THE RÔLE OF THE LYMPHOCYTE IN ANTIBODY FORMATION*

By T. N. HARRIS, M.D., E. GRIMM, M.D., E. MERTENS, M.D., AND W. E. EHRICH, M.D.

(From the Children's Hospital of Philadelphia, the Philadelphia General Hospital, and the Departments of Pediatrics and Pathology, the School of Medicine, University of Pennsylvania, Philadelphia)

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In a previous paper(1) the production of antibodies in the popliteal lymph node of the rabbit was compared with the output of lymphocytes in the efferent lymph vessel of the node and with the morphological changes which took place within the node itself. Antibodies first appeared in the efferent lymph 2 to 4 days after the injection of the antigen and reached their highest titer after 6 days. This antibody formation was preceded and accompanied by a rise in the output of lymphocytes in the efferent lymph which ranged from 15,000 to 20,000 per c. mm. to 60,000 to 80,000 per c. mm. or more. At the same time hyperplasia of the lymphatic tissue within the node occurred, resulting in some experiments in a weight increase of the node of 0.2 gm. to 1.0 gm. or more. The fact that the cellular response during antibody formation was chiefly lymphocytic suggested that the lymphocyte may play an important rôle in antibody formation.

The present investigation deals with the rôle in antibody formation played by the lymphocyte itself. Instead of comparing antibody production and cellular response in the pad of the hind foot of the rabbit (which was the site of injection of antigen), the lymph contained in the afferent lymph vessel, the popliteal lymph node (the only node regional to the site of injection), the efferent lymph, and the serum, we now determined antibodies within the lymphocytes and the supernatant fluid of the efferent lymph collected during the period of antibody formation in the lymph node. The results of this study not only stregthen the conclusions drawn in the previous paper, but appear to show for the first time that the lymphocyte is engaged in antibody formation.

Methods

The antigens were prepared as follows: Typhoid bacilli, strain H 901, were taken from an 18 hour nutrient broth culture, washed 3 times by centrifugation in saline, heated in a water bath at 56°C. for 1 hour, and then diluted with an equal volume of saline to make a suspension of approximately 50 per cent. Sheep erythrocytes, obtained from blood drawn within the week, were washed 3 times and diluted with saline to make an approximately 50 per cent suspension. In all cases, 0.2 cc. of antigen was injected subcutaneously into the plantar surface of the hind feet of 2000 gm. Chinchilla rabbits, and the puncture wounds were closed with collodion.

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Lymph was collected, as described in the earlier paper, in a "tuberculin" syringe containing a small amount of either sodium citrate or heparin solution. Immediately after collection the lymph was centrifuged at 1600 R.P.M. for 10 minutes. The lymph plasma was then drawn off as completely as possible, and the cell sediment kept in the original tube.

Before centrifugation the approximate total volume of the cells in the specimen was determined as follows: A sample of the well mixed lymph was drawn into a capillary glass tube of constant diameter, about 20 cm. in length. The tube was spun at 1600 R.P.M. until the height of the cell column became constant. Then the heights of the cell column and of the total column were read and their ratio used as the relative cell volume of the lymph specimen. This ratio, multiplied by the total volume of lymph, was taken as the volume of the lymphocytes. Since this quantity, usually a few thousandths of a cubic centimeter, was of course too small for any immunologic manipulation, the cell sediment was treated as follows:—

From the cell volume, as derived above, the minimum degree of dilution which would provide enough material for the immunologic tests was calculated. The dilutions being calculated were in powers of two. The volume of saline necessary for such a dilution was then added to the lymph cell sediment. The cells were gently shaken into the saline until the suspension was uniform. The tube was then placed alternately in an alcohol–dry-ice bath (approximately −70°C.) and in a water bath a 37°C. until the suspension had frozen and thawed 3 times. After the cellular debris had been removed by centrifugation, the supernatant fluid was used as the lymph cell extract at the calculated dilution. Microscopic examination of the discarded sediment showed no whole cells.

The determination of antibody concentration presented a technical problem, because of the small amounts of material available. For lymph plasma and the higher dilutions of lymph cell extract the dilutions were made in 0.2 cc. quantities. For the lower dilutions of lymph cell extract, the tests were performed with one-quarter of this quantity. All dilutions were carefully made with pipettes graduated in 0.001 cc. amounts. The tests were performed as follows:

1. The typhoid bacillus agglutination was done by incubating equal quantities of the dilution of lymph plasma or lymph cell extract overnight at 50°C. in stoppered agglutination tubes, 10 mm. × 75 mm. The antigen was prepared from the suspension used for injection, the density corresponding to a reading of 70 in the Klett-Summerson colorimeter, filter number 54. The tests involving 0.05 cc. each of antigen suspension and lymph cell extract were very satisfactorily read with the aid of an agglutinoscope with a sixfold magnification.

2. The hemolysis test was done by adding to 0.2 cc. of the specimen 0.8 cc. of a mixture of 1 part of 5 per cent suspension of sheep erythrocytes, 1 part of 1:10 pooled guinea pig complement, and 2 parts of physiologic saline solution. After incubation at 37°C. for 30 minutes, the test was read. Complete hemolysis was taken as the end point, fairly strong hemolysis was considered half a step, and a trace of hemolysis was disregarded. For the smaller quantities of lymph cell extract dilution, 0.2 cc. of the mixture was used, and the results were as clearly readable as in the standard test. Control tests were made for normally occurring anti-sheep-erythrocyte antibodies in a number of the rabbits.

The Relative Antibody Titers in Lymph Plasma and Lymph Cell Extract

In a first series of experiments we compared the concentration of antibodies in the lymph cell extract with that of the lymph plasma. Rabbits were injected with either the typhoid antigen or sheep erythrocytes and the efferent lymph from the popliteal lymph node was collected during the period of rising titer. Experiments carried out on lymph collected within less than 5 days after the injection of antigen were unsatisfactory because the antibody titers were too low. As the interval between injection and collection increased, however,

both the titer of the antibodies in the lymph and the volumes of the specimens increased so that the experiment became technically feasible.

TABLE I

The Comparison of Antibody Titer in Lymph Plasma and Lymph Cell Extract

Antigen	Time after injection of antigen	Rabbit No.	Lymph	Cells per c. mm. of undiluted lymph	Anti-coagulant	Cell volume	Titer of lymph plasma	Titer of lymph cell extract	Ratio of titers
	days		*cc.*		*cc.*	*cc.*			
		6 Rt.	0.50	52,200	0.10	0.0055	16	128	1:8
		7 Rt.	0.50	61,200	0.10	0.0058	16	128	1:8
		7 Lt.	0.95	38,200	0.15	0.0042	32	192	1:6
		8 Lt.	1.00	91,700	0.15	0.0178	24	24	1:1
		11 Rt.	1.05	25,700	0.15	0.0052	64	512	1:8
		11 Lt.	1.00	40,500	0.30	0.0084	32	128	1:4
	5	15 Rt.	1.00	43,700	0.15	0.0080	64	512	1:8
		15 Lt.	0.30	65,350	0.10	0.0046	32	512	1:16
		17 Rt.	0.50	134,400	0.10	0.0132	32	128	1:4
		17 Lt.	0.50	100,800	0.10	0.0100	32	256	1:8
Sheep		43 Rt.	0.37	62,000	0.08	0.0065	48	192	1:4
erythro-		43 Lt.	0.22	46,400	0.08				
cytes		45 Pool	0.85	20,100	0.13	0.0038	32	384	1:12
		2 Rt.	0.20	46,000	0.00	0.0032	256	768	1:2.5
	6	2 Lt.	0.35	63,000	0.00	0.0056	256	1024	1:4
		3 Rt.	0.20	43,750	0.00	0.0032	256	384	1:1.5
		4 Rt.	0.25	22,500	0.00	0.0014	48	96	1:2
		4 Lt.	0.40	42,000	0.00	0.0030	32	128	1:4
	7	5 Rt.	0.55	46,100	0.10	0.0049	64	128	1:2
		5 Lt.	0.40	47,500	0.10	0.0038	64	96	1:1.5
		20 Rt.	0.85	67,050	0.15	0.0110	64	128	1:2
		12 Rt.	1.00	50,600	0.15	0.0098	64	512	1:8
		16 Rt.	1.00	60,950	0.15	0.0118	1024	4096	1:4
		16 Lt.	1.00	57,500	0.15	0.0114	1024	6144	1:8
	5	42 Rt.	0.60	20,900	0.10	0.0062	256	1532	1:6
Typhoid		42 Lt.	0.50	37,600	0.10				
bacilli		44 Rt.	0.90	74,600	0.10	0.0144	256	1024	1:4
		44 Lt.	1.10	53,000	0.10	0.0116	192	384	1:2
	7	19 Rt.	0.45	67,350	0.15	0.0122	2048	4096	1:2
		19 Lt.	0.90	51,350	0.15	0.0174	1024	4096	1:4

Rt refers to the right leg, Lt to the left leg.

The results of these experiments are summarized in Table I. It can be seen that the titers in the cell extract were consistently higher than those in the

lymph plasma and that the difference was frequently of considerable magnitude. It is of some interest to note that the highest ratios of lymph cell antibody titer to lymph plasma titer were found at the time at which the antibody titer in lymph as a whole has been shown to rise most sharply; *i.e.*, at 5 days (1).

Differential counts of the cells contained in the efferent lymph, both by the film method and by supravital stain, showed results similar to those presented in our previous study. In seven animals (rabbits 22 to 28) studied 5 to 6 days after injection of typhoid vaccine or sheep erythrocytes, the percentage of monocytes was less than 1 except in one experiment where they may have amounted to 2 per cent. These results agree with the differential cell counts made by Murakami on efferent lymph of the popliteal lymph node of rabbits (2) and with counts made by Thorne and Evans (3), Kindwall (4), Murakami (5), Hall and Furth (6), and Medawar (7) on lymph of the thoracic duct of rabbits. Yoffey and Drinker (8) and Yamagishi (9) observed only a negligible number of monocytes in the efferent lymph as compared with the large number of these cells in the afferent lymph. These observations illustrate again the filter function of the lymph node which was studied so well by Drinker and his associates (10).

The Effect of Continued Contact between Lymph Cells and Lymph Plasma

The question now arose of whether the antibodies were formed within, or on the surface of, the lymphocytes and passed from the cells into the fluid about them, or whether the antibodies in the lymph were derived from some other source and were secondarily absorbed or adsorbed by the lymphocytes.

As a preliminary experiment, a few specimens of lymph were drawn as for the experiment above. Of each specimen one half was withdrawn and kept for 24 hours in the refrigerator before centrifugation. The remaining half of the lymph specimen, was as usual, separated immediately and the cells and lymph plasma were also stored in the refrigerator. On the next day the aliquots were centrifuged and the supernatant fluid drawn off. The cell extract and lymph plasma of each half of the specimens of lymph were tested.

The results of the tests, shown in Table II, indicate that on prolonged contact of the lymphocytes with the lymph plasma *in vitro* there was an approach to equilibrium of antibody concentration between them, the titer in the cell contents falling sharply to approach that of the supernatant lymph fluid. However, since the relative volume of the cells was small, and since antibodies of the same species were present in the lymph plasma before the incubation, the rise in antibody titer of the lymph plasma as it approached equilibrium with the cells was necessarily very slight.

In Vitro Experiments on the Absorption or Adsorption of Antibodies by Lymphocytes

The experiments summarized above indicate that antibodies which had been within the lymphocytes or on their surface had escaped into the lymph plasma

under the conditions of the experiment. However, equilibrium was not reached, except in one case, and the possibility that lymphocytes might absorb or adsorb antibodies from lymph surrounding them had not been tested. Accordingly, a series of experiments was performed in which lymphocytes containing one type of antibody, *e.g.* antityphoid, were suspended in lymph plasma containing another antibody, anti-erythrocyte. Each mixture was agitated frequently to increase the probability of reaching equilibrium of antibody concentration. The cells and lymph fluid were then tested separately. The procedure was as follows:—

One rabbit was injected with typhoid antigen in each hind foot, another rabbit was injected with sheep erythrocyte antigen, and a third was kept as a control animal. After 5 days, lymph was collected from the efferent popliteal lymph vessel. The specimens were

TABLE II

The Effect of Prolonged Contact between Cells and Lymph Fluid on the Titer of Lymph Cell Extract and of Lymph Plasma

Antigen	Time after injection	Specimen centrifuged immediately			Specimen allowed to stand overnight		
		Lymph plasma	Lymph cell extract	Ratio	Lymph plasma	Lymph cell extract	Ratio
	days						
Erythrocytes.....	5	64	512	1:8	64	128	1:2
Erythrocytes.....	5	64	512	1:8	96	128	1:1.3
Erythrocytes.....	5	32	512	1:16	32	96	1:2.7
Typhoid.....	5	64	512	1:8	64	64	1:1

divided into halves, each of which was immediately separated into cell sediment and supernatant. The components of one-half of each specimen were saved for measurement of antibodies to the appropriate antigen.

Half of the remaining lymph plasma from the right leg of the rabbit immunized against typhoid bacilli was added to the cell sediment from the right leg of the rabbit immunized against sheep erythrocytes, and a duplicate preparation was provided by specimens from the left legs. Correspondingly, half of the remaining lymph plasma from each leg of the rabbit immunized against sheep erythrocytes was added to the cell sediment from each leg, respectively, of the rabbit immunized against typhoid bacilli. Each cell sediment was shaken up into the lymph plasma to which it had been added and the suspensions were gently agitated at intervals. As a result, lymphocytes containig antityphoid antibodies were allowed to come into intimate contact with lymph plasma which contained anti-sheep-erythrocyte antibodies but no antityphoid antibodies, and *vice versa*. Incubation was carried out either at room temperature or in the refrigerator and after 24 hours these suspensions were centrifuged, the cells were removed and quickly washed with saline solution, and then cells and lymph plasma were each tested for antibody content with each of the antigens. Lymphocytes obtained from the uninjected rabbit were also incubated with small samples of lymph plasma from each of the injected rabbits.

The results of a typical experiment are shown in Table III. It will be seen that no uptake of antibodies by lymphocytes occurred even when the fluid in

which incubation took place had a high titer. Thus "anti-erythrocyte" cells, although incubated in fluid of an antityphoid titer of 1024 showed no trace of reaction in 1:64 dilution of the cell extract after incubation, 64 being the lowest dilution possible for this volume of cells. Correspondingly, in no case was there any decrease of titer in lymph plasma during the incubation, as would be expected if the lymphocytes took up antibodies from the fluid about them.

TABLE III

The Failure of Lymphocytes to Absorb or Adsorb Antibodies in Vitro

	Material	Antityphoid antibodies		Anti-erythrocyte antibodies	
		Supernatant fluid	Lymph cell extract	Supernatant fluid	Lymph cell extract
Original specimen	T, right leg	1024	4096	<3	—
	T, left leg	1024	6144	<3	—
	E, right leg	—	—	32	128
	E, left leg	—	—	32	256
After incubation as indicated	T cells plus E lymph plasma, right leg	128	128	32	<48
	T cells plus E lymph plasma, left leg	256	128	32	<48
	E cells plus T lymph plasma, right leg	1024	<48	6	<48
	E cells plus T lymph plasma, left leg	1024	<48	4	<48
	N cells plus E lymph plasma, right leg	—	—	32	<48
	N cells plus T lymph plasma, left leg	1024	<48	—	—

T indicates rabbit injected with typhoid bacilli.
E indicates rabbit injected with sheep erythrocytes.
N indicates normal control.
— indicates no test.
<3 and <48 mean that no antigen-antibody reaction was obtained with dilutions of 1:4 and 1:64, the smallest dilutions used in these tests.

On the other hand, the data show that the lymphocytes in each case lost antibodies to the supernatant fluid. In each case the titer of the contents of the lymphocytes fell to a value in the range consistent with the relative volumes of cells and surrounding fluid. The antityphoid cell sediments, incubated with about 25 times their volume of anti-erythrocyte supernatant lymph fluid fell in titer to 1:32 and 1:48 of the control values, respectively.

Finally, in the case of these artificial suspensions antibodies released from the lymphocytes were demonstrable in the lymph plasma after incubation. Here again the final titers were consistent with the ratio of volumes which were used. Thus, the antityphoid lymph plasma reached a titer of 6 and 4

against sheep erythrocytes respectively, or about 1/30 of the titer of the control cell extract. It had not been possible to measure this effect in the experiment shown in Table II, since antibodies were passing from the cells into a fluid containing those antibodies in titer of 1:128.

The time of incubation was not varied in this series of experiments, so the equality of concentration between cell extract and supernatant of the artificial suspensions incubated may be fortuitous, since we are probably not dealing with a dynamic equilibrium. It may be that on more prolonged contact the titer of cell extract might fall to a lower range of values than that of the supernatant fluid.

In Vivo Experiments on the Absorption or Adsorption of Antibodies by Lymphocytes

Althought the results of the experiments described thus far are consistent with the hypothesis of the formation of antibodies by the lymphocyte, rather than the selective absorption or adsorption by the lymphocyte from the lymph fluid, the evidence presented was open to the criticism that opportunity was given to lymphocytes to take up antibodies only under non-physiologic conditions: *in vitro*, not at body temperature, and in the presence of anticoagulants. In order to test directly this possibility, a series of experiments was performed as follows:—

Rabbits which had been injected with 0.2 cc. of typhoid vaccine into each foot 6 days previously were anesthetized and the popliteal lymph node and its efferent lymph vessel were exposed. A ligature was placed about the efferent lymph vessel at approximately 1.5 cm. proximal to the node. This procedure did not interfere with the viability of the lymphatic tissue (11). Rabbit serum exhibiting a high titer of antibodies against sheep erythrocytes was then injected into the lymph node. In order to promote the possible uptake of antibodies by the lymphocytes, periods varying from 3 to 5½ hours were allowed to elapse before the lymph was aspirated from the efferent lymph vessel. The specimens were centrifuged but the cells were not washed. Simultaneous titrations for hemolysin were made on: the injected serum, the supernatant lymph fluid recovered, and the lymphocytes. In one case (rabbit 37) the wound was closed in the interim and the animal was allowed to move about at will after it recovered from the anesthesia. The results of these experiments are shown in Table IV.

The ratios of cell extract titer to lymph plasma titer are strikingly different from those in Table I. Indeed, the actual ratios were even smaller than recorded, because some of the antibodies recovered from the lymphocytes were undoubtedly the results of contamination of cell extract by lymph plasma among the cells and on the walls of the tubes. This factor would not be significant in the experiments quoted in Table I, where the cell extract titer was substantially higher, whereas it would be appreciable in these experiments in which the contaminating lymph plasma had a higher titer than the lymphocyte extract.

DISCUSSION

A number of investigations have been reported in recent years on the site of formation of antibodies and the cells concerned with this process. An inherent difficulty in the interpretation of most of these studies is that the conclusions drawn depended on indirect evidence, such as time relations of observed phenomena. In the earlier paper of this series, it was suggested that the lymphocyte may have an important part in the formation of antibodies, because, after injection of an antigen, lymphatic hyperplasia occurred in the regional node and the lymphocyte count of the efferent lymph from that node increased

TABLE IV

In Vivo Incubation of Normal Lymphocytes in Lymph Containing Antibodies

Rabbit No.	Incubation time interval	Amount of antibody injected	Amount of lymph collected	Cells per c.mm. of undiluted lymph	Anticoagulant	Lymph cell volume	Hemolysin titer			Ratio of lymph cell extract titer: lymph plasma titer
							Serum injected	Lymph plasma	Lymph cell extract	
	hrs.	*cc.*	*cc.*		*cc.*	*cc.*				
32	3	0.30	0.40	61,200	0.25	0.0055	2048	256	<16	<1:16
33 Rt.	3½	0.25	1.05	1,100	0.25	0.0002	2048	768	68*	
33 Lt.	3½	0.25	0.80	6,750	0.40	0.0013	2048	768	<16	<1:48
36 Rt.	4¼	0.25	0.90	13,200	0.30	0.0026	1024	128	80	1:1.5
36 Lt.	4¼	0.25	0.85	6,800	0.30	0.0010	1024	192	160	1:1.3
37	5½	0.25	0.90	49,400	0.30	0.0092	1024	16	<10	<1:1.5
38 Rt.	3¼	0.25	0.70	29,800	0.30	0.0043	1024	40	20	1:2
38 Lt.	3¼	0.25	0.70	23,000	0.30	0.0034	1024	<32	<8	

* Lymphocytes caught in a fine coagulum of fibrin.

sharply at the same time that antibodies appeared and increased in the efferent lymph. In the present study, we have been able to make a finer analysis of a small and relatively isolated system, and to present evidence of a direct nature of the natural history of the antibodies measured.

It is not the purpose of this discussion to consider the broader implications of the experiments described. These will be discussed in another paper (12). We shall consider here only the validity of the experimental observations and the deductions made from them.

Validity of Experimental Methods.—The fundamental experiment in this study was the comparison of antibody titers in the lymph cell extract with those in the lymph plasma. These comparisons were in every case made as part of a single test, using the same preparations of the reagents.

It is well to review the quantitative technics used in preparing the lymphocyte extract. The cell volume was derived from measurements of columns of packed cells, which certainly included some lymph plasma between the cells,

so that the apparent cell column as used in obtaining the titer, was larger than the actual volume of lymphocytes present. Accordingly, the error in the titration due to this effect, if it was appreciable in a titration by steps of two, was in the direction of producing a lower titer for the cell extract than was actually present. It may be that some of the ratios of cell extract titer to lymph plasma titer were actually higher than they appeared to be.

The cell volumes in cubic centimeters, as derived in this way, were found to be directly proportional to the volume of lymph collected and to the cell count of the lymph. Plotted against these variables, it was found that the observed volume of lymphocytes in any specimen followed approximately the empirical equation:

$$\text{Total cell volume} = 0.0002\ T\ V \text{ cc.}$$

where T = cell count in thousands per c. mm.
V = volume of lymph, in cc.

Theoretically, assuming the small lymphocytes to comprise all of the cells in the lymph, rather than approximately 95 per cent[1] as found, and assuming that these cells are spheres with a diameter of 6 micra, their volume would be given by the expression $0.000113\ T\ V$, using the quantities defined above, not allowing for any fluid between the cells. The agreement with the theoretical value is reasonable in view of the small number of larger cells and the range in diameter of small lymphocytes, and especially in view of the fluid caught among the cells in the hematocrit tube.

The small quantities of materials used do not present difficulties in interpreting the experiments. Titrations were made carefully, using Kahn pipettes, and although the accuracy was probably somewhat less than in the case of larger amounts, no error is to be expected on this account in a titration by discrete steps of two. Although some tubes of cell extract dilutions contained as little as 0.05 cc. of each reagent, most of the end points occurred beyond the third tube of the titration, where the amounts of each reagent were 0.2 cc. This was certainly true of all of the higher titers obtained for lymphocyte extract.

Interpretation of Results.—The unequivocal difference in titer between lymph plasma and cell extract as observed in our experiments seems to offer only two possible interpretations. The antibodies might enter the lymph from the lymphocyte or be selectively absorbed or adsorbed by the cell from the surrounding fluid. The following observations and experiments in this study support the former hypothesis.

First, although there is no precise pattern in the ratios between the respective titers of lymph plasma and cell extract, it is of considerable interest to

[1] Most of the remaining cells were medium or large lymphocytes.

note that the ratio is greatest on the 5th day, which is the time of the greatest rate of increase in antibody titer in the lymph. A brief tabulation shows this:

	Average ratio of titers		
	5th day	6th day	7th day
Erythrocytes	7	2.6	2.3
Typhoid	5		3

This observation is of particular interest in view of the fact that the titers in the lymph plasma are higher on the 6th and 7th days than on the 5th. The findings are thus consistent with a primary appearance of antibodies within or on the surface of the lymphocyte, and inconsistent with what would be expected if the opposite were true.

Second, the experiments summarized in Tables II and III offer evidence of the escape of antibodies from the lymphocytes into the surrounding medium, but no evidence of uptake of antibodies by these cells. In interpreting these experiments, it is important to realize that the conditions of incubation were not physiologic, and that the lymphocytes were probably no longer living cells at the conclusion of the experiment. Accordingly, one might not expect an acquisition of antibodies by the lymphocytes in the experiment even if such absorption or adsorption were a physiologic mechanism.

Neither of these questions enters into the interpretation of the results of our last series of experiments which are summarized in Table IV. Here the antibody was in contact with cells which were living during the entire period of incubation, and there was sufficient time for adsorption or absorption to take place. However, again, none was demonstrable.

SUMMARY

Following the injection of typhoid antigen or sheep erythrocytes into the pad of the rabbit's hind foot, lymph from the efferent lymphatic of the popliteal lymph node was collected and analyzed for antibody content.

On separating the lymphocytes from the lymph plasma, it was found that the antibody titer of the cell extract was substantially and consistently higher than that of the surrounding fluid. This difference was greatest at the time of greatest rate of increase of antibody titer in the whole lymph, rather than when the antibody titer of the lymph plasma was highest.

These results can only be interpreted to mean that the lymphocytes either produce antibodies or take them up from the lymph plasma. Incubation *in vitro* of lymphocytes containing one species of antibody with lymph plasma containing another showed that antibodies pass from the cells to the supernatant lymph fluid to reach approximate equilibrium; acquisition of antibody from supernatant lymph fluid was not observed. Similar results were obtained when normal lymphocytes were allowed to incubate *in vivo* in their own lymph fluid to which antibodies had been added. It was again found that antibodies

were not absorbed or adsorbed by lymphocytes. These results seem to indicate that lymphocytes are instrumental in the formation of antibodies.

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