viruses and, to our knowledge, no studies previously reported have shown such marked differences in influenza virus hemagglutinins closely related antigenically.

As might be anticipated from other experiments concerned with physical agents and influenza virus, inactivation of infectivity and hemagglutinin does not occur coincidentally. Part of the aim of the present experiments was to determine whether pressure inactivation might be useful as a technic to prepare killed-virus vaccines. Unfortunately, the results indicate that antigenicity is lost at pressures requisite for infectivity inactivation so that, at least with the influenza virus strains tested, pressure inactivation for vaccine preparation would not appear feasible.

Summary. High compression pressures attained at below freezing temperatures have been studied for their effects on influenza virus hemagglutinin, infectivity and antigenicity. Influenza virus hemagglutinin from the virus strains tested varies markedly in its resistance to pressure inactivation.

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Separation of Lymphocytes in Human Blood by Means of Glass Wool Column.* (25237)

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With the exception of the magnetic technic of Levine(1), previous procedures for separation of lymphocytes from the other white cells found in human blood(2,3) have been based on differences in density of the various formed elements. This report describes the successful application of the principle of differential adsorption to separation of human lymphocytes. In this technic, granulocytes are removed from whole blood by passage through a short column of siliconized glass wool, while the lymphocytes in good yield and apparently intact are recovered in the effluent.

Materials and methods. The column and attachments are shown in Fig. 1. All glass surfaces were siliconized with a mixture of monomethyltrichloro- and dimethyldichlorosilane.‡ The vapor of this mixture (obtained by forcing air through a bubbler) was passed through the glass wool for 1 minute and then quickly flushed out with air. The glass wool was washed 10 times with water, heated 30 minutes at 110°C, soaked in water 30 minutes, rinsed many times with distilled water, and finally air dried. The glass wool used was Pyrex fiber No. 3950.§ Two grams of glass wool were packed into the column and held under firm pressure overnight by a rubber stopper fitted with a glass rod. The column was first conditioned by passing through it about 10 ml of fresh serum under 5% carbon dioxide, 85% nitrogen, 10% oxygen. Preliminary experiments carried out since the series reported in this paper indicate that this conditioning may be unnecessary. A freshly drawn 30 ml sample of the same subject's blood was then obtained, heparinized with 3.0 mg of heparin, and added to the column. No attempt was made to control the gas phase over the whole blood. The flow rate was adjusted to about 0.8 ml/minute by turning the screw clamp on the vent tube. The first 4 ml of effluent blood were discarded, and the next

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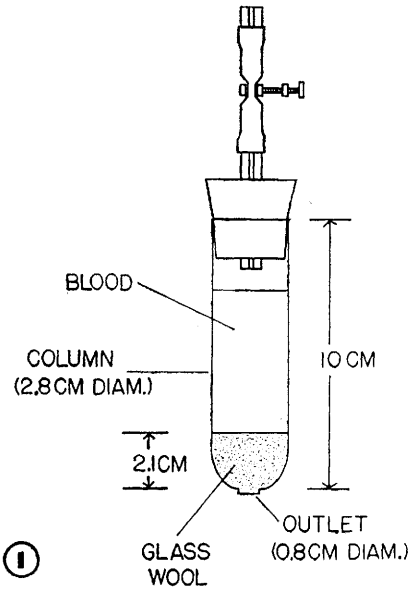

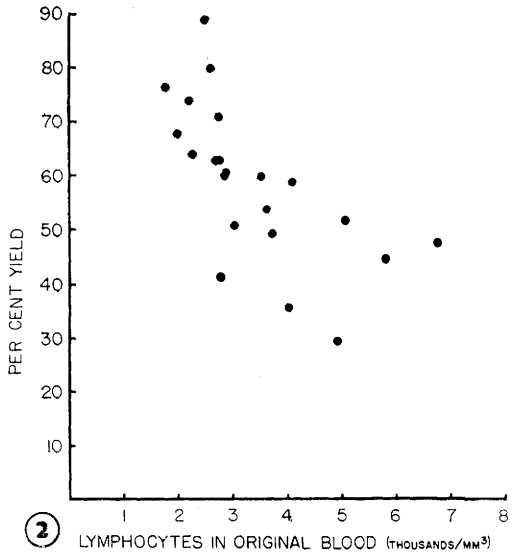

FIG. 1. Diagram of glass wool column.

FIG. 2. Relationship between % yield* of lymphocytes and total lymphocyte count in original blood samples.

* $\frac{\text{Lymphs. in 24 ml of effluent blood}}{\text{Lymphs. in 30 ml of original blood}} \times 100 = \%$ yield.

24 ml were collected for study. The effluent, which consisted essentially of whole blood minus the granulocytes, was examined for its cellular composition. All differential counts were made from smears on chemically clean glass slides and stained with Wright's stain. Two hundred cells were counted in the first 11 experiments and 100 cells were counted in the second 11 experiments. In counting lymphocytes a category of "distorted" lymphocytes was included although such forms may be seen regularly in smears of normal blood. This category of lymphocytes included cells with the following alterations: 1) cytoplasm partially pulled away from the nucleus, 2) nucleus markedly elongated of deformed, and 3) cytoplasm present only in wisps or leaves about the nucleus. Degenerated cells were infrequent and not enumerated.

TABLE I. Cellular Composition of Blood before and after Passage through a Column of Siliconized Glass Wool.

	Original blood	Effluent blood
Total white blood count (thousands/mm³)	5.3-10.6* 8.2†	1.8-4.3 2.6
% lymphocytes	24-62 41	67-100 90
% polymorphonuclear neutrophils	35-72 57	0-33 9.8

* Range † Avg

Results. The results of 22 consecutive experiments using blood from normal human adults are summarized in Table I. Removal of the granulocytes was somewhat more effective than suggested by the table since in 12 of these experiments the proportion of lymphocytes in the effluent blood was 95% or higher. Platelets were seen in abundance in the effluent smears but no quantitative measurements were made. Using a water-jacketed column, there was no difference between results obtained at 27°C and 37°C. However, at 16°C and 4°C large numbers of granulocytes were found in the effluents.

The integrity of the lymphocytes obtained in these effluents was judged primarily upon morphological bases. Ten "distorted" lymphocytes were found in counting 954 lymphocytes in the smears from original blood for a ratio of 1.05 "distorted" lymphocytes per 100 lymphocytes counted. Twenty-four "distorted" lymphocytes were found in counting 2009 lymphocytes in smears from effluent blood for a ratio of 1.15 "distorted" lymphocytes per 100 lymphocytes counted. The

small difference between the 2 groups supports the impression that lymphocytes were relatively undamaged in their passage through the column. Typical smears from effluents were examined by a hematologist,‖ and were judged to be normal in all respects. Phase contrast microscopy of lymphocytes in fresh blood and effluent blood also showed no detectable differences in appearance. Per cent yield of lymphocytes was inversely related to absolute lymphocyte count in the original blood (Fig. 2).

Discussion. This procedure offers promise as a means of preparing lymphocytes for metabolic study, especially since no colloidal or other foreign matter is required to remove the granulocytes. Blood can be processed quickly to remove granulocytes, then one of the methods for removal of erythrocytes can be applied.

The mechanism by which the granulocytes appear to be adsorbed upon the glass wool is unknown. The temperature dependence suggests that the cells must be metabolically active, but it is not clear whether some further features of the interaction of the cell surface with the adsorbant are involved. A count of band form neutrophils in both original and effluent bloods was made in the first 11 experiments. No band forms were found in any of the effluent bloods although from 0-6% were found in the original bloods. This suggests that the young forms are no less sticky than the more mature ones.

The tendency for a greater proportion of lymphocytes to be retained on the column when the original blood has a high lymphocyte count is also unexplained. Several of the subjects with high original lymphocyte counts had just recovered from upper respiratory infections. This relationship to previous disease is currently being studied.

Summary. The method of differential adsorption has been successfully applied to separation of lymphocytes from granulocytes in human blood. When whole blood was passed through a short column of siliconized glass wool, the granulocytes were almost quantitatively retained on the column, and uninjured lymphocytes in 30-89% yield appeared in the effluent blood.

‖ We are indebted to Dr. Walter A. Rambach, Dept. of Hematology, Northwestern University Medical School for making these evaluations.

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Effect of Endotoxin on Plasma Catechol Amines and Serum Serotonin.* (25238)

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The exact role of the pressor amines in endotoxin shock has not been clearly defined, though it has often been observed that systemic effects of endotoxin appear to be associated with generalized sympathomimetic responses. The work of Zweifach, Nagler and Thomas led to the conclusion that an abnormal response to the catechol amines may be an important factor in the lethal effects of endotoxin(1). However Meyer and Ballin have been unable to confirm this finding(2). The effects of serotonin (5-hydroxytryptamine) are far more obscure. Gordon and Lipton demonstrated a protective effect of 5-hydroxytryptamine (5-HT) in endotoxin shock and later pointed out an "antagonism" between this substance and noradrenaline(3,4). Haddy *et al.* showed that serotonin has a vasodilatory effect on small vessels and a vasoconstrictor effect on large vessels(5). In situations where these vessels are maximally constricted, the net effect is vasodilatation.

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