STUDIES ON THE BLOOD PROTEINS.

I. The Serum Globulins in Bacterial Infection and Immunity.

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INTRODUCTION.

For a number of years much study has been devoted to the origin and the chemical nature of the antibodies which may develop within the organism during the course of an infection, or which may be elaborated within it by the various methods of immunization. The efforts to establish the chemical identity of antibodies have naturally been centered about a study of the possible relationship subsisting between the proteins of the blood and the immune bodies demonstrable in it by various serologic tests. A great stimulus to these investigations has come from the discovery of new methods of separating and of chemically identifying the different fractions which go to make up the blood proteins. Of these additions to our knowledge the method, introduced by the Hofmeister school, of separating the various protein constituents by fractional precipitation with different salts has, perhaps, produced the most far reaching results.

For some time it has been a well established fact that diphtheria antitoxin, for instance, is precipitable from serum by any precipitants which throw down the globulins. The early observations of Brodie (1), Seng (2), and Hiss and Atkinson (3) have been confirmed and extended by a number of later workers (4).

Considerable work has been done also to establish the chemical nature of bacterial antibodies. Probably one of the earliest contributions to this subject was made by Pfeiffer and Proskauer (5), who separated cholera immune serum into its globulin and albumin fractions, and showed that the cholera immune bodies which give rise to Pfeiffer's phenomenon are present only in the globulin fraction. This important study was later amplified by the classical experiments of Pick (6), who demonstrated conclusively that cholera and typhoid agglutinins also occur in one or another of the globulin fractions depending upon the species

of animal employed for immunization and the nature of the antibody studied. To these observations should be added those of Rodhain (7) and of Moll (8). According to the work of Rodhain, the immune bodies of antistreptococcus serum occur in the euglobulin fraction; and according to Moll, the development of precipitins following immunization with a foreign protein is also associated with a rise in the serum globulins of the immunized animal.

The above observations have been variously interpreted by different investigators. Whereas some workers strongly incline to the view that the antibodies in question are a form of blood globulin, others entertain the possibility that the antibody, by analogy with bacterial poisons, enzymes, and similar bodies, is mechanically carried down by the precipitate of globulin.

We believed that the only satisfactory method of procuring reliable data on the globulin-antibody problem was to make quantitative estimations of the immune bodies and of the blood proteins, not at random periods during the experiment, but at frequent and well timed intervals during the process of immunization. In this way alone is it possible to determine whether an increase in the antibodies and in the globulins parallels one another, or whether either the globulin content or the concentration of immune bodies may increase independently of one another.

Methods.

Healthy Belgian hares kept under constant conditions of diet and activity were used for all the experiments. The animals were fed once daily, the diet consisting of alfalfa hay, toasted bread, oats, and at times small amounts of cabbage. All were allowed a liberal amount of water.

Obtaining Blood.—Specimens of blood, varying in amounts from 5 to 7 cc. were obtained from fresh incisions made in the ear vein. In all instances the use of local applications of xylol or other substances that might cause stasis was avoided. At times the presence of a low blood pressure made bleeding difficult, but in such cases a free flow of blood was obtained by suspending the rabbit by its hind legs. The blood obtained in small sterile tubes was immediately centrifugalized in order to obtain a clear serum. Separation of the serum from the clot was effected as soon as possible, for as will be shown later, serum allowed to remain in contact with the clot is not suitable for accurate determinations of the proteins. The specimens before use were kept in sterile, stoppered vials in the refrigerator.

Tests for Agglutination.—The antigen used for the agglutination tests consisted of a 24 hour carbolized or formalinized Liebig's or rabbit broth cul-

ture, which had been properly controlled by tests with immune sera of known antibody content. To a series of tubes containing the clear, untreated serum in amounts ranging from 0.1 cc. to 0.00005 cc. was added 1 cc. of antigen. After incubating the mixtures for 2 hours at 37° C. or at room temperature for 12 hours readings were made. The final readings were always made after the lapse of 12 or 14 hours. The highest dilution of the serum in which complete agglutination occurred was taken to represent the agglutination titer of that serum, and only these readings are recorded in the tables.

Tests for Complement Fixation. Antigens.—Cultures of the various organisms grown upon lemco broth[1] for 18 to 20 hours[2] were found to be most suitable for this purpose. They were killed by heating for ½ hour at 60°C., and preserved with 0.5 per cent carbolic acid and 1 per cent glycerol. When kept in the refrigerator and protected from the light such antigens may be ready for use after a period of 2 months. Some, however, may become anticomplementary after a period of 4 weeks, and these must be discarded.

The dose of antigen employed was four times the antigenic unit as determined by preliminary titrations of the antigen with a standard amount of immune serum; either 0.1 or 0.2 cc. This dose was at least one-quarter to one-fifth of the anticomplementary unit as determined by repeated titrations. The range of the specific antigenic properties of each antigen was further controlled by tests with sera of known antibody content.

Sera.—Dilutions of inactivated serum (62°C. for 30 minutes) in descending doses from 0.2 to 0.003 cc. were used. In selecting such dilutions it frequently occurred that the gradations were not well chosen. This made it difficult to express the results absolutely in terms of the highest dilution of serum which gave definite fixation. For this reason the signs >< are employed. Thus the notation > 0.005 indicates that complete fixation of the complement would probably have occurred in a serum dilution of 0.004 or 0.003 cc., since in a dilution of 0.002 cc. of the serum only 50 per cent fixation was obtained. In the tables only those dilutions of the serum are recorded which with the proper dose of the antigen caused a complete fixation of the complement.

Hemolytic System.—The anti-sheep hemolytic system was used. Complement was furnished by the pooled sera of several guinea pigs, and was employed in a dosage of 0.05 cc. of a dilution of 1 to 4 in salt solution. The red corpuscles were used in 1 per cent suspension. The hemolytic unit was determined by a preliminary test using 0.05 cc. of complement and 0.5 cc. of a 1 per cent suspension of fresh sheep cells. In the titrations of the antigens as well as for the actual complement fixation tests two hemolytic units (about 0.2 to 0.1 cc. of a dilution of 1 to 100 in saline solution) were employed.

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[1] Eyre, J. W. H., The Elements of Bacteriological Technique; a Laboratory Guide, Philadelphia and London, 2nd edition, 1913, 163.

[2] Cultures less than 18 hours old are frequently inactive, and those older than 24 hours may be anticomplementary in doses of 0.5 cc.

Technique.—Preliminary tests were first made to rule out any anticomplementary activity of the different antigens used. These were carried out as follows: To each of a series of tubes containing decreasing doses of antigen diluted with 1.5 cc. of isotonic salt solution was added 0.05 cc. of complement in a dilution of 1 to 4. The tubes were then incubated at 37° C. either in a water bath for ½ hour or in an incubator for 1 hour. To each tube was then added a previously prepared mixture of two units of hemolysin and 0.5 cc. of the corpuscle suspension. After mixing and incubating for 1 or 2 hours, sedimentation of the red cells was hastened by placing the tubes in the refrigerator so as to make the readings more precise.

After determining the dosage of antigen to be used in the final test, the latter is carried out in the following manner: To each of a series of tubes containing the inactivated serum in descending doses is added 0.05 cc. of complement in 1.5 cc. of saline solution followed by the proper dose of antigen. After incubation for a period of ½ to 1 hour, the sensitized corpuscle suspension is added to each tube in the dosage already given, the mixture reincubated for 1 or 2 hours, and the readings are made as already indicated.

The customary controls for the serum, antigen, and hemolytic system were employed.

Non-specific fixations of the complement by rabbit sera, so frequently observed with bacterial antigens, must, of course, be kept in mind. Such a possibility, however, was ruled out by careful preliminary tests of the serum before immunization with numerous antigens, a procedure recommended by Kolmer and Trist (9). For various reasons it was not possible to select only those rabbits whose sera showed at the outset negative reactions. But we consider that this is unnecessary in serial studies, inasmuch as non-specific fixation does not interfere with specific deviations of the complement due to the presence of immune bodies.

Tests for Antistaphylolysin.—The staphylolysin used in the tests was prepared from a recently isolated strain of *Staphylococcus aureus* grown on a medium having an ionization equal to the value $P_H^+ = 7.7$. Two units of this hemotoxin suspended in isotonic salt solution were added to a series of test-tubes containing the inactivated serum in descending doses. The total volume was now made up to 2 cc., and the mixtures were incubated for 15 minutes in a water bath at 37° C. To each tube was then added 0.05 cc. of a suspension of red blood corpuscles prepared by washing the cells and adding an amount of saline solution equal to the original blood volume. The mixtures were now incubated for 1 hour. The readings were made at the end of 2 hours and again after 12 hours, the figures in the tables indicating the dilutions of serum in which the lytic activity of the staphylohemotoxin was completely inhibited.

Quantitation of Serum Proteins.—Those who have heretofore studied the problem of the relationship of the blood proteins to immunity have in the main obtained their data by precipitation of the globulins and the subsequent Kjeldahl determinations of the nitrogen contained in the precipitate and in the coagu-

lated proteins of the whole serum. Other workers have resorted to the less accurate method of weighing the precipitates. Neither of these procedures is applicable to a systematic study requiring frequent observations upon small animals because of the need of large quantities of blood and the time-consuming character of these procedures.

All the determinations of the albumin, globulin, and non-protein constituents in the blood of the animals experimented upon by us were made by the micro-refractometric method of Robertson (10). As the author has shown in numerous publications, the results obtained by this method are in accord with those obtained by the older methods, and the procedure possesses the important advantages of being less laborious and of being applicable to small quantities of serum.

In brief the method is as follows:[3] Blood is collected in centrifuge tubes, allowed to clot, and centrifugalized to obtain a clear serum. The blood should be obtained before a feeding, since lipemic sera are read with more difficulty. Furthermore, the serum should not be allowed to remain in contact with the clot for any length of time, nor should bacterial contamination be permitted, especially if it is desired to keep the serum for 24 or 48 hours before analyzing it.

By actual experiment we became assured that serum, and more particularly immune serum, may dissolve out substances from the clot which may considerably alter its refractive index. It has been found, for instance, that after 48 hours a sterile immune serum[4] kept in contact with the clot at low temperatures already showed a reduction in the protein quotient. On the other hand, the clear serum, immediately separated from the clot and kept under similar conditions, showed no marked changes after a period of 72 hours. A little over 1.5 cc. of serum is sufficient for the determination of the four fractions.

The tests are carried out in glass tubes having an inside diameter of about 5 mm. and walls about 1 mm. thick. These are sealed at one end.

For the determination of the albumin and globulin, 0.5 cc. of a saturated solution of ammonium sulphate is introduced with the aid of a graduated pipette into one of the tubes, about 10 cm. in length. With the same pipette, which has been cleaned by washing with water, alcohol, and ether, and dried by passing through it a stream of cold air, is added the same amount of clear serum. For purposes of mixing, a piece of silver wire is dropped into the tube, a stopper—consisting of a piece of sealed glass tubing inserted into a piece of rubber tubing—is affixed and the mixture of serum and sulphate is now shaken thoroughly. The precipitate of globulin is sedimented by centrifugalization, the clear fluid is diluted with a graduated pipette to one-half and its refractive index determined.

[3] For details concerning the various steps in the method, and for a discussion of the reasons for them and of the manner of calculating the results, reference should be made to Robertson (10).

[4] This immune serum exhibited a high antibody content. On May 8 the serum agglutinated in a dilution of 1:4,000, and fixed the complement in a dilution of 0.001 cc. of serum.

This reading corrected for the ammonium sulphate gives the total albumin plus the non-protein.

The non-protein value is determined by mixing in a similar glass tube 0.5 or 1 cc. of the clear serum with an equal volume of 0.04 N acetic acid solution. A short piece of silver wire is now dropped into the tube, the upper end is sealed off in the flame, the mixture shaken, and coagulated by placing the tubes in a beaker of water heated to boiling for several minutes. This precipitate also is sedimented by centrifugalizing and the refractive index of the clear supernatant fluid is determined.

Lastly the refractive index of the whole serum is determined. From this reading the refractive index of the total globulin is obtained by subtracting the refractive reading of the albumin from that of the whole serum after deducting the value of the non-proteins.

The readings were made with a Pulfrich refractometer, and the calculations of the percentages of the various constituents were carried out in the manner presented in detail by Robertson. The results are expressed not only in percentage but also in the per cent of total protein. For purposes of graphic presentation, it was thought well to express the ratio of albumin to globulin in the form of a quotient. This was obtained by dividing the percentage of albumin by that of globulin. Thus a fall in the quotient would indicate a rise in the blood globulins, and *vice versa*.

From a large series of determinations, numbering several hundred, we have become convinced of the accuracy of this method, provided the sources of error are understood and proper care is exercised in the manipulations. The method is especially recommended on account of the rapidity with which the determinations can be made and the small quantities of serum required.

EXPERIMENTAL.

Observations on the serum proteins were made in normal, infected, immunized, and hyperimmunized animals. With the exception of some of the infected animals, a parallel study was made also of the degree of immunity present during different periods of the experiment. As a typical example of an acute infection, staphylococcus pyemia was chosen. Infections with the tubercle bacillus and with sporothrix were selected as types of chronic infections.

For purposes of immunization living and killed cultures of *Bacillus typhosus* and *Bacillus dysenteriæ* (Shiga) and *Staphylococcus pyogenes aureus* were used. In addition to the classical method of immunization, a study was made also of the effect of massive inoculations in normal and immune animals upon the albumin-globulin ratio and

upon antibody formation. To these were added several observations upon the changes produced in the serum proteins by the inoculation of bacterial endotoxins and inflammatory irritants.

Serum Proteins of Normal Rabbits.

Observations on the serum proteins were made on a dozen normal rabbits kept under constant conditions of diet and activity. Notwithstanding the constancy of the conditions, it is apparent that individual animals may show considerable variations in the percentage of the serum proteins. Thus the total proteins may vary from 5 to 7 per cent. The albumin fraction may show fluctuations from 3.1 to 5.5 per cent, and the globulins from 0.8 to 2.7 per cent. But the averages of all the readings yield values which are in fair accord with those of other workers (11), especially in so far as the albumin-globulin ratio is concerned. Such fluctuations as have been observed in the protein quotient in normal animals are small in comparison with the marked diminution in the quotient which has been noted in the pathological conditions studied. Whereas the quotient in normal animals averaged 3.5, and in most instances did not fall below 1.5, infected and immunized animals have at one period or another shown a quotient below 1.0. Furthermore, it is essential in all these experiments to determine the percentages of the serum proteins existing in the normal animal before proceeding to determine the variations which may follow the establishment of a pathological condition.

Infection.

Experiment 1. Infection. Pyemia.—Two rabbits were inoculated intravenously with potato cultures of *Staphylococcus pyogenes aureus*. One of the animals (Rabbit 1) received on February 17 two loopfuls of a culture, and died 8 days later of a bilateral fibrinous pleurisy, and abscesses in the lungs and kidneys. The second animal (Rabbit 2) was inoculated on March 2 with only one loopful of a potato culture. This animal emaciated gradually, losing about 600 gm. in weight, and was killed 13 days later.

Autopsy.—Animal anemic and emaciated. Pleural and peritoneal cavities contain a considerable amount of fluid. Atrophy of mesenteric fat. Slight engorgement of liver and spleen. Abscesses and infarcts in both kidneys. Large abscess in muscles of right hind leg. Fibrinopurulent arthritis of right coxofemoral joint. Bone marrow gelatinous and deep red in color.

The serum proteins were studied at frequent intervals during the course of the infection, and the results obtained showed clearly the fluctuations which may occur in the blood proteins in a typical infection with *Staphylococcus aureus*. The progress of the infection in both animals studied was accompanied by some antibody response as evidenced in the formation of antistaphylolysins. These developed to an equal degree in both rabbits. In Rabbit 2, however, the changes in the percentages of the various constituents, and especially in the albumin-globulin ratio, were not marked. The striking result of this experiment was the precipitous drop in the protein quotient observed in Rabbit 1, which received a massive inoculation of staphylococci. Of interest, too, is the fact that this rise in the total globulins was associated with a parallel fall in the total albumin.

The loss in weight in both animals was considerable, but it is of interest that this loss occurred more rapidly in Rabbit 1 (3 days), whereas as in Rabbit 2 it was more gradual. The significance of this observation will become more apparent in connection with other experiments.

Experiment 2. Tuberculosis.—On February 17 a rabbit weighing 2,600 gm. was inoculated intravenously with 0.1 mg. of a 72 day culture of bovine tubercle bacillus grown on tuberculin agar. During the progress of the infection, frequent analyses of the serum proteins were made, and the albumin-globulin ratio was determined. These observations are presented in Table I.

The animal emaciated only moderately, and was killed about 2½ months later.

Autopsy.—Extensive pulmonary tuberculosis with cavity formation. Numerous cheesy foci and miliary tubercles. Tuberculosis of mediastinal lymph nodes. Tubercles in spleen and kidneys.

Experiment 3. Infection with Sporotrichum schenckii and beurmani.—A rabbit weighing 1,750 gm. was injected intraperitoneally with 2 cc. of a cream suspension of sporothrix (No. 3725, 1912) grown in 4 per cent glucose broth since October 25, 1915. The animal gained in weight and 10 days later 4 cc. of the same culture were injected. On the 30th day a third and final injection of 6 cc. was given. On March 17 three nodules about the size of large cherries were felt at the site of inoculation.

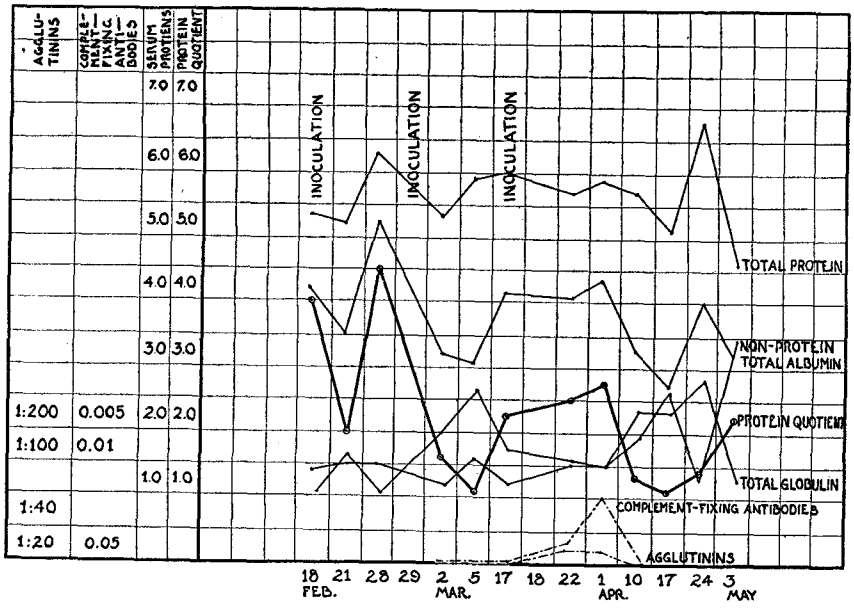
Frequent observations were made upon the agglutinins and complement-fixing antibodies as well as upon the serum proteins (Text-fig. 1). The animal was killed about 2½ months later.

Autopsy.—At the site of the injections there were many pea-sized nodules. These were present both in the subfascial layers and in the abdominal muscles.

TABLE I.

Experiment 2. Chronic Infection. Tuberculosis. Rabbit 3.

Date.	Weight.	Organisms inoculated.	Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
	gm.		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
Feb. 17..	2,600	0.1 mg. intravenously.	6.6	5.3	1.3	80	20	1.2	4.0	Bovine strain grown on tuberculin agar, 72 days old.
" 21..	2,400	—	6.0	4.6	1.4	76	24	1.5	3.1	
" 25..	2,625	—	—	—	—	—	—	—	—	
" 28..	2,400	—	6.5	4.7	1.8	72	28	1.6	2.9	
Mar. 9..	2,700	—	6.0	4.2	1.8	70	30	1.4	2.3	
" 17..	2,400	—	6.8	4.6	2.2	68	32	1.4	2.1	
Apr. 1..	2,550	—	7.2	4.4	2.8	61	39	1.5	1.6	
" 10..	2,550	—	6.0	3.1	2.9	51	49	2.6	1.0	
" 17..	2,450	—	5.2	2.1	3.1	40	60	3.5	0.66	
" 24..	2,350	—	6.9	3.6	3.3	52	48	1.7	1.1	
May 3..	2,300	—	5.0	0.5	4.5	10	90	3.5	0.1	Animal killed. Blood for examination obtained from heart.



TEXT-FIG. 1. Infection with sporothrix.

Similar ones were found also in the omentum, between the loops of intestine and between the liver and diaphragm. Histologically, they were found to show the presence of typical sporotrichotic granulation tissue. From one of these nodules a positive culture was obtained.

Both the tubercular and mycotic infections were characterized by a long chronic course of several months associated with only slight wasting. Immediately following the inoculation, in the one instance with the tubercle bacillus and in the other with the sporothrix, each animal showed a slight rise in the total globulins as evidenced by a fall in the protein quotient. But it is of interest that the latter continued at a fairly constant level, and that the fluctuations observed usually followed the intraperitoneal injections. Thus it will be noticed the protein quotient did not show such a precipitous fall in the chronic as in the acute infections.

In the animal infected with sporothrix a slight grade of immunity developed, but neither the agglutinins nor the complement-fixing antibodies ever rose to a high level. Notwithstanding this lack of response, the serum proteins still showed striking fluctuations. The curve of total albumin for the most part paralleled closely the curve of total proteins. The globulins showed a tendency to rise when the albumin curve fell, but this was not the case for all periods of the experiment. Both of these features are well represented by the fluctuations of the protein quotient as shown graphically in the text-figure.

Immunity.

Experiments 1 and 2. Immunization with Bacillus typhosus.—Two healthy rabbits were inoculated with *B. typhosus*. One animal received inoculations of stock strain and the other Army vaccine. Serum samples were obtained before the injection for a study of the agglutinins, complement-fixing antibodies, and serum proteins. Similar observations were made at frequent intervals following the inoculations. The details of Experiment 2, in which Army vaccine was used, are recorded in Table II and Text-fig. 2.

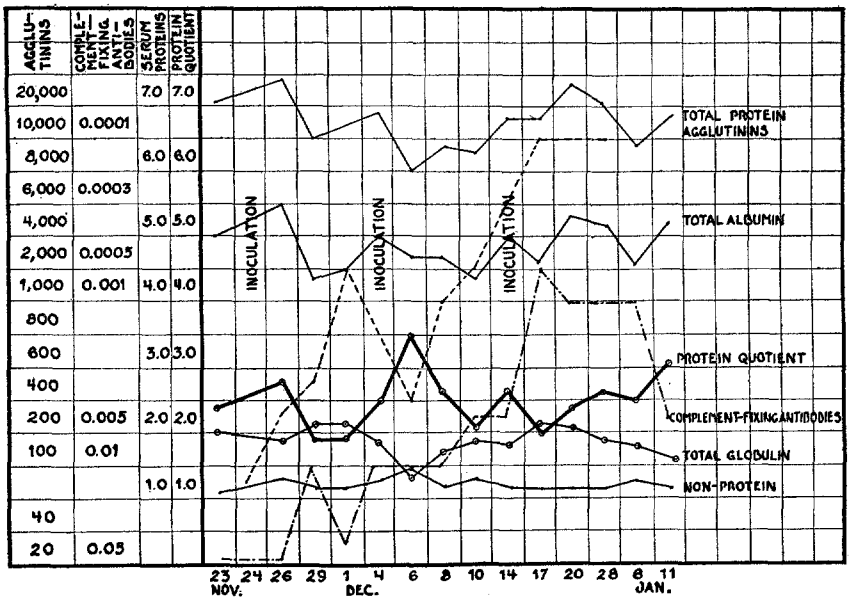
In both of the experiments on typhoid immunization the inoculations were given in such dosage and at such intervals as to develop within the organism a maximum degree of immunity without causing any marked metabolic disorder. Both animals maintained their weight at a normal level throughout the period of immunization, and

TABLE II.

Experiment 2. Typhoid Immunization. Rabbit 4.

Date.	Organisms inoculated.	Antibodies.		Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
		Agglutinins.	Complement-fixing antibodies.								
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
Nov. 23.....	—	1:20	0.05	7.0	5.0	2.0	71	29	1.1	2.4	
" 24.....	0.5 cc. (250,000,000) intravenously.	1:80	0.05	—	—	—	—	—	—	—	Army vaccine. Weight 3,800 gm.
" 26.....	—	1:320	0.05	7.4	5.5	1.9	74	26	1.3	2.8	
" 29.....	—	1:500	<0.01	6.5	4.3	2.2	66	34	1.2	1.9	Weight 3,400 gm.
Dec. 1.....	—	1:2,000	0.03	6.7	4.5	2.2	67	33	1.2	2.0	
" 4.....	—	1:800	0.01	6.9	5.0	1.9	72	28	1.3	2.5	
" 5.....	1.0 cc. (500,000,000) intravenously.	—	—	—	—	—	—	—	—	—	
" 6.....	—	1:400	<0.01	6.0	4.7	1.3	78	22	1.4	3.5	
" 8.....	—	1:1,000	<0.01	6.4	4.7	1.7	73	27	1.2	2.7	
" 10.....	—	1:2,000	0.003	6.3	4.4	1.9	69	31	1.3	2.2	
" 14.....	—	1:6,000	0.003	6.8	5.0	1.8	73	27	1.2	2.7	Weight 3,800 gm.
" 15.....	1.0 cc. (500,000,000) intravenously.	—	—	—	—	—	—	—	—	—	
" 17.....	—	1:10,000	<0.0005	6.8	4.6	2.2	67	33	1.2	2.0	
" 20.....	—	1:10,000	0.001	7.4	5.3	2.1	71	29	1.2	2.4	
" 28.....	—	1:10,000	0.001	7.1	5.2	1.9	73	27	1.1	2.7	Weight 3,500 gm.
Jan. 6.....	—	—	0.001	6.4	4.6	1.8	72	28	1.3	2.5	
" 11.....	—	—	0.003	6.8	5.2	1.6	76	24	1.1	3.1	

the immunity developed after the third inoculation was of a high grade in each instance. In one of these animals (Rabbit 5), the agglutinins showed a tendency to fall at one period following an intercurrent infection resulting from an abortion. Soon after this reduction in the agglutination titer, there occurred also a definite rise in the serum globulins. The association of the development of a pyemia with a high globulin content has been a frequent observation. Its significance will be discussed in subsequent paragraphs.



TEXT-FIG. 2. Typhoid immunization.

In neither animal was it possible to demonstrate any direct parallelism between the rise in the immune bodies and the fluctuations in the serum globulins. The latter showed a tendency to rise (fall in protein quotient) 24 to 48 hours following an inoculation, and a tendency to return to a normal level in the following several days.

Text-fig. 2 represents these fluctuations graphically for Experiment 2. It will be observed that after a period of about 3 weeks following the initial inoculation the value of the protein quotient showed no tendency to change materially, although the development of immune

bodies had reached its highest point. The total proteins and total albumins showed parallel fluctuations but no definite tendency to rise during the process of immunization.

Experiment 3. Immunization with Dysentery Bacillus (Shiga).—A rabbit weighing 2,450 gm. was inoculated intravenously with increasing doses of living dysentery bacilli (Strain Do, Pasteur Institute, December, 1913) suspended in salt solution. The first inoculation was given on January 27 when 0.01 of a loop (20,000 organisms) was inoculated. The same dose was given 10 days later. 20 days after the first inoculation the animal received ten times this number of organisms. This was increased to 100 times the dose on the 30th day. On the 40th and 50th days, two and one-third and eight loopfuls, respectively, were inoculated. These injections were all well tolerated and were not followed by loss in weight. The degree of antibody response and the change in the serum proteins are recorded in detail in Table III and Text-fig. 3.

8 weeks after the beginning of the experiment, the animal died of exsanguination following prolonged bleeding from the ear artery.

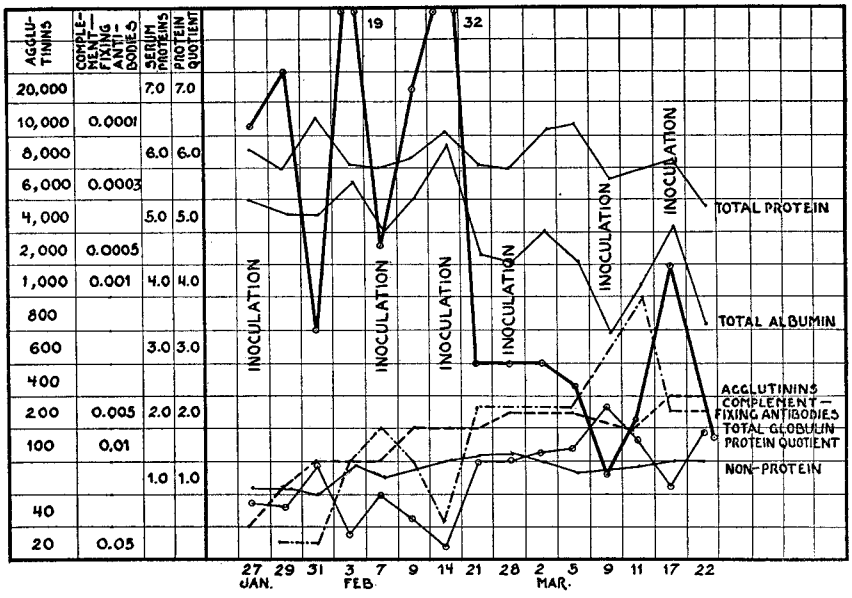
The striking changes in the blood globulins brought about by the inoculation of living dysentery bacilli are well shown in the text-figure. It will be observed that following the first two inoculations both the albumin and globulin curves showed wide fluctuation, and that only after the third inoculation did the globulins show a gradual upward course and the albumin a gradual downward course. During two periods of the experiment (February 3 and 14) the albumin fraction rose to a high level. A similar observation was made upon an animal immunized with living staphylococci. Apart from the explanation that the injection of living organisms may give rise to a marked metabolic disorder, the reasons for such extreme variations in the curve are not clear, unless it is assumed that the active multiplication of bacteria may bear some relation to these fluctuations.

The agglutinins and complement-fixing antibodies rose gradually reaching their highest level during the 5th week. But as a careful analysis of the antibody and globulin curves will disclose, there is a marked fluctuation of the latter curve throughout its entire course. The most striking discrepancy was noted on March 17, when the concentration of antibodies had reached its maximum; whereas the globulin content was beginning to return to its initial level.

TABLE III.

Experiment 3. Immunization with Dysentery Bacillus (Shiga). Rabbit 6.

Date.	Organisms inoculated.	Antibodies.		Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
		Agglutinins.	Complement-fixing antibodies.								
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
Jan. 27.....	0.01 loop (20,000) intravenously.	0	0.03	6.3	5.5	0.8	87	13	1.2	6.7	Weight 2,450 gm.
" 29.....	—	1:80	0.03	6.0	5.3	0.7	88	12	1.2	7.5	
" 31.....	—	1:100	0.03	6.8	5.3	1.5	78	22	1.0	3.5	Weight 2,100 gm.
Feb. 3.....	—	1:100	0.01	6.1	5.8	0.3	95	5	1.5	19	
" 7.....	0.01 loop intravenously.	1:100	0.005	6.0	5.0	1.0	83	17	1.3	4.8	Weight 2,300 gm.
" 9.....	—	1:200	0.01	6.2	5.5	0.7	88	12	1.4	7.3	
" 14.....	—	1:200	>0.02	6.6	6.4	0.2	97	3	1.5	32	Weight 2,450 gm.
" 17.....	0.1 loop intravenously.	—	—	—	—	—	—	—	—	—	
" 21.....	—	1:200	<0.003	6.1	4.6	1.5	75	25	1.6	3.0	
" 28.....	—	1:300	<0.003	6.0	4.5	1.5	75	25	1.5	3.0	Weight 2,650 gm.
" 29.....	1 loop intravenously.	—	—	—	—	—	—	—	—	—	
Mar. 2.....	—	—	—	6.6	5.0	1.6	75	25	1.5	3.0	
" 5.....	—	1:300	>0.003	6.7	4.6	1.7	73	27	1.3	2.7	
" 9.....	—	—	—	5.8	3.4	2.4	58	42	1.3	1.4	Weight 2,600 gm.
" 11.....	2.5 loops intravenously.	1:200	0.001	6.0	4.1	1.9	68	32	1.4	2.1	
" 17.....	8 loops intravenously.	1:400	0.003	6.3	5.2	1.1	82	18	1.5	4.5	48 hour culture suspended in 2 cc. of saline solution.
" 22.....	—	1:400	0.003	5.4	3.6	1.8	66	34	1.5	1.9	Weight 2,650 gm.
" 23.....	—	—	—	—	—	—	—	—	—	—	Autopsy. Marked anemia.



TEXT-FIG. 3. Immunization with living dysentery bacilli.

Experiments 4 and 5. Immunization with Living and Killed Staphylococci.— Two healthy animals were inoculated intravenously with *Staphylococcus pyogenes aureus*. One rabbit received 0.001 of a slant (2,000,000 organisms) at the beginning and two subsequent inoculations of 0.02 and 0.1 of a slant. This animal at autopsy showed an osteomyelitis of the sternum, an adhesive pericarditis, and thrombophlebitis of the deep femoral vein. The second animal was injected with cultures of staphylococci killed by heating at 60° C., for one or more hours. In all, four inoculations of 0.01, 0.2, 0.5 and 1 slant were given. The animal died on the 40th day of a septicemia following a hypopyon due to accidental injury.

Some interesting differences have been observed between the effects produced by the intravenous inoculation of living and of killed staphylococci. The injection of living organisms is followed immediately by marked fluctuations in the protein quotient (Text-fig. 3), whereas following the injection of killed organisms the globulins first diminish and then the quotient shows a gradual downward course.

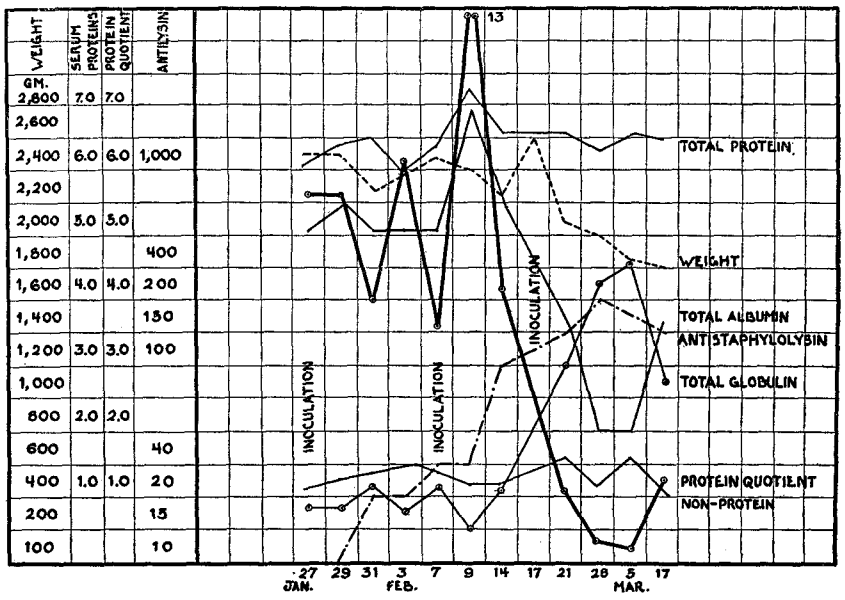
In Experiment 4 the injection of living organisms was followed on the 13th day by a marked rise in the albumin fraction. A similar

TABLE IV.

Experiment 5. Immunization with Killed Staphylococci. Rabbit 7.

Date.	Weight.	Organisms inoculated.	Antibodies.		Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
			Agglutinins.	Complement-fixing antibodies.								
	gm.				*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
Jan. 27.....	2,400	0.01 slant intravenously.	0	0	5.8	3.7	2.1	64	36	1.2	1.7	
" 29.....	2,400	—	0	0	5.7	4.2	1.5	73	27	1.5	2.7	
Feb. 3.....	2,350	—	0	0	5.4	4.1	1.3	76	24	1.5	3.1	Injury on left eye.
" 7.....	2,450	0.2 slant intravenously.	1:10	<0.2	—	—	—	—	—	—	—	
" 9.....	2,250	—	1:40	<0.2	6.6	5.7	0.9	86	14	1.4	6.1	
" 14.....	2,300	—	1:100	0.2	6.6	5.9	0.7	90	10	1.4	9.0	
" 21.....	2,600	—	1:60	>0.2	5.9	4.5	1.4	76	24	1.5	3.1	
" 22.....	2,850	0.5 slant intravenously.	—	—	—	—	—	—	—	—	—	
" 28.....	2,550	—	1:100	0.05	6.7	4.2	2.5	62	38	1.6	1.6	
" 29.....	2,600	1 slant intravenously.	—	—	—	—	—	—	—	—	—	Suspension heated for 1 hr. at 60°C.
Mar. 2.....	2,600	—	—	—	—	—	—	—	—	—	—	
" 6.....	2,300	—	1:200	0.01	7.8	2.4	5.4	31	69	1.6	0.45	

event occurred also in Rabbit 7 inoculated with killed organisms. But this animal had developed a suppurative condition of the left eye about the time that this sudden rise occurred. These two observations taken in conjunction with the one following the inoculation of living dysentery bacilli suggest the possibility that such extreme fluctuations may be explained by the active multiplication of living organisms within the animal.



TEXT-FIG. 4. Immunization with living staphylococci.

Both experiments illustrate still another point which will be considered more fully later; namely, that the increase in globulins is associated with a diminution in the albumin fraction. The total proteins exhibit a slight upward course in each animal, whereas the non-protein constituents show no significant variation.

A gradual rise in antistaphylolysins took place in Rabbit 8 inoculated with living organisms. For the most part, this rise appears to parallel the increase in globulins, but we feel that there is another consideration to be kept in mind in the interpretation of this result. The experimental evidence would seem to indicate that marked

alterations in weight, such as occurred in this animal, may be associated with a great increase in the globulins of the blood independently of a rise in immune bodies.

A Comparison of the Effect of the Inoculation of Living Typhoid Bacilli upon the Normal and Immune Animal.

The observations upon the fluctuations in the serum globulins and in the antibody response in normal animals immunized with living organisms suggested the problem of the possible effect of inoculating living organisms into the typhoid immune animal. That the immunized organism because of the sensitization of its fixed tissue cells may possess a more responsive defensive mechanism is now well known (12). This power of defense may become manifest by a rapid mobilization of antibodies and by a large increase in the number of circulating leukocytes. It was our purpose in the experiments of this series to ascertain whether this protective reaction was in any manner related to the changes in the blood proteins, and more especially whether any parallelism existed between the rise in leukocytes and the increase in the blood globulins.

Experiments 1, 2, 3, 4, and 5.—Five animals already possessing a basic immunity against the typhoid bacillus were chosen for this study. In three of the animals (Rabbits 9, 10, and 4) a record was made at intervals of hourly periods of changes in the leukocytes, antibodies, and serum proteins. In one of the experiments (Rabbit 10) the observations were extended over a period of 12 days. The results of this experiment are representative of the others in this series. These are given in detail in Table V and Text-fig. 5.

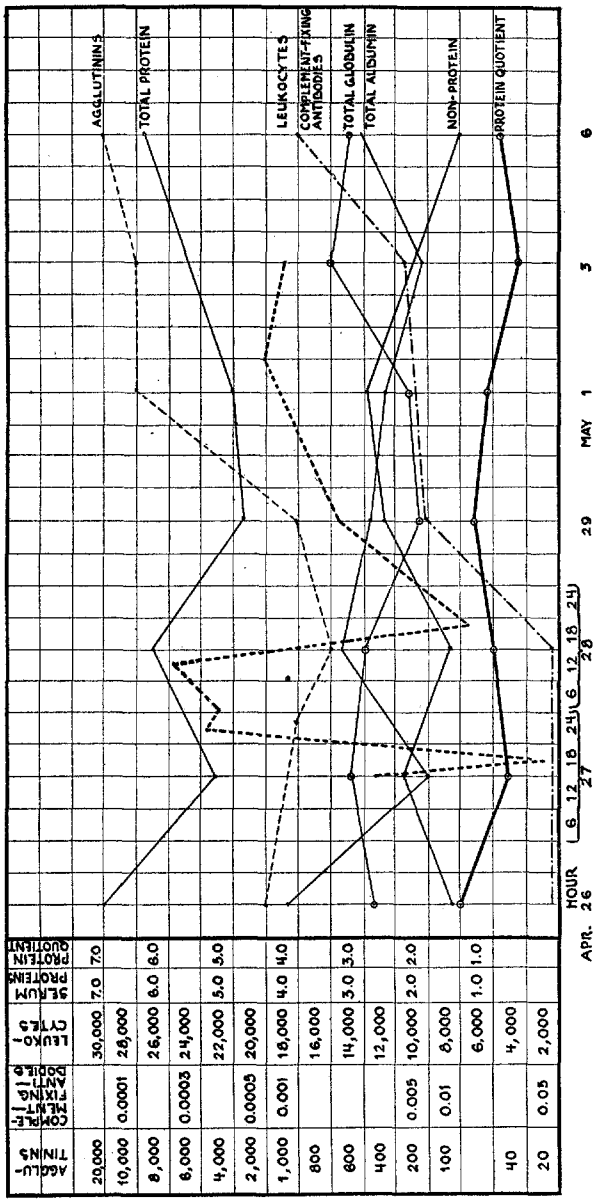
Experiment 6.—A normal rabbit weighing 2,800 gm. was inoculated intravenously with 0.25 of a slant of living typhoid bacilli (Strain H 125) on May 8. The animal tolerated the injection well, and gave a good leukocytic and antibody response. Observations on the leukocytes, antibodies, and serum proteins were made at hourly periods during the first 48 hours, and then at intervals over a period of 16 days.

The observations given in the protocols, tables, and text-figures of this series support the general conclusions that the immune animal responds more quickly to the inoculation of living organisms with a leukocytosis, a rise in immune bodies, and an increase in the blood globulins; and that the changes noted in the blood proteins bear no relation to the hyperleukocytosis.

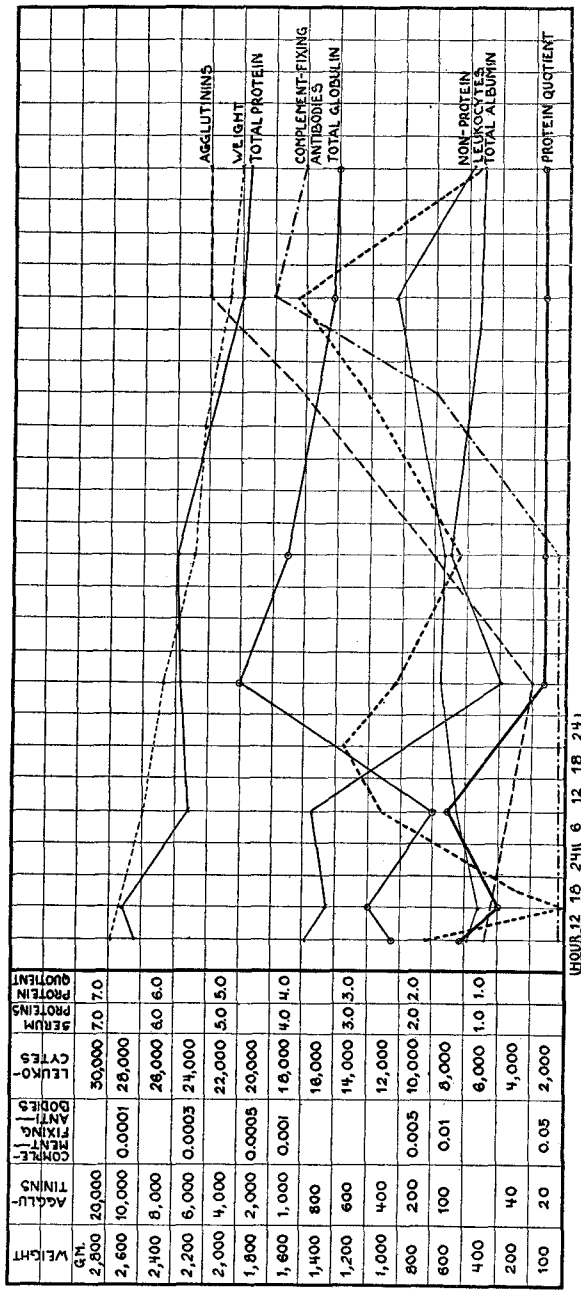
TABLE V.

Experiment 4. Rabbit 10.

Date.	Time.	Weight.	Antibodies.		Leukocyte count.	Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
			Agglutinins.	Complement-fixing antibodies.									
		gm.				*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
Apr. 26.....	—	—	1: 2,000	0.05	—	7.0	4.1	2.9	60	40	1.6	1.5	
" 27.....	12 m.	3,475	—	—	12,300	—	—	—	—	—	—	—	Inoculation of 0.25 slant of 20 hour typhoid culture intravenously.
	4 p.m.	—	—	—	3,770	—	—	—	—	—	—	—	
	5.30 p.m.	—	1: 1,000	0.1++	—	5.3	2.0	3.3	37	63	2.4	0.6	
	8.30 p.m.	—	—	—	23,000	—	—	—	—	—	—	—	. *typhosus* in blood.
" 28.....	9 a.m.	—	—	—	24,600	—	—	—	—	—	—	—	" " " "
	12.10 p.m.	—	1: 800	0.05	18,600	6.2	3.2	3.0	51	49	1.7	1.0	
	6 p.m.	3,350	—	—	7,400	—	—	—	—	—	—	—	
" 29.....	—	—	1: 1,000	0.005++	14,900	4.9	2.8	2.1	57	43	2.6	1.3	
May 1.....	—	3,750	1: 10,000	0.003+++	19,700	5.0	2.7	2.3	54	46	2.7	1.1	
" 3.....	—	—	1: 10,000	0.003+++	19,000	5.7	2.2	3.5	38	62	2.4	0.6	
" 6.....	—	—	1: 20,000	0.001	—	6.3	3.0	3.3	47	53	1.5	0.88	



TEXT-FIG. 5. Injection of living typhoid bacilli. Immune animal.



TEXT-FIG. 6. Injection of living typhoid bacilli. Normal animal.

In the main the details of some of the experiments are given in Table V and Text-figs. 5 and 6, but a few points relating to the individual experiments deserve special mention. From a comparison of the results obtained in the experiments with Rabbits 11 and 12 it would appear that the rapidity of response bears some relation to the degree of the initial basic immunity. The first animal showed a fall in the agglutination titer immediately after the injection, and in this animal the rise in globulins occurred only after 72 hours. In Rabbit 12, however, the antibodies rose steadily, and in this instance a rise in the concentration of the serum globulins took place more rapidly within a period of 24 hours.

Experiments 3 and 4 are more complete since in them the observations were extended over a longer period. That the degree of hyperleukocytosis is dependent in part at least upon the number of organisms inoculated is clear from a comparison of the results obtained in Rabbits 9 and 10. In the former where the more marked reaction occurred, one-half of a standard agar slant had been inoculated; whereas, the latter received only one-quarter of a slant. The highest leukocytic reactions were observed in Rabbit 9 (44,000) and in Rabbit 4 (58,000). In this respect our observations coincide with those of McWilliams (12). The usual leukopenia which immediately follows the intravenous injection occurred in both the normal and immune animal as is graphically shown in Text-figs. 5 and 6.

By referring to these charts it will be noted that in neither animal was there any parallelism between the leukocytic response, the rise in the immune properties of the serum, and the increase in globulins. Whereas the curve showed periods in which a rise in globulins occurred simultaneously with a leukocytosis, the latter remained low even during the periods of leukopenia. Nor was any direct correspondence demonstrable between the rise in immune bodies and the increase in the concentration of the serum globulins. Both in the normal and the immune animal the latter took place long before any appreciable rise in the agglutinins and complement-fixing antibodies had occurred. In Rabbit 10, for instance, a fall in the protein quotient (globulin rise) was demonstrable within 24 hours after the inoculation at a time when the antibody content was at its lowest level; and similarly in Rabbit 13, the normal control, the serum globulins rose mark-

edly within 48 hours, while the antibodies rose to their maximum height only after 4 days. In both instances the globulin content remained high throughout the period of immunization while the antibody curve continued to rise independently of the globulins.

The advantage of such frequent determinations made at different periods during the process of immunization as compared with isolated observations made at random intervals is well illustrated in these experiments. If, for instance, a determination of the globulin fraction should show an increase at a time when the immune bodies had reached a high level, the conclusion would naturally follow that fluctuations in the two parallel one another; whereas, as we have pointed out, more frequent observations demonstrated that such parallelism was not of constant occurrence.

The tabulated results emphasize still another point of importance. To be of absolute value the albumin-globulin ratio must be expressed in terms of their quotient. This takes into consideration also fluctuations which have been found to take place in the total proteins during the course of an infection and during the process of immunization.

The Effect of the Injection of Bacterial Toxin upon the Serum Proteins.

The experimental data already presented afford evidence that both living and killed cultures of various bacteria when inoculated into an animal give rise to marked changes in the serum proteins, and more especially to an upset of the normal albumin-globulin ratio. This phenomenon may well be attributed to the changed condition of the animal's metabolism resulting from the multiplication of bacteria within its body, to the liberation of toxic products from the disintegration of the bacterial bodies, or to both causes. The part which the autolyzed bacterial bodies themselves may play in bringing about the results observed is well shown in the following experiment.

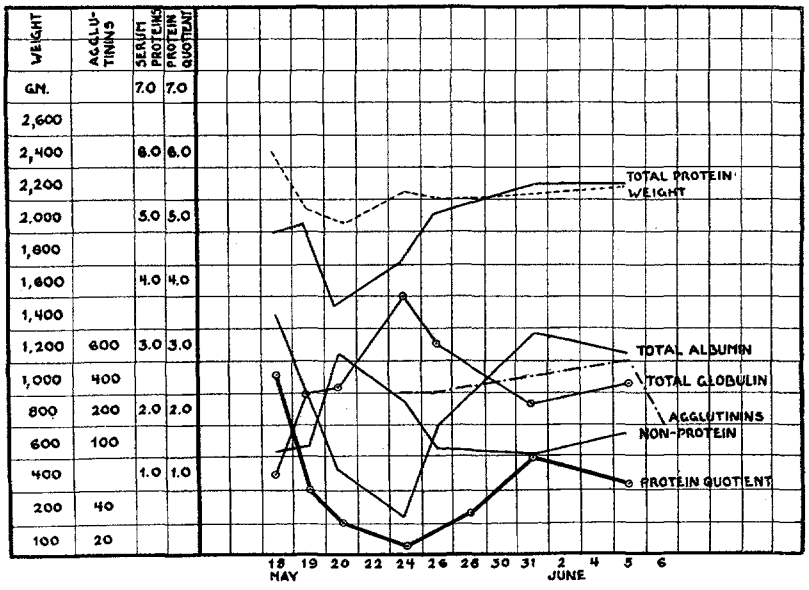
The Action of Bacterial Endotoxin.—A rabbit weighing 2,500 gm. was injected intravenously on May 18 with 1 cc. of the toxin of fowl typhoid. This toxin was prepared by growing the organisms on Martin's broth for 14 days, after which the culture was centrifugalized and filtered through a Berkefeld filter.

Specimens of 5 cc. of blood were taken 24 hours after the injection and at varying intervals until June 6. The alterations in the serum proteins following the injection are given in Table VI and Text-fig. 7.

TABLE VI.

The Effect of the Injection of Bacterial Toxin (Fowl Typhoid). Rabbit 14.

Date.	Weight.	Amount of toxin injected.	Agglutinins.	Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
	gm.			*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
May 18...	2,500	1 cc. intravenously.	—	5.0	3.7	1.3	74	26	1.6	2.8	Strain 605 grown on Martin's broth for 14 days. Centrifugalized and filtered once through a Berkefeld filter.
" 19...	2,175	—	—	5.1	2.6	2.5	51	49	1.7	1.0	
" 20...	2,100	—	—	3.9	1.3	2.6	33	67	3.1	0.5	
" 24...	2,275	—	1:400	4.6	0.6	4.0	13	87	2.4	0.15	
" 26...	2,225	—	1:400	5.3	2.0	3.3	37	63	1.7	0.6	
" 31...	—	—	—	5.7	3.4	2.3	60	40	1.6	1.5	
June 5...	2,300	—	1:600	5.7	3.1	2.6	54	46	1.9	1.1	
" 6...	—	—	1:200	—	—	—	—	—	—	—	



TEXT-FIG. 7. The effect of the injection of bacterial toxin.

The dosage of endotoxin in this experiment was apparently well chosen, for although the animal lost moderately in weight, the amount of toxin was not sufficient to prevent a gradual return of the serum proteins to a more or less normal state. The most striking effect of the inoculation, shown graphically in Text-fig. 7, was the gradual increase in the serum globulins at the expense of the albumin fraction, and a reduction in the percentage of the total proteins. This rise in globulins was already appreciable 72 hours following the injection, and 6 days later the albumin-globulin ratio still showed an inversion of the normal formula. On the 8th day the total per cent of proteins had returned to normal, and continued to rise somewhat above the normal level during the subsequent 11 days. At this time the protein quotient, however, still remained low, although the albumin-globulin ratio was beginning to revert to its normal state.

The tendency in this instance for an alteration in the blood protein fractions to readjustment is of interest. In this respect this observation is unique, for in the majority of the experiments an upset of the normal ratio continued with some fluctuations for a long period of time due to subsequent reinoculations. The readjustment of conditions in this animal may be attributed to the absence of bacterial invasion to perpetuate the process. It may be assumed that after its initial effect upon the animal, the toxin was spent, as may be inferred from the appearance of antibodies in the blood, and that the organism was then able to readjust itself.

A Comparison of the Effect of the Intraperitoneal Injection of Bacterial and of Inflammatory Irritants.

In some experiments which will be recorded at a later date, we observed that the intraperitoneal injection of red blood corpuscles gives rise to an alteration in the albumin-globulin ratio which is both rapid in its occurrence and marked in its degree. In fact such injections usually resulted in a complete inversion of the ratio within a period of 24 hours. Because we were dealing here with non-bacterial protein and with a different route of injection, it seemed worth while to ascertain whether it was the nature of the inoculated material or the route of the inoculation which was responsible for

the changes observed. With this purpose in view, the following two experiments were carried out.

Experiment 1.—On May 22 a rabbit weighing 2,525 gm. was injected intraperitoneally with 0.2 of a slant of a killed culture of staphylococci. The organisms were killed by heating for an hour at 60° C., and 1 cc. of the suspension was used. An analysis of the serum proteins was made at stated intervals following the injection. These are recorded in Table VII.

TABLE VII.

The Effect of the Injection of Killed Staphylococci Intraperitoneally. Rabbit 15.

Date.	Weight.	Organisms inoculated.	Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
	gm.		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
May 19..	2,500	—	5.8	3.2	2.6	60	40	1.7	1.5	
" 22..	2,525	0.2 slant intraperitoneally.	—	—	—	—	—	—	—	24 hour culture killed by heating for 1 hr. at 60°C. suspended in 1 cc. of saline solution.
" 23..	2,500	—	4.7	1.3	3.4	28	72	2.9	0.4	
" 24..	2,600	—	4.7	1.8	2.9	38	62	2.8	0.6	
" 26..	2,600	—	4.9	0.8	4.1	16	84	1.8	0.2	
" 31..	—	—	5.7	2.0	3.7	35	65	1.6	0.5	
June 6..	—	—	5.8	1.8	4.0	31	69	1.3	0.45	

Experiment 2.—2 cc. of an aleuronat suspension in saline solution were injected intraperitoneally into a rabbit weighing 2,150 gm. The suspension of aleuronat was so made that it corresponded in density to that of the killed staphylococci used in the first experiment. Following the injection, observations were made upon the serum proteins. These are recorded in Table VIII. The animal lost gradually in weight, and died 7 days after the injection.

Autopsy.—Small masses of unabsorbed aleuronat were found adherent to the peritoneum. There was a definite intestinal paralysis with coprostasis, chiefly in the large bowel. There was considerable injection of the peritoneum and an enteritis of the small bowel.

TABLE VIII.

The Effect of the Injection of Aleuronat Intraperitoneally. Rabbit 16.

Date.	Weight.	Amount of aleuronat injected.	Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
	gm.		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
May 19..	2,075	—	5.1	3.1	2.0	60	40	1.6	1.5	
" 22..	2,150	2cc.	—	—	—	—	—	—	—	Aleuronat (Merck) suspension made in saline solution and of about same density as that of staphylococci (Table VII).
" 23..	2,025	—	4.7	1.8	2.9	38	62	2.8	0.6	
" 24..	2,025	—	4.2	1.7	2.5	40	60	3.1	0.66	
" 26..	1,900	—	4.7	1.5	3.2	32	68	2.6	0.5	
" 29..	1,450	—	—	—	—	—	—	—	—	Death.

Both experiments would seem to support the view that the route of injection rather than the nature of the substance injected is responsible for the rapid inversion in the albumin-globulin ratio. 24 hours following the intraperitoneal injection of killed staphylococci the quotient fell from 1.5 to 0.4, indicating an increase in globulins to more than three times the initial value. This upset in the ratio continued with slight fluctuations for a period of about 2 weeks. The animal injected with aleuronat showed a change similar in every respect.

The retardation in response noted after intravenous inoculations must therefore be attributed to the protective properties of the blood which enable it to delay the action of the bacteria or toxin upon the body tissues.

Another point brought out by this experiment deserves emphasis; namely, that agents other than bacteria or their toxins may cause an upset in the serum proteins. The manner in which an inflammatory irritant and leukotactic substance like aleuronat may produce this result offers some difficulty of explanation. It is not unlikely, however, that the rapid absorption of toxic protein products result-

ing from the disintegration of leukocytes and fixed tissue cells produces a profound metabolic disturbance of which the heaping up of blood globulins is one of the resultant phenomena. A further consideration of the factors which may give rise to this result will be presented later.

DISCUSSION.

The experimental evidence presented does not support the views held by a number of workers concerning the relationship of the blood globulins to the resistance developed in bacterial infection and immunity. From a large number of observations, continued over a long period of time, we have become convinced that other causes are responsible for the rise in globulins observed in these conditions.

Our observations have shown with considerable certainty that a heaping up of globulins in the blood during the development of an infection is more apt to occur in those instances where the infection has been overwhelming and associated with extensive suppuration and wasting. We have found, in fact, that animals which succumb to such an acute process have usually developed only a moderate resistance as far as the development of immune bodies is concerned. On the other hand, a mild chronic infection may continue over a long period of time, and may register only slight changes in the blood globulins until the animal begins to emaciate and to lose in weight. This point has been discussed in connection with the tubercular and the mycotic infections.

The main points of interest have come from a study of the serum globulins during the process of immunization. Contrary to the results of a number of workers (13), our experiments have shown that immunization with bacteria causes a rise in globulins only when the animals react severely to the inoculation. Immunization carried out carefully and with a well controlled dosage is not usually accompanied by an increase in the serum globulins, although the immune bodies may attain a high concentration. The inoculation of massive doses, however, either into a normal animal or into an animal already possessing a basic immunity results in most instances in a marked rise in the globulins. This may occur, indeed, before the animal has responded by the production of antibodies. It would seem,

therefore, that no direct parallelism exists between the two phenomena. In fact we have come to regard the heaping up of serum globulins supervening during the process of immunization as an index of a metabolic disorder unfavorable to the attainment of the best immunologic results. And it is not unlikely that observations on the blood globulins may serve as an important practical guide to careful immunization.

Any attempt to explain the cause of the rise in globulins observed in infection and immunity is difficult. At best all such explanations must be of a hypothetical character until we have learned more concerning the origin of the various protein fractions, their function,[5] and their chemical nature. One important conclusion may be derived from the experimental evidence presented; namely, that the increase in blood globulins is usually accompanied by a marked metabolic disorder. This observation has been made also by other workers (14). Clinically, the metabolic disturbance is manifested by a febrile reaction, intoxication, and rapid emaciation. The extensive destruction of body protein which is going on is further evidenced by an increase in the nitrogen elimination (15). That such marked proteolytic activity may be initiated by the intravenous injection of bacteria, bacterial toxins, and protein split-products has been clearly shown by Jobling and his coworkers (16). They attribute this active proteolysis to a more or less marked mobilization of ferments, both protease and lipase. Apart from the consideration of the cause of this disturbance in metabolism, it seems reasonable to assume that it must register a change in the proteins of the blood.

More difficult to explain, however, are the facts that an inversion of the albumin-globulin ratio can be so readily produced, and that the change markedly affects the globulins. The possible explanations which may be offered for these phenomena have some basis in experiment. Moll (17) has shown, for instance, that under optimum conditions of reaction and temperature crystalline albumin can be converted *in vitro* into a substance whose chemical and physical

[5] Friedemann (*Z. Hyg. u. Infectionskrankh.*, 1910, lxvii, 279) thinks that the globulins and albumins of normal serum are in antagonism, the albumins preventing certain reactions, such as complement fixation, in which the former become active as soon as the albumins are removed or diminished.

properties correspond in every particular to a globulin. On the basis of this observation one would have to assume that the more rapid conversion of albumin into globulin within the body is only a part of the accelerated metabolism which takes place in infected and immunized animals.

This is only one of many hypotheses which might be advanced to explain the difficult questions which the recorded observations offer for consideration. But at the present time it is better to adhere to those views which have an experimental basis until further additions to our knowledge make them untenable.

SUMMARY.

The progress of an infection is usually associated with marked changes in the serum proteins. There may be an increase in the percentage of the total protein during some stage of the infection, and there is usually a change in the albumin-globulin ratio with an increase in the total globulins. This rise may antedate the development of any resistance by a considerable period of time.

The non-protein constituents of the blood show fluctuations with a tendency to rise as the infection progresses.

The process of immunization is in almost all instances associated with a definite increase in the globulins of the blood, and in some cases with a complete inversion of the normal albumin-globulin ratio. This may be produced both by living and dead organisms and by bacterial endotoxins. Massive doses usually result in an upset which shows no tendency to right itself during the period of observation.

A rise in the globulins has been shown to occur long before the animal develops immune bodies in any appreciable concentration; and where the globulin curve and antibody curve appear to parallel one another, it can be shown by a careful analysis of both curves that there is a definite lack of correspondence at various periods of the experiment.

Animals possessing a basic immunity show a more rapid rise in the globulin curve following inoculation.

There is no parallelism between the leukocytic reaction and the

globulin reaction. During periods of leukopenia the globulins may be as high as during the period of a leukocytosis.

Bacterial endotoxins produce as striking an increase in the serum globulins as do living and killed bacteria. This would seem to indicate that a bacterial invasion of the organism is not absolutely essential for the globulin changes, and that the toxogenic factor in infection and immunity must play a part in the production of the changes noted.

Inflammatory irritants injected intraperitoneally also result in a globulin increase. In this case the changes produced may best be explained by the toxogenic effect produced by the protein split products resulting from the inflammatory condition.

Intraperitoneal injections of killed bacteria give rise to a more rapid increase in the serum globulins. The rapidity of the response following intraperitoneal as compared with intravenous injections doubtless stands in intimate relationship to the neutralizing power possessed by the blood serum and perhaps to the more extensive surface of absorption following injection by the intraperitoneal route.

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