fixation of dicumarol in the liver and the duration of the prothrombinopenia in the blood. Vitamin K appeared to interfere with the fixation of dicumarol in the liver in such a way that the dicumarol was displaced from the organ more quickly.

Since no radioactivity appeared in the urine for some hours after intravenous injection of labelled dicumarol, it is evident that in mice and rabbits, the dicumarol was not excreted by the kidney. This has also been reported by Weiner, Axelrod, Shapiro and Brodie(3) in man. However, the activity appeared in the bile and gastro-intestinal tract shortly after administration and much later it was found in the urine, indicating metabolism of the dicumarol. In the rabbit, all of the labelled groups in the dicumarol was reabsorbed and excreted in the urine but in the mouse, a part of it appeared in the feces. The nature of the product excreted is now under investigation. Thus far, it has been shown that the activity in the urine is not dicumarol, agreeing with the suggestion above that the dicumarol has been metabolised. In order to ensure complete absorption, the dicumarol used was administered intravenously. However, experiments have been conducted with the material given orally with essentially the same results.

Summary. Dicumarol, containing C^{14} in the methylene bridge was synthesized and given intravenously to mice and rabbits. The activity disappeared rapidly from the blood and was recovered in the liver, bile, intestinal contents and later in the urine, but not from other tissues. About 10% of the activity became fixed in the liver. It was demonstrated by isolation using the isotope dilution technique that this activity was due to unchanged dicumarol. This dicumarol remained fixed in the mouse liver for 16 hours, in rabbit liver for 3 days. The corresponding duration of hypoprothrombinemia was 4 days and 9 days. The presence of extra vitamin K resulted in the dicumarol disappearing more rapidly from the liver. It is suggested that the period of time that dicumarol remains in the liver is related to the effectiveness of the agent in interfering with the formation of prothrombin. Dicumarol was not excreted by the kidney but evidence of excretion of metabolic products was found in the urine and feces for the mouse and in the urine only for the rabbit.

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Relation of Adrenal Cortical Steroids to Antibody Release. (17839)

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That the lymphocyte is concerned with the manufacture and storage of antibodies has been demonstrated by a number of investigators(1,2,3,4,5). The administration to normal animals of those adrenal cortical steroids oxygenated at C_{11} or adrenotrophic hormone produces involution of the thymus, degeneration of lymphocytes in lymphoid tissue, a peripheral leucopenia accompanied by a granulocytosis and a decrease of lymphocytes in thoracic duct lymph(6,7,8,9,10,11,12,13). The lymphocyte has been shown to contain

* This work was submitted in thesis form by the senior author as a partial requirement for the degree of Doctor of Philosophy at the University of Illinois Graduate College, Chicago, Ill.

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2. Ehrich, W. E., and Harris, T. N., *J. Exp. Med.*, 1942, v76, 335.

3. Harris, T. N., Grimm, E., Mertens, E., and Ehrich, W. E., *J. Exp. Med.*, 1945, v81, 73.

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normal serum gamma globulin(14,15) the release of which is under adrenotrophic control(15). Furthermore, it has been demonstrated that adrenal cortical hormones or adrenotrophic hormone administered to an immune or hyperimmune animal produces a marked rise in antibody titer(16,17,18). The anamnestic reaction has been produced in intact animals by means of adrenal cortical steroids or adrenotrophin(19). Whether or not adrenal cortical hormones will induce a disintegration of circulating lymphocytes is not known(9,20). The present investigation was undertaken in an attempt to answer this question.

The initial step was the reproduction of the work reported by Dougherty(19) in regard to inducing the anamnestic response by means of adrenal cortical steroids. Eight albino rabbits of both sexes weighing between 4-5 lb. were immunized against sheep erythrocytes in exactly the fashion described by Dougherty *et al.*(19). Hemolysin and agglutinin titers were determined on each animal before and at intervals during the period of immunization. The average peak titer obtained after 2½ months was 1:3200. The animals were then kept until their titers fell to approximately the initial levels. At that time 4 of them were given a "booster" dose of antigen consisting of 2 cc of a 5% suspension of sheep red blood cells administered intravenously. Each of the remaining 4 was given subcutaneously into the skin of the back 5 cc of Upjohn's Lipo-Adrenal Cortex. Blood samples were obtained from individual rabbits by slitting the marginal ear vein with a razor blade and collecting the blood in a small test tube. Samples were secured at 3, 6, 9 and 24 hours after the administration of the antigen or the cortical steroids, and hemolysin determinations were made. The results are recorded in Fig. 1. The peak titer following the injection of cortical steroids was not as high as that reported by Dougherty *et al.*(19) and it was reached at 9 hours rather than 6 hours. It was definitely much higher, however, than that secured with the antigen. Whereas it had fallen to its initial level within 24 hours, the titer obtained after the "booster" dose of antigen was still at its peak at 24 hours. The work of Dougherty, Chase and White(19) having been confirmed, the body of the experiment was undertaken.

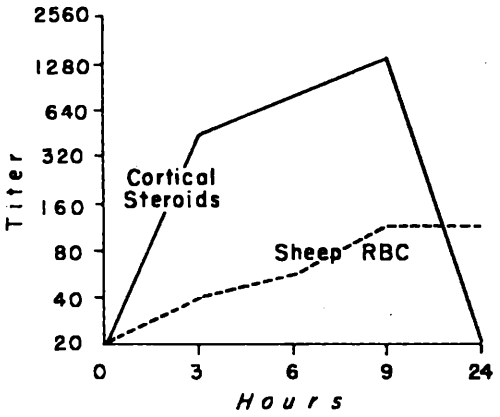

FIG. 1.
Anamnestic response induced by means of the antigen and adrenal cortical steroids in oil.

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7. Dougherty, T. F., and White, A., PROC. SOC. EXP. BIOL. AND MED., 1943, v53, 132.

8. Simpson, M. E., Li, C. H., Reinhardt, W. O., and Evans, H. M., PROC. SOC. EXP. BIOL. AND MED., 1943, v54, 135.

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13. Yoffey, J. M., and Baxter, J. S., *J. Anat.*, 1946, v80, 132.

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15. White, A., and Dougherty, T. F., *Endocrin.*, 1945, v36, 207.

16. Fox, C. A., and Whitehead, R. W., *J. Immunol.*, 1936, v30, 51.

17. Dougherty, T. F., White, A., and Chase, J. H., PROC. SOC. EXP. BIOL. AND MED., 1944, v56, 28.

18. Chase, J. H., White, A., and Dougherty, T. F., *J. Immunol.*, 1946, v52, 101.

19. Dougherty, T. F., Chase, J. H., and White, A., PROC. SOC. EXP. BIOL. AND MED., 1945, v58, 135.

20. Selye, H., *Textbook of Endocrinology*, Acta Endocrinologica, Montreal University, Montreal, Canada, 1947.

TABLE I.
Normal Rabbits Receiving 4 cc of "Immune" Lymphocytes.

Rabbit No.	Wt, lb	Lymphocytes received: No./mm³ (in 1000's)	Total No.	No./cc rabbit bld.	% lymphs in suspension
C5	4	500	200×10^7	2083×10^4	99
C6	4½	650	260×10^7	2708×10^4	98
C7	4	1,090	436×10^7	4541×10^4	96
C8	4	920	368×10^7	3833×10^4	95
Normal Rabbits Receiving 4 cc of "Immune" Lymphocytes and 5 cc of Cortical Steroids.					
C9	4½	550	220×10^7	2291×10^4	98
C10	4	420	168×10^7	1750×10^4	99
C11	4	720	288×10^7	3000×10^4	96
C12*	4	530	212×10^7	2208×10^4	97

* Died after cells inj.

The number of cells per cc of rabbit blood calculated by determining the blood vol. of each rabbit (48 cc/kg body wt) and dividing that into the total number of cells received.

All animals used in the experiment were female albino rabbits weighing between 4-5 lb. Hemolysin tests were made on each rabbit previous to immunization, periodically during immunization and as indicated on recipient rabbits. Lederle amboceptor was employed and the complement was either Lyovac or was secured by bleeding 3 guinea pigs from the heart and pooling the sera. Amboceptor and complement were titrated in the usual manner prior to each hemolysin test done. Sixteen rabbits were injected intravenously on alternate days with 2 cc of a 5% suspension of fresh, washed sheep red blood cells for a 2½ month period. At the end of this time the animals were killed in pairs by anesthetizing them lightly with ether and then exsanguinating them by heart puncture. The thymus, large mesenteric lymph node and appendix were removed from each rabbit. The appendix was slit and thoroughly washed free of fecal material with warm saline. All tissues were rinsed 3 times in separate beakers of physiologic salt solution standing in a 37°C water bath. They were then placed in 50-75 cc of warm saline and were minced as finely as possible with a scissors. The milky supernatant saline was strained through 2 layers of surgical gauze to remove the gross pieces of tissue and the cells were washed 3 times in warm salt solution by centrifugation at 1500 r.p.m. for 10 minutes. A sample of the third washing was saved for hemolysin determination as a check that all free hemolysin had been removed. Between each washing, after the saline had been added and the cells resuspended, the mixture was stirred slowly with wooden applicators to remove fibrin. This procedure continued until no further fibrin collected around the applicators. After the last washing the packed cells were suspended in 5 cc of warm saline. A count of the cells contained in this amount was done by means of a red blood cell counting pipette, and a smear was made and stained with Giemsa to determine the percentage of lymphocytes present (Tables I and II). One cubic centimeter was then removed and placed in a separate test tube. These cells were frozen and thawed 3 times to extract antibody from the lymphocytes. Following the third thawing the material was centrifuged slowly at 1500 r.p.m. for 5 minutes to remove cellular debris and hemolysin tests were made on the supernatant fluid to determine the amount of antibody present per cubic centimeter of cellular suspension injected. The remaining 4 cc of lymphocytic suspension were injected very slowly intravenously into a normal rabbit as soon as possible after recovery from the original animals. Leucocyte and differential counts were made 15, 30 minutes, 1, 3, 6, 9 and 24 hours following the injection of cells, and samples of blood for hemolysin determinations were secured at 3, 6, 9 and 24 hours following the administration of the cells. Of the 16 immune animals, 8 donated cells to 4 normal rabbits which received only the cells, and 8 donated cells to 4 normal rabbits each of

TABLE II.
Immunized Rabbits Receiving "Immune" Lymphocytes.
Immune Controls.

Rabbit No.	Wt, lb	No. cc	Lymphocytes received: No./mm³ (in 1000's)	Lymphocytes received: Total No.	No./cc rabbit bld	% lymphs in suspension
C20*	4	4½	240	100 × 10⁷	1050 × 10⁴	97
C21*	4	10	145	145 × 10⁷	1510 × 10⁴	96
C1	5½	4	540	216 × 10⁷	1800 × 10⁴	97
C2(H)	5½	4	900	360 × 10⁷	3000 × 10⁴	98
C3*	5	4	810	324 × 10⁷	2700 × 10⁴	95
C4*	5	4				96
Immunized Rabbits Receiving "Immune" Lymphocytes and 5 cc of Cortical Steroids.						
C13	5½	4	790	316 × 10⁷	2550 × 10⁴	95
C14(H)	4	4	760	304 × 10⁷	3166 × 10⁴	96
C15	5½	5	390	195 × 10⁷	1625 × 10⁴	99
C16*	4	4	700	280 × 10⁷	2916 × 10⁴	97
C17	4	6	470	282 × 10⁷	2937 × 10⁴	98
C18	4	3½	84.6	29 × 10⁷	308 × 10⁴	98
C19	4	4½	330	148 × 10⁷	1546 × 10⁴	96

* Died after cells injected.
(H) Received heparin.

TABLE III.
Hemolysin Titers in Normal Animals Receiving "Immune" Lymphocytes and Cortical Steroids.

Rabbit No.	Initial	3 hr	6 hr	9 hr	24 hr	96 hr	6 days	14 days	Lymph ext.	3rd washing	Donor rabbits	
C13	0	0	1:20	1:40	1:100		1:100	1:40	1:1600	0	No. 83	1:3200
											No. 100	1:1600
C19	0	1:20	1:20	1:20	1:20				1:1280	0	No. 17	1:1280
C14(H)	0	0	0	0	0	0			1:3200	0	No. 94	1:1600
											No. 95	1:6400
C15	1:40	1:40	1:40	1:40	1:40				1:1600	0	No. 77	1:800
											No. 86	1:3200
C17	1:20	1:20	1:20	1:20	1:20						No. 9	1:800
C18	0	0	0	0	0				1:640		No. 21	1:2560
C16*	0									0	No. 81	1:3200
											No. 84	1:3200
C15'*	0								1:1600	0	No. 77	1:800
											No. 86	1:3200

* Died after cells inj. (H) Received heparin. C15' received 1 cc lymphocytic suspension and died. The remaining 3½ cc diluted to 7 cc and C15 received 5 cc of this.

which was given subcutaneously into the skin of the back 5 cc of Lipo-Adrenal Cortex 30 minutes before the cells were injected.

In addition, 2 control groups of normal animals were done. Each of 4 normal rabbits received intravenously lymphocytes secured in the aforementioned manner from 2 normal rabbits. Each of 4 additional rabbits received intravenously lymphocytes secured from 2 normal rabbits and 5 cc of Lipo-Adrenal Cortex injected subcutaneously 30 minutes before the cells. Leucocyte counts and differentials were done on these animals and blood samples were obtained at the same times as for the test rabbits.

As can be observed in Table III animals C17, C18 and C19 each received lymphocytes secured from one immune rabbit. These animals were used as a preliminary experiment. Thereafter cells from 2 animals were pooled in order to insure having a sufficient number of lymphocytes.

The fate of the circulating lymphocyte is unknown. There are, however, several theories as to their disposition, one of which is that they pass through the mucosa of the gastrointestinal canal and are lost(21). Erf (22) has shown that this is probably not the case. When gastro-enterectomized rabbits were injected intravenously with autogenetic

or homogenetic lymphocytes an immediate leucopenia accompanied by a lymphocytosis and granulopenia which persisted for 30 minutes was observed. At the end of this time there occurred a leucocytosis associated with a granulocytosis and a lymphopenia which was observed for 6 hours. Erf concluded the injected lymphocytes circulated for 30 minutes.

Faludi(23), however, after administering polymorphonuclear leucocytes intravenously to rabbits also observed an immediate leucopenia accompanied by a lymphocytosis and a granulopenia which persisted for 30 minutes. Later the leucocytosis, granulocytosis and lymphopenia occurred as described by Erf (22).

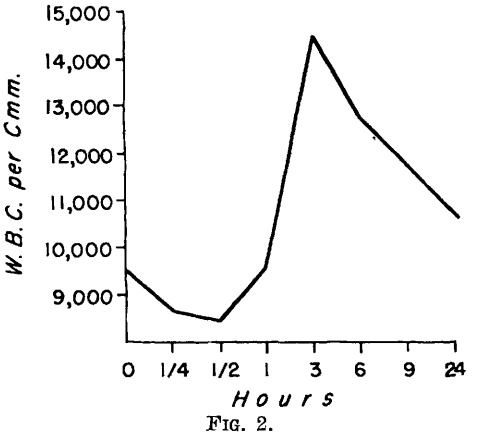

FIG. 2.
Leucocyte count following the intravenous injections of lymphocytes. Average of 4 animals.

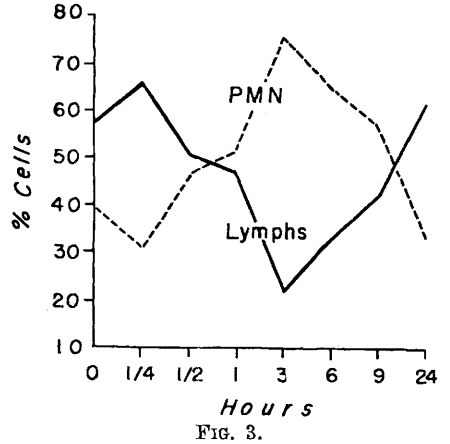

FIG. 3.
Polymorphonuclear and lymphocyte percentages following the intravenous injection of lymphocytes. Average of 4 animals.

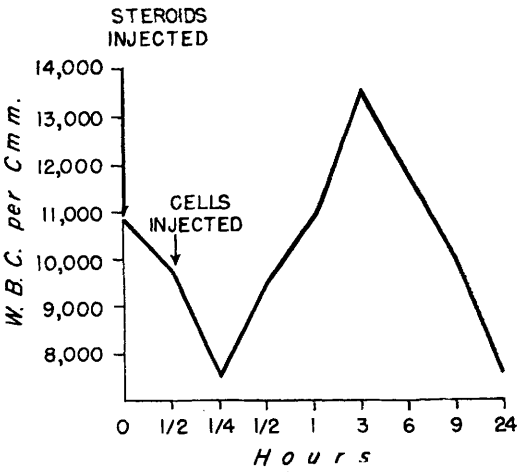

FIG. 4.
Leucocyte count following the injection of adrenal cortical steroids in oil and lymphocytes. Average of 3 animals.

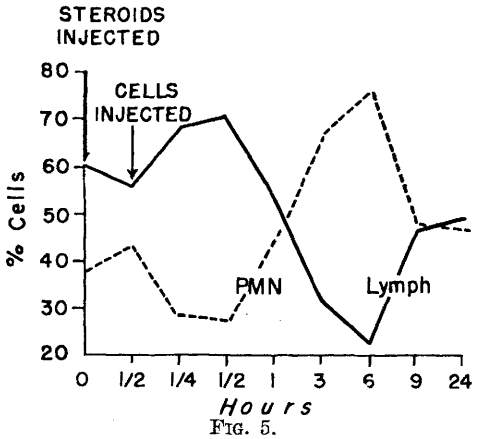

FIG. 5.
Polymorphonuclear and lymphocyte percentages following the injection of adrenal cortical steroids in oil and lymphocytes. Average of 3 animals.

Both of these reactions might be explained on the basis of parenterally administered protein which has been shown to produce a lymphocytosis(24,25).

21. Drinker, C. K., and Yoffey, J. M., *Lymphatics, Lymph, and Lymphoid Tissue*, Harvard University Press, Cambridge, Mass., 1941.
22. Erf, L. A., *Am. J. Med. Sci.*, 1940, v200. 1.
23. Faludi, F., *Folia Hematol.*, 1938, v59, 357.
24. Rich, A. R., Lewis, M. R., and Wintrobe, M. M., *Bull. Johns Hopkins Hosp.*, 1939, v65, 311.
25. Wiseman, B. K., *J. Exp. Med.*, 1931, v53, 499.

TABLE IV.
Hemolysin Titers in Normal Animals Receiving "Immune" Lymphocytes.

Rabbit No.	Initial	3 hr	6 hr	9 hr	24 hr	Lymph ext.	3rd washing	Donor rabbits
C20*	0							No. 18 1:3200
C21*	0							No. 19 1:3200
C1	0	0	0	0	0	1:3200	0	No. 80 1:1600
								No. 87 1:6400
C2(H)	0	0	0	0	0	1:3200	0	No. 76 1:3200
								No. 91 1:6400
C3*	0					1:3200	0	No. 92 1:6400
								No. 93 1:3200
C4*	0					1:1600	0	No. 88 1:1600
								No. 89 1:3200

* Died after cells inj.
(H) Received heparin.

It became necessary, therefore, to establish how long the injected lymphocytes under consideration circulated. Figs. 2 and 3 are averages of blood counts observed in 4 rabbits each receiving lymphocytes pooled from 2 normal rabbits. As can be seen, the observations of Erf(22) and Faludi(23) are confirmed. Figs. 4 and 5 are averages of 3 rabbits which received Lipo-Adrenal Cortex 30 minutes before lymphocytes pooled from 2 normal rabbits were injected. Thirty minutes after the administration of cortical steroids the leucocyte count fell and simultaneously there occurred a lymphopenia with agranulocytosis. This is in agreement with previous reports(7,8,9,10,11,13). Upon injecting the cells the leucopenia became more pronounced but the lymphopenia and granulocytosis were replaced by a lymphocytosis and a granulopenia which persisted for 30 minutes.

No antibody could be detected in the sera of the 8 normal rabbits receiving intravenously lymphocytes from normal rabbits. The administration of cortical steroids did not alter these results.

In Tables III and IV are recorded the results of hemolysin titer determinations made on both groups of animals receiving "immune" lymphocytes. Two animals which received cells and cortical steroids were positive, one very definitely. That this was a true transfer of antibody is indicated by the fact that it persisted so long. Previous workers(26,27) who transferred antibody by means of emulsifying spleen from an immune animal and injecting the cells intraperitoneally into a normal animal reported being able to detect antibody in the normal animal for as long as 30 days.

In spite of the 2 positive rabbits, it is concluded that under the conditions of the experimental method adrenal cortical steroids do not cause circulating lymphocytes to disintegrate and release antibody. The peak titer in the definitely positive animal (C13) did not come until 24 hours after the cells were injected. The effect of a single dose of cortical steroids is completely expended in 24 hours(17,18). Furthermore, the injected lymphocytes do not circulate that long. Since the cortical steroids did not have to penetrate lymphoid tissue to produce their effect in this instance but simply needed to be in the blood stream, the peak should have come much earlier. The steroids were injected 30 minutes before the lymphocytes. That a sufficient amount had been absorbed to affect lymphocytes in lymphoid tissue is indicated by the leucopenia and lymphopenia which were detectable in 30 minutes. Therefore, adequate amounts should have been in the circulating blood stream to act on the injected cells. These cells circulated only 30 minutes while the cortical steroids depressed the animal's own lymphocytes. Consequently, the lymphocytosis which occurred for 30 minutes after the injection of cells was proba-

26. Luckhart, A. B., and Becht, F. C., *Am. J. Physiol.*, 1911, v28, 257.

27. Topley, W. W. C., *J. Path. Bact.*, 1930, v33, 339.

bly due to the injected cells.

A striking phenomenon was encountered during the course of the experiment. Of 8 animals receiving cells from normal rabbits, 1 died following the administration of cells or 12%. As can be observed from Tables III and IV of 13 animals receiving lymphocytes from immune rabbits, 6 died or 45%. The deaths were sudden and occurred 1-2 minutes after the cells were injected. The animal's ears became cold, it developed cyanosis, marked respiratory distress, and convulsions followed by death. The heart beat after the animal ceased to breathe but artificial respiration or adrenalin given either intravenously or intracardially was of no avail. The deaths were not due to embolism apparently. The injected cells were evenly dispersed and were not agglutinated by the recipient rabbit's serum. Fibrin was known to be present. Thromboplastin was present in large amounts due to the use of the thymus as a source of cells. That should have been removed during the washing process, however. Nevertheless, 2 animals were heparinized before the cells were injected and these rabbits survived. One cc of Abbott's Sodium Heparin containing 10 mg of purified sodium heparin standardized to contain 1000 provisional International Units was injected intravenously 5 minutes before the cells. According to Jorpes (28) heparin neutralizes complement and may be concerned in the immunologic process. It was felt that a variable of unknown quantity was being introduced and the use of heparin was discontinued.

It became evident that the greater percentage of deaths followed the injection of "immune" cells and might be due to shock of the anaphylactic type. None of the animals showed right heart dilatation, however, and no gross pathology was found except in one animal whose intestinal and mesenteric vessels appeared engorged. This animal showed clots in both chambers of the right heart also. Histologic examination of tissues from one animal were made. The only pathologic finding was constriction of the pulmonary arteries. This finding plus the type of death could very well indicate shock.

An explanation of shock on the basis of Forssman antigen was considered but the rabbit has no Forssman antigen. According to Landsteiner(29), the occurrence of immunologically similar substances in the cells of unrelated species is not limited to Forssman antigen. The presence of such an antigen in the rabbit and its antibody in the "immune" lymphocytes would explain a shock reaction. This problem should be investigated further.

Conclusions. 1. The anamnestic reaction was produced by means of subcutaneously administered adrenal cortical steroids in oil to rabbits immunized against sheep erythrocytes.

2. Lymphocytes obtained from the appendix, thymus and mesenteric node of rabbits when injected intravenously into a normal rabbit circulate for a period of about 30 minutes only.

3. Lymphocytes obtained from the appendix, thymus and mesenteric node of rabbits immunized against sheep erythrocytes when injected intravenously into a normal rabbit do not release antibody under the conditions of the experimental method employed.

4. Adrenal cortical steroids administered to a normal rabbit which also receives lymphocytes intravenously from rabbits immunized against sheep erythrocytes do not induce a release of antibody from these cells while they are circulating according to the experimental method employed.

The authors are indebted to the Upjohn Co., Kalamazoo, Mich., for 40 cc of the 80 cc of Lipo-Adrenal Cortex used in the experiment.

28. Jorpes, J. E., *Heparin in the Treatment of Thrombosis*, Oxford University Press, London, New York, Toronto, 1946.

29. Landsteiner, K., *The Specificity of Serological Reactions*, Harvard University Press, Cambridge, Mass., 1947.

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