2. Gyorgy, P., Moro, E., Witebsky, E., *Klin. Wschr.*, 1930, v9, 1012.
3. Strobl, A., Wasitsky, A., *Mschr. Kinderheilk.*, 1932, v52, 95.
4. Lippard, V. W., *Am. J. Dis. Child.*, 1939, v57, 524.
5. Grabar, M. M. P., Bourduas, A., Holtzer, A., *Acta allergol.*, 1952, v5, 30.
6. Coombs, R. R. A., Moffat, J. L., Whittle, C. H., *Int. Arch. Allergy*, 1955, v6, 122.
7. Larose, C., Delorne, P. J., Richter, M., Rose, B., *J. Allergy*, 1962, v33, 306.

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Effects of Phytohemagglutinin on Rat and Normal and Leukemic Human Blood Cells.* (28317)

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Nowell(1) found that phytohemagglutinin (PHA) initiated mitosis in cultures of normal human leukocytes and produced cells which were undifferentiated or blast-like. He stated (2) that the undifferentiated cells were presumably derived from monocytes and possibly young lymphocytes, while other studies(3,4) indicated that the cells represented transformed lymphocytes.

In the present work, the effect of PHA was studied on 1) leukocytes of normal blood, 2) lymphocytes, concentrated and purified, from normal blood, 3) blood cells from patients with chronic lymphocytic leukemia, and 4) leukocytes from rat blood. The objectives of this work were to study the origin and morphology of the living blastlike cells produced by PHA, and to compare them with macrophages.

Methods. The slide chamber method to study white blood cells has been described (5). The cells were suspended in a mixture of equal parts of homologous serum and TC 199 (Difco). Human blood was obtained from non-leukemic patients with normal hemograms and from 10 patients with chronic lymphocytic leukemia. The phytohemagglutinin used was PHA-P of Difco Laboratories. The contents of a vial were dissolved in 5 ml of distilled water. The dilutions were expressed as μl of stock solution per ml of final suspension.

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Results. Addition of PHA, 0.5 to 10 μl/ml, to suspensions of normal human blood cells resulted in small and large clumps of white and red blood cells, platelets and debris. Large blast-like cells developed after 2 to 4 days of incubation at 37°C and these were called "PHA-cells." The number of PHA-cells in 4 days was usually 2 to 20% of the original number of mononuclear cells. In suspension 3787 (Table I) an unusually large number of PHA-cells developed.

The living PHA-cell as seen in the slide chamber was 12 μ or more in diameter (Fig. 1). Usually the cell was irregular in shape or elongated with pseudopod formation. The nucleus was large and vesicular and comprised about half the cross sectional area of the cell. The pale, rather homogeneous nucleoplasm was enclosed in a sharply defined nuclear membrane. Each cell had one or two large, dark, usually irregularly shaped nucleoli which often appeared to be attached to the nuclear membrane. The cytoplasm was homogeneous with a few coarse dark granules, presumably mitochondria. Pseudopods were large and free of granules.

The viable PHA-cells could be readily differentiated from macrophages. Viable macrophages, as shown previously(6), were much larger, had numerous granules and tended to flatten on the glass slide (Fig. 2). The PHA-cells resembled somewhat lymphosarcoma-cells (Fig. 4) observed in blood of patients with lymphosarcoma cell leukemia(7). Both types

TABLE I. Effects of PHA on Blood Cells of Non-Leukemic and Leukemic Patients and of Rat.

Source of blood cells		PHA, μl/ml	Days of incubation	No. in standard area (10 × .04 mm) Lympho-cytes	PHA-cells	Granulo-cytes	Mono-cytes	Macro-phages	Clumping of cells†
Patients with normal blood cell counts	#3787	0	0	131	0	146	23	0	
		0	4	55	0	2	0	18	—
		1	4	27	45	2	0	4	+
	#3592	0	0	116	0	225	26		
		0	3	92	0				—
		1	3	21	17				+
	#3592 p*	0	0	211	0	20	1		
		0	3	146	0		0	0	—
		1	3	60	20		0	0	+
Patients with chronic lymphocytic leukemia	#4052	0	0	240	0				
		0	4	140	0				—
		1	4	49	22				+
	#4051	0	0	279	0				
		0	4	179	0				—
		1	4	186	0				±
Rat	#45	0	0	243	0				
		0	3	63	0	26	4	5	—
		2	3	30	0	5	4	15	+

* p = purified.

† + indicates a small percentage of leukocytes in small clumps in which individual cells usually could not be identified and counted. Accordingly, corresponding cell counts are low.

Blank spaces in table indicate that no counts were made.

of cells had large dark nucleoli and homogeneous gray nucleoplasma. The PHA-cells, however, were larger and more irregular in shape with larger pseudopods. In addition, lymphosarcoma-cells were observed within the first hour of incubation(7) while the PHA-cells developed only after 2 to 4 days of incubation.

Daily observations of the slide chambers showed intermediate stages between lymphocytes and PHA-cells during the first and second day of incubation. On the other hand, intermediate stages between monocytes and macrophages were seen even in the presence of PHA. These observations gave the impression that PHA-cells were derived from lymphocytes, and not monocytes.

After 3 days of incubation, a few cells were seen to be in mitotic division. These cells were large, spherical and were presumably PHA-cells, although no direct observations were made of a PHA-cell going into mitotic division. A few dividing cells were studied for several hours by direct observation and by time-lapse cinemicrography. The resulting daughter cells were moderately large with large irregular shaped nuclei which after a few hours acquired small nucleoli. These daughter cells were not large enough to be classified as PHA-cells but were intermediate in size and morphology between PHA-cells and lymphocytes.

Doses of 0.5 to 3 μl/ml of PHA-P were effective in producing PHA-cells. With a dose of 0.5 μl/ml, normal transformation of monocytes to macrophages occurred, as described previously(6). With doses of 1-3 μl/ml, transformation of monocytes was impaired and relatively unchanged monocytes persisted without transforming to macrophages (Fig. 3). Thus, at 3 μl/ml many PHA-cells were formed with persistence of monocytes, whereas at 0.5 μl/ml both macrophages and PHA cells were seen. Accurate counts of monocytes and macrophages could not be obtained since these cells in particular were involved in clumps in the PHA-treated cultures.

Passage of suspensions of white blood cells through Garvin's columns of glass beads(8) resulted in removal of almost all the monocytes and granulocytes with resultant suspensions of 90 to 97% lymphocytes. Counts on an unpurified control suspension 3592 (Table I) showed 116 lymphocytes and 26 mono-

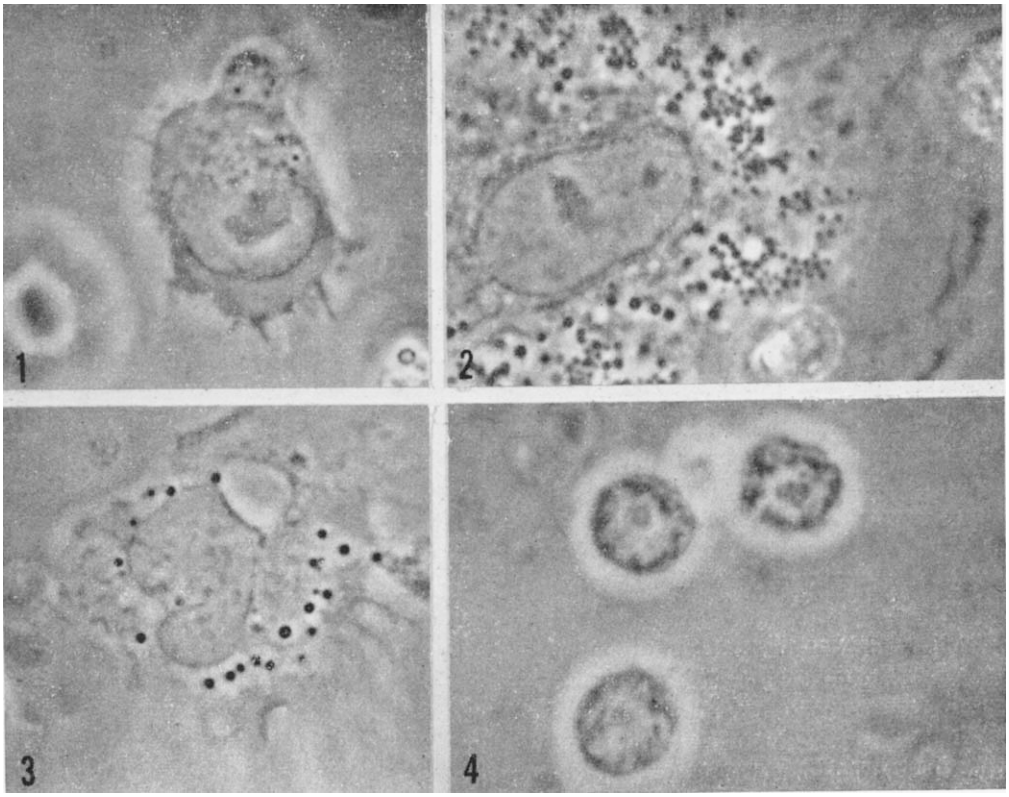

FIG. 1-4. Viable, unstained cells in slide chambers, photographed with an inverted phase contrast microscope. Magnification 2100 ×.

FIG. 1. PHA-cell derived after 4 days of incubation from normal blood cells incubated with 2 μl/ml of PHA-P.

FIG. 2. Macrophage derived from normal blood cells, incubated for 10 days.

FIG. 3. Flattened monocyte observed in a slide chamber of normal blood cells incubated for 5 days with 3 μl/ml of PHA-P.

FIG. 4. Lymphosarcoma-cells from blood of patient with lymphosarcoma in leukemic phase. Incubated 5 hr.

cytes per standard area while counts on the glass purified preparation had 211 lymphocytes and only one monocyte. Addition of 1 μl/ml of PHA to the purified suspension resulted in formation of many PHA-cells in 3 days. It was evident that PHA-cells developed from lymphocytes in the almost complete absence of monocytes.

Suspensions were prepared of white blood cells from 10 patients with chronic lymphocytic leukemia. Addition of PHA to these suspensions usually resulted in clumping of cells although a greater percentage of cells remained isolated, as compared to suspensions of normal cells. In suspensions from some patients, such as 4052 (Table I), it was definite that a moderate number of PHA-cells developed. In some cases, it was difficult to determine whether PHA-cells were present due to their resemblance to lymphosarcoma cells. PHA-cells, however, did not develop in suspensions of blood cells from most patients with chronic lymphocytic leukemia (as 4051, Table I).

Suspensions of white blood cells of the rat incubated with PHA caused clumping of red and white blood cells but PHA-cells did not develop (Table I). It should be noted that in the control suspensions without PHA, the rat lymphocytes survived only 2 to 4 days, while human lymphocytes survived 5 to 10 days(9). In suspensions from rat blood, macrophages developed from monocytes in 3 to 5 days.

Discussion. Our glass purification experiments have indicated an origin of macrophages from monocytes(6) and of PHA-cells from lymphocytes. It is our impression that macrophages represent highly differentiated, specialized end cells. The PHA-cell, on the other hand, would seem to be a reversion to a less differentiated cell type. Its formation from lymphocytes is significant in the light of the opinions of such observers as Yoffey and Courtice(10) and Maximow(11) that the lymphocyte is a multipotential cell capable of becoming the stem blood cell. The PHA-cell may be related to hematological blast cells, but there is no evidence for its ability to transform into a more differentiated cell. We are in accord with McCulloch and Parker (12) who suggested caution in applying hematological terminology to altered cells in tissue culture.

We observed that blood cells of patients with chronic lymphocytic leukemia usually failed to transform to PHA-cells. If PHA-cells are derived from lymphocytes, it may be concluded that leukemic lymphocytes had an impaired ability to transform into blastlike PHA-cells.

Further experiments are in progress to study the PHA-cell and it has been found that a similar type of cell could be obtained with tuberculin-purified protein derivative(13).

Summary. Suspensions of normal human blood cells were incubated at 37°C with phytohemagglutinin (PHA) in slide chambers. In 2 to 4 days, the reagent stimulated the formation of large blast-like cells (PHA-cells) which could be readily differentiated from macrophages. PHA-cells developed in purified suspensions of lymphocytes and many intermediate cells were seen between PHA-cells and lymphocytes. PHA-cells did not develop in blood cell suspensions of some patients with chronic lymphocytic leukemia nor in suspensions of rat blood cells. The findings suggest that PHA-cells were derived from normal human lymphocytes and should be differentiated from macrophages.

1. Nowell, P. C., *Cancer Res.,* 1960, v20, 462.
2. ———, *Exp. Cell Res.,* 1960, v19, 267.
3. MacKinney, A. A., Jr., Stohlman, F., Jr., Brecher, G., *Blood,* 1962, v19, 349.
4. Berman, L., Stulberg, C. S., *Lab. Invest.,* 1962, v11, 1322.
5. Schrek, R., *AMA Arch. Path.,* 1958, v66, 569.
6. Rabinowitz, Y., Schrek, R., PROC. SOC. EXP. BIOL. AND MED., 1962, v110, 429.
7. Schrek, R., Donnelly, W. J., *Blood,* 1961, v18, 561.
8. Garvin, J. E., *J. Exp. Med.,* 1961, v114, 51.
9. Schrek, R., *Am. J. Clin. Path.,* 1959, v31, 112.
10. Yoffey, J. M., Courtice, F. C., *Lymphatics, Lymph and Lymphoid Tissue,* Harvard Univ. Press, Cambridge, 1956.
11. Maximow, A., PROC. SOC. EXP. BIOL. AND MED. 1927, v24, 570.
12. McCulloch, E. A., Parker, R. C., in *Canadian Cancer Conference,* R. W. Begg, Ed., Academic Press, Inc., New York, 1957, v2, 152.
13. Schrek, R., *Am. Rev. Resp. Dis.,* in press.

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Chlorpromazine in Protection from Acute Effects of Extensive High Temperature Skin Injury in Rats.* (28318)

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The mortality from high temperature skin injury can be greatly reduced by proper fluid management. Thermal skin injury may nevertheless, despite extensive fluid therapy and according to the degree and area of the burn, result in a gradual impairment of homeostasis which progresses to a point of insufficiency similar to the fatal peripheral circulatory collapse following mechanical trauma and hemorrhage. In common with other forms of shock,

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