EXPERIMENTAL STUDIES UPON LYMPHOCYTES.

II. The Action of Immune Sera upon Lymphocytes and Small Thymus Cells.

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Plate 13.

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In a recent paper[1] brief reference was made to the production of cytotoxic sera for lymphocytes derived from human tonsils and from the rat thymus. It is desired to report here upon further experiments which have been carried out with these sera, and which appear to bear directly upon the general problem of the specificity of cytotoxins, and upon the important question of the biological identity of the small thymus cells with the lymphocytes found in the lymphoid tissues and in the circulating blood.

The method used in these studies has been described in detail in a previous paper. Briefly stated, it consists in subjecting suspensions of thymus or tonsil cells in salt solution or Locke's fluid for a given period, to the action of whatever toxic agent is chosen, and then adding trypan blue in appropriate dilution. The percentage of diffusely stained cells affords a quantitative measure of the injury produced, when compared with a control suspension maintained under similar conditions. The factors of error, and the precautions necessary in order to make the determinations of value are related in the previous paper.

Brief reference should be made to the more important studies of previous workers, using other methods.

Metchnikoff[2] in 1899 induced leukotoxic sera by injecting spleen and lymph node emulsions of rat and guinea pig into rabbits. He observed the agglutinative

[1] Pappenheimer, A. M., The reactions of lymphocytes under various experimental conditions, *J. Exp. Med.*, 1917, xxv, 635.

[2] Metchnikoff, E., Études sur la résorption des cellules, *Ann. Inst. Pasteur*, 1899, xiii, 737.

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and lytic changes which followed when suspensions were exposed to the action of the immune serum, and described the hydropic swelling of the mononuclears "by which they become transformed into transparent vesicles, the nucleus thereby being rendered very visible." He found that his sera were toxic only for the cells of the species used as antigen, but that there was no evidence of specificity for any particular type of leukocyte, polymorphonuclears and mononuclears being equally affected by anti-lymph-gland serum.

Besredka[3] continuing these studies found that the toxic properties of the leukotoxic sera were destroyed by heating to 55°C. for 30 minutes, and that emulsions of cells heated to 60°C. lost their antigenic properties. He also observed that his sera were mildly hemolytic, although the injected suspensions were macroscopically blood-free. Injections of leukotoxic sera into normal animals produced toxic, or, in large doses, lethal effects. The blood showed an initial hypoleukocytosis followed by hyperleukocytosis.

Flexner[4] has contributed to the subject a detailed study of the lesions produced in the hematopoietic tissues by myelotoxic and lymphotoxic sera. In the lymph glands the principal changes produced were hyperplasia of the follicles, swelling of the germinal centers, and degeneration of the large cells in the center of the follicles. The degenerative changes in general, however, are described as "minimal and trifling."

Bunting,[5] continuing the work of Flexner, immunized geese with lymph glands and bone marrow of the rabbit, and obtained sera which were "to a large extent specific both in their action on the tonsils and on the circulating blood." One of the sera of the two geese used as controls, however, showed well marked hemolytic powers, and both were agglutinative and lytic for suspensions of lymph gland cells *in vitro*. This action was, however, less intense than that of the serum from a goose immunized against lymph glands.

Christian and Leen[6] have used the cessation of motion of leukocytes observed in a warm chamber as an index of toxicity. They found that sera having both hemolytic and leukotoxic properties could be produced by immunizing with a variety of somatic cells, as those of the liver, spleen, kidney, and cardiac muscle.

The production of thymotoxic sera has been attempted by Moorhead[7] and by

[3] Besredka, La leucotoxine et son action sur le système leucocytaire, *Ann. Inst. Pasteur*, 1900, xiv, 390.

[4] Flexner, S., The pathology of lymphotoxic and myélotoxic intoxication, *Univ. Penn. Med. Bull.*, 1902, xv, 287.

[5] Bunting, C. H., The effects of lymphotoxins and myelotoxins on the leucocytes of the blood and on the blood-forming organs, *Univ. Penn. Med. Bull.*, 1903–04, xvi, 200.

[6] Christian, H. A., and Leen, J. F., Some further observations on leucocytoxins, *Boston Med. and Surg. J.*, 1905, clii, 397.

[7] Moorhead, T. G., The thymus gland, *Practitioner*, 1905, lxxv, 733.

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Ritchie.[8] Moorhead states briefly that sera from rabbits immunized with guinea pig thymus did not agglutinate thymus cells *in vitro*, and had no leukolytic action. Ritchie, however, using complement fixation methods found that the sera of ducks immunized with suspensions of guinea pig thymus contained an immune body having an affinity for the receptors of the guinea pig thymus and lymph glands, spleen, and bone marrow. In the presence of this immune body, guinea pig complement became fixed, so that there was no hemolysis in the hemolytic system used as a test. The sera showed no affinity for the receptors of the liver, adrenal, thyroid, lung, etc. The serum was not hemolytic, and its binding powers were not affected by heating.

The cytotoxic sera were prepared by injecting rabbits intravenously with washed suspensions of rat thymus cells or of tonsil lymphocytes. The former could be obtained almost blood-free by exsanguinating the rat, carefully dissecting off the superficial blood vessels from the gland, and washing the suspended cells in one or more changes of salt solution. The tonsil suspensions were usually more or less admixed with red blood cells.

EXPERIMENTAL.

The following experiments illustrate the cytotoxic and cytoagglutinative properties of these sera, when tested against the appropriate antigen.

Experiment 1.—Dec. 5, 1916. Serum C (normal control), Serum H (immunized against human tonsil lymphocytes), and Serum R (from rabbit immunized against rat thymus cells) were added in the proportion of 1:25 to a suspension of rat thymus cells. The tubes were then placed in the incubator for 35 minutes, trypan blue was added in 1:5,000 dilution, and the percentage of stained cells determined. Duplicate counts were made.

Mixture.	Stained cells.	Unstained cells.	Percentage of stained cells.	Agglutination.
Serum C, 0.02 cc.	195	417	31.8	0
Thymus cells, 0.5 "	212	469	31.1	
Serum H, 0.02 "	228	208	52.3	0
Thymus cells, 0.5 "	322	329	49.5	
Serum R, 0.02 "	309	243	55.9	++
Thymus cells, 0.5 "	284	194	59.4	++

[8] Ritchie, W. T., The specificity and potency of adrenolytic and thymolytic sera, *J. Path. and Bacteriol.*, 1908, xii, 140.

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Although the highest count is obtained with Immune Serum R all the tubes show a high proportion of stained cells. Agglutination occurred only in Serum R.

The sera were then diluted 1:10 and inactivated for 30 minutes at 58°C. A fresh suspension of thymus cells from a healthy young rat was prepared and the following tubes were set up and incubated for 15 minutes at 37°C.

Mixture.	Stained cells.	Unstained cells.	Percentage of stained cells.	Agglutination.
Serum C, 0.5 cc.	17	473	3.4	0
Thymus cells, 0.2 "				
Serum H, 0.5 "	38	433	8.0	0
Thymus cells, 0.2 "				
Serum R, 0.5 "	27	438	5.8	++
Thymus cells, 0.2 "				

The toxicity of the serum is thus destroyed by heating. Agglutination by Serum R is still marked.

To each tube was added 0.5 cc. of fresh 1:10 guinea pig complement and the tubes were replaced in the thermostat. Counts were made after 1 hour and 15 minutes.

Mixture.	Stained cells.	Unstained cells.	Percentage of stained cells.	Agglutination.
Serum C, 0.5 cc.	30	515	5.5	0
Thymus cells, 0.2 "				
Complement, 0.5 "				
Serum H, 0.5 "	60	427	12.3	0
Thymus cells, 0.2 "				
Complement, 0.5 "				
Serum R, 0.5 "	113	150	42.9	+++
Thymus cells, 0.2 "				
Complement, 0.5 "				

Under the influence of the immune serum the thymus cells are markedly agglutinated, so that there are comparatively few free cells. They are highly irregular in shape, and a great proportion of them are diffusely stained with trypan. Some of the cells show a hydropic swelling of the cytoplasm, the stained nucleus appearing suspended in a clear vesicle. The control sera produced no such effect. The results are shown graphically in Text-fig. 1.

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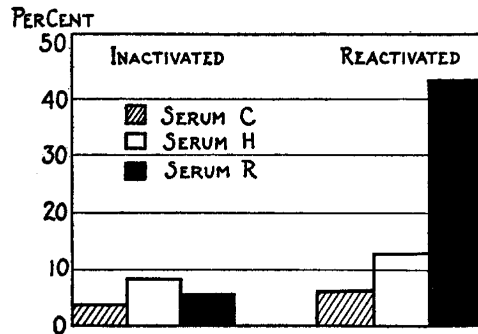
Numerous experiments have given uniform results. Serum R was last tested on January 30, 1917, 26 days after the rabbit had received an injection of thymus cells. The toxicity and agglutinative power were even more marked than in the experiment cited.



Text-Fig. 1. Experiment 1. The effect of Thymotoxic Serum R upon the stainability of rat thymus cells.

The following protocol illustrates a similar action of the serum from Rabbit H, immunized with a suspension of human tonsil lymphocytes, when tested against the appropriate antigen.

Experiment 2.—Dec. 12, 1916. Serum from Rabbit H, which had received four intraperitoneal and two intravenous injections of human tonsil lymphocytes, and Serum C from a control normal rabbit were tested against suspensions of human lymphocytes. The suspension, twice washed and centrifuged, had stood over night in the ice box. Sera were inactivated for 30 minutes at 58°C. and diluted to 1:10.

Mixture.	Stained cells.	Unstained cells.	Percentage of stained cells.	Agglutination.
Serum H (1:10), 1.0 cc. Tonsil lymphocytes, 0.2 "	26	283	8.4	+
Serum C (1:10), 1.0 " Tonsil lymphocytes, 0.2 "	27	363	6.9	0

0.5 cc. of a 1:10 dilution of fresh guinea pig complement was added and the tubes were replaced in the thermostat. Counts made after 45 minutes showed the following:

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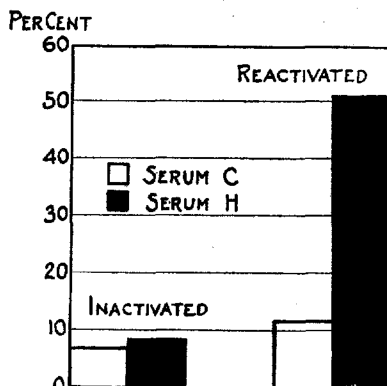
Mixture.	Stained cells.	Unstained cells.	Percentage of stained cells.	Agglutination.
Serum H (1:10), 1.0 cc. Tonsil lymphocytes, 0.2 "	163	156	51.0	++
Serum C (1:10), 1.0 " Tonsil lymphocytes, 0.2 "	40	273	12.7	0

There is thus a striking increase in the proportion of stained cells under the influence of the immune serum, accompanied by macroscopic agglutination, and the morphological changes described above. The results are shown in Text-fig. 2.



TEXT-FIG. 2. Experiment 2. The effect of lymphocytoxic serum upon the stainability of human tonsil lymphocytes.

Repeated observations having assured us of the constancy of this cytotoxic action of Anti-thymus Serum R, the effects produced *in vitro* were compared with those which might be caused by injection into the living animal.

Experiment 3.—Dec. 14, 1916. Two young rats were etherized and a fragment of thymus was removed from each for histological control. Sections showed a normal structure.

Dec. 19. Rat A was injected intraperitoneally with 1 cc. of native Anti-thymus Serum R. The control, Rat B, received normal rat serum. After 24 hours the rats were killed and suspensions in Locke's fluid were made from a portion of each thymus gland, the remainder being fixed and sectioned. Counts of the suspensions, with and without the addition of rat serum, showed no significant

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differences which would indicate an increased fragility on the part of the cells of the rat which had received the cytotoxic serum. Correspondingly, no histological changes were found in sections of the tissue, as compared with the control. The experiment, therefore, was negative.

Experiment 4, however, in which the sera were first inactivated and complement was injected simultaneously gave definite indication of cytotoxic action *in vivo*, and also confirms the validity of the method used in determining cell injury.

Experiment 4.—Dec. 21, 1916. Rats A and B, well nourished young animals of approximately the same age and weight, were etherized and fragments of thymus excised from each. Portions of the fragments were fixed in Zenker's fluid, sectioned, and found to show a normal structure. The remainder was teased in Locke's solution and counts were made in the usual manner.

Mixture.	Stained cells.	Unstained cells.	Percentage of stained cells.
Rat A thymus cells in Locke's fluid.........	30	551	5.1
" B " " " " "	44	442	9.0

Dec. 26. Wounds healing and uninfected; rats in good condition. Rat A received 1.5 cc. of Anti-thymus Serum R (inactivated) and 1 cc. of fresh guinea pig complement (1:10) intraperitoneally. Rat B received 1.5 cc. of Serum C (normal rabbit inactivated) and 1 cc. of fresh guinea pig complement (1:10) intraperitoneally.

Rats killed after 24 hours. The thymus of each was divided into two parts; one was used for determining the stainability of cells, the other fixed and sectioned. The count shows the following, after 1 hour at 37°C.

Mixture.	Stained cells.	Unstained cells.	Percentage of stained cells.
Rat A thymus cells in 0.85 per cent salt solution................................	112	156	41.7
Rat B thymus cells in 0.85 per cent salt solution................................	58	424	12.0

The cells of Rat A, which before the injection of cytotoxic serum were somewhat more resistant than those of Rat B, have now been rendered much more fragile, as shown by the greatly increased number of stained cells (Text-fig. 3).

The study of the histological preparations indicates that the thymus of Rat A has been severely damaged by the cytotoxic serum

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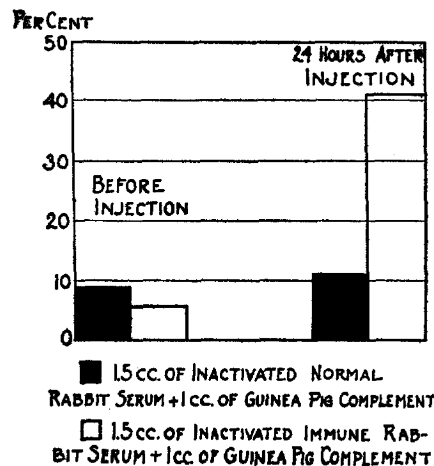
(Fig. 1). The gland which macroscopically was smaller and firmer than the control shows a great rarefaction of the cortex, fragmentation of the small thymus cells, and clumps of bluish staining material (remains of altered chromatin) in and amongst the conspicuous reticular cells. The interlobular septa are edematous and contain wandering cells of different types. There are no hemorrhages.

There is thus a convincing correspondence between the lesions produced and the increased fragility of the cells under the influence of the immune serum *in vitro*. The negative results of the previous experiments, in which the native serum was injected, are not ex-



TEXT-FIG. 3. Experiment 4. The stainability of rat thymus cells before and after the injection of immune serum.

plained. Later repetitions of this experiment have not been uniformly successful. In some instances we have observed a pronounced destruction of small thymus cells with phagocytosis of the reticular epithelium; in other cases no effects were produced. It is evident that there are variable factors which have not yet been determined. It is perhaps in accord with the well known phenomenon that, as Zinsser[9] states, "the alexin of an animal is entirely impotent or but weakly capable of producing hemolysis of the sensitized cells of its own species."

[9] Zinsser, H., Infection and resistance, New York, 1914, 154.

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This possible explanation, however, was tested by comparing the complementary action of guinea pig serum and rat serum, in the presence of anti-thymus cell amboceptor and washed thymus cells. It was found that whereas 0.2 cc. of guinea pig serum was sufficient to cause a maximal cytotoxic effect in the presence of immune serum (0.5 cc.) and thymus cell suspension 0.2 cc., 0.4 cc. or double the amount of rat serum was required. Whether this slight difference in the complementary action, which was determined in only a single experiment, is a constant one, cannot be stated. It hardly affords a sufficient explanation for the negative experiment, and further studies are necessary to clear up this point.

Previous workers with leukotoxic and lymphotoxic sera have invariably found a moderate hemolytic activity upon the red cells of the species furnishing the antigen. The sera studied by us have also been moderately hemolytic, as shown by the following protocol.

Experiment 5.—Serum H from a rabbit which had been injected with human tonsil lymphocytes was tested against washed human erythrocytes.

Serum H (1 : 10) inactivated.	5 per cent red blood cells.	Salt solution.	10 per cent guinea pig complement.	Hemolysis.	Agglutination.
cc.	*cc.*				
1.0	0.5	0.5	0.5	++	+
0.5	0.5	1.0	0.5	+	0
0.1	0.5	1.4	0.5	0	0
0.05	0.5	1.45	0.5	0	0
0.01	0.5	1.49	0.5	0	0
0.00	0.5	2.00	0.5	0	0

Readings after 1 hour at 37°C. and 18 hours in the ice box showed that the serum caused slight hemolysis in a dilution of 1:50. The thymotoxic serum (R) was also feebly hemolytic for rat corpuscles.

Jan. 29, 1917. *Experiment 6.*

Serum R (1 : 10) inactivated.	5 per cent red blood cells.	Salt solution.	10 per cent guinea pig complement.	Hemolysis.	Agglutination.
cc.	*cc.*				
1.0	0.25	0.75	0.5	+	0
0.5	0.25	1.25	0.5	+	0
0.1	0.25	1.65	0.5	0	0
0.0	0.25	1.75	0.5	0	0

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This brings up at once the question as to whether the thymotoxic and thymoagglutinative factors are distinct from the hemolytic ones. That the sera should be mildly hemolytic was to be expected not only from the concurrent experience of previous workers, but from the fact that the cell suspensions could not be rendered wholly blood-free even by repeated washing.

We have attacked the problem in two ways: first, by determining whether, after complete absorption of the hemolysin, the serum still retained to a degree its thymotoxic and thymoagglutinative properties; and secondly, by studying the effects upon the thymus cells of a serum prepared by immunizing a rabbit against rat erythrocytes.

Experiment 7 illustrates the fact that both thymotoxic and thymoagglutinative factors persist after complete absorption of the hemolysin and hemagglutinin.

Experiment 7.—Jan. 29, 1917. Anti-thymus Serum R from the rabbit whose last injection with thymus cells had been on Jan. 5 was inactivated. The hemolytic activity of the serum was first tested, and it was found that 0.1 cc. of serum in the presence of 0.5 cc. of 10 per cent complement completely hemolyzed 0.25 cc. of washed rat erythrocytes.

A preliminary test was also made of its cytotoxic property for thymus cells. The percentage of stained cells rose after reactivation of the serum from 6.2 to 100 per cent and agglutination was marked.

To absorb out the hemolytic factor 2 cc. of 5 per cent red cells were added to 2 cc. of inactivated serum. After 1 hour at 37°C. the red cells were removed by centrifugation, and 2 cc. of red blood cells were again added. The erythrocytes were then allowed to remain in contact with the serum in the ice box for 18 hours. On the following day the serum was pipetted off and tested both for its hemolytic activity and for its thymotoxic action.

0.1 cc. of the absorbed serum now completely failed to hemolyze 0.25 cc. of washed erythrocytes in the presence of 0.5 cc. of complement. A definite though diminished toxicity for the thymus cells could still be demonstrated, the percentage of stained cells after reactivation rising from 12.8 to 37.0 per cent. A control normal serum, tested against the same suspension, showed no increase in the percentage of stained cells, after addition of complement. These relations are shown graphically in Text-fig. 4.

Several other experiments conducted in the same manner have given comparable results. One may conclude from them that either (*a*) there is a toxic factor distinct from the hemolytic one or (*b*) the hemolytic factor persists in the absorbed serum in minimal

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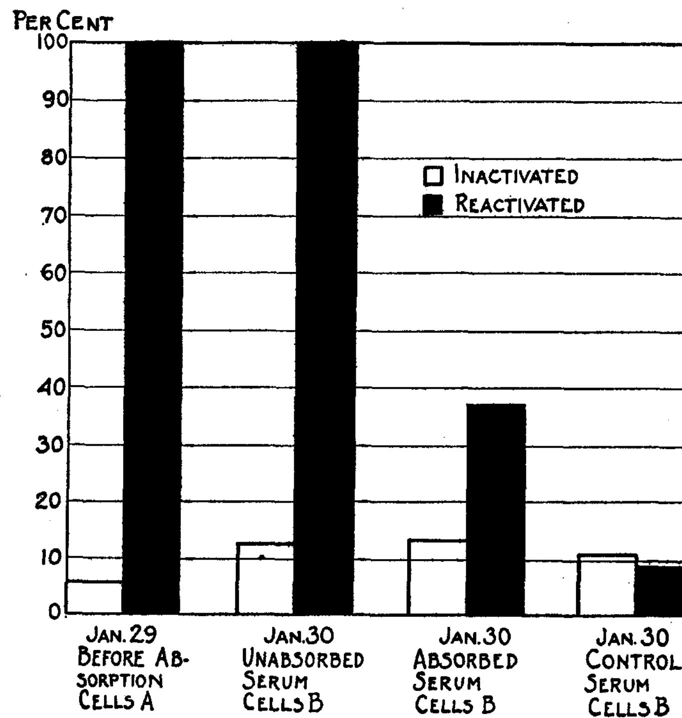
amount, which is impotent to cause laking of the erythrocytes, but is still sufficient so to alter the permeability of the lymphocytes that the percentage of stained cells is increased. There seems at present no way of deciding which of these two suppositions is the correct one.

In the hope of obtaining further information upon the question of the identity of hemolytic and thymotoxic substances, a hemolytic serum was prepared by injecting a rabbit with washed rat erythro-



TEXT-FIG. 4. Experiment 7. Persistence of thymotoxins and thymoagglutinins after the absorption of hemolysin from thymotoxic serum.

cytes. The toxicity of this serum for thymus cells was then tested, and the separation of the two factors by absorption with red cells and thymus cells, respectively, attempted.

Experiment 8.—Jan. 23, 1917. Serum from Rabbit H which had received four spaced injections of washed rat corpuscles intravenously was obtained 12 days after the last injection.

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A. Preliminary Hemolytic Titration.

Serum (1 : 10) inactivated.	Red blood cells.	Complement.	Hemolysis.	Agglutination.
cc.	*cc.*			
1.0	0.25	0.5	+	+++
0.5	0.25	0.5	+	+++
0.25	0.25	0.5	+	++
0.12	0.25	0.5	+	+
0.0	0.25	0.5	0	0
1.0	0.25		0	0

The serum is moderately hemolytic in dilution up to 1:400 although the hemolysis is masked somewhat by the strong agglutination.

B. Preliminary Test for Thymotoxic and Thymoagglutinating Properties.

Mixture.	20 min. in incubator.			30 min. after the addition of 0.5 cc. of 10 per cent guinea pig complement.
	Stained cells.	Unstained cells.	Percentage of stained cells.	Percentage of stained cells.
Serum (1: 10) inactivated, 0.05 cc. Thymus cells, 0.1 "	27	458	5.5	94.5

C. Absorption of Hemolysin and Hemagglutinin.

Inactivated serum (1: 10)..3 cc.
5 per cent rat erythrocytes ....3 "

In the incubator 1 hour at 37°C. Centrifuged serum was pipetted off and 1 cc. of fresh suspension added. Placed in the ice box over night. The following morning the completeness of the absorption was tested. It was found that 0.1 cc. produced neither hemolysis nor agglutination of 0.25 cc. of red corpuscles in the presence of 0.5 cc. of 10 per cent complement. The absorption of the hemolysis was, therefore, apparently complete.

The toxicity of the absorbed and unabsorbed sera for rat thymus cells was then compared, a fresh suspension from a healthy young animal being taken (Text-fig. 5).

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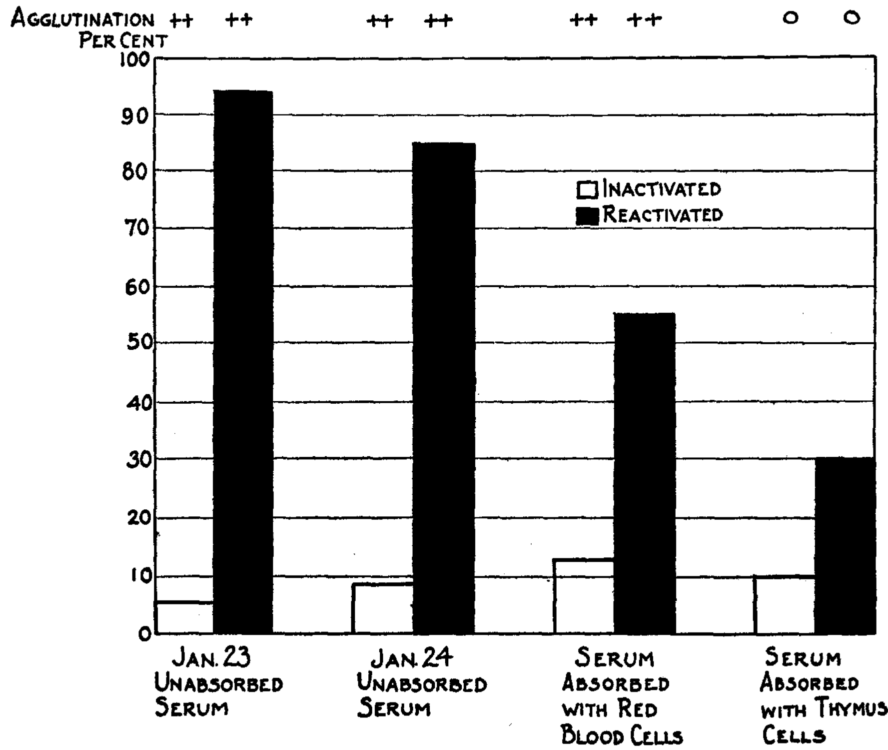

TEXT-FIG. 5. Experiment 8. Persistence of the thymotoxic factor in hemolytic serum after absorption.

Mixture.	Stained cells.	Unstained cells.	Percentage of stained cells.	Agglutination.
Absorbed serum (1: 10) inactivated, 1.0 cc. Thymus cells, 0.1 cc.	40 min. in incubator.			
	73	456	13.8	— —
	45 min. after the addition of 0.5 cc. of 10 per cent guinea pig complement.			
	315	251	55.7	
Unabsorbed serum (1: 10) inactivated, 1.0 cc. Thymus cells, 0.1 cc.	40 min. in incubator.			
	50	457	9.8	++
	0.5 cc. of 10 per cent guinea pig complement added. 45 min. in incubator.			
	388	67	85.3	

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The serum, after apparently complete absorption of hemolysin and hemagglutinin, is still strongly agglutinative and toxic for thymus cells.

To determine whether any of the agglutinin for thymus cells had been removed by exposure of the serum to red blood corpuscles a rough quantitative comparison of the agglutinin present in the absorbed and unabsorbed serum was made.

Unabsorbed serum (1:10).			Absorbed serum (1:10).		
Serum.	Salt solution.	Agglutination.	Serum.	Salt solution.	Agglutination.
gtt.	*gtt.*		*gtt.*	*gtt.*	
10	0	+++	10	0	+++
8	2	++	8	2	+++
6	4	++	6	4	++
3	7	±	3	7	++
1	9	0	1	9	0
0	10	0			

1 drop of a thick suspension of thymus cells to each tube was used. Macroscopic readings were made after 1 hour at 37°C. There appears to be no quantitative diminution in the amount of agglutinin for thymus cells.

Experiments were undertaken to absorb out the hemagglutinative and hemolytic factors by exposing the serum to suspensions of washed thymus cells. This was done, an equal volume of a thick suspension being added, and after 1 hour in the incubator, the serum was centrifuged and pipetted off and fresh cells were allowed to stand in contact with the serum for 18 hours in the ice box.

The serum was then tested for its hemolytic and hemagglutinative powers. These were found to have been practically unaffected, 0.1 cc. upon the addition of complement still causing complete hemolysis and strong agglutination of 0.25 cc. of rat corpuscles. The thymoagglutinative and thymotoxic factors, though much reduced were still present.

To sum up this experiment, which has been repeated with similar results, one finds that (1) hemolytic serum obtained by immunizing rabbits with washed rat erythrocytes is both toxic and agglutinative for small thymus cells; (2) the thymotoxic and thymoagglutinative factors, however, cannot be completely removed by absorption of the hemolysins and hemagglutinins; (3) the hemolysin and hemagglutinin cannot be absorbed by exposure of the serum to suspensions of thymus cells.

The evidence seems to favor the view that we are dealing with two distinct factors. If we assume that the thymus cells are identical with the lymphocytes of the circulating blood, it seems probable

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that specific immune bodies have been produced in response to the lymphocytes injected with the washed erythrocytes. The alternative view is the one already suggested; namely, that minute amounts of hemolysin, insufficient to cause laking of the red cells, are still able to produce the striking cytotoxic effects upon the thymus cells recognizable by the trypan blue method.

Although we have not been able to work with lymphocytes from the circulating blood, we have studied the action of thymotoxic serum upon suspensions of lymphocytes derived from lymph glands. The following illustrative protocol shows that, as far as their reaction to the immune serum is concerned, thymus cells and lymph gland lymphocytes are identical.

Experiment 9.

Jan. 30, 1917.

<table>
<tr><th>Mixture.</th><th>Stained cells.</th><th>Unstained cells.</th><th>Percentage of stained cells.</th><th>Agglutination.</th></tr>
<tr><td rowspan="4">Anti-thymus serum (1: 10), 1.0 cc.
Lymph gland lymphocytes, 0.2 "</td><td colspan="4">55 min. in incubator.</td></tr>
<tr><td>112</td><td>431</td><td>20.6</td><td>— —</td></tr>
<tr><td colspan="4">45 min. after the addition of 0.5 cc. of complement.</td></tr>
<tr><td>493</td><td>27</td><td>94.8</td><td></td></tr>
<tr><td rowspan="4">Anti-thymus serum (1: 10), 1.0 cc.
Thymus cells, 0.2 "</td><td colspan="4">55 min. in incubator.</td></tr>
<tr><td>62</td><td>477</td><td>11.5</td><td>— —</td></tr>
<tr><td colspan="4">1 hr. after the addition of 0.5 cc. of complement.</td></tr>
<tr><td>487</td><td>25</td><td>95.1</td><td></td></tr>
</table>

Both lymph gland lymphocytes and small thymus cells are severely injured after exposure to the activated anti-thymus serum (Text-fig. 6). It is not possible to secure from the small lymph nodes of the rat sufficiently large suspensions to make absorption tests satisfactory. The experiment as it stands, however, seems strongly to point to a close biological relationship between the thymus cells and lymphocytes from other sources.

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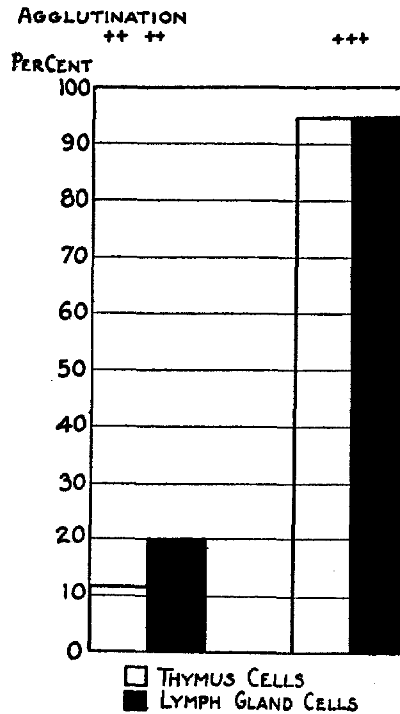

TEXT-FIG. 6. Experiment 9. Comparison of the action of thymotoxic serum upon thymus cells and lymph gland lymphocytes.

DISCUSSION AND SUMMARY.

The work of previous investigators gives the impression that it is easy to produce sera which both *in vitro* and upon injection are leukotoxic. At the same time the specificity of these leukotoxic sera for the particular type of cell used as antigen, and even for leukocytes in general, has been doubtful. The methods used have made certain possible factors of error unavoidable. Even careful washing of an organ or suspension cannot render it wholly blood-free, so that it is not surprising that the sera should be moderately hemolytic and hemagglutinative. Pearce[10] has shown that the injection of very

[10] Pearce, R. M., Concerning the specificity of the somatogenic cytotoxins, *J. Med. Research*, 1904, xii, 1.

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small amounts of blood is sufficient to evoke the production of immune hemolysins. When such sera are injected the lesions, as Pearce states, may be due in part to the production of hemagglutinative thrombi, although this hardly seems to apply to the changes in lymphoid tissue described by Flexner. On the other hand, the lymphotoxic effect of hemolytic sera may be due to the lymphocytes injected with the red cells.

Our own experiments indicate that the lymphotoxic and agglutinative factors are to a considerable degree distinct from the hemolytic and hemagglutinative ones, since they can be separated from one another by absorption.

Further evidence is presented that the small thymus cells are biologically related to, if not identical with the lymphocytes derived from lymph glands.

I wish to acknowledge the assistance of Miss Kate Brogan in the technical part of the work.

EXPLANATION OF PLATE 13.

FIG. 1. Rat A. Thymus 24 hours after the injection of immune serum and complement.

FIG. 2. Rat B. Thymus 24 hours after the injection of normal serum and complement.

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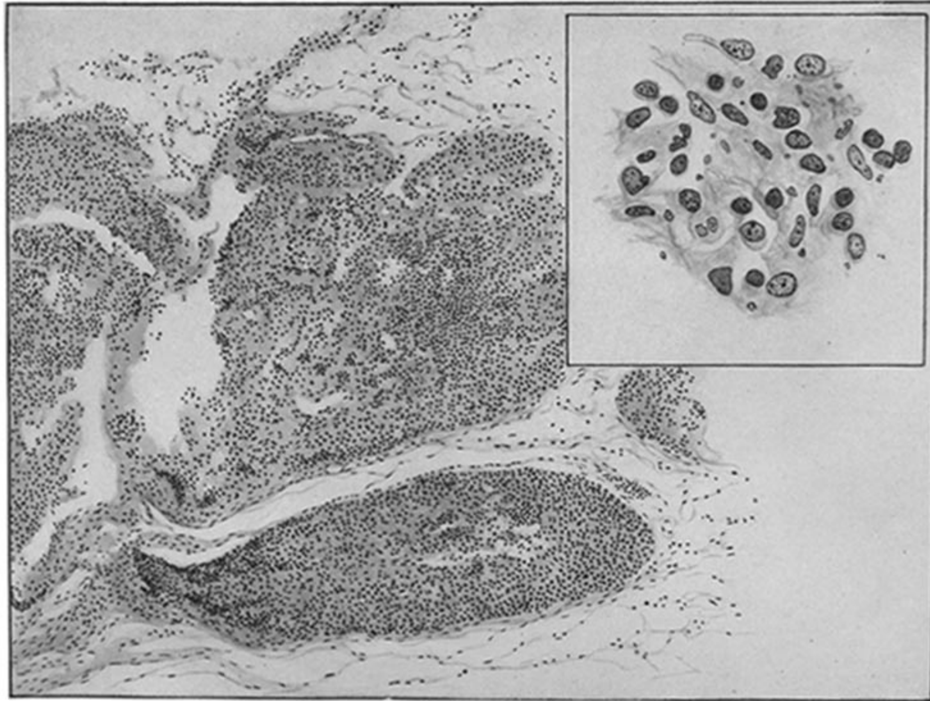

FIG. 1.

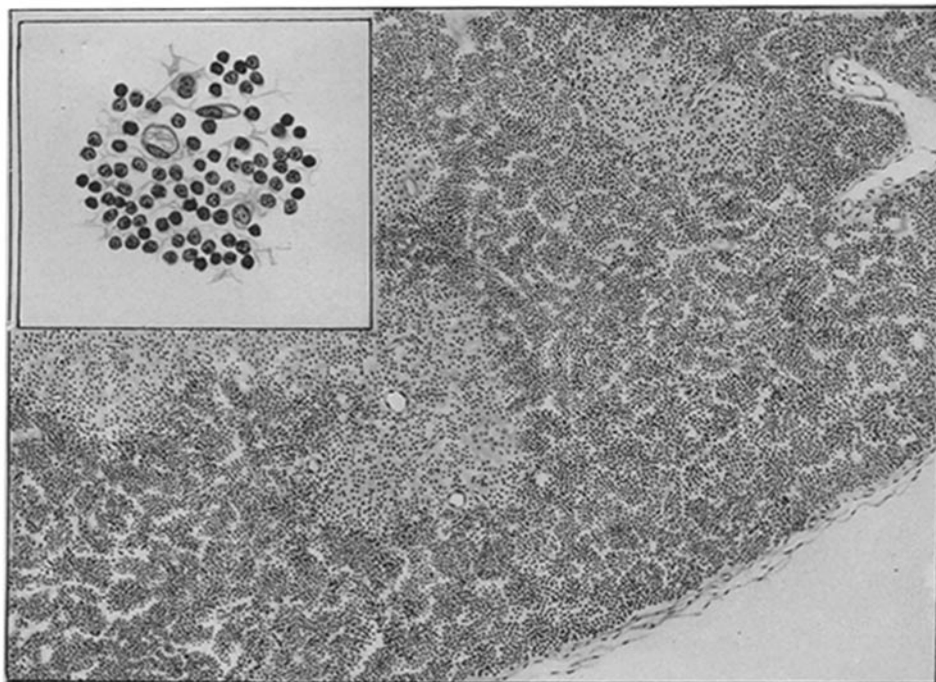

FIG. 2.

(Pappenheimer: Experimental studies upon lymphocytes. II.)

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