vitamin K1. For comparison, sulfadiazine and sulfathiazole were given once every 24 hours to one group of mice and every 6 hours to another group. The results of these experiments are given in Tables I and II. For simplicity, only the results at daily intervals with *D. pneumoniæ* and *S. schottmülleri* are given. Essentially the same findings were obtained with *Strep. hemolyticus* and *S. aertrycke*.

When all 3 drugs were given once daily, sulfaquinoxaline was superior to sulfadiazine or sulfathiazole, in that a single daily dose of 40 mg per mouse protected 72.5% of the mice infected with *D. pneumoniæ* Type I. Under the same conditions sulfadiazine and sulfathiazole protected only 27% and 0% respectively. Moreover, single daily doses of sulfaquinoxaline in severe pneumococcal infections compared favorably with multiple daily doses of sulfadiazine or sulfathiazole (Table I). This was found when 20 mg of sulfaquinoxaline was given as a daily dose, as compared with 20 mg of sulfadiazine or sulfathiazole divided into four 6-hourly doses of 5 mg per mouse. Both drugs protected about 50% of the mice.

Tests performed to determine the efficacy of sulfaquinoxaline when given at intervals other than every 24 hours, showed that if treatment was given every 36 or 48 hours, a dose of 40 mg/mouse of sulfaquinoxaline was not sufficient to protect mice from severe infection.

Blood Levels. The striking therapeutic effects obtained with single daily doses of sulfaquinoxaline may be explained by the fact that this drug remains in the blood for long periods of time and consequently, therapeutic blood concentrations can be maintained by infrequent drug administration. Thus, applying the method of Bratton and Marshall,[7] it was found that a concentration of 3 to 7 mg% was still present in the blood of mice 24 hours after the administration of a single dose of 20 mg per 20 g mouse. Even after 72 hours, small quantities of the drug could be detected in the blood. When the interval between treatments was extended beyond 24 hours, the blood concentration of sulfaquinoxaline fell below the effective range, with the resulting death of the animal.

Summary. Sulfaquinoxaline was found to be active against certain bacteria both *in vitro* and *in vivo*. Single daily doses of this drug were more effective than single daily doses of sulfadiazine or sulfathiazole. These findings may be explained by the fact that sulfaquinoxaline remains in the blood for long periods of time.

[7] Bratton, A. E., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

14789

The Demonstration of Antibodies in Lymphocytes.*

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The presence in extracts of lymphocytes of normal rabbits of a protein probably identical with the gamma globulin of normal rabbit serum[1] has led to the search for immunologically labeled globulin in lymphocytes of immunized animals. Reticulo-endothelial cells in general[2] and lymphoid tissue in par-

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[1] White, A., and Dougherty, T. F., *Abstracts of Proc. Meetings of Am. Chem. Soc.*, New York, September, 1944.

[2] Sabin, F. R., *J. Exp. Med.*, 1939, **70**, 67.

ticular[3,4] have been suggested as a probable site of antibody production, and we have recently shown[5] that the rate of liberation of antibody from lymphoid tissue is under the control of pituitary-adrenal cortical secretion. The evidence therefore points to lymphocytes as a storehouse of antibody protein. This is now confirmed by the present work which demonstrates the presence of antibodies in lymphocytes.

Experimental. Normal mice of both sexes (NHO strain, Strong), 6 to 8 weeks old at the beginning of the study, received intraperitoneal injections of a 4% solution of fresh, washed sheep erythrocytes on alternate days for 5 weeks. Agglutinin titers were done at weekly intervals on heart blood. Each animal was discarded after being bled once. After 5 weeks of immunization, 2 groups of 5 mice each were bled from the heart and sacrificed. Inguinal, axillary, cervical, and mesenteric lymph nodes, and the thymi were removed and carefully freed from surrounding connective tissues. This lymphoid tissue from each group of 5 mice was pooled and finely minced. The mince was suspended in 3 times its weight of physiological saline, the mixture stirred, and centrifuged in order to free the cells from lymph. This washing was repeated 3 times. Following the last washing, the cells were examined microscopically and found to be essentially normal in appearance. Differential count revealed that at least 90% of the cells were lymphocytes. The cellular mass was lysed by grinding thoroughly with one and one-half times its weight of distilled water. An equal volume of 2% saline was then added and mixing continued for a few minutes. This material was used for analysis.

As controls, the cells from the same lymphoid structures of a group of 5 non-immunized mice of the same strain and age were extracted as described above. For further controls, extracts of muscle tissue and of salivary glands of the immunized mice were

TABLE 1.
Agglutinin and Hemolysin Titers in Sera and in Tissue Extracts of Normal and of Immunized Mice.

Material titrated	Mg nitrogen/ml sera or extract			Agglutinin titers			Hemolysin titers		
	Normal mice	Immunized mice I	Immunized mice II	Normal mice	Immunized mice I	Immunized mice II	Normal mice	Immunized mice I	Immunized mice II
Lymphocyte extract	2.26	2.00	2.10	0*	1-2560	1-2560	0	1-3000	1-3000
Serum	7.70	7.39	8.21	0	1-1280	1-1280	0	1-2000	1-2000
First lymphocyte washings	—†	—	—	0	0	0	0	1-10	1-10
Second " "	—	—	—	0	0	0	0	0	0
Third " "	—	—	—	0	0	0	0	0	0
Salivary gland extract	—	—	3.22	—	0	0	—	0	0
Muscle extract	—	—	2.37	—	0	0	—	0	0

* 0 indicates absence of titer in any dilution.
† — indicates determination not done.

[3] McMaster, P. D., and Hudack, S. S., *J. Exp. Med.*, 1935, **61**, 783.

[4] Ehrich, W. E., and Harris, T. N., *J. Exp. Med.*, 1942, **76**, 335.

[5] Dougherty, T. F., White, A., and Chase, J. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 28.

obtained in the same manner as those from lymphoid tissue.

Micro-Kjeldahl determinations were made on samples of each extract and on the pooled sera of each group of 5 animals. Agglutinin and hemolysin titers were done on these same extracts and sera. A 2% suspension of fresh, washed sheep erythrocytes was used in all titrations. The titer was read as that dilution of antibody demonstrating complete macroscopic agglutination or hemolysis.

Results. The data are summarized in Table I. The agglutinin and hemolysin titers obtained with the extracts of lymphoid cells from both groups of immunized mice demonstrate that these extracts contained significant quantities of antibody protein. The titers in the extracts of lymphoid cells from immunized mice were approximately eight-fold higher than those in the corresponding sera on the basis of nitrogen contents. The absence of titer in the final washings of the lymphoid cells is proof that the antibody titer in the extracts was derived from cells and not from adherent lymph. Salivary gland or muscle tissue, from the same immunized mice which had yielded antibody-containing lymphoid cells, showed no extractable agglutinins or hemolysins. Also, lymphoid cell extracts and sera from non-immunized mice were negative when tested for antibodies.‡

Discussion. Preliminary attempts were made to extract lysed lymphoid cells with 10% saline, a better solvent for globulins than the 1% salt solution necessary for antibody estimations. However, viscous masses were obtained which contained approximately five milligrams of nitrogen per milliliter. On dilution with water, in order to decrease the salt concentration to 1%, a precipitate of protein formed. After removal of the precipitate, the solutions had nitrogen contents comparable to those found in 1% saline extracts of lymphoid cells, *i.e.*, 2 to 2½ mg nitrogen per milliliter. Therefore, the more dilute saline was directly employed as an extraction medium since the mixtures obtained were easier to manipulate. It is obvious, however, that not all of the cellular globulins, including antibody, were present in the final solutions used for nitrogen and antibody estimations. The titer in the extracts may represent only a fraction of the total antibody of the lymphoid tissue. The considerably higher titers of the lymphocyte extracts, when compared to the corresponding sera on the basis of nitrogen content, indicate a concentration of antibodies within lymphoid cells.

The data substantiate previous suggestions of antibody production in lymph nodes. A significant portion of antibody protein is present in lymphocyte elements of immunized animals. Sites of antibody production and concentration other than lymph nodes may exist, *e.g.*, bone marrow, spleen, liver, and other organs containing high proportions of reticulo-endothelial cells. These have not been examined in the present investigation.

The term "lymphoid cells" has been used in this paper because it is recognized that small numbers of cells other than lymphocytes, designated by the term "lymphoid," exist in the lymph tissue studied. However, the number of non-lymphocyte cells was exceedingly small, as shown by differential count. The antibody nitrogen contributed by reticulum cells, macrophages, or fibroblasts must be insignificant. This is further substantiated by the fact that these cell types are present in the minced salivary gland and muscle tissue of the immunized mice. Extracts of these tissues gave negative titers. The evidence obtained is overwhelmingly in support of the conclusion that the antibody is concentrated chiefly within lymphocytes. The actual production of antibodies by lymphocytes has not been established.

The titer present in the first washings of the minced lymphoid tissue must be derived not only from lymph but also from cells injured by the mincing process. This suggestion is confirmed by the absence of antibody in the subsequent washings. The negative findings with both glandular tissue containing large numbers of cells, and with other tissue from immunized mice, indicate that the antibody in extracts of washed lymphoid cells is present within these cells and is not adsorbed on their surfaces.

The rate of release of certain immune

‡ These extracts and sera were titrated in a one-to-one dilution.

bodies from lymphocytes is under pituitary-adrenal-cortical control.[5] The lymphocyte functions as a carrier of antibody and yields this protein at an increased rate in circumstances of augmented adrenal cortical secretion. The hormonal control of an important lymphocyte function integrates the role of the lymphocyte and the adrenal cortex in maintaining certain normal protective mechanisms of the organism.

Summary. Agglutinin and hemolysin titers have been obtained from extracts of washed cells secured from selected lymph nodes and thymi of mice immunized to sheep erythrocytes. The titers of similar extracts of salivary glands and muscle of the same immunized animals were negative in all dilutions although these extracts were higher in nitrogen content. Per unit of extractable nitrogen, lymphoid tissue had significantly higher agglutinin and hemolysin titers than did the sera of the same animals. It is concluded that antibodies are concentrated in lymphocytes.