STUDIES IN NON-SPECIFIC COMPLEMENT FIXATION *

IV. THE RELATION OF SERUM LIPOIDS AND PROTEINS TO NON-SPECIFIC COMPLEMENT FIXATION WITH NORMAL RABBIT AND DOG SERA

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Since ether and chloroform during anesthesia have been found to influence the substances in normal rabbit and dog sera responsible for absorbing complement in a non-specific manner,[1] probably through some influence on the serum lipoids, we have continued this investigation to determine more definitely the relation of these lipoids to the process by extractions of serum with lipoid solvents, as ether and chloroform, and by feeding and immunizing experiments with various lipoids.

Landsteiner and v. Eisler[2] found that blood corpuscles and serum contained lipoids that exerted antihemolytic action against serum hemolysins. They believed the action of these lipoids to be anti-amboceptoric, in the sense that they constitute the receptors, which in the intact corpuscles anchor the amboceptors. Bang and Forssmann[3] extracted ox corpuscles with ether and obtained hemolytic and antihemolytic substances, the latter being acetone-soluble and the insoluble residue containing the former. Noguchi[4] found the thermostabil anticomplementary principles of the blood to be closely identified with the serum lipoids, and by means of ether extracted a soluble fraction which was markedly antilytic for the susceptible blood corpuscles of different animals. A salt solution of this substance, to which Noguchi applied the name "protectin," was found to resist temperatures as high as 90 C. Zinsser and Johnson,[5] in a study of heat-sensitive, or thermolabil, anticomplementary bodies in human serum, extracted serum with ether, but were unable to extract the anticomplementary substances in this manner. On the other hand, according to them, the thermolabil body was removed by precipitation of the globulins.

Since in rabbit and dog sera the property of absorbing complement with various lipoidal and bacterial antigens may be due to sub-

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[1] Jour. Infect. Dis., 1916, 18, p. 32.
[2] Wien. klin. Wchnschr., 1904, 27, p. 676. Centralbl. f. Bakteriol., I, O., 1905, 39, p. 309.
[3] Centralbl. f. Bakteriol., I. O., 1905, 40, p. 150.
[4] Jour. Exper. Med., 1906, 8, p. 726.
[5] Ibid., 1911, 13, p. 31.

stances closely allied to those regarded as antihemolytic, we have studied the serum lipoids and proteins with the object of determining which contained the antilytic and complement-absorbing substances, the result of our work strongly indicating the more important role of lipoids in these processes.

I

THE RELATION OF SERUM LIPOIDS TO THE ANTILYTIC AND COMPLEMENT-FIXING POWERS OF NORMAL RABBIT AND DOG SERA

Briefly, the plan of study included antilytic and Wassermann tests with rabbit and dog sera in a fresh and active state, and after heating at 56 C. for half an hour, before and after extraction with ether and chloroform. Other lipoid solvents, as alcohol, acetone, and benzol, were employed, but these caused such pronounced physical changes of the serum that most of our work was conducted with ether as the solvent.

TECHNIC

Sera were extracted by diluting with 4 parts of sterile salt solution, adding 5 parts of ether, and shaking in a mechanical shaker for 2 hours. The mixtures were placed in a refrigerator over night; then the supernatant ether was pipetted off, and the serum filtered repeatedly through fat-free paper until all odor of ether had been removed. Each cubic centimeter of the serum residue represented 0.2 c.c. of the original amount, and this dose and fractions of it were regularly used in the antilytic and complement-fixation tests to determine comparative values. Sera extracted with chloroform, acetone, etc., required centrifugation, after which the diluted serum could be removed and filtered.

The Wassermann tests were conducted with 5 different antigens: an alcoholic extract of heart re-enforced with cholesterin; an alcoholic extract of syphilitic liver; an extract of acetone-insoluble lipoids of heart; an emulsion of staphylococci (human) and colon bacilli (human). The lipoidal tissue extracts were employed in doses varying from 6 to 12 times less than their anticomplementary doses, according to the extract and titrations; the bacterial antigens were titrated each day and used in amounts equal to one-fourth of their anticomplementary doses.

Complement was furnished by the mixed sera of at least 2 guinea-pigs and used in constant dosage of 0.05 c.c. (1 c.c. of 1:20 dilution). Antisheep amboceptor was titrated against this dose of complement and 1 c.c. of a 2.5% suspension of washed cells and used in the titrations, and in the antilytic, and Wassermann tests in an amount equal to 2 hemolytic units.

Antilytic tests were conducted by mixing serum and complement and incubating at 37 C. for an hour; then 1 c.c. of corpuscle suspension and 2 units of hemolysin were added; after re-incubation for an hour the results were read.

Complement-fixation tests were conducted by incubating together for one hour serum, antigen, and complement; corpuscles and 2 units of hemolysin were then added and re-incubation continued for 1 hour, after which the results were read.

ANTILYTIC ACTIVITY OF EXTRACTED SERA

The antilytic activity of dog and rabbit sera was determined with sera in a fresh or active condition, and again after heating at 56 C. for 30 minutes. Portions of each of these sera were extracted with ether after the method described, and the antilytic tests repeated with the unheated and heated serum residues. Portions of sera were also heated and then extracted, and antilytic tests conducted with the serum residues before and after reheating at 56 C.

TABLE 1

ANTILYTIC ACTIVITY OF PLAIN AND EXTRACTED DOG SERUM (DOG 538)

Amt. c.c.	Fresh Serum	Heated Serum	Extracted Serum, Not Heated	Extracted Serum, Heated	Heated Serum, Extracted, Not Reheated	Heated Serum, Extracted, Reheated
0.1	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	No hemolysis (strongly antilytic)	Complete hemolysis (not antilytic)	No hemolysis (strongly antilytic)	Complete hemolysis (not antilytic)
0.2	Complete hemolysis (not antilytic)	Marked hemolysis (slightly antilytic)	No hemolysis (strongly antilytic)	Complete hemolysis (not antilytic)	No hemolysis (strongly antilytic)	Complete hemolysis (not antilytic)
0.4	Complete hemolysis (not antilytic)	No hemolysis (strongly antilytic)	No hemolysis (strongly antilytic)	Marked hemolysis (slightly antilytic)	No hemolysis (strongly antilytic)	Marked hemolysis (slightly antilytic)
0.6	Complete hemolysis (not antilytic)	No hemolysis (strongly antilytic)	No hemolysis (strongly antilytic)	Marked hemolysis (slightly antilytic)	No hemolysis (strongly antilytic)	Slight hemolysis (moderately antilytic)
0.8	Complete hemolysis (not antilytic)	No hemolysis (strongly antilytic)	No hemolysis (strongly antilytic)	Slight hemolysis (moderately antilytic)	No hemolysis (strongly antilytic)	Slight hemolysis (moderately antilytic)
1.0	Complete hemolysis (not antilytic)	No hemolysis (strongly antilytic)	No hemolysis (strongly antilytic)	No hemolysis (strongly antilytic)	No hemolysis (strongly antilytic)	No hemolysis (strongly antilytic)

This plan of study was rendered necessary by the fact that antilytic and complement-absorbing powers are increased in rabbit and dog sera heated at temperatures between 55 and 60 C. for half an hour. This constitutes an important characteristic feature of these sera and one to which we have endeavored to draw particular attention both from practical and theoretical standpoints.

The results with a dog and a rabbit serum are shown in Tables 1 and 2; these are types of the results observed with similar titrations of 5 different dog, and 4 different rabbit sera.

Titrations of 3 dog, and 2 rabbit sera, after chloroform extractions, have yielded results similar to those obtained after extraction with ether. These results may be summarized as follows:

1. Heating normal rabbit and dog sera greatly increases the antilytic activity.

2. Sera extracted with ether and chloroform and tested at once are highly antilytic.

3. Sera extracted with ether and chloroform and then heated at 56 C. for 30 minutes usually possess a diminished antilytic activity.

TABLE 2

ANTILYTIC ACTIVITY OF PLAIN AND EXTRACTED RABBIT SERUM (RABBIT 65)

Amt. c.c.	Fresh Serum	Heated Serum	Extracted Serum, Not Heated	Extracted Serum, Heated	Heated Serum, Extracted, Not Reheated	Heated Serum, Extracted, Reheated
0.1	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	No hemolysis (strongly antilytic)	Complete hemolysis (not antilytic)	No hemolysis (strongly antilytic)	Complete hemolysis (not antilytic)
0.2	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	No hemolysis (strongly antilytic)	Complete hemolysis (not antilytic)	No hemolysis (strongly antilytic)	Complete hemolysis (not antilytic)
0.4	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	No hemolysis (strongly antilytic)	Complete hemolysis (not antilytic)	No hemolysis (strongly antilytic)	Complete hemolysis (not antilytic)
0.6	Complete hemolysis (not antilytic)	Marked hemolysis (slightly antilytic)	No hemolysis (strongly antilytic)	Marked hemolysis (slightly antilytic)	No hemolysis (strongly antilytic)	Marked hemolysis (slightly antilytic)
0.8	Complete hemolysis (not antilytic)	Slight hemolysis (moderately antilytic)	No hemolysis (strongly antilytic)	No hemolysis (strongly antilytic)	No hemolysis (strongly antilytic)	Marked hemolysis (slightly antilytic)
1.0	Complete hemolysis (not antilytic)	No hemolysis (strongly antilytic)	No hemolysis (strongly antilytic)	No hemolysis (strongly antilytic)	No hemolysis (strongly antilytic)	No hemolysis (strongly antilytic)

4. Sera first heated and then extracted with ether and chloroform are markedly antilytic; when the extracted sera are again heated, the antilytic activity is about the same as in fresh sera after extraction and heating.

5. According to the method of extraction employed, ether and chloroform extract from dog and rabbit sera a portion, but not all, of the antilytic substances, probably lipoidal in nature. Since extraction of fresh and that of heated sera yielded similar results, it is probable that the lipoid substances are unchanged by heating, in so far as

solubility in ether and chloroform are concerned, altho heating a serum results in increasing its antilytic activity either by changing the nature of the lipoidal or protein constituents or by the liberation of the antilysin from a combination with other bodies in normal serum.

COMPLEMENT-FIXATION TESTS WITH EXTRACTED SERA

Similar procedures were carried out with normal dog and rabbit sera, except that the tests were conducted with various antigens in place of antilysin titrations. In a number of instances both antilysin and complement-fixation tests were conducted with the same sera for purposes of comparison. The results are shown in Tables 3 and 4.

These results may be summarized as follows:

1. Fresh sera that are extracted with ether or chloroform without subsequent heating are so highly antilytic that complement-fixation reactions can not be conducted with them. This observation, which has been previously described in this paper, shows that extraction of sera with these substances increases the antilysin content of sera either by releasing the antilysin from combination with other serum constituents or by some alteration of the latter. These apparently newly formed antilysins are thermolabil; heating extracted sera removes a large portion of them.

2. The results obtained with chloroform extraction were similar to those observed with extraction by ether, except that chloroform tends to remove the antilysin either less completely or else alters the protein or other serum constituents in such manner as to increase antilytic activity.

3. Extraction of normal rabbit and dog sera with ether tends to remove a large portion of the serum constituents that are responsible for the complement fixation with non-specific lipoidal and bacterial antigens. At one time we thought that this could be developed into a practical method for overcoming the difficulty of non-specific complement fixation with these sera, but the results were somewhat too irregular, and furthermore, as will be shown later, the process of extraction tends to remove or destroy to some extent the specific amboceptors in serum.

4. Sera that are highly antilytic, as some of those shown in Table 4 (Dogs 1 and 2; Mules 1, 3, and 4), are slightly or not at all affected by extraction with ether. The same was true after extraction with chloroform, and it is probable that not all antilysins are lipoidal and

TABLE 3

RESULTS OF COMPLEMENT-FIXATION TESTS WITH ANIMAL SERA BEFORE AND AFTER EXTRACTION WITH ETHER AND HEATED AT 56 C. FOR 30 MINUTES

Amount of Serum in c.c.	Plain Heated Serum						Serum Extracted and Heated					
	Cholesterinized Alcoholic Extract of Heart	Alcoholic Extract of Syphilitic Liver	Extract Acetone Insoluble Lipoids of Heart	Staphylococci	Colon Bacilli	Serum Control	Cholesterinized Alcoholic Extract of Heart	Alcoholic Extract of Syphilitic Liver	Extract Acetone Insoluble Lipoids of Heart	Staphylococci	Colon Bacilli	Serum Control
	A. Normal Dog Serum						A. Normal Dog Serum					
0.01	—	—	—	—	—	—	—	—	—	—	—	—
0.05	—	—	—	+	+	—	—	—	—	—	—	—
0.1	+	—	—	++	++	—	—	—	—	—	—	—
0.15	++	+	—	+++	++	—	—	—	—	—	—	—
0.2	++	++	+	++++	++++	—	—	—	—	—	—	—
0.3	+++	++	+	++++	++++	±	±	—	—	+	+	—
	B. Normal Rabbit Serum						B. Normal Rabbit Serum					
0.01	—	—	—	+	+	—	—	—	—	—	—	—
0.05	—	—	—	+++	+++	—	—	—	—	—	—	—
0.1	+	—	—	++++	++++	—	—	—	—	—	—	—
0.15	++	+	+	++++	++++	—	—	—	—	+	+	—
0.2	+++	++	++	++++	++++	—	±	—	—	++	+++	—
0.3	++++	++	++	++++	++++	±	+	±	—	+++	+++	—

KEY TO TABLES 3, 4, 5, 6, 7, 8, 9 and 11

++++ = complete inhibition of hemolysis (strongly positive).
+++ = 75% inhibition (moderately positive).
++ = 50% inhibition (weakly positive).
+ = 25% inhibition (weakly positive).
± = less than 25% inhibition (doubtfully positive).
— = complete hemolysis (negative).

TABLE 4

RESULTS OF COMPLEMENT-FIXATION TESTS WITH DOG, RABBIT, MULE, AND HORSE SERA BEFORE AND AFTER EXTRACTION WITH ETHER AND HEATED AT 56 C. FOR 30 MINUTES

Serum	Heated Serum (0.2 c.c.)						Serum Extracted and Heated (0.2 c.c.)					
	Cholesterinized Alcoholic Extract of Heart	Alcoholic Extract of Syphilitic Liver	Extract Acetone Insoluble Lipoids of Heart	Staphylococci	Colon Bacilli	Serum Control	Cholesterinized Alcoholic Extract of Heart	Alcoholic Extract of Syphilitic Liver	Extract Acetone Insoluble Lipoids of Heart	Staphylococci	Colon Bacilli	Serum Control
Dog 1	++++	++++	++++	++++	++++	+++	++++	++++	++++	++++	++++	++
Dog 2	+++	++	++	++++	++++	±	+	+	+	+++	++	—
Dog 3	+++	+++	+++	++++	++++	—	++	++	++	+++	+++	—
Dog 4	++	+	+	+++	+++	—	+	±	±	+	++	—
Dog 5	++++	++++	++++	++++	++++	++	++++	++++	++++	++++	++++	++
Dog 66	+++	++	++	++++	++++	±	++	+	+	+++	++	—
Rabbit 1	++	++	++	+++	+++	—	+	+	±	++	++	—
Rabbit 2	+++	+++	+++	++++	++++	—	—	—	—	—	—	—
Rabbit 3	+++	+++	+++	++++	++++	—	—	—	—	—	—	—
Rabbit 4	++++	++++	++++	++++	++++	—	+	+	±	++	++	—
Rabbit 5	++++	—	—	++++	++++	—	—	—	—	—	—	—
Rabbit 6	++++	++	++	++++	++++	±	+	—	—	++	++	—
Mule 1	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Mule 2	+++	+++	+++	++++	++++	+	++	++	++	+++	+++	—
Mule 3	++++	++++	++++	++++	++++	++++	++	+++	+++	+++	+++	++
Mule 4	++++	++++	++++	++++	++++	++++	++++	+++	+++	++++	++++	+++
Horse 1	+++	++	++	+	++++	+	—	—	—	++	+++	—
Horse 2	+	+	+	±	+	±	—	—	—	—	—	—

soluble in these solvents, but that antilysins may be of other structure and identified with the protein constituents.

5. As shown in Table 5, sera may possess very slight or no antilytic power and yet absorb complement to a well-marked degree in the presence of a lipoidal antigen (cholesterinized alcoholic extract of heart) and an indifferent bacterial antigen (staphylococci from human lesions). As repeatedly stated, this is the most important factor in complement fixation with rabbit, dog, and mule sera, as the serum control tubes containing the maximal amount of serum may show complete hemolysis, and yet this amount of serum or less is capable of absorbing or fixing complement in a non-specific manner in the presence of various indifferent antigens.

TABLE 5

RESULTS OF ANTILYTIC AND COMPLEMENT-FIXATION TESTS WITH NORMAL DOG AND RABBIT SERA HEATED AT 56 C. FOR 30 MINUTES

Amount of Serum in c.c.	Dog 10			Dog 15			Rabbit 20		
		Complement Fixation			Complement Fixation			Complement Fixation	
	Antilytic Test	Cholesterinized Alcoholic Extract of Heart	Staphylococci	Antilytic Test	Cholesterinized Alcoholic Extract of Heart	Staphylococci	Antilytic Test	Cholesterinized Alcoholic Extract of Heart	Staphylococci
0.01	—	—	—	—	—	—	—	—	+
0.05	—	—	+	—	—	—	—	±	+++
0.1	—	+	++	—	±	—	—	+	++++
0.15	—	++	+++	—	+	—	—	++	++++
0.2	—	++	++++	—	++	—	—	+++	++++
0.3	±	++	++++	—	++	±	±	++++	++++

In this table the — and ± signs in the antilytic tests indicate "complete hemolysis" and "almost complete hemolysis," respectively.

6. As shown in Table 6 extraction of the sera of syphilitics (human) with ether and chloroform tends to remove thermostabil antilytic substances (Nos. 4, 5, and 8), as shown by Noguchi, and also to some extent the Wassermann antibody or reagin (Nos. 3, 6, and 8). This action, however, was not constant in either direction, as the antilytic power of some sera was slightly increased (Nos. 1 and 2) and the Wassermann reagin undisturbed (Nos. 1, 2, 4, and 5), as the result of extractions. When similar tests were made with the sera of glanderous mules with specific glanders antigen, the same tendency toward removal of specific amboceptors was noted. On the other hand, it may be that the removal of antilysin was responsible for the weaker complement fixation and that the specific amboceptors were not removed.

7. The tendency of ether and chloroform to remove in vitro the substances responsible for complement fixation with non-specific antigens is similar to the action of these substances in vivo when administered as anesthetics; for, as shown by Kolmer and Pearce, the tendency for normal rabbit and dog serum to yield these non-specific complement fixations is removed or decreased after the administration of ether and chloroform, probably as the result of a solvent action on the lipoid constituents of the serum.

TABLE 6

RESULTS OF COMPLEMENT-FIXATION TESTS WITH HUMAN WASSERMANN-POSITIVE SERA BEFORE AND AFTER EXTRACTION WITH ETHER AND CHLOROFORM AND HEATED AT 56 C. FOR 30 MINUTES (DOSE 0.2 C.C.)

No.	Plain Heated Serum				Ether Extracted and Heated				Chloroform Extracted and Heated			
	Cholesterinized Alcoholic Extract of Heart	Alcoholic Extract of Syphilitic Liver	Extract Acetone Insoluble Lipoids of Heart	Serum Control	Cholesterinized Alcoholic Extract of Heart	Alcoholic Extract of Syphilitic Liver	Extract Acetone Insoluble Lipoids of Heart	Serum Control	Cholesterinized Alcoholic Extract of Heart	Alcoholic Extract of Syphilitic Liver	Extract Acetone Insoluble Lipoids of Heart	Serum Control
1	++	+	+	—	+++	++	++	+	+++	++	++	+
2	++++	++++	++++	—	++++	++++	++++	±	++++	++++	++++	+
3	++++	+++	++++	—	+	+	+	—	—	—	—	—
4	++++	++++	++++	+	++++	++++	++++	—	++++	++++	++++	—
5	++++	+	++	—	++++	+	+	—	++++	±	±	—
6	++++	+++	++++	+	++	+	+	—	0	0	0	0
7	++++	++++	++++	—	+	+	+	±	0	0	0	0
8	++++	++++	++++	+	±	±	±	—	+	+	+	—

THE FEEDING OF LIPOIDS IN RELATION TO NON-SPECIFIC COMPLEMENT FIXATION

As our previous experiments indicated the important rôle of serum lipoids as antilysins and their responsibility in some degree for non-specific complement fixation, it appeared of interest to determine whether feeding lipoids to selected animals would cause the appearance in the blood serum of antilysins, as the high antilytic and complement-absorbing power of the sera of these animals may bear some relation to the diet. (This portion of the work was conducted with the co-operation of Dr. Richard M. Pearce, of the John Herr Musser Department of Research Medicine of the University of Pennsylvania.) Rabbits the sera of which repeatedly failed to fix complement with the various lipoidal antigens, were selected for these experiments.

Three lipoids were administered: (a) cholesterin isolated from human gallstones and Merck's preparation in doses of 0.5 to 0.13 gram; (b) Merck's lecithin in dosage of 0.5 to 0.13 gram; (c) glymol (a purified paraffin oil) in dosage of 10 c.c. These lipoids were administered in capsule, and by the stomach tube, and in one series were injected in the form of an emulsion or were placed under the skin.

The antigens employed in the complement-fixation tests consisted of the 3 usual lipoidal extracts—cholesterinized heart extract, alcoholic extract of syphilitic liver, an extract of acetone-insoluble lipoids of heart muscles—and solutions of cholesterin (0.4%), lecithin (Merck), (0.5%), and glymol (10%) in absolute ethyl alcohol. These 3 preparations were diluted with normal salt solution, repeatedly titrated, and used in amounts equal to one-quarter of their antilytic dose. The lecithin solution was especially difficult to titrate because of the marked hemolytic properties of the preparation.

The complement-fixation tests were conducted with the same technic as previously described.

The results of these experiments are shown in Tables 7, 8 and 9, and may be summarized as follows:

1. The feeding of cholesterin, lecithin, and glymol to normal rabbits appears to be followed by an increased power of the serum for the absorption or inhibition of complement. This was especially evident after the feeding of lecithin and much less so with glymol.

2. This increased power of the serum for complement absorption or inhibition was in evidence practically only with heated serum. No changes were apparent when fresh, unheated serum was used.

3. The increased power of the sera for complement absorption or inhibition was apparent within 48 hours after the administration of the lipoids; after several days the sera returned to their former states.

4. The degree of complement absorption was highest with the antigen of lecithin, next highest with that of cholesterin, and least with that of glymol. Specific reactions were not observed; that is, the administration of cholesterin was followed by an increased power for the absorption of complement not only with an antigen of cholesterin, but likewise with that of lecithin. The stronger reactions with the antigens of cholesterin, lecithin, and glymol may be the result of using these in doses equal to one-fourth of their anticomplementary doses,

TABLE 7

RESULTS OF COMPLEMENT-FIXATION TESTS WITH THE SERA OF NORMAL RABBITS BEFORE AND AFTER THE ADMINISTRATION OF CHOLESTERIN

Rabbit	Cholesterin	Active Serum (0.2 c.c.)							Inactivated Serum (0.2 c.c.)						
		Cholesterinized Alcoholic Extract of Heart	Alcoholic Extract of Syphilitic Liver	Extract Acetone Insoluble Lipoids of Heart	Cholesterin	Lecithin	Glymol	Serum Control	Cholesterinized Alcoholic Extract of Heart	Alcoholic Extract of Syphilitic Liver	Extract Acetone Insoluble Lipoids of Heart	Cholesterin	Lecithin	Glymol	Serum Control
25	11 days before	—	—	—	0	0	0	—	—	—	—	0	0	0	—
25	8 days before	—	—	—	0	0	0	—	—	—	—	0	0	0	—
25	2 days before	—	—	—	—	—	—	—	—	—	—	—	—	—	—
25	0.5 gm. cholesterin (stomach)														
25	2 days later	—	—	—	—	—	—	—	—	—	±	++	+++	+	—
25	7 days later	—	—	—	—	—	—	—	—	—	—	+	+	—	—
25	0.5 gm. cholesterin (stomach)														
25	2 days later	—	—	—	—	—	—	—	—	—	—	++	++	—	—
26	11 days before	—	—	—	0	0	0	—	—	—	—	0	0	0	—
26	8 days before	—	—	—	0	0	0	—	—	—	—	0	0	0	—
26	2 days before	—	—	—	—	—	—	—	—	—	—	—	—	—	—
26	0.5 gm. cholesterin (stomach)														
26	2 days later	—	—	—	—	—	—	—	—	—	—	++	++	—	—
26	7 days later	—	—	—	—	—	—	—	—	—	—	±	+	—	—
26	0.5 gm. cholesterin (stomach)														
26	2 days later	—	—	—	—	—	—	—	—	—	—	++	++	—	—
83	5 days before	—	—	—	—	—	—	—	—	—	—	—	—	—	—
83	2 days before	—	—	—	—	—	—	—	—	—	—	—	—	—	—
83	0.13 gm. cholesterin (stomach)														
83	1 day later	—	—	—	—	—	—	—	—	—	—	—	—	—	—
65	2 days before	—	—	—	—	—	—	—	+	+	+	+	++	+	—
65	0.13 gm. cholesterin (subcutaneously)														
65	1 day later	—	—	—	—	—	—	—	+++	++	+++	+++	++++	++	—
65	3 days later	—	—	—	—	—	—	—	+	+	+	+	++++	+	—
65	4 days later	—	—	—	—	—	—	—	+	+	+	+	++++	+	—

TABLE 8

RESULTS OF COMPLEMENT-FIXATION TESTS WITH THE SERA OF NORMAL RABBITS BEFORE AND AFTER THE ADMINISTRATION OF LECITHIN

Rabbit	Lecithin	Active Serum (0.2 c.c.)							Inactivated Serum (0.2 c.c.)						
		Cholesterinized Alcoholic Extract of Heart	Alcoholic Extract of Syphilitic Liver	Extract Acetone Insoluble Lipoids of Heart	Cholesterin	Lecithin	Glymol	Serum Control	Cholesterinized Alcoholic Extract of Heart	Alcoholic Extract of Syphilitic Liver	Extract Acetone Insoluble Lipoids of Heart	Cholesterin	Lecithin	Glymol	Serum Control
36	11 days before	—	—	—	0	0	0	—	—	—	—	0	0	0	—
36	8 days before	—	—	—	0	0	0	—	—	—	—	0	0	0	—
36	2 days before	—	—	—	—	—	—	—	—	—	—	—	—	—	—
36	0.5 gm. lecithin (stomach)														
36	2 days later	—	—	—	—	—	—	—	—	—	—	+	+	—	—
36	7 days later	—	—	—	—	—	—	—	—	—	—	+	±	—	—
36	0.5 gm. lecithin (stomach)														
36	2 days later	—	—	—	—	—	—	—	+	+	—	++	++	—	—
29	11 days before	—	—	—	0	0	0	—	—	—	—	0	0	0	—
29	8 days before	—	—	—	0	0	0	—	—	—	—	0	0	0	—
29	2 days before	—	—	—	—	—	—	—	—	—	—	—	—	—	—
29	0.5 gm. lecithin (stomach)														
29	2 days later	—	—	—	—	—	—	—	+	+	—	++	++	—	—
29	7 days later	—	—	—	+	—	—	—	—	—	—	+	+	—	—
29	0.5 gm. lecithin (stomach)														
29	2 days later	—	—	—	—	+	—	—	+	+	—	++	++	—	—
68	5 days before	—	—	—	—	—	—	—	—	—	—	—	±	—	—
68	2 days before	—	—	—	—	—	—	—	—	—	—	—	±	—	—
68	0.13 gm. lecithin (stomach)														
68	1 day later	—	—	—	—	—	—	—	—	—	—	—	±	—	—
68	3 days later	—	—	—	—	—	—	—	+	+	+	+	++++	+	—
68	4 days later	—	—	—	—	—	—	—	+	+	+	+	++++	+	—
68	7 days later	—	—	—	—	—	—	—	—	—	—	—	+++	—	—
69	5 days before	—	—	—	—	—	—	—	—	—	—	—	++	—	—
69	2 days before	—	—	—	—	—	—	—	—	—	—	—	++	—	—
69	0.13 gm. lecithin (subcutaneously)														
69	1 day later	—	—	—	—	—	—	—	—	—	—	—	++	—	—
69	3 days later	—	—	—	—	—	—	—	—	—	—	—	++++	—	—
69	4 days later (died)	—	—	—	—	—	—	—	—	—	—	—	++++	—	—

TABLE 9

RESULTS OF COMPLEMENT-FIXATION TESTS WITH THE SERA OF NORMAL RABBITS BEFORE AND AFTER THE ADMINISTRATION OF GLYMOL

Rabbit	Glymol	Active Serum (0.2 c.c.)							Inactivated Serum (0.2 c.c.)						
		Cholesterinized Alcoholic Extract of Heart	Alcoholic Extract of Syphilitic Liver	Extract Acetone Insoluble Lipoids of Heart	Cholesterin	Lecithin	Glymol	Serum Control	Cholesterinized Alcoholic Extract of Heart	Alcoholic Extract of Syphilitic Liver	Extract Acetone Insoluble Lipoids of Heart	Cholesterin	Lecithin	Glymol	Serum Control
38	11 days before	—	—	—	0	0	0	—	—	—	—	0	0	0	—
38	8 days before	—	—	—	0	0	0	—	—	—	—	0	0	0	—
38	2 days before	—	—	—	—	—	—	—	—	—	—	—	—	—	—
38	10 c.c. glymol (stomach)														
38	2 days later	—	—	—	—	—	—	—	—	—	—	—	—	—	—
38	7 days later	—	—	—	—	—	—	—	—	—	—	—	—	—	—
38	15 days before	—	—	—	0	0	0	—	—	—	—	0	0	0	—
38	7 days before	—	—	—	0	0	0	—	—	—	—	0	0	0	—
38	10 c.c. glymol (stomach)														
38	2 days later	—	—	—	—	—	—	—	+	+	—	++	++	—	—
66	5 days before	—	—	—	—	—	—	—	—	—	—	—	++	—	—
66	2 days before	—	—	—	—	—	—	—	—	—	—	—	++	—	—
66	5 c.c. glymol (stomach)														
66	1 day later	—	—	—	—	—	—	—	—	—	—	—	++++	—	—
66	3 days later	—	—	—	—	—	—	—	+	+	+	+	++++	+	—
66	4 days later	—	—	—	—	—	—	—	+	+	+	+	++++	+	—
67	5 days before	—	—	—	—	—	—	—	—	—	—	—	+	—	—
67	2 days before	—	—	—	—	—	—	—	—	—	—	—	+	—	—
67	5 c.c. glymol (subcutaneously)														
67	1 day later	—	—	—	—	—	—	—	—	—	—	—	+	—	—
67	3 days later (died)	—	—	—	—	—	—	—	+	+	+	+	++++	+	—

whereas the regular Wassermann antigens were used in doses equal to one-sixth to one-twelfth of their anticomplementary doses.

5. The subcutaneous administration of cholesterin, lecithin, and glymol was followed by slow absorption and with less influence upon the serum reactions.

Several of these sera were extracted with ether and the complement-fixation tests repeated with the antigens after heating the sera at 56 C. for half an hour. In practically all instances the complement-absorbing power of the serum was reduced, as a result, apparently, of the removal of ether-soluble substances.

These experiments indicate, therefore, that following the feeding of lipoids some absorption occurs, resulting in an increase of the complement-absorbing power of the serum and further indicating the rôle that serum lipoids play in the process.

II

THE RELATION OF THE SERUM PROTEINS TO THE ANTILYTIC AND NON-SPECIFIC COMPLEMENT-FIXATION POWERS OF NORMAL RABBIT AND DOG SERA

Since in our experiments the extraction of lipoids from normal rabbit and dog sera did not serve to remove all the substances responsible for the antilytic and non-specific complement-fixation reactions, it is probable that other serum constituents are also concerned. The proteins of the serum may act in this capacity, since it is well known that solutions of proteins in sufficient concentration possess antilytic or anticomplementary properties. My particular object was to ascertain, if possible, whether these properties were contained in the globulin or in the albumin fractions, or in both, particularly since, according to Zinsser and Johnson, the globulin fraction of human serum contains the thermolabil anticomplementary substances, while Noguchi has identified the thermostabil anticomplementary substances of human serum with the serum lipoids.

Two specimens each of normal rabbit and dog sera were fractionated and the antilytic and complement-fixing powers determined. Five cubic centimeters of fresh serum were diluted with 45 c.c. of sterile normal salt solution, and an equal amount (50 c.c.) of a saturated solution of ammonium sulfate was added. The precipitated globulins were removed by repeated filtration through 5 thicknesses of fine filter paper. All the precipitate on the filters was then dissolved in 50 c.c. of normal salt solution, containing 0.25% tricresol, and the solution dialyzed against running water for 72 hours in order to remove the ammonium

sulfate; practically all the globulins were removed, and, when they were redissolved in the same bulk, 1 c.c. represented very closely the globulins contained in 0.1 c.c. of undiluted serum.

TABLE 10

RESULTS OF ANTILYTIC TESTS WITH FRESH, HEATED, AND ETHER-EXTRACTED SERA OF A NORMAL DOG AND THE GLOBULIN AND ALBUMIN FRACTIONS OF THESE

Dose c.c.	Fresh Serum	Heated Serum	Ether Extracted Serum	Globulin of Fresh Serum	Globulin of Heated Serum	Globulin of Ether Extracted Serum	Filtrate of Fresh Serum	Filtrate of Heated Serum	Filtrate of Ether Extracted Serum
.05 (.005)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)
.1 (.01)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)
.2 (.02)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)
.5 (.05)	Complete hemolysis (not antilytic)	Marked hemolysis (slightly antilytic)	Complete hemolysis (not antilytic)	Marked hemolysis (slightly antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)	Complete hemolysis (not antilytic)
1.0 (0.1)	Marked hemolysis (slightly antilytic)	No hemolysis (strongly antilytic)	Complete hemolysis (not antilytic)	Slight hemolysis (moderately antilytic)	Marked hemolysis (slightly antilytic)	Complete hemol- (not antilytic)	Marked hemolysis (slightly antilytic)	Complete hemol- (not antilytic)	Complete hemol- (not antilytic)
2.0 (0.2)	Slight hemolysis (moderately antilytic)	No hemolysis (strongly antilytic)	Marked hemolysis (slightly antilytic)	No hemolysis (strongly antilytic)	Marked hemolysis (slightly antilytic)	Marked hemolysis (slightly antilytic)	No hemolysis (strongly antilytic)	Marked hemolysis (slightly antilytic)	Comhemolysis (slightly antilytic)

Tricresol to 0.25% was added to the filtrate to prevent bacterial growth during dialyzation, and the whole dialyzed against running water for 72 hours. One cubic centimeter of the filtrate represented 0.1 c.c. of undiluted serum.

After this period of dialyzation practically all the ammonium sulfate had been removed as determined by tests with barium chlorid. In both the globulin

solutions and filtrates, however, traces remained. Several solutions became cloudy and showed a small amount of precipitate. These were filtered before being used in the hemolytic tests. As all the sodium chlorid had dialyzed, each solution was made isotonic (0.85) by the addition of sodium chlorid.

Several controls composed of equal parts of ammonium sulfate and salt solution were dialyzed at the same time. This solution possesses marked antilytic properties, but after dialyzation the minute trace of the remaining salt had no effect in doses ranging from 0.05 to 2 c.c.

The antilytic titer of each whole serum (diluted 1:10), filtrate, and solution of globulins was determined by titrating in 6 doses; namely, 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 c.c., corresponding, respectively, to 0.005, 0.01, 0.02, 0.05, 0.1, and 0.2 c.c. of undiluted serum.

Complement-fixation tests were conducted with these doses, with as antigen an alcoholic extract of beef heart re-enforced with cholesterin in dose equal to one-twelfth of its anticomplementary dose.

These tests were conducted with unheated serum, filtrates, and globulins, and again after these had been heated at 56 C. for half an hour. The antilytic and complement-fixing values were lower with the heated than with the unheated solutions, presumably on account of the removal of thermolabil anticomplementary substances which had developed during the process of dialyzation.

TABLE 11

RESULTS OF COMPLEMENT-FIXATION TESTS WITH FRESH, HEATED AND ETHER-EXTRACTED SERA OF A NORMAL DOG AND THE GLOBULIN AND ALBUMIN FRACTIONS OF THESE

Dose c.c.	Fresh Serum	Heated Serum	Ether Extracted Serum	Globulin of Fresh Serum	Globulin of Heated Serum	Globulin of Ether Extracted Serum	Filtrate of Fresh Serum	Filtrate of Heated Serum	Filtrate of Ether Extracted Serum
.05 (.005)	—	—	—	—	—	—	—	—	—
.1 (.01)	±	+	—	—	—	—	—	—	—
.2 (.02)	++	+++	—	—	—	—	—	—	—
.5 (.05)	+++	++++	—	+	—	—	—	—	—
1.0 (0.1)	++++	++++	++	++	+	—	++	++	—
2.0 (0.2)	++++	++++	+++	++++	++	+++	++++	++	++

With each of 2 rabbit and 2 dog sera the antilytic and complement-fixing values of each of the following were determined:

1. Fresh unheated serum.
2. Serum heated at 56 C. for half an hour.
3. Serum extracted with ether, according to the method previously described, and heated.
4. The globulin and filtrate of fresh serum.
5. The globulin and filtrate of heated serum.
6. The globulin and filtrate of ether-extracted serum.

The results observed with one of the dog sera are shown in Tables 10 and 11, and these illustrate the results obtained with the other three sera. They may be summarized as follows:

1. Heating the sera at 56 C. for half an hour increases the antilytic and complement-fixing powers.

2. Extraction with ether serves to remove a large percentage of the antilytic and complement-fixing substances.

3. Both the globulin and albumin (filtrate) portions of rabbit and dog sera possess antilytic and complement-fixing properties. In general, the globulin fractions show these properties to a slightly greater degree.

4. The antilysins contained in the globulin and albumin (filtrate) fractions are thermostabil.

5. According to these results I must conclude that the protein constituents of serum represent a portion of the antilytic and complement-fixing substances in normal rabbit and dog sera and that both the globulin and albumin fractions are concerned, the former to a slightly greater degree.

ANTILYSINS NOT DIALYZABLE

Two rabbit and two dog sera were diluted 1:10 with normal salt solution, 0.25% tricresol added, and the whole dialyzed against running water for 72 hours. Antilytic and complement-fixation tests with these sera compared with similar tests with non-dialyzed sera showed that none of the antilytic substances had been removed; indeed, the dialyzed sera were slightly more antilytic.

CONCLUSIONS

Both the serum lipoids and proteins are concerned in the antilytic and non-specific complement-fixation reactions with normal rabbit and dog sera.

The rôle of the serum lipoids in these processes is indicated in the observation that extraction of suitable sera with ether and chloroform usually diminished the antilytic and complement-fixing powers of a serum, whereas the enteral and parenteral administration of lipoids increased the antilytic and complement-fixing powers.

Sera that are extracted with ether are rendered primarily more antilytic; after heating an extracted serum the antilytic titer is generally reduced, as compared with the titer of plain heated serum.

Both the globulin and albumin (filtrate) fractions of normal rabbit and dog sera possess thermostabil antilytic and complement-fixing properties, usually the former to a slightly greater degree.

The antilytic and complement-fixing substances of normal rabbit and dog sera are not dialyzable.