It may be seen that a 50% increase in heart weight occurs after approximately 12 days of treatment. Van Liere and Feder obtained an increase in heart weight of only 24% after 41 days of treatment employing reduced atmospheric pressure alone.

Summary. Graded cardiac hypertrophy has been produced in the albino rat following exposure to 37°C ± 1°C and 25,000 ± 1,000 ft. for 4 hours a day. After 12 days of treatment a 50% increase in heart weight is produced by this means.

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Purification of Human Leukocytes.* (23859)

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Attracted by availability of human white blood cells (WBC) as compared to other cellular elements of body, numerous investigators have utilized a variety of methods for leukocyte isolation. Most technics for separating white cells from whole blood have emphasized leukocyte yields. However, as more exacting measurements are required, it becomes increasingly important to obtain preparations which are free of other formed elements. In these situations purity rather than yield must be the criterion for evaluating the merit of an isolation procedure. Of many technics we examined, the most promising for obtaining high white cell purity were fibrinogen sedimentation method of Buckley, Powell, and Gibson (1) as modified by Skoog and Beck(2) and the phytohemagglutinin method of Li and Osgood(3). The present report summarizes our experience with these 2 methods and introduces several modifications to facilitate evaluation and to increase purity of white cells.

Methods. Phytohemagglutinin prepared from McCaslan Pole Bean (*Phaseolus vulgaris*) by the method of Boyd(4)† was used and found to be a panagglutinin. The bean extract, at its optimal concentration for leukocyte isolation, has a titer of 1/256 with fresh erythrocytes of varying blood group and Rh types. Venous blood is collected with a few drops of potassium oxalate solution serving as anticoagulant. All syringes, pipettes, and other glassware are siliconed. The bean extract (0.3 ml) is added to 10 ml blood in a 15 x 100 mm test tube at room temperature. The contents are mixed by inversion, and the glass surface above blood is cleaned with cotton swab. The tubes are centrifuged (International Size 1, Type C centrifuge, Head No. 246 or International Clinical Centrifuge, Model CL) at 50 g for 4 min. The supernatant is removed and centrifuged at 100 g for 10 min. to sediment the leukocytes. The WBC are resuspended in 5-10 ml of phosphate buffer. This buffer is prepared from 2 g sodium acetate, 0.003 g ascorbic acid, and 1.42 ml 85% phosphoric acid; the mixture is diluted to one liter after adjusting to pH 7.45 with NaOH. (Tullis(5) pointed out the advantages of acetate and ascorbate.) The cell suspension is centrifuged at 90 g for 7 min., and the final button is resuspended in the same phosphate buffer. With experience, this procedure from time of collection to preparation of final phosphate suspension should require 45-60 min. WBC counts are prepared immediately using Toisson's solution as diluting fluid. (Toisson's solution is composed of 20 ml glycerin, 160 ml distilled water, 8 g sodium sulfate, 1 g calcium chloride, and 0.025 g methyl violet.) Slides for differential leukocyte counts are prepared by adding a

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† Phytohemagglutinin was a gift from Dr. C. W. Cotterman.

TABLE I. Evaluation of Leukocyte Purification by the Phytohemagglutinin Technic in Conjunction with the Use of Phosphate Buffer.

	No. determinations	WBC recovery		WBC purity		
		Mean %	S.E.	% of cell population Mean	S.E.	No. WBC/No. RBC (mean)
Supernatant plasma						
Normal	10	30.9	3.22	72.7	5.64	2.7
Leukemia	5	44.8	6.76	96.5	1.48	27.6
After PO_4 treatment						
Normal	12	18.5	1.30	93.8	1.95	15.1
Leukemia	20	38.4*	7.66	98.8	.48	82.3

* Data available from 12 determinations.

drop of 8% albumin to a drop of phosphate suspended WBC. The method of Skoog and Beck(2) for isolating WBC by sedimenting erythrocytes with fibrinogen was employed.

Results. With the phytohemagglutinin technic leukocyte purity has been evaluated after initial removal of erythrocytes when WBC were still suspended in their own plasma and again after treatment with phosphate buffer. Results summarized in Table I show high purity leukocyte suspensions in the supernatant plasma. With the elevated white cell counts of leukemic bloods higher WBC purity (96.5%) was obtained at this point in procedure than with normal bloods (72.7%). Utilizing the differential lytic effect of phosphate buffer, WBC purity was increased to 93.8% and 98.8% for normal and leukemic bloods, respectively. After phosphate treatment, 31 of 32 preparations exhibited cell populations that were at least 90% leukocytes. Chi-square analysis (χ^2) of differential counts revealed no significant variation in distribution of WBC types in whole blood compared to phosphate suspensions of isolated cells.

The results of applying fibrinogen sedimentation of erythrocytes to isolation of leukocytes are presented in Table II. In each of 5 instances decided improvements in purity were obtained by washing isolated cells with phosphate buffer. Before treatment, the cell populations had a mean leukocyte content of 25.9% (WBC : RBC = 0.35); after treatment, 82% (WBC : RBC = 4.56).

Discussion. Several criteria have been employed for evaluating the merit of some 10-15 leukocyte separation procedures. We have insisted that leukocytes be essentially free of erythrocytes, that white cell yield be adequate and that the cells be morphologically intact and freely suspendable for counting.

The phytohemagglutinin technic(3) has been superior to other methods. Several modifications have been presented to facilitate evaluation and to improve further the purity of WBC. Use of Toisson's solution for staining white cells has simplified differentiating leukocytes from erythrocytes in the counting chamber under unusual circumstances where white cells greatly outnumber the red. In preparation of slides for differential counts on isolated leukocytes, it was advantageous to suspend the cells in albumin. In aqueous medium they are frequently ruptured when smeared on the slide. That distribution of

TABLE II. Leukocyte Recovery and Purity following Fibrinogen Sedimentation of Erythrocytes of Normal Whole Blood.

No. determinations	Vol fibrinogen/vol blood	Fibrinogen conc. (g/100 ml)	WBC recovery		WBC purity		
			Mean %	S.E.	% of cell population Mean	S.E.	No. WBC/No. RBC (mean)
2	2	4			30.8	21.65	.45
3	1	4	65.0	9.34	29.7	13.76	.42
4	1	6	57.2	3.97	21.1	4.96	.27
3	.5	4	57.8	10.22	35.5	16.61	.55
2	.5	6	40.5	8.50	17.1	4.80	.21

WBC types is the same after isolation as in whole blood argues strongly that the cells are a representative rather than a resistant population. In this study yields have been sacrificed for the sake of purity. Leukocyte purities greater than 90% have been uniformly achieved by washing and resuspending isolated WBC in a phosphate buffer which lyses residual red cells while leaving white cells intact. This result is considerably better than the optimal 50% purity recorded by Skoog and Beck(2) for the fibrinogen technic or the 52.5% and 83.8% for normal and leukemic bloods, respectively, calculated from data of Li and Osgood(3). The phytohemagglutination technic with phosphate buffer is recommended to those workers who require white cell suspensions of high purity.

Summary. Methods for preparing human leukocytes from venous blood have been investigated. Sedimentation of erythrocytes by fibrinogen or phytohemagglutinin of *Phaseolus vulgaris* have given best results. With white cell purity as a goal, the phytohemagglutination method has been clearly superior, giving preparations of 72.7% and 96.5% from normal and leukemic bloods, respectively. These values have been increased to 93.8% and 98.8% by utilization of a phosphate buffer which preferentially lyses red cells while leaving white cells intact.

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Effect of Divalent Cations on Proteolysis of Fibrinogen.* (23860)

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Calcium, in conjunction with an unidentified serum factor, exerts an effect on the fibrinogen-fibrin conversion such that the final clot differs significantly from that formed in the absence of one or both of these components. The clots differ in solubility in dilute acid(1), in concentrated urea(2), and in concentrated lithium bromide(3). They differ also in tensile strength(4) and in ease of conversion from α- to β-keratin structures, as demonstrated by x-ray diffraction patterns(5). It has also been demonstrated(6) that the intermediate polymers formed in the conversion are longer and that the rate and extent of their dissociation is diminished in the presence of Ca. Recently, Thomas† observed the inhibition by Ca of streptokinase-induced clot lysis. The conditions of the experiment were such that the effect of Ca could have been on the nature of the clot formed; Ca does not inhibit activity of plasmin(7).

Since these effects might have a common origin in the initial proteolytic attack of thrombin on fibrinogen, it appeared of interest to investigate the effect of Ca on hydrolysis of fibrinogen by trypsin. Both thrombin(8) and plasmin, the streptokinase-induced fibrinolysin(9), possess proteolytic specificity similar to trypsin. Chymotrypsin, a proteolytic enzyme of differing specificity, was studied for comparison.

Methods and materials. Fibrinogen, 90-94% clottable by the method of Laki(10), was prepared from bovine plasma fraction I‡ by the methods of Morrison(11) and Laki (12). Other reagents were obtained commercially. The assay was similar to that of Kunitz(13). A 0.9 ml aliquot of a solution

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† W. A. Thomas, personal communication.

‡ Generously supplied by Armour Laboratories, Chicago, Ill.