. E. H. Cooper, P. Barkhan, A. J. Hale, *ibid.* **1961-II**, 210 (1961).
41. L. Beckman, K. E. Fichtelius, S. C. Finley, W. H. Finley, K. Lindahl-Kiessling, *Hereditas* **48**, 619 (1962).
42. A. Lima-de-Faria, J. Reitalu, S. Bergman, *ibid.* **47**, 695 (1961); J. W. Byron and L. G. Lajtha, *Blood* **20**, 102 (1962); M. A. Bender and D. M. Prescott, *Exptl. Cell Res.* **27**, 221 (1962); ———, *Blood* **20**, 103 (1962); J. F. Jackson, *Clin. Res.* **10**, 26 (1962); A. Michalowski, *Exptl. Cell Res.* **32**, 609 (1963); L. B. Epstein and F. Stohlman, Jr., *Blood* **24**, 69 (1964).
43. P. C. Nowell, *Exptl. Cell Res.* **19**, 267 (1960).
44. C. Bernard, A. Geraldes, M. Boiron, *Lancet* **1964-I**, 667 (1964).
45. It should be noted that when cultures are made from the peripheral blood of patients with very high lymphocyte counts, the high concentration of lymphocytes is often diluted with cell-free plasma before being cultured. During this dilution the concentration of erythrocytes, platelets, and other cells is correspondingly reduced, and they will be

present in the leukemic cultures at a concentration lower than that in most cultures of normal peripheral blood. Experiments (for example, with isolated, single cell cultures of normal lymphocytes) have not yet been performed to determine whether the presence of a certain concentration of intact platelets, erythrocytes, or some other cell is required for a normal lymphocyte to undergo blastogenesis. Thus, the possibility that the impaired blastogenesis in the leukemic cultures is due to a deficiency of some other cell type has not yet been ruled out.

46. "Tissue Culture and Virus Propagation," *Difco Laboratories, Mich., Publ. No. 140* (1961), pp. 50–52.
47. W. J. Mellman, H. D. Klevit, P. S. Moorhead, *Blood* **20**, 103 (1962).
48. K. Hirschhorn, F. Bach, R. L. Kolodny, I. L. Firschein, N. Hashem, *Science* **142**, 1185 (1963); F. Bach and K. Hirschhorn, *Exptl. Cell. Res.* **32**, 592 (1963); R. L. Kolodny and K. Hirschhorn, *Nature* **201**, 715 (1964); K. Hirschhorn, R. L. Kolodny, N. Hashem, F. Bach, *Lancet* **1963-II**, 305 (1963).
49. C. T. Nordman, A. de la Chapelle, R. Grasbeck, *Acta Med. Scand. Suppl.* **412**, 49 (1964).
50. L. Beckman, *Nature* **195**, 582 (1962).
51. T. Punnett, H. H. Punnett, B. N. Kaufman, *Lancet* **1962-I**, 1359 (1962).
52. P. Genest, *ibid.* **1963-I**, 828 (1963).
53. D. A. Rigas and E. A. Johnson, *Ann. N.Y. Acad. Sci.* **113**, 800 (1964).
54. J. Borjeson, R. Bouveng, S. Gardell, A. Norden, S. Thunell, *Biochim. Biophys. Acta* **82**, 158 (1964).
55. R. Marshall and B. Capon, *Lancet* **1961-II**, 103 (1961); L. Brandt, J. Borjeson, A. Norden, I. Olsson, *Acta Med. Scand.* **172**, 459 (1962); B. Hall, *Lancet* **1962-II**, 1026 (1962); J. H. Edwards, *ibid.* **1963-I**, 725 (1963).
56. A. de la Chapelle, *Lancet* **1961-I**, 1348 (1961).
57. P. Barkhan and A. Ballas, *Nature* **200**, 141 (1963).
58. M. Tunis, *Federation Proc.* **23**, 510 (1964).
59. J. H. Robbins and A. W. Wachtel, *Lancet* **1963-II**, 406 (1963); M. Spitz, *Nature* **202**, 902 (1964).
60. G. Pearmain, R. R. Lycette, P. H. Fitzgerald, *Lancet* **1963-I**, 637 (1963).
61. R. Schrek, *Am. Rev. Respirat. Diseases* **87**, 734 (1963).
62. W. H. Marshall and K. B. Roberts, *Lancet* **1963-I**, 773 (1963).
63. D. C. Cowling, D. Quaglino, E. Davidson, *ibid.* **1963-II**, 1091 (1963).
64. M. W. Elves, S. Roath, M. C. G. Israels, *ibid.* **1963-I**, 806 (1963).
65. The blastogenic substances include typhoid-paratyphoid vaccine, pertussis vaccine, diphtheria toxoid-antitoxin floccules, smallpox vaccine lymph, staphylococcal filtrate, streptolysin O, polyvalent extract of grass pollens, Sabin vaccine, penicillin, dilantin, and rabies vaccine.
66. N. R. Ling and E. M. Husband, *Lancet* **1964-I**, 363 (1964).
67. N. S. Matsaniotis and C. J. Tsenghi, *ibid.*, p. 989.
68. R. R. Lycette and G. E. Pearmain, *ibid.* **1963-II**, 386 (1963).
69. P. Holland and A. M. Mauer, *ibid.* **1964-I**, 1368 (1964); N. Hashem and M. L. Barr, *ibid.* **1963-II**, 1029 (1963).
70. M. W. Elves, S. Roath, G. Taylor, M. C. G. Israels, *ibid.* **1963-I**, 1292 (1963).
71. *Fluorescent Protein Tracing*, R. C. Nairn, Ed. (Livingstone, Edinburgh, 1962), pp. 116–122.
72. S. V. Boyden, in *Cell-bound Antibodies*, B. Amos and H. Koprowski, Eds. (Wistar Institute Press, Philadelphia, 1963), pp. 7–14; E. Sorkin, in Ciba Foundation Study Group No. 16, *The Immunologically Competent Cell: Its Nature and Origin* (Little, Brown, Boston, 1963), pp. 38–53; A. P. Ridges and R. Augustin, *Nature* **202**, 667 (1964).
73. R. N. Hamburger, D. A. Pious, S. E. Mills, *Immunology* **6**, 439 (1963).
74. R. Robineaux and J. Pinet, in *Ciba Foundation Symposium on Cellular Aspects of Immunity* (Little, Brown, Boston, 1960), pp. 17–18.
75. B. Bain, M. Vas, L. Lowenstein, *Federation Proc.* **22**, 428 (1963); *Blood* **23**, 108 (1964).
76. N. Hashem and D. H. Carr, *Lancet* **1963-II**, 1030 (1963).
77. N. Hashem and F. S. Rosen, *ibid.* **1964-I**, 201 (1964).
78. F. Bach and K. Hirschhorn, *Science* **143**, 813 (1964); A. L. Rubin, K. H. Stenzel, K. Hirschhorn, F. Bach, *ibid*, p. 815.
79. R. Grasbeck, C. Nordman, A. de la Chapelle, *Lancet* **1963-II**, 385 (1963).
80. ———, *Acta Med. Scand. Suppl.* **412**, 39 (1964).
81. J. Newsome, *Lancet* **1963-II**, 91 (1963).
82. A. Fagraeus, *J. Immunol.* **58**, 1 (1948); ———, *Acta Med. Scand. Suppl.* **204**, 3 (1948); E. H. Leduc, A. H. Coons, J. M. Connolly, *J. Exptl. Med.* **102**, 61 (1955); A. H. Coons, *Harvey Lectures Ser.* **53**, 113 (1959).
83. R. A. Good, in *Host-Parasite Relationships in Living Cells*, H. M. Felton, Ed. (Thomas, Springfield, Ill., 1957), pp. 78–160; A. Fagraeus, *Acta Haematol.* **20**, 1 (1958); P. D. McMaster, in *The Cell*, J. Brachet and A. E. Mirsky, Eds. (Academic Press, New York, 1961), vol. 5, pt. 2, pp. 323–404; A. Solomon, J. L. Fahey, R. A. Malmgren, *Blood* **21**, 403 (1963).
84. L. G. Ortega and R. C. Mellors, *J. Exptl. Med.* **106**, 627 (1957); R. C. Mellors and L. Korngold, *ibid.* **118**, 387 (1963).
85. H. Braunsteiner, K. Fellinger, F. Pakesch, *Blood* **8**, 916 (1953).
86. J. L. Binet and G. Mathe, *Ann. N.Y. Acad. Sci.* **99**, 426 (1962).
87. G. Astaldi, in *Ciba Foundation Symposium on Haemopoiesis* (Little, Brown, Boston, 1960), pp. 99–127; E. H. Reisner, Jr., *Ann. N.Y. Acad. Sci.* **77**, 487 (1959).
88. W. McFarland and F. Soehnlein, *Proc. Soc. Exptl. Biol. Med.* **115**, 563 (1964).
89. The marrow "lymphocyte" is a cell in mammalian bone marrow which morphologically resembles a small lymphocyte. There is evidence from in vivo studies [see G. Cudkowicz, M. Bennett, G. M. Shearer, *Science* **144**, 866 (1964)] that this cell may give rise to the erythroid and granulocytic cells. Since this cell can be obtained free from most of the other bone marrow cells, it should be possible to determine whether PHA will induce it to undergo blastogenesis in vitro, and, if so, whether the resulting blastoid cells can differentiate in vitro into the different cell types.
90. M. W. Elves and J. F. Wilkinson, *Nature* **194**, 1257 (1962).
91. R. J. Scothorne, *Ann. N.Y. Acad. Sci.* **64**, 1028 (1957); J. A. Andre, R. S. Schwartz, W. J. Mitus, W. Dameshek, *Blood* **19**, 313 (1962).
92. A. R. Rich, M. R. Lewis, M. M. Wintrobe, *Bull. Johns Hopkins Hosp.* **65**, 311 (1939); J. L. Turk and S. H. Stone, in *Cell-bound Antibodies*, B. Amos and H. Koprowski, Eds. (Wistar Institute Press, Philadelphia, 1963), p. 51; H. L. Langevoort, R. M. Asofsky, E. B. Jacobson, T. de Vries, G. J. Thorbecke, *J. Immunol.* **90**, 60 (1963); R. Schrek and S. S. Stephani, *J. Natl. Cancer Inst.* **32**, 507 (1964).
93. P. S. Vassar and C. F. A. Culling, *Nature* **202**, 610 (1964).
94. A. Michalowski, J. Jasinska, W. J. Brzosko, A. Nowoslawski, *Exptl. Cell Res.* **34**, 417 (1964).
95. S. O. Freedman, R. Turcotte, A. J. Fish, A. H. Sehon, *J. Immunol.* **90**, 52 (1963).
96. Experiments have not yet been performed to determine whether the lymphocytes from a donor who is not sensitized to tuberculin and whose cells, therefore, do not undergo blastogenesis in the presence of PPD will undergo blastogenesis in the presence of PPD after they are made to possess "cell-fixed" antibodies to PPD by incubation with serums containing such antibodies.
97. T. A. Witten, W. L. Wang, M. Killian, *Science* **142**, 596 (1963).
98. R. W. Dutton and G. Harris, *Nature* **197**, 608 (1963).
99. H. S. Lawrence, in *Ciba Foundation Symposium on Cellular Aspects of Immunity* (Little, Brown, Boston, 1960), p. 243; ———, in *Cell-bound Antibodies*, B. Amos and H. Koprowski, Eds. (Wistar Institute Press, Philadelphia, 1963), p. 6.
100. W. W. Nichols and A. Levan, *Blood* **20**, 106 (1962); M. R. Schwarz, *Anat. Record* **148**, 333 (1964).
101. D. C. Cowling, D. Quaglino, P. K. M. Barrett, *Brit. Med. J.* **1964-I**, 1481 (1964).