A COMPARISON OF THE EFFECTS OF X-RAY AND DRY HEAT ON ANTIBODY FORMATION.

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The location within the body of antibody formation has remained undetermined in spite of extensive investigation. However, much of the collected data points to the lymphoid tissue as importantly concerned therein.

The significant point in this relation is the fact that antibodies during immunization are found earlier and in greater concentration in the spleen than in the blood serum,[1] and the further observation that removal of the spleen in the early stages of immunization causes a retardation in antibody production.[2-4] The results of splenectomy have failed to convince, as the severity of the operation alone might easily account for the falling off in antibody production. The fact that animals immunized after recovery from splenectomy develop antibodies much as do normal animals has little of value for it is well known that other lymphoid structures quickly compensate for the loss of the spleen.

Hektoen,[4,5] in an attempt to throw light on the subject, has utilized the fact that x-ray produces a destructive effect on the lymphoid structures, and has shown that antibody formation is restrained to a marked degree in x-rayed animals. He concluded that antibodies are formed in the spleen, lymphatic tissue, and bone marrow since these are the structures most affected by x-ray.

[1] Pfeiffer, R., and Marx, *Z. Hyg. u. Infectionskrankh.*, 1898, xxvii, 272. Wassermann, A., *Berl. klin. Woch.*, 1898, xxxv, 209. Castellani, A., *Z. Hyg. u. Infectionskrankh.*, 1901, xxxvii, 381. Cantacuzène, J., *Ann. Inst. Pasteur*, 1902, xvi, 522. Tsurumi, M., and Kohda, K., *Z. Immunitätsforsch., Orig.*, 1913, xix, 519.

[2] Deutsch, L., *Ann. Inst. Pasteur*, 1899, xiii, 689.

[3] Hektoen, L., *J. Infect. Dis.*, 1909, vi, 78.

[4] Hektoen, L., *J. Infect. Dis.*, 1920, xxvii, 23.

[5] Hektoen, L., *J. Infect. Dis.*, 1915, xvii, 415; 1918, xxii, 28.

Our own earlier studies have developed the facts (1) that x-ray, in properly graded doses, depletes the lymphoid tissues without damage to the bone marrow,[6] and (2) that dry heat stimulates the activity of those organs.[7] It therefore seemed to us that in these two contrary effects on the lymphoid structure of the body, we possess means of determining quite directly the part they play in antibody formation.

The plan we pursued was the following. Extensive preliminary experiments were carried out in order to determine the number and intensity of x-ray doses required to reduce the amount of lymphoid tissue in rabbits without damage to the bone marrow. The method eventually evolved was to give from three to five daily exposures to x-ray, the dosage being governed by the following factors: spark-gap 3 inches, milliamperes 10, target distance 6 inches. The duration of the exposure was 4 minutes, 2 to the upper half of the animal and 2 to the lower. This series of three or more daily exposures was given prior to or immediately following the first injection designed to elicit antibodies and was repeated weekly throughout the experiments. For the stimulation of the lymphoid structures the rabbits were given a 15 minute exposure to dry heat at a temperature ranging from 50–52°, one exposure before the immunizing injections were started and others thereafter at weekly intervals. Particular care was taken, in working out the dosage of these two physical agents, that the treatments caused no loss of weight or other evidence of interference with the general health of the animals.

Precipitin Formation.[8]

Experiments 1 to 3.—Since practically the same methods were used in these three tests they will be reported together. The experimental groups were made up of (1) rabbits depleted by x-ray, (2) normal rabbits, and (3) rabbits stimulated by

[6] Murphy, Jas. B., *J. Am. Med. Assn.*, 1914, lxii, 1459. Murphy, Jas. B., and Ellis, A. W. M., *J. Exp. Med.*, 1914, xx, 397. Murphy, Jas. B., and Taylor, H. D., *J. Exp. Med.*, 1918, xxviii, 1. Taylor, H. D., Witherbee, W. D., and Murphy, Jas. B., *J. Exp. Med.*, 1919, xxix, 53.

[7] Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1919, xxix, 1. Nakahara, W., *J. Exp. Med.*, 1919, xxix, 17.

[8] We wish to express our appreciation to Dr. O. T. Avery for material help in the planning and carrying out of the experiments reported in this paper.

dry heat. All of these animals were injected with 10 cc. of horse serum intravenously 1 or 2 days after the first series of x-ray treatments and 3 or 4 days after the heat exposure. A second injection of 5 cc. of horse serum was given 1 week later and a third of 2 cc. after still another week. The heat and x-ray treatments were continued at weekly intervals throughout the experiment. All of the rabbits were bled from the heart 14 days after the final injection of antigen, and the serum was separated. For each of the sera to be tested a set of tubes was prepared containing 0.5 cc. of the various dilutions of the antigen. To each of these was added 0.5 cc. of the rabbit serum and the tubes placed in a water bath at 37°C. for 2 hours, after which a preliminary reading was made. The final observation was recorded after 12 hours in the ice box. Suitable controls were carried for each test, consisting of horse serum with salt solution, the individual rabbit sera, both with salt solution and with several dilutions of guinea pig serum. The results are given in Table I.

While the difference in potency of the precipitin in the sera from the three groups is striking, the difference in the amount of precipitate formed is even more so. In the majority of instances the amount of precipitate thrown down by the sera from the heated animals in antigen dilutions of 1:4,000 was equal in amount to that formed in dilutions of 1:40 from the control rabbits.

Bacterial Agglutinins and Protective Antibodies.

For the following experiments we have used Pneumococcus Type I for the antigen and the technique for immunization in use at the Hospital of The Rockefeller Institute.[9]

Experiments 4 and 5.—In Experiment 4, three groups of rabbits were prepared in the same manner as in the above described experiments; *i.e.*, one group with the lymphoid tissue reduced by repeated exposure to x-ray, another group stimulated by dry heat, and a third made up of untreated animals. A series of five daily x-ray exposures was given in this experiment instead of three as in the previous ones, and the immunizing injections were started 2 days prior to the first x-ray and heat applications. Experiment 5 was similar except that no x-rayed animals were included. As the technical procedures were the same the two experiments will be described together.

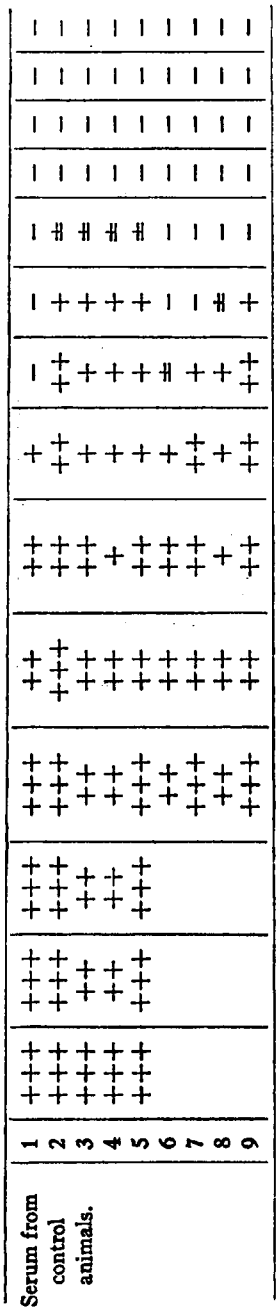
All of the rabbits received six daily intravenous injections of 1 cc. of a Type I pneumococcus vaccine, consisting of heat-killed organisms suspended in a sufficient amount of physiological salt solution so that 1 cc. was equal in bacterial count to 1 cc. of the original culture. 7 days later the rabbits were bled from the

[9] Cole, R., and Moore, H. F., *J. Exp. Med.*, 1917, xxvi, 537.

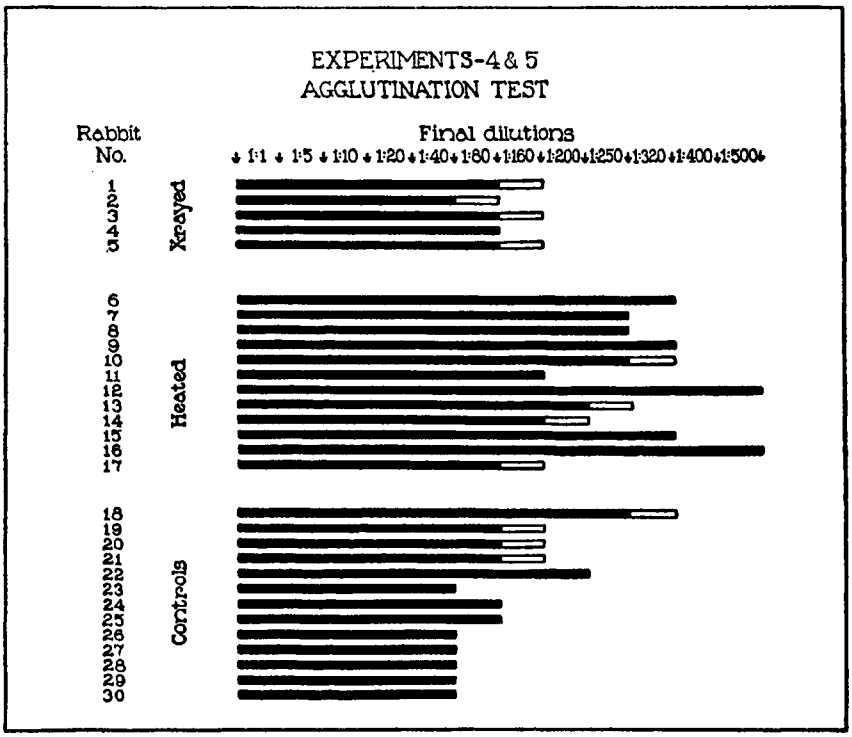
TABLE I.

Precipitin Value of Sera from X-Rayed Rabbits, Heated Rabbits, and Controls.

		Antigen dilution.													
		1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,000	1:2,000	1:4,000	1:8,000	1:16,000	1:20,000	
		Final dilution.													
		1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,000	1:4,000	1:8,000	1:16,000	1:32,000	1:40,000	Controls.
Serum from x-rayed animals.	1	+++	++	++	+	−	−	−	−	−	−	−	−	−	−
	2	++	++	+	−	−	−	−	−	−	−	−	−	−	−
	3	++	++	++	++	++	+	−	−	−	−	−	−	−	−
	4	++	++	++	++	++	+	−	−	−	−	−	−	−	−
	5	++	++	++	+	+	+	+	−	−	−	−	−	−	−
	6				±	±	−	−	−	−	−	−	−	−	−
	7				−	−	−	−	−	−	−	−	−	−	−
	8				+	+	±	±	−	−	−	−	−	−	−
	9				++	++	+	+	+	−	−	−	−	−	−
Serum from heated animals.	1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	−	−	−
	2	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+	−	−	−
	3	++++	++++	++++	+++	+++	++	++	++	++	++	++	+	−	−
	4	++++	++++	++++	+++	+++	+++	++	++	++	++	+	+	−	−
	5	++++	++++	+++	+++	+++	+++	+++	+++	++	++	++	+	−	−
	6	+++	+++	+++	+++	+++	+++	+++	++	++	+	+	−	−	−
	7				+++++	+++++	+++++	++++	+++	+++	++	+	±	−	−
	8				++++	++++	++++	+++	+++	++	+	+	+	±	−
	9				++++	++++	++++	+++	+++	++	+	+	+	+	−
	10				+++	+++	+++	+++	++	+	+	+	±	−	−


Serum from control animals.
1
2
3
4
5
6
7
8
9

marginal vein of the ear and the agglutinating power of the sera was tested. After the elapse of this 7 day rest period a second series of six daily injections of vaccine was given, and then, after yet another rest period a third series of six injections was made. Throughout the experiment the heat treatments were repeated at weekly intervals and the course of five x-ray exposures was repeated every 2nd week. 9 days after the last vaccine dose all of the rabbits were bled from the heart and the sera collected.



TEXT-FIG. 1.

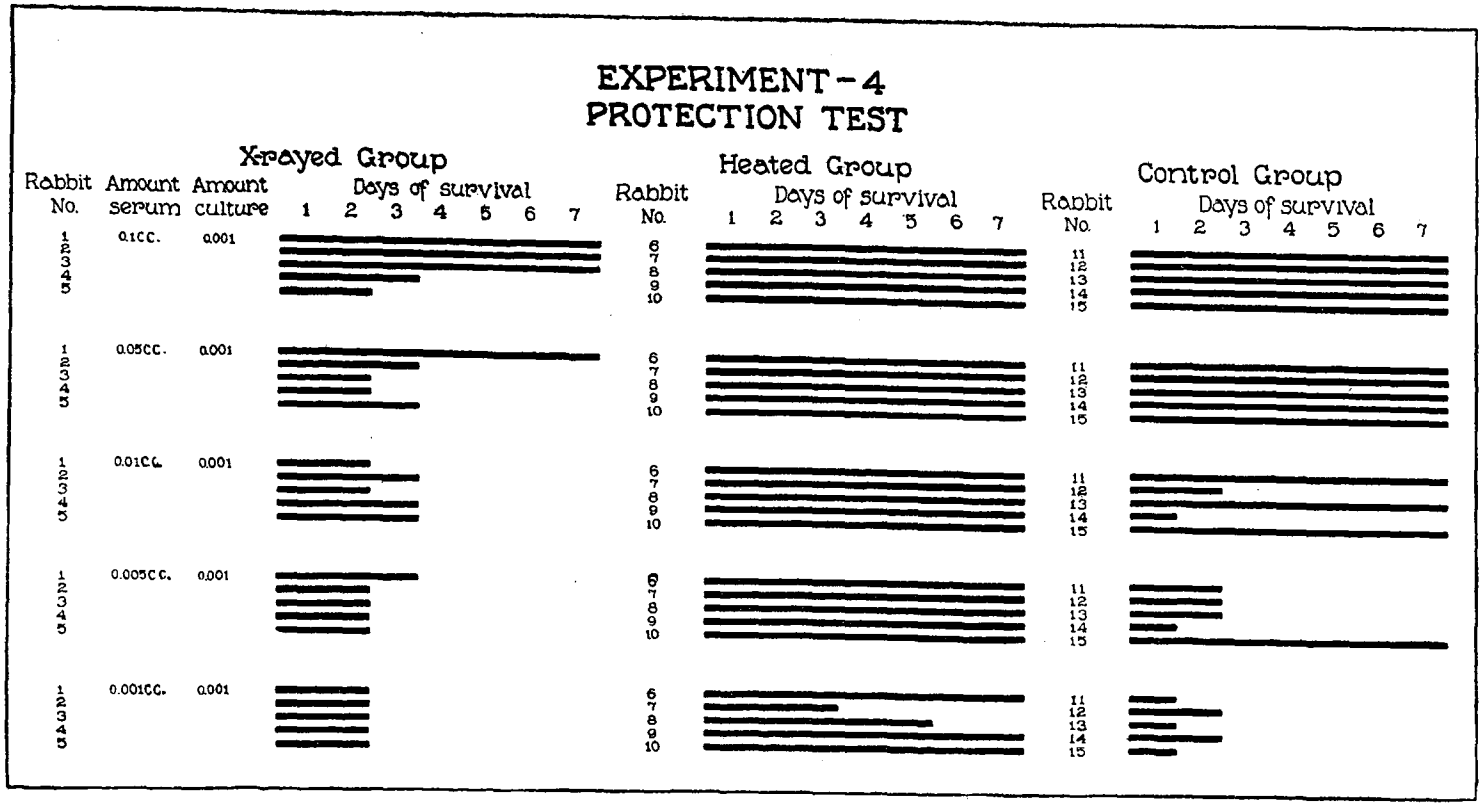
Agglutinins.—To test-tubes containing 0.5 cc. of the various sera diluted so as to give a range of from 1:5 to 1:250, was added 0.5 cc. of a suspension of Type I pneumococcus. After a thorough shaking the tubes were placed in a water bath at 37.5°C. for 2 hours and then in the ice box overnight. The final readings made at this time are recorded in Text-fig. 1.

Protective Antibodies.—The sera from all of the rabbits were also tested for their protective power against infection. A Type I pneumococcus of the Neufeld strain was subjected to two animal passages, thereby increasing its virulence to a

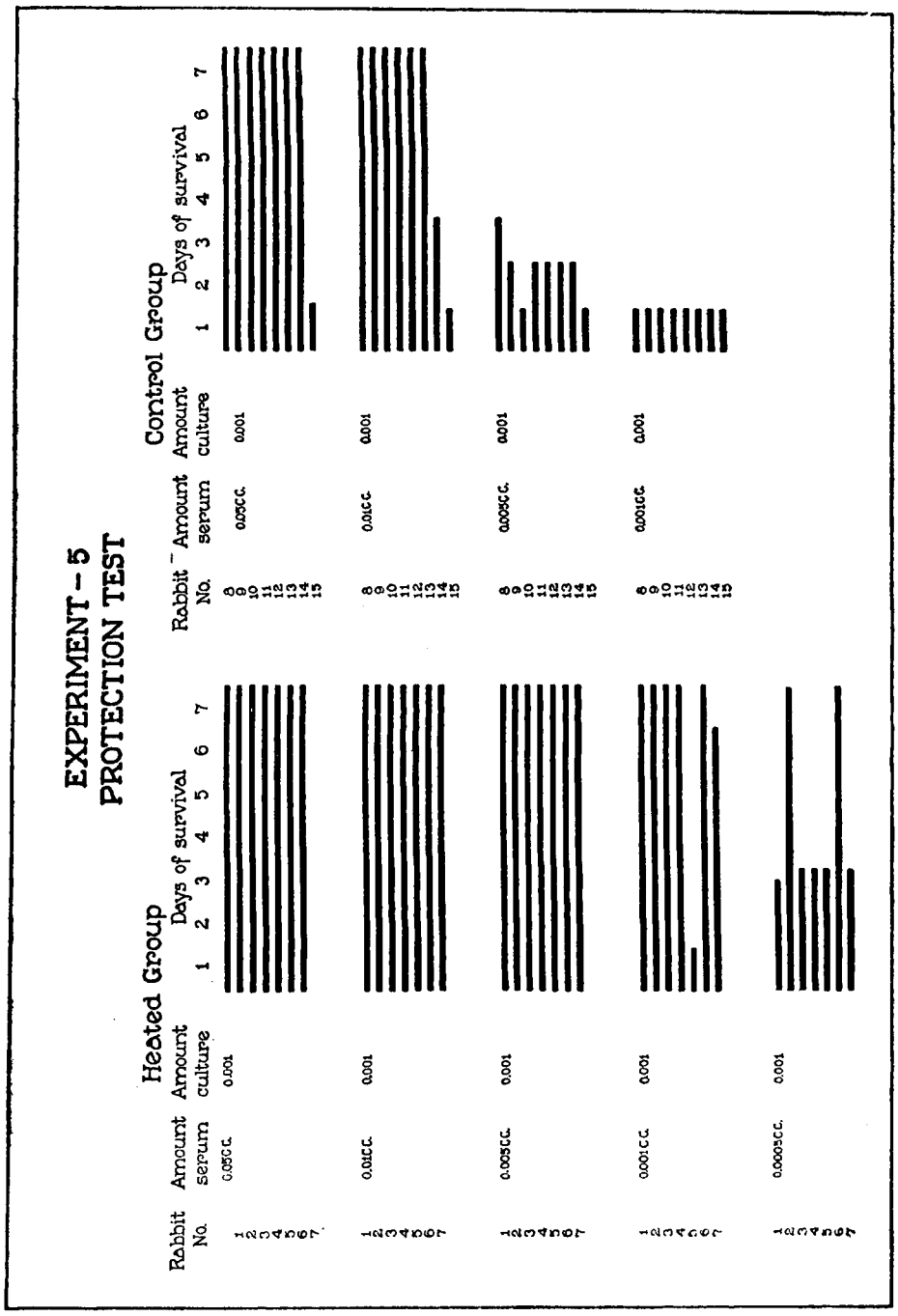
point where 0.000001 of a 12 hour broth culture killed mice within 48 hours and 0.001 uniformly caused death within 18 hours. For each serum to be tested for its protecting power, six normal white mice weighing from 18 to 20 gm. were given an intraperitoneal injection of 0.001 of a 12 hour bouillon culture of the organism mixed with graded amounts of the immune sera ranging from 0.1 to 0.001 cc. In some instances, when the serum protected in these amounts, additional tests were carried out with smaller amounts. Mice which survived for 7 days were considered effectively protected from infection. The results of this experiment are given in Text-figs. 2 and 3.

The agglutinating power of the sera from the three groups of rabbits, even in specimens taken after the first series of vaccine injections, showed distinct differences. The titer of the sera from the heated rabbits was almost uniformly higher and that from the x-rayed rabbits lower than in the case of the controls. In the final test, the sera from two heated animals agglutinated in dilutions as high as 1:500 with the average for the group of about 1:320. On the other hand, sera from the control rabbits failed to agglutinate in dilutions above 1:80 in nine out of thirteen specimens and in only one instance did agglutination take place in dilutions higher than 1:200. The sera from the x-rayed group was somewhat less potent than that from the controls but not strikingly so. From these results it would appear that the heat treatment not only gives rise to an increase in the amount of circulating antibodies but also increases the rate of production.

The protection test demonstrated quite as clearly the difference in the strength of the sera of the three groups. That from the x-rayed animals was not uniformly protective even in 0.1 cc. amounts, whereas the sera from the controls gave little protection when less than 0.01 cc. was used. On the other hand, 0.005 cc. of sera from heated rabbits uniformly protected the mice against infection, and as little as 0.001 cc. of eight out of the twelve specimens was found to be sufficient. Two of these sera gave complete protection even when so small an amount as 0.0005 cc. was injected with the infecting organism. In other words, the heat treatment effected a tenfold increase or an even greater one in the rabbits' ability to develop protective antibodies, whereas the x-ray treatment has inhibited the production to about an equal amount.


EXPERIMENT-4
PROTECTION TEST
X-rayed Group
Heated Group
Control Group
Rabbit No.
Amount serum
Amount culture
Days of survival
1 2 3 4 5 6 7
0.1CC.
0.05CC.
0.01CC.
0.005CC.
0.001CC.
0.001

TEXT-FIG. 2.


EXPERIMENT – 5
PROTECTION TEST
Heated Group
Rabbit No.
Amount serum
Amount culture
Days of survival
1 2 3 4 5 6 7
1 2 3 4 5 6 7
0.05cc.
0.01cc.
0.005cc.
0.001cc.
0.0005cc.
0.001
Control Group
Rabbit No.
Amount serum
Amount culture
Days of survival
1 2 3 4 5 6 7
8 9 10 11 12 13 14 15
0.05cc.
0.01cc.
0.005cc.
0.001cc.
0.001

TEXT-FIG. 3.

DISCUSSION.

The experiments here reported confirm and extend the observations of Hektoen and others that x-ray in large doses given before and during the immunizing process retards definitely the production of such antibodies as precipitins, bacterial agglutinins, and protective antibodies. The present work shows that x-ray will effect this retardation when given in such a manner as not to damage the bone marrow while seriously injuring the lymphoid tissue. The evidence pointing to the lymphoid tissue as the manufacturer of antibodies is strengthened by the further observation that an animal's ability to form such bodies may be greatly increased by the stimulation of the lymphoid tissues by dry heat.

There are not sufficient data at hand to justify final deductions from Gay and Clark's[10] observation that animals vitally stained with trypan blue develop antibodies with extreme slowness. This fact these authors interpret as evidence that the reticulo-endothelial system, which is principally affected by the dye, is the site of antibody formation. It is true that the endothelial cells of the lymphoid organs following destructive doses of x-ray are engorged with the remains of the lymphoid cells, but granted that such engorged cells are incapacitated for antibody formation, the total number of them in the lymphoid system represents such a minute fraction of the total number in the animal body that their incapacity would scarcely explain the results of our experiments. Furthermore, there is no evidence that dry heat affects endothelial cells in the slightest.

Gay and Clark make the point in favor of the reticulo-endothelial system as the source of antibodies that its wide distribution throughout the body would account for the phenomenon of local immunity. The same argument may be presented in favor of the lymphoid tissue, for small deposits of this tissue are scattered throughout the body and it is well known that the small local accumulations may be quickly augmented either by multiplication of the existing cells or by migration from the blood vessels.

While the lymphoid organs would seem to be concerned in the responses which we have detected in the course of the present work the

[10] Gay, F. P., and Clark, A. R., *J. Am. Med. Assn.*, 1924, lxxxiii, 1296.

possibility cannot be excluded that other organs and physiological processes are influenced by the x-ray and heat and affect the results. At the moment we have no way of settling this matter. With this limitation we can conclude that along with the manifest changes in the lymphoid organs, of depletion on the one and stimulation on the other hand, there are correlated decreases and increases in the production respectively of precipitating, agglutinating, and protecting antibodies.

SUMMARY.

Rabbits x-rayed in doses sufficient to reduce the amount of their lymphoid tissue without damage to the bone marrow showed a definite deficiency in the production of precipitins, bacterial agglutinins, and protective antibodies. On the other hand, rabbits subjected to exposures of dry heat sufficient to increase the activity of the lymphoid organs, on immunization develop antibodies in larger quantity than do untreated animals immunized by the same process.