mates. The *l-* isomers of pronethalol and MJ-1999 were 2-3 times more potent than the *dl-* isomers. The 2 naphthyl compounds (propranolol and pronethalol and their isomers) produced significant depression of myocardial contraction and electrical properties (refractory period and excitability). In equal or higher concentrations, MJ-1998 and MJ-1999 showed no significant effect on the electrically driven atria. DCI produced a significant and sustained increase in force of contraction in a concentration of 1.35×10^{-6} M. A higher concentration (1.35×10^{-4} M) produced a transient increase in force of contraction followed by a rapid decrease leading to asystole. In a concentration of 1.35×10^{-5} M, only *dl-* and *d-*propranolol produced a significant decrease in heart rate of spontaneously beating right atrial preparations. DCI produced a significant increase in rate. On the basis of the compounds studied, there does not appear to be any simple interrelationship between (a) chemical structure, (b) direct inotropic and chronotropic effects, (c) *beta*-blocking potency, and (d) ability to depress the electrical properties of the myocardium.

The authors wish to thank Dr. A. Sahagian-Edwards of Ayerst Laboratories and Dr. S. Stephen of Imperial Chemical Industries (England) for kindly supplying pronethalol and propranolol isomers. We are also grateful to Dr. P. M. Lish of Mead Johnson Laboratories for the generous gift of MJ-1998 and MJ-1999. Dr. A. Engelhardt of Boehringer Sohn, Ingelheim, Germany, kindly donated the compound Kö-592.

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Received January 3, 1966. P.S.E.B.M., 1966, v122.

Culture of Leucocytes from Rabbit Blood and Lymph: Effect of Phytohemagglutinin and Growth of Macrophages.* (31139)

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Phytohemagglutinin (PHA)(1) has been shown to be mitogenic for human peripheral blood lymphocytes *in vitro*. The mitogenic effect is preceded by and associated with the

* Supported in part by USPHS Grant AI-01821-08 and by the Commission on Immunization, Armed Forces Epidemiological Board, and in part by the Office of The Surgeon General, Dept. of the Army, Washington, D. C.

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TABLE I. Seventy-Two Hour Cultures of Rabbit Blood and Lymph Leucocytes.

		Mitosis	Lymphocytes	Blasts	Macrophages	Polymorphs
		%				
Blood	Control	.07 (0 – .4)*	74 (65–89)	2.3 (1– 5)	12.2 (5–18)	11.5 (4–20)
	PHA	1.4 (.7–2.4)	41.4 (24–50)	41.5 (28–61)	11.0 (4–22)	6.1 (1– 8)
Mesenteric lymph	Control	.14 (0 – .2)	96.7 (95–98)	3.3 (2– 5)	0	0
	PHA	1.32 (.8–1.7)	72 (71–78)	28 (22–29)	0	0

* Percentage figures are arithmetical mean of different experiments. Figures in parentheses indicate range.

enlargement of small lymphocytes into blast-like cells capable of division(2-5) and of synthesis of RNA and DNA(5-8). PHA has also been shown to stimulate blastogenesis and mitosis in animal lymphocytes(9-11).

This report describes the morphological changes that occur in rabbit lymphoid cells derived from blood and mesenteric lymph when they are cultured *in vitro* in the presence of PHA. Observations on the origin of macrophages in cultures of peripheral blood are also reported.

Materials and methods. Rabbit blood and mesenteric lymph were used as sources of leucocytes. Lymph was obtained by carefully cutting a mesenteric duct with fine scissors and removing lymph with a Pasteur pipette; results of examination of a Wright's stained smear of the cells from such lymph excluded contamination with blood. Leucocytes were cultered *in vitro* for 72-120 hours, after which the cells were harvested according to the method of Hirschhorn(12). Three- and five-day-old cultures were also stained with Wright's stain; in the case of blood cultures, red cells were first lysed by a one-minute exposure to distilled water. Differential counts were made on 200-400 cells.

The presence of phagocytes was determined by studying the uptake of opsonized bacteria (*S. paratyphi* B.) by cells in blood and in culture before they were harvested, and by morphological criteria(13). In this paper, mononuclear phagocytes in cultures will be called macrophages; mononuclear phagocytes in blood will be called monocytes. In contrast to monocytes, the macrophages that appeared in culture were very large cells (15-40 μ in diameter) with coarse granules as well as vacuoles in their cytoplasm.

In experiments designed to investigate the origin of macrophages, rabbit blood was filtered through one inch of tightly packed sterile glass wool. Leucocytes from filtered and non-filtered blood were isolated by mixing the specimens with dextran (one part dextran M.W. 184,000, Pharmachem. Corp. and 9 parts of blood) and removing clumped red blood cells. Blood filtered through glass wool was found to be virtually free of monocytes. 500-1,000 stained cells were examined to determine each differential count.

Results. Table I summarizes the results of the 72-hour-old blood and lymph leucocyte cultures with and without PHA, from 10 different rabbits. At the end of 72 hours, control cultures from blood had an average of 0.07% mitotic figures while PHA-stimulated cultures showed an average of 1.4%. Blast forms were repeatedly observed in the PHA-stimulated cultures as early as 48 hours and more strikingly at 72 hours after culture. The criteria used for defining blast cells were the same as those adopted by others in similar studies using human cells(8,14). Blast cells occurred primarily within small or large clumps. The nucleus occupied a large proportion of the cell and was stained lightly and homogeneously with orcein. Prominent nucleoli were seen in some cells.

In orcein-stained smears of cells from blood a number of cells resembling blast cells were observed to have a high cytoplasm-to-nucleus ratio. Furthermore, their nuclei were predominantly kidney shaped or bi-lobed. When similar smears were stained with Wright's stain after exposure of the culture to opsonized bacteria, these cells proved to be macrophages. The percentage of macrophages appeared to be unrelated to the presence of PHA in the cultures. It was also observed that polymorphonuclear leucocytes persisted

TABLE II. Five-Day Cultures of Rabbit Blood and Lymph Leucocytes.

		Mitosis	Blasts	Macrophages
		%		
Blood	Control	1.6 (.9–3.3)*	32 (20–25)	10.8 (5–25)
	PHA	1.8 (1.6–2.8)	44 (35–70)	11.6 (6–19)
Mesenteric lymph	Control	2.2 (1.2–3.0)	10 (6–16)	0
	PHA	2.5 (1.0–4.0)	14.6 (9–24)	0

* Percentage figures are arithmetical mean of different experiments. Figures in parentheses indicate range.

TABLE III. Development of Macrophages in Cultures of Monocyte-Depleted Blood.

		Total No. of cells			
		Start of culture		6 Days of culture	
Exp	Treatment of cells	WBC	Monocytes	WBC	Macrophages
I	Unfiltered	2.5×10^6	175,000	$.9 \times 10^6$	48,000
	Filtered	2.5×10^6	62,500	$.55 \times 10^6$	3,000
II	Unfiltered	2.0×10^6	32,000	$.76 \times 10^6$	8,000
	Filtered	2.0×10^6	<4,000	$.64 \times 10^6$	<1,300

in 72-hour cultures whether or not PHA was present. This finding differs from observations of human cultures, in which the majority of polymorphonuclear leucocytes degenerate by the end of 24 hours(15).

After 72 hours, PHA-stimulated cultures of lymph cells also showed blast forms (22-29%) and mitoses (0.8-1.7%) in contrast to control cultures, which had less than 5% blasts and only rare mitoses. In contrast to the blood cultures, no macrophages were observed in the lymph cell cultures.

Unlike 72-hour cultures, 5-day cultures of both blood and lymph leucocytes showed spontaneous enlargement of lymphocytes into blast cells. There was no significant difference between the number of blast cells and mitotic figures occurring in the control and in the PHA-stimulated cultures. Table II summarizes these results. The substitution of other sera (decomplemented fetal calf, calf, commercial or autologous rabbit or guinea pig sera) for fetal calf serum did not alter the results. On the other hand, horse and pig sera were found to be toxic for the cells.

To investigate further the origin of macrophages, blood was cultured after having been depleted of monocytes by being filtered through glass wool. The results of 2 representative experiments are demonstrated in Table III. In both experiments, there was a correlation between the number of monocytes at the start of culture and the number observed after 6 days. In the second experiment, in which monocytes were not observed in the filtered blood, macrophages were not detected in the 6-day cultures.

Discussion. The effect of PHA on rabbit lymphocytes appears to be similar to its effect on human lymphocytes: enlargement into blast cells, followed by mitosis. In contrast to human lymphocytes, however, rabbit lymphocytes from blood or lymph undergo some spontaneous blastogenesis after 5 days of culture. Sabesin *et al*(16) recently reported similar findings with cells from rabbit blood. One possible explanation of spontaneous blastogenesis is that blood monocytes release commonly encountered antigens during incubation and that the antigens stimulate lymphocytes that were previously sensitized to such antigens; this could result in the characteristic 5-day blastogenesis. Our results with lymph cultures that do not contain monocytes exclude this possibility.

The growth of macrophages in human peripheral blood cultures was previously reported by Berman(2). On the sole basis of morphological observations made at intervals, Berman attributed the origin of such macrophages to small lymphocytes. In contrast, Schrek and Rabinowitz(17,18) concluded that such macrophages arise from monocytes, on the ground that the removal of monocytes

from the blood by filtration through glass wool prevented the appearance of macrophages. More recently, Volkman and Gowans(19) used Rebuck's skin-window technique(20) in the rat, together with isotopic labeling of cells, to investigate the origin of macrophages at inflammatory sites. Their experiments showed that the macrophages were derived from blood and originated in bone marrow; macrophages did not arise from small lymphocytes that traversed the thoracic duct.

Our report further supports this conclusion. Macrophages did develop in cultures of whole blood but did not develop from cultures of either mesenteric lymph or monocyte-free blood. It is possible, however, that our culture conditions may only have been sufficient to support the minor morphological change of monocytes to macrophages and may not have been optimal for the stimulation and differentiation of all potential macrophage precursors.

Summary. 1. Rabbit blood or mesenteric lymph leucocytes cultured *in vitro* for 72 hours with PHA show blast forms and mitoses. 2. Spontaneous blastogenesis and mitosis of such cells takes place at the end of the fifth day. 3. Macrophages develop in 5-day blood cultures whether or not phytohemagglutinin is present. Macrophages do not arise from leucocyte cultures of monocyte-free blood or from lymph. This supports the view that macrophages arise from monocytes in the peripheral blood, and not from lymphocytes.

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Received January 13, 1966. P.S.E.B.M., 1966, v122.

Effect of Iodide-Deficiency on Thyrocalcitonin Secretion.* (31140)

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It has been demonstrated that thyrocalcitonin, the recently identified hypocalcemic principle(1) is released from the thyroid under experimental conditions of induced high calcium levels and is effective in increasing the rate of fall of serum calcium levels following experimental elevation(2). Perfusion of the thyroparathyroid complex, or of the isolated thyroid gland was reported to induce a

*Aided in part by grants from A.E.C. and N.I.D.R.

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