tion might be the differences in antigen dose employed, since mice in the BSA group received 1.25 mg BSA weekly while the HRBC group received inocula of 0.8 ml packed red cells. In this connection, a number of possible mechanisms could be invoked to account for the protective effect of HRBC administration. For example, HRBC may have stimulated formation of antibodies cross reacting with RAUSCHER virus. Alternatively, a non-specific protective effect might be obtaining, such as stimulation of interferon production[10] by the red cells or by virus contaminating these cells. Delayed deaths may also have been the consequence of decreased availability of cells susceptible to virus infection due to their prior commitment along immunologic pathways[7].

METCALF[11], employing weekly injections of BSA and *Salmonella flagellar* antigen, reported an increased incidence of reticular tumors in C3H mice. Increased reticular tumors were also demonstrated in animals following transfer of spleen cells from parent to F1 hybrid recipients[12] and between strains of mice differing at the H-1 histocompatibility locus[13]. Possible mechanisms suggested have included activation of oncogenic virus and stimulation of immunocompetent cells to neoplastic proliferation. DAMESHEK[14] has proposed that certain forms of leukemia may be due to abnormalities in immunoproliferation. Such differences from those findings of the present study may be due to differences in the host cell types involved in the neoplastic responses. If, as speculated[2-5], the target for RAUSCHER virus infection is a stem cell, availability of such cells may be influenced by the systemic requirements for differentiated cells. On this basis, it would be proposed that the demand for differentiated cell types participating in the response to foreign red cell antigens was sufficiently great to cause a diminution in numbers of precursor cells available for viral infection. Further studies are required to clarify the mechanism of this retardative effect on viral leukemogenesis and to determine its relationship, for example, to antigen dose and structure[15].

Zusammenfassung. Die Mortalität an Leukämie bei BALB/c-Mäusen wurde durch wöchentliche i.p. Injektionen mit menschlichen Erythrozyten herabgesetzt, wenn sie während 8 Monaten vor der Impfung mit RAUSCHER-Leukämievirus vorbehandelt waren. 7 Monate nach der Virusinfektion zeigten 20% dieser Mäuse keine Zeichen von Leukämie. Kontrollversuche mit der gleichen Serie von Injektionen von Ochsenserumalbumin zeigten keinen Schutz.

B. V. SIEGEL and JANE I. MORTON

Department of Pathology and Division of Immunology, University of Oregon Medical School, Portland (Oregon 97201, USA), 21 October 1968.

[10] M. Ho, in *Interferons* (Ed. N. B. FINTER; W. B. Saunders Co., Philadelphia 1966).
[11] D. METCALF, Br. J. Cancer *15*, 769 (1962).
[12] R. S. SCHWARTZ and L. BELDOTTI, Science *149*, 1511 (1965).
[13] R. L. WALFORD, Science *152*, 78 (1966).
[14] W. DAMESHEK, Ann. N.Y. Acad. Sci. *124*, 6 (1965).
[15] Supported by U.S.P.H.S. Grant No. CA-10089 and U.S.A.E.C. Contract No. RLO-1927-32.

Blastomitogenic Agents in Leguminosae and Other Families

Except for the pokeweed mitogen of *Phytolacca americana*[1], plants which have been reported to contain substances capable of stimulating blastomitogenesis in lymphocyte cultures have been limited to the family Leguminosae[2-5]. It is known that a plasmacytoid cell type appears in pokeweed stimulated cultures[6] in addition to the blast cell produced by both phytohemagglutinin (*Phaseolus vulgaris*) and pokeweed[2,6]. In searching for a possible differential effect of blastomitogenic agents from different sources, upon refractory lymphocytes, we have screened seed extracts from a variety of plant families for blastomitogenic activity. In doing so we have discovered 4 such agents in the seeds of plants from the families Compositae, Ephedraceae, Clusiaceae and Solanaceae.

Lymphocyte cultures from several healthy donors were prepared by gelatin sedimentation of defibrinated blood as previously described[7]. Saline extracts of the seeds were prepared and filtered through Millipore filters (0.45 μ). A 1 or 2 ml suspension of lymphocytes (1×10^6 lymphocytes/ml) in TC-199 containing 100 U of penicillin-streptomycin mixture and 20% autologous serum was cultured in each tightly stoppered Bellco disposable glass tube. The seed extracts were added to the tubes in volumes of 0.01 ml and 0.1 ml. For each group of cultures an extract of red kidney beans (*P. vulgaris*), prepared in the same manner, was used as the reference mitogen. Tubes to which no extract was added served as controls. The cultures were sacrificed at 48 or 72 h and smears were prepared and stained with Wright's stain. 500–1000 cells were counted on smears from 72 h cultures which appeared to contain more blasts than the control cultures. Cells categorized as transformed were 'blasts', i.e. large cells with fine chromatin, single or multiple large nucleoli, and abundant, blue cytoplasm, often containing vacuoles. These cells could be distinguished from the macrophages that were present in some of the cultures. Lymphocytes with increased cytoplasm, but little or no nuclear change were not classified as transformed.

Ninety seed extracts, representing 38 families were tested for blastomitogenic activity[8]. Those extracts demonstrating significant blastomitogenic activity at 72 h are listed in the Table.

[1] P. FARNES, B. E. BARKER, L. E. BROWNHILL and H. FANGER, Lancet *2*, 1100 (1964).
[2] B. E. BARKER and P. FARNES, Nature *215*, 659 (1967).
[3] J. MUSTIER and M. COULET, C. r. Séanc. Soc. Biol. *161*, 1067 (1967).
[4] M. KRÜPE, W. WIRTH, P. NIES and G. ENSGRABAR, Z. Immun. Forsch. exp. Ther. *135*, 19 (1968).
[5] H. J. DOWNING, G. C. KEMP and M. A. DENBOROUGH, Nature *217*, 654 (1968).
[6] S. DOUGLAS, P. HOFFMAN, J. BORJESON and L. CHESSIN, J. Immun. *98*, 17 (1967).
[7] J. W. PARKER and R. J. LUKES, Third Annual Leucocyte Culture Conference (9–11 November 1967, Iowa City, Iowa), in press.
[8] To conserve space, the seeds tested are not included. Interested readers may obtain the list from the authors (J.W.P.).

Several other extracts showed slight activity, but there was usually insufficient material, because of the scarcity of seeds, to run dilution curves to determine optimum concentration, or to attempt removal of possible inhibitors or cytotoxic factors. Thus, we cannot be certain of their potency as blastomitogenic agents. The extracts of *P. vulgaris* consistently produced the highest percentage of blast cells. However, a comparison of specific activities of the various blastomitogenic extracts was not possible in the absence of purified preparations.

Several of the extracts produced pronounced erythroagglutination, but no lymphocyte transformation; and, although the great majority of blast cells in positive cultures tended to occur in clumps, often detected only microscopically, there were numerous instances of lymphoagglutination without transformation. Thus, lymphoagglutination alone does not appear to be sufficient to produce lymphocyte transformation. Furthermore, reports of prevention of leukoagglutination without depression of transformation[9], lack of agglutination by agents such as staphylococcal filtrates[10], and transformation of isolated lymphocytes in agar[11] indicate that agglutination is not essential for transformation. However, our own experience has been that blasts in mixed lymphocyte cultures and those stimulated by staphylococcal filtrates occur, for the most part, in loosely adherent microscopic clumps. This agglutination occurs prior to cell division so that the aggregates are not the result of adherent clones of daughter cells. It is our impression that although agglutination may not be essential for transformation, the degree of transformation in cultures is enhanced by increased cell contact which may be produced either by high cell density[12] or agglutination.

KRÜPE et al.[4], in surveying plant hemagglutinins from 6 families, found only 3 'new' species with mitogenic activity and these were all from the family Leguminosae. These authors concluded that the mitogenic and agglutinating agents were identical, whereas DOWNING et al.[5] have suggested that the mitogenic factor in some plant agglutinins may be independent of both erythroagglutinating and leukoagglutinating activity. However, the nature of the relationship between these 3 properties is still obscure[5].

Both KRÜPE and DOWNING found no mitogenic activity in plant agglutinins with ABO blood group specificity. Among the seed extracts included in our own study were 4 with specific blood group agglutinating activity – *Ulex europaeus* (anti-H), *Dolichos biflorus* (anti-A), *Iberis amara* (anti-M) and *Vicia graminea* (anti-N)[13]. These did not produce transformation of lymphocytes from donors with these antigens, confirming the lack of relationship between specific blood group agglutinins and blastomitogenic activity reported by the above workers[4,5].

The blast cells produced by the extracts listed in the Table were indistinguishable from those produced by PHA, but the plasmacytoid cells reported in pokeweed stimulated lymphocyte cultures[6] were not observed. However, electron microscopic studies are in progress to better characterize the cells produced by mitogenic extracts.

The results of this study confirm the existence of multiple species of plants, within the family Leguminosae, with seeds that contain blastomitogenic agents, and also indicate that apparently not all legumes possess these agents. However, the latter is less certain because of the possibility that inhibitors or cytotoxic agents coexist in the crude extracts of some seeds.

In addition, this survey has revealed blastomitogenic activity in 4 species from 4 other families, i.e. Solanaceae, *Datura discolor*; Compositae, *Trixis californica*; Ephedraceae, *Ephedra nevadensis*; and Clusiaceae, *Mammea sajra*. This wide distribution of phytomitogens in nature raises interesting questions about the chemical similarity of the agents, the mechanism of blastomitogenic action, the role of the agents in plant physiology, and their evolutionary significance. Of potentially more practical value is the possibility that blastomitogens from different sources will produce varying degrees of effect upon abnormal lymphocytes, e.g. chronic lymphocytic leukemia, which show depressed and/or delayed transformation when exposed to PHA[14,15].

Seed extracts with blastomitogenic activity

Genus and species	Family	% Transformation[a] 72 h
Phaseolus vulgaris (red kidney bean)	Leguminosae	49.6–65.8
Datura discolor (shrub)	Solanaceae	42.8
Crotolaria breviflora (rattle-box)	Leguminosae	40.6
Trixis californica (American trixis)	Compositae	38.6
Canavalia ensiformis (jackbean)	Leguminosae	30.6
Ephedra nevadensis (Mexican tea, shrub)	Ephedraceae	22.5
Mammea sajra (mammee-apple)	Clusiaceae	19.4
Pisum sativum (pea)	Leguminosae	17.4
Phaseolus sp. (Japanese red bean)	Leguminosae	6.0
Control (no extract)		0–2.0

[a] 500–1000 cell counts.

Résumé. Des agents blastomitogéniques furent décelés non seulement dans les extraits de 4 genres propres à la famille des Légumineuses, mais aussi dans les graines d'espèces déterminées appartenant aux familles des Solanacées, Composées, Ephédracées et Clusiacées. Ces 4 dernières sources, non mentionnées au préalable, témoignent de la répartition variée de ces agents dans la nature et donnent lieu à des conjectures aux points de vue de leur affinité chimique, du mécanisme de leur action blastomitogénique, de leur rôle dans la physiologie végétale et de leur importance dans l'évolution.

J. W. PARKER, JOAN STEINER, ANDREA COFFIN, R. J. LUKES, KATHLEEN BURR and LAURA BRILLIANTINE

Department of Pathology, University of Southern California School of Medicine, Los Angeles (California 90033, USA) and Biology Department, Immaculate Heart College, Los Angeles (California, USA), 8 October 1968.

[9] J. BORJESON, L. CHESSIN and M. LANDY, Int. Archs Allergy appl. Immun. *31*, 184 (1967).

[10] N. R. LING, E. SPICER, K. JAMES and N. WILLIAMSON, Br. J. Haemat. *11*, 421 (1965).

[11] A. S. COULSON, A. TURK, P. R. GLADE and L. N. CHESSIN, Lancet *1*, 89 (1968).

[12] B. LEVANTHAL and J. OPPENHEIM, Third Annual Leucocyte Culture Conference (9–11 November 1967, Iowa City, Iowa), in press.

[13] These 4 seeds were kindly supplied by Hyland Laboratories, Los Angeles, California.

[14] We would like to thank Dr. M. GARDNER, University of California, Berkeley, for his interest and assistance in obtaining many of the seeds.

[15] This work was supported by grants from the U.S. Public Health Service (No. CA 08654) and American Cancer Society (No. T-374A). One of us (J.W.P.) is an American Cancer Society Senior Dernham Fellow in Oncology.