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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TABLE I. Effect of Endotoxin on Plasma Catechol Amines and Serum Serotonin.

	Dogs surviving less than 12 hr								
	Epinephrine (μg/l)			Norepinephrine (μg/l)			Serotonin (μg/ml)		
	Control (mean)	After 5 min.	Terminal	Control (mean)	After 5 min.	Terminal	Control (mean)	After 5 min.	Terminal
	.16	65.81	89.80	2.58	36.48	21.98	1.12	.13	.63
	1.82	.00	180.72	2.48	32.80	49.26	.48	.01	.24
	.00	.10	90.81	.00	30.33	27.28			
	1.44	20.14	457.20	.24	18.00	92.87	.47	.19	.28
	3.46	55.40	287.70	2.36	19.10	84.00	.43	.10	
	.91		144.75	2.92		55.40			
	.67		53.10	4.76		9.85	.24		.14
Mean	1.21	28.29	186.30	2.19	27.34	48.66	.55	.11	.32

This might account for the protective effect of 5-HT in endotoxin shock. More recently, it has been shown that serum serotonin decreases following administration of endotoxin(6) and that a marked depletion of serotonin stores occurs as part of the anaphylactic reaction (7). These findings might explain an altered response of blood vessels to epinephrine and norepinephrine on the basis of a serotonin deficiency. These studies were designed to determine change in concentration of plasma catechol amines and serum serotonin following administration of endotoxin in order to help clarify the role of these substances in producing the aforementioned effects.

Materials and methods. Mongrel dogs weighing approximately 20 kg were sedated with morphine and given a lethal dose (7.5-10 mg/kg) of *E. coli* cell suspension intravenously.† Venous blood samples were obtained before administration of endotoxin and at regular intervals during the shock state. Blood loss incurred by sampling was replaced. The following determinations were performed; plasma catechol amines by Weil-Malherbe's method(8), serum serotonin by Davis' method(9), platelet count and hematocrit.

Results. It was found that the plasma catechol amine concentration generally reflected the blood pressure, *i.e.*, when the latter fell, concentration of epinephrine and norepinephrine (particularly the former) rose. The plasma catechol amines usually increased immediately after administration of endotoxin, simultaneously with the initial sudden fall in blood pressure (Table I). By 30 minutes, concentrations approaching control levels were found, and they remained unchanged until the animal began to deteriorate and the blood pressure began to fall. Extremely high values were obtained when the animal was in extremis (Table I). A marked fall in serum serotonin concentration occurred following administration of endotoxin (Table I) which persisted throughout the shock state. There was a tendency for the concentration to recover somewhat as time progressed. In an animal which survived the experiment normal levels were reached. However, in those that succumbed, 5-HT fell back to the level it had attained immediately after endotoxin was administered.

Discussion. These studies would indicate that endotoxin can incite the release of toxic amounts of epinephrine into the circulating blood. It seems, however, that the extremely high levels which are attained become manifest after the animal has suffered metabolic and physiologic derangements which produce its eventual demise. The significance of the initial elevation of plasma catechol amines is hard to evaluate. During most of the interval between administration of endotoxin and death, plasma catechol amine concentration is only slightly elevated. Serum serotonin, however, is always far below its pre-shock level. In view of the mean decrease in serotonin to 18.3% of the control level, the elevated levels of epinephrine and norepinephrine may have a physiologic effect greater than their concentrations would indicate. The fall in serotonin concentration is consistent. We have also found it to occur in rabbits following administration of a lethal dose of endotoxin. The question of how these changes are produced by endotoxin remains to be answered. Since

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anaphylactic reactions have also been shown to be accompanied by low levels of 5-HT in the blood and a generalized sympathomimetic response, the above data further demonstrate the similarity between these two phenomena (10).

Summary. Elevated levels of epinephrine are found immediately after administration of a lethal dose of endotoxin. Following this, plasma catechol amine concentration is only slightly above control levels. Prior to death, toxic levels of epinephrine are attained. Serum serotonin concentration falls immediately after administration of endotoxin and remains low throughout the shock state.

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Variation with Age of Antistreptococcal Antibodies and Their Correlation With Antistreptolysin O Titers in Human Sera.* (25239)

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Halbert, Swick and Sonn(1) found that sera from non-rheumatic individuals gave from 0-4 bands, when tested by Ouchterlony's technic(2,3), against a crude ammonium sulfate concentrate of the supernate from a Group A streptococcus culture (C 203 S). Sera from active cases of rheumatic fever gave 2 to 7 bands when similarly tested. Harris, Harris and Ogburn(4) obtained similar results using Oudin's method(5) and the concentrated supernate of a type 4 culture. Halbert *et al.*(6) also observed a correlation between migration rate of the heaviest bands, antistreptolysin O (ASO) titers of the sera and intensity of the precipitate supposedly produced by the ASO system. This study is concerned with the immunogenic response to streptococcal antigens of normal individuals of different ages in a tropical environment. Possible correlations between precipitins, as evidenced by gel diffusion, and ASO titers have been investigated.

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Materials and methods. Human sera comprised 9 taken from the umbilical cord of infants at birth, 74 from infants 1 to 12 months old, many of whom were suffering from diarrheal disease, and 331 from apparently normal adults aged 17 to 70 years, selected randomly at various factories, blood banks and public health dispensaries. The antigen used consisted of a type 3, group A, beta hemolytic streptococcus inoculated into 500 ml of trypticase soy broth and incubated at 37°C for 18 hours. Culture was divided into 50 ml portions, lyophilized and each dissolved in 5 ml of sterile saline just before use. Same batch of lyophilized material was used in all gel diffusion tests(1). Usually antigen was placed in the center and sera in the peripheral wells. Plates were placed in the refrigerator and precipitin bands read at intervals of 3, 7, 14 and 21 days. Antistreptolysin O assay was done with Bacto Streptolysin O reagents (Difco) and results expressed in Todd units.

Results. All sera from umbilical cord blood revealed the presence of 1 to 3 bands with an