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AUTHOR Stanley Marcus

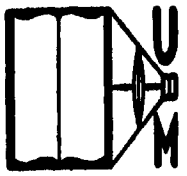
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SOME ASPECTS OF THE KAHN REACTION WITH ANIMAL SERA AND NONSYPHILITIC HUMAN SERA

Stanley Marcus

A Dissertation

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I. INTRODUCTION

The present investigation is concerned with certain aspects of positive reactions given by serological tests for syphilis with nonsyphilitic sera. As is well known, all tests for syphilis give, under various conditions, positive reactions in the absence of this disease. These reactions are common with certain animal sera and less so with human sera, and have been encountered by many investigators in their studies of, or in their endeavors to improve upon the dependability of tests for syphilis. In view of the practical importance of these tests as aids in the diagnosis of syphilis, every effort has been made to reduce nonsyphilitic reactions to a minimum. This effort has led to considerable success in reducing the number of nonsyphilitic reactions in serological practice, but has resulted in the accumulation of relatively little experimental data concerning these reactions. In this thesis, an attempt has been made to investigate nonsyphilitic serological reactions as a distinctive biologic phenomenon.

The experiments are presented in four parts. Part One is concerned with factors affecting the flocculation (Kahn) reaction with nonsyphilitic human and animal sera. Four factors were studied: 1) Effect on the degree of flocculation of variation in temperature at which reactions were carried out. Three temperatures were employed; namely, 37°C., room

temperature (21°-24°C., occasionally higher during summer months), and 1°C. 2) Effect on flocculation of variations in the state of the serum employed, i.e., whether unheated or heated at 56°C. for 30 minutes. 3) Effect on flocculation of variation in the sensitivity of the antigen employed. 4) The use of serum protein fractions in place of the serum in the reactions. Combinations of these effects were also studied.

In Part Two, an attempt has been made to investigate the fixation of complement by serum-antigen (Kahn) mixtures, employing both animal and nonsyphilitic human sera. Serum-antigen mixtures were used in which flocculation was obtained at 1°C. but not at 37°C. Two aspects of the problem have been considered. The first was whether these mixtures at 37°C., which were free from floccules still possessed the capacity to fix complement. The second aspect studied was whether serum-antigen mixtures were able to fix complement after the floccules had been dispersed by heating at 56°C.

In Part Three, investigations have been directed to flocculation reactions at 37°C., room temperature and at 1°C. with certain animal sera (rabbits) during their growth period. The fact that mature rabbits give especially marked flocculation reactions at 1°C., raised the question as to whether young rabbits, generally seronegative, might give

reactions at 1°C. Serological reactions in young animals are also considered in relation to the problem of antibody formation in the young.

Part Four is also concerned with serological reactions at 37°C., room temperature and at 1°C. given by rabbits. The problem investigated has been to determine to what extent serological reactions of rabbits at these three temperatures are affected by inoculation of the animals with Spirochaeta pallida. It is known that in human syphilis the pattern of serological reactivity at the three temperatures is different from that shown by nonsyphilitic animals (Kahn, 1940). It was therefore desired to find whether the serological pattern of the rabbits would change following infection with syphilis.

II. NONSYPHILITIC SEROLOGICAL REACTIONS - HISTORICAL REVIEW

Nonsyphilitic serological reactions obviously cannot be considered apart from serological tests for syphilis. Indeed, the development of complement-fixation and flocculation tests for syphilis has carried with it the growth of the problems of nonsyphilitic reactions. Many reviews discussing the historical aspects of tests for syphilis have been written (Bruck, 1911; Wassermann, 1921; Browing, et al., 1924; Bruck, 1924; Bruck, 1931; Sachs, 1931; Klopstock, 1933; Kahn, 1925, 1942; Eagle, 1937; Wicks, 1939; Vogelsang, 1940). In this review an attempt will be made to stress the evolution of knowledge concerning nonsyphilitic serological reactions and means which have been described for the recognition of such reactions.

1. Nonsyphilitic Serological Reactions with Human Sera

By nonsyphilitic serological reactions are meant reactions which result in flocculation or in fixation of complement, in a test for syphilis, with a serum obtained from a nonluetic source. Such reactions are often referred to as "nonspecific." Attitudes toward these reactions, in the serology of syphilis, have varied from time to time.

Mohr (1941) states: "Opinion as to the specificity of serological tests for syphilis, since their first introduction in 1906, has passed through two periods and is well on its way into a third. For the first ten to fifteen years it was generally thought that positive results might occur in relation to many diseases other than syphilis, and there is a vast literature reporting "false positive" reactions in cases of almost every known disease. In the following two decades the pendulum swung in the reverse direction. It was generally felt that, except for the results in yaws, leprosy, malaria, infectious mononucleosis and perhaps one or two other conditions, serological tests for syphilis were amazingly specific. Technical errors excluded, when positive results were obtained either in the case of an apparently normal person or in the case of one with a clearly non-syphilic disease, these results were regularly assumed to be due to associated asymptomatic syphilitic infection.

"In more recent years doubt has again been thrown on thespecificity of serological tests for syphilis. There is now considerable evidence, not only that positive results may be produced with varying frequency by a number of diseases other than syphilis, but that even nonsyphilitic, clinically normal persons may occasionally yield serum that give biologic false positive reactions."

Kraus and Volk (1906), speaking of the first publication by Wassermann, Neisser and Bruck (1906) of the successful application of the Bordet-Gengou phenomenon of complement-fixation to the serodiagnosis of syphilis, claimed that they had previously done unsuccessful work along these lines. This lack of success they believed was due to the inability of any complement-fixation procedure to detect circulating antibodies in the blood stream with any degree of consistency. They had obtained positive reactions in the absence of the disease and negative reactions in the presence of the disease. Michaelis (1907) also reported two positive complement-fixation reactions and one weakly positive reaction in a small series of 26 non-syphilitic patients.

Bruck (1911) has summarized the literature on non-syphilitic serological reactions through 1910. He reviewed the work of 33 authors. According to these authors, 4377 nonsyphilitic patients showed negative reactions and 57 showed positive reactions. Bruck found 2 of 596 nonsyphilitic patients who showed false positive reactions. It was generally agreed at that time that yaws, trypanosoma infections, leprosy, malaria and scarlet fever would show high percentages of positive reactions in the absence of syphilis.

Evaluations of serological tests such as those summarized by Bruck have continued to the present day. More dependable evaluations however have resulted from the "Serological Conferences" sponsored by the League of Nations Health Organization (1923, 1928, 1931) and the United States Public Health Service (1935, 1941). It has been shown by these conferences that false positives occur in appreciable numbers. The first League of Nations Conference (1923) included 885 nonsyphilitic control sera. Boas reported 5 (0.6%) and 3 (0.3%) false positive reactions with the Sachs-Georgi and Sigma reactions, respectively. The second, and more elaborate, conference of the Organization (1928) included sixteen serologists. Seven of these performed complement-fixation tests and nine performed flocculation tests. A total of 435 nonsyphilitic sera were examined. False positives ranged from 29 (5.5%) to 0; false "doubtfuls" from 40 (9.0%) to 1 (0.2%). The American serological conferences have elicited similar information.

As noted by Mohr, (1941) reports in the literature on the occurrence of false positive reactions in practically every known disease as well as in normal human beings are relatively numerous. Eagle (1937) has summarized much of this literature. Among recent reports of this type, the following may be mentioned: Rothbart (1937) reports false

positives in 12 children suffering from acute or chronic infections. Krag and Lonberg (1938) give an account of clinical findings in 53 cases of nonspecific Kahn and Wassermann reactions observed among 120,000 examinations of patients with no sign of syphilis. Berstein (1938) reports 6 of 37 cases of infectious mononucleosis exhibited transitory positive reactions. Chung and Chang (1939) report 7.95 per cent of 88 patients with relapsing fever showing positive reactions. According to Lane (1939), 8 of 56 employees of the Civilian Conservation Corps with previously negative serological reactions, developed positive Kahn reactions from 4 to 8 days after showing clinical signs of measles. Crawford and Ray (1939) report that 14.3 per cent of 335 positive Hinton flocculation reactions obtained on admission of 2,862 patients to the Massachusetts General Hospital were false positives. Kitchen, Webb and Kupper (1939) report that 30 nonsyphilitic patients inoculated with malaria developed positive flocculation and complement-fixation reactions some time after inoculation. According to Beck (1939), 6 of 496 (1.2%) nonsyphilitic patients showed positive reactions in a complement-fixation test in which spirochetal antigen was employed. Kahn (1940a) reports 10 patients with various diseases other than syphilis, showing false positive reactions. Lynch, Boynton and Kimball (1941), report that 43 (16%) of 263 cases of vaccinia

developed positive reactions in various tests for syphilis. The above may be looked upon as representative reports, but they represent only a minority of the publications on false positives which have appeared in the literature since 1937.

This section on false positive reactions given by human beings might be summarized by stating that, although our present knowledge concerning these reactions is fragmentary, they appear to exist in significant numbers.

2. Nonsyphilitic Serological Reactions With Animal Sera.

The original observations made by Landsteiner, Müller and Pötzl (1907b, c) with the complement-fixation test on positive reactions in normal rabbits have been repeatedly confirmed both by complement-fixation and flocculation procedures. With regard to flocculation reactions, the first observation concerning positive results with animal sera was reported by Sachs and Georgi (1923) in rabbits. This finding was confirmed by Mackie and Watson (1926) who used rabbits as well as other animals. Kemp, Fitzgerald and Shepherd (1940) have summarized the literature on serological reactions given by animals. From the data obtained by different authors, certain tentative conclusions may be drawn:

1) The sera of certain species of mammals and birds

and the pressed out juices of certain plants (Breazeale, Reusser and Breazeale, 1941) yield positive flocculation and complement-fixation reactions. Percentages of positive reactions in any species vary widely with different investigators (Kemp, et al., 1940).

2) Reptiles and amphibians tested have almost invariably been found to be seronegative.

3) No phylogenetic relationship exists between the species studied and the serological reactions obtained. Frogs and monkeys are persistently negative, whereas animals such as sheep, horses and goats are positive in a high percentage of instances.

4) The range of quantitative titers (Kahn) encountered in animal sera is relatively low (0 to 160), while the range of quantitative titers encountered in syphilitic sera is relatively broad, reaching 1000 units or more.

3. Efforts to Differentiate Nonsyphilitic from Syphilitic Serological Reactions

a. Methods proposed by Wassermann and Citron, Sachs and Georgi, Witebsky, Rytz. The presence of numerous serological reactors among a number of animal species has led some investigators to attempt to serologically distinguish the animal reactions from those exhibited by syphilitic individuals. Others have attempted to distinguish between

true positive reactions and those occasionally encountered in normal persons or in individuals infected with other diseases such as malaria, trypanosomiasis, measles, etc. The first effort of this type was that of Wassermann and Citron (1922). Their method was based on experimental observations (data not given) which led these investigators to believe that the "antigen-antibody" aggregates formed by the reaction of syphilitic serum and extract antigen could be "broken" by filtering through very fine Kieselguhr filters. If the aggregates were associated with syphilis, the antigen was retained in the filter, while the active serum fraction passed through the filter; on retesting, the filtrate plus antigen would be found to fix complement. Normal human sera or sera which were not derived from a syphilitic source did not fix complement following such treatment. This procedure, to our knowledge, has not been further evaluated.

Sachs and Georgi (1923) described a procedure which they believed would distinguish between true and false positive reactions in rabbits. To 1.0 cc. of inactivated blood serum is added 8.2 cc. of N/250 HCL; the mixture is centrifugated and the sediment discarded. To the supernatant fluid, 0.8 cc. of 10 per cent NaCl solution is added and the final product is examined with the Wassermann test. Gloor and Klinger are cited by the authors as having shown

that it was unnecessary to neutralize the weak acid solution before performing the complement-fixation test since the precipitated protein draws the acid down with it. Sachs and Georgi reported that syphilitic rabbits give positive Wassermann reactions with this procedure while nonsyphilitic rabbits give negative reactions.

This method is apparently not applicable to flocculation procedures since it involves a 1:10 dilution of the rabbit serum. Such dilution was reported by Walker (1927) to so weaken the serological reactivity of seropositive normal rabbits as to render them Kahn-negative. Mahoney, Bryant and Pichard (1930), in a study of 179 rabbits of which 54 per cent were seropositive, found only two animals which gave reactions in a 1:10 dilution with the Kahn test. Furthermore, according to Wakerlin and Carrol (1925) and Kemp, Chesney and Poole (1926) experimentally infected syphilitic rabbits show a decrease in titer which may result in negative serological reactions on 1:10 dilution of the serum within 20-24 weeks after infection. The value of the Sachs and Georgi procedure in complement-fixation tests has not been reported upon with regard to human beings.[1]

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[1]This procedure has been found to be satisfactory in the treatment and use of anticomplementary human sera in the complement-fixation test (Kolmer, 1940; Gold, 1940). The dilution factor is overcome by the use of 1.0 cc. of the HCl treated serum which is equivalent to the 0.1 cc. amount used in the usual procedure. However, as the dilution factor cannot be corrected for in a flocculation procedure, the test amounts to determining the reactivity of a 1:10 dilution of the serum.

It may be of interest to digress at this point with reference to the problem of nonsyphilitic serological reactions in rabbits, which is in a highly controversial state. It has been claimed that such reactions differ from syphilitic human reactions in several aspects; 1) The rabbits reactions are comparatively "labile," being eliminated if the Sachs-Georgi HCl (1923) procedure is employed (Blum, 1924); 2) These reactions are associated only with the euglobulin fraction of the serum protein (Mackie and Watson, 1926), whereas syphilitic reactions are apparently associated with both the euglobulin and pseudoglobulin fractions (although the data show that syphilitic human sera of low titer also showed reactions with euglobulin fractions only); 3) The reacting substance in rabbit sera is thermolabile to a greater extent than that in syphilitic sera (Griffith and Scott, 1920).

Conflicting data throw doubt on the validity of these differences in the reactivity of syphilitic human and nonsyphilitic rabbit sera. The fallacy in the use of the Sachs-Georgi HCl procedure in flocculation tests has been considered above. Sherwood, Bond and Clark (1939) have found that the reactive portion of beef, sheep, horse, rabbit, guinea pig, dog, hog, and chicken sera is present in equal concentration in the euglobulin and pseudoglobulin portions of the inactivated serum. It should be noted that Mackie and Watson used the CO_2 fractionation method, while Sherwood and his co-workers used Howe's Na_2SO_4 fractionation procedure. Finally, Mackie and Watson (1926) found nonsyphilitic rabbit sera fully as stable to the effect of heat as were syphilitic human sera.

Eagle (1932) has claimed that nonspecifically reacting rabbit sera will also fix complement in the presence of "almost any colloidally dispersed lipid suspension, including some which have not the slightest reactivity with human syphilitic serum, such as egg lecithin, milk lipoid, sheep red blood cell lipoid, or cholesterol." But these experiments include results obtained with only nine normal rabbits and nine sera of syphilitic humans of which the data of one experiment are shown. Again, it has been claimed that the majority of these Wassermann-positive rabbit sera show entirely negative flocculation tests (Bachmann, 1922). The results of many investigators (Kemp, 1940; Hoag and Leven, 1932) contradicts this claim.

Before returning to the discussion of other efforts to differentiate nonsyphilitic from syphilitic serological reactions we might summarize this brief discussion on nonsyphilitic serological reactions in rabbits by adding that little is known concerning the relationship of these rabbit reactions to the reaction in syphilis.

Witebsky (1931) described a procedure for distinguishing between positive reactions given by rabbits inoculated with Spirochaeta pallida from positive reactions given by rabbits injected with syphilitic serum-antigen mixtures containing floccules. The technique is as follows: A serum is examined with a flocculation test. The tube containing the formed floccules is placed in a water bath at 56°C. (time not specified). The floccules are washed 20 to 30 times with saline, then suspended in saline, the tube heated at 56°C., and centrifugated while contained in a water jacket at 56°C. The supernatant fluid is tested either by flocculation or complement-fixation. Positive findings indicate a specific reaction. The dilution error is allowed for by replacing the aqueous supernatant fluid with a volume of saline equal to the original volume of serum. The procedure was claimed to be applicable to the determination of the specificity of serologic/al reactions given by human beings. This publication was followed by a more extensive article (Witebsky, 1933). The procedure

was somewhat modified. "Citochol" or Kahn antigen is employed. To 1.0 cc. of serum is added 0.5, 0.25 and 0.125 cc. of antigen suspension properly diluted. The mixture is shaken vigorously and incubated in the cold room overnight. On the following morning the tubes are centrifugated for 10 minutes at high speed, and the sedimented floccules washed three times with ice cold saline. The volume is replaced to 1.0 cc. with saline and the tube heated at 56°C. for 30 minutes. Centrifugation at high speed in water jackets at 56°C. follows. The supernatant fluid is then tested as previously described, i.e., by complement-fixation or flocculation. Witebsky stated that the reaction did not distinguish false positive reactions occurring in trypanosoma infections or in malaria.

D'Allessandro and Sofia (1933) further modified the procedure by adding a small amount of formalin to the saline when washing the floccules. These authors found that they could separate the reactive portion of syphilitic sera giving positive flocculation and complement-fixation reactions from the reactive portions of the same sera giving positive complement-fixation reactions in tuberculosis and gonococcal infections. However, they were not able to differentiate positive reactions in syphilis from those given

by patients with malaria and by one patient with typhoid fever. Bier and Trapp (1941) modified the Witebsky procedure by taking the washed floccules up in 10 per cent saline solution before heating at 56°C. They were unable to distinguish between positive syphilitic and leprosy sera by their method. No further publications concerning the method have appeared.

Rytz (1939, 1942) has described a differential method. Serums used must not contain hemoglobin. To 0.15 cc. of the serum is added 0.3 cc. of a 2 per cent copper sulfate solution. The contents are mixed and centrifugated at about 2000 r.p.m. for five minutes. To 0.15 cc. of the supernatant fluid is added 0.05 cc. of half saturated ammonium sulfate solution. After thoroughly shaking, 0.03 cc. of Rytz antigen emulsion is added to the tube. The tube is then shaken and centrifugated at about 2000 r.p.m. for five minutes. After centrifugation, 2.0 cc. of distilled water is added to each tube. The tubes are slowly inverted twice and recentrifugated. The supernatant fluid is discarded, the tubes allowed to drain for a moment or two, then placed upright and 1.0 cc. of 1:20,000 solution of potassium oxalate in distilled water is added. Tubes are allowed to stand at room temperature for 15 minutes and then read for degree of flocculation. According to Rytz,

only syphilitic sera show flocculation. No reports other than that published by Rytz have appeared in the literature.

b. Use of Spirochetal Antigen (Mohr, Moore and Eagle; Gaehtgens). It has been suggested (Mohr, Moore and Eagle, 1941) that the complement-fixation test with spirochetal antigen (Pallida reaction of Gaehtgens) might serve as a procedure for differentiating syphilitic from non-syphilitic serological reactions. Beck (1939), however, reports 1.2 per cent false positives in 500 control sera using the Reiter strain of cultured spirochetes; 1,8 per cent with the Kasan strain; 1.7 per cent with the Kroó strain and 4.4 per cent false positives with a mouth spirochete strain. Eagle, Hogan, et al. (1941) report complete lack of specificity of spirochetal complement-fixation tests in the case of positive serological reactions associated with malaria. Capelli (1939) reports lack of specificity in nonsyphilitic leprosy patients. Vogelsang reports (1940) 1.2 per cent false positive reactions with spirochetal antigen (Gaehtgens) in 1587 nonluetic sera. The studies of Kolmer, Kast and Lynch (1941a) are especially significant. These authors cultured Reiter's strain of nonvirulent spirochetes and used it as antigen in complement-fixation tests. They found that these gave positive reactions in malaria and in leprosy in the

absence of syphilis and also in a high percentage of normal sera (13.5% of 260 sera). They suggest that these reactions are due to a natural antibody to Spirochaeta macrodentium and Spirochaeta microdentium which may be present in many human beings.

Gaehtgens (1930) claimed that nonspecific "pallida" reactions were usually thermolabile (62° - 63°C. for 30 minutes), while syphilitic sera were thermostable and would react in the complement-fixation test with the spirochetal antigen after being heated at this temperature. These findings could not be confirmed by either Hoeltzer and Ssuschkowa (1930) or Eagle and Hogan (1940).

c. Differential Temperature Method Proposed by Kahn. Kahn (1940) has described a differential serological procedure (verification test) based on the observation that syphilitic sera show optimum flocculation at 37°C., while nonsyphilitic sera show optimum flocculation at 1°C. The procedure is similar to that of the standard Kahn test except that it is carried out at 37° and 1°C. Kahn has limited this procedure to the diagnosis of latent (asymptomatic) syphilis. Some recent clinical studies (Mohr, Moore and Eagle, 1941; Chargin and Rein, 1941; Jacobsthal, 1941) indicate some promise for this method.

1) Effect of Temperature on Serological Reactions. In view of the fact that temperature studies play a major role in this thesis, it seemed advisable to present a brief review of some previous studies on the relation between temperature and serological reactions.

The effect of temperature on serological reactions in vitro can be broadly considered from at least two points of view: (1) the effect of temperature on the reaction when the reactants are treated for definite periods preceding and/or following mixing, and (2) the effect of temperature on a reaction in which the components have not previously been exposed to temperature-time relations known to affect their serological reactivity. These two factors can each be further considered as to the effect of temperature on the product of the interaction of the reactants. This plan has been used as a general, nonrigid, approach to the discussion of findings of different experimenters.

The recognition by early workers of bactericidal properties of unheated whole blood (Nuttall, 1883), and of blood serum (Buchner, 1890), which could be destroyed by heating at temperatures which brought about no visible changes in these fluids, are among the first studies concerned with the effect of temperature on immunological reactions. Fodor (1887) had found that whole blood of a rabbit inoculated with a culture of anthrax bacilli, allowed to

incubate for 10, 30, 60, and 120 minutes had an inhibitory effect on the growth of these organisms in direct relation to the length of incubation. This effect was demonstrated in gelatin cultures.[1]

Nuttall (1888) found that the defibrinated whole blood (at 38°C.) of mice, rabbits, pigeons and a ram was bactericidal for anthrax bacilli. The defibrinated blood began to lose this bactericidal property on standing at 38°C. or at room temperature (19°-21°C.) for about six hours. Nuttall claimed that this antibacterial property of blood was not due to a "fixed disinfectant substance," but due to a heat-labile substance which had a ferment like action. The lability of the substance was demonstrated by the destruction of the bactericidal property on heating the defibrinated blood. Heating dog blood ten to thirty minutes at 52°C. destroyed its bactericidal action for the anthrax bacillus. Rabbit blood heated for 45 minutes at 55°C. also lost its activity, although ten minutes heating at 48° to 50°C. only weakened the germicidal property.

Buchner and his associates (1890) confirmed and extended Nuttall's findings. Dog and rabbit sera were

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[1] Buchner (1890) quotes Fodor as saying that the decrease in the bacterial count might have been due to the mechanical occlusion of organisms in the clotted blood and not to the bactericidal property.

used against cholera, anthrax and typhoid organisms in their experiments. The authors found that the serum expressed from clotted blood retained the full bactericidal activity of the defibrinated whole blood and that freezing and thawing did not destroy this activity. The antibacterial action diminished on standing at room temperature, but retained some activity after being left for twenty days in a cold room (4° - 6°C.).

Pfeiffer and Issaeff (1894) observed that the fresh serum of guinea pigs, immunized against the cholera organism, would lyse these organisms *in vitro*. Serum heated from 60° - 70°C. lost this lytic power, although exhibiting protective and lytic properties if it were introduced into normal guinea pig peritoneal cavity along with cholera organisms.

Bordet (1895) confirmed and furthered these observations. He found that fresh, unheated serum of guinea pigs, which alone was only "slightly bactericidal," and anticholera serum heated at 60°C. for one hour (*i.e.*, serum which had no obvious action against the vibrios *in vitro*[1]) would, when brought together, form a mixture which was "very antiseptic," bringing about complete destruction of the cholera organisms in his experiments. He concluded, therefore, that the lysis of the cholera vibrio was due to two

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[1] In a footnote, Bordet states that ". . . . les microbes rassemblés en amas."

substances in immune serum: the specific preventive substance which could withstand heating at 55° to 64°C. for one hour, and another substance (alexin or complement) which was contained in fresh serum of non-immunized animals. Bordet was thus able to separate antibody and complement effects as a result of his temperature studies. Later (1898), Bordet extended his studied to hemolysis. Guinea pigs immunized with defibrinated rabbit blood developed the capability of lysing rabbit erythrocytes injected intraperitoneally. The fresh sera of these animals lysed rabbit blood cells also in vitro, but after heating from 10 to 20 minutes at 56°C., the lytic property was lost.

Bordet and Gengou (1901) applied these studies on hemolysis in a practical manner with a description of the complement-fixation test.[1] The fixation period employed was five hours at room temperature (15° - 20°C.). Systems used by them included: a) horse antiplague-bacillus serum, and a saline suspension of the corresponding antigen, b) guinea pig antianthrax bacillus serum and a saline suspension of the organisms as antigen. Other antigen-antibody systems were also employed. The complement in these systems was obtained from fresh guinea pigs.

The method of complement-fixation was adapted by a

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[1] The use of the term "complement-fixation" is not Bordet's, but conforms with contemporary usage. Bordet and Gengou did coin the expression "fixation d'alexine" in this communication.

number of workers (Wassermann, Neisser and Bruck, 1906; Detre, 1906; Wassermann, et al., 1906) to the serodiagnosis of syphilis. The major technical modifications introduced were the use of aqueous tissue extracts for antigens and fixation periods of one hour at 37°C. Jacobsthal (1910) showed that fixation at icebox temperatures resulted in more sensitive results than did incubation at 37°C. Until the description by Kahn (1923a,b) of his flocculation procedure, all tests for syphilis embodied an incubation period following admixture of the reagents. Kahn found (1923a,b) that results of high sensitivity could be obtained in a flocculation test for syphilis by employing brief shaking periods (2-5 minutes) instead of long periods of incubation.

Preheating of sera to be tested with a complement-fixation procedure is necessary to destroy native complement. Theoretically, this should not be necessary if the hemolytic indicator is not used, as in flocculation tests. However, it is an old observation that sera used in flocculation tests for syphilis give more sensitive and specific results if first heated at 56° for 30 minutes.

Nishio, working in Kahn's laboratory, attempted to elucidate (1929) the difference in behavior of heated and unheated syphilitic sera in the flocculation test. He found that the globulin fractions of heated and unheated syphilitic sera behaved alike in the Kahn test. Unheated serum albumin, added to the globulin, inhibited the reaction, while if the

albumin was first heated at 56°C. for 30 minutes and then added to the globulin no inhibition of reactivity occurred. This finding suggested that native serum albumin exerted some "protective" action on the globulin; hence, it was the albumin fraction which was responsible for the low reactivity of unheated sera. Curiously enough, in spite of the importance of the observation, the problem, to our knowledge, has not been reinvestigated by other workers.

Many studies have been made on the temperature-time treatment of sera preceding their use in flocculation tests for syphilis but the classical 56°C. incubation for 30 minutes remains predominant. Attempts to introduce higher temperatures for shorter periods (Hardesty and Jurischk, 1932; Rein and Hazay, 1940) have not met with favor, although some serologists have suggested the use of three- to four-minute heating periods at 60° to 62°C. in emergency procedures. (Strauss, 1937; Eagle, 1940; Hinton, 1940; Kline, 1940)

It was mentioned above that syphilitic sera of human beings tested in an unheated state are usually nonreactive. This finding does not apply to positive animal sera which may give strong flocculation reactions in an unheated state. Little attempt has been made to study serologic/al reactions with unheated animal sera. Greene and Breazeale (1941) found that 119 cow sera gave positive flocculation reactions whether heated or unheated. The heated sera showed a higher quantitative titer than the unheated sera.

Any studies of the effect of increased temperature on antibody or antigen function *in vitro* are, of course, limited by the temperature at which the substance used is altered or destroyed. Antibody (*i.e.*, serum) proteins are destroyed by heating at 70° to 80°C. for short periods (Hartley, 1931). This generalization is not invariable. It is notable that different antisera display varying lability to heating. For example, Jones (1927) showed that heating rabbit antityphoid (flagellar and somatic) serum and rabbit amboceptor at 65°C. for 20 minutes lowered their activity disproportionately, the latter antibody being the more stable. Jones emphasizes also that comparisons cannot be made between similar antibodies in different species since somatic (typhoid) agglutinin in rabbit serum resisted 70°C. for 20 minutes, while the same agglutinin in cow serum was destroyed at 65°C.

Kroó, Schulze and Zander (1929) reported that rabbit antispirochetal antibody (cultured *Spirochaeta pallida*) was resistant to heating at 63°C. for 30 minutes, whereas reactivity to Wassermann antigen was destroyed at this temperature. Gaehtgens (1930) confirmed this claim. He found that nonsyphilitic sera giving "pallida" reactions were usually thermolabile (62°-63°C. for 30 minutes), while syphilitic sera were thermostable and would react positively after being heated at this temperature. These findings could not be corroborated by Hoeltzer and Ssuschkowa (1930), or by Eagle and Hogan (1940).

Antigens which are of protein nature may have their specificity altered and a new antigenic specificity conferred upon them by the use of heat (Furth, 1925; Landsteiner, p. 29, 1936). Furth has reviewed this work and found also that heating at 70°C. partially transforms rabbit blood serum into the "cocto-antigen." The antigen used in tests for syphilis is relatively heat stable. It can be prepared by extracting dried beef heart with boiling alcohol in a reflux condenser for one to three hours (Ecker and Sasano, 1919), or by extracting with boiling ether and then alcohol in a reflux condenser (Kolmer, 1928), or in a Soxhlet apparatus (Kolmer, 1928; Hunter, 1936).

Some interesting studies of temperature effects on the hemolytic system have been made. Kahn and Lyon (1921) examined the affinity of sheep erythrocytes for antisheep hemolysin at different temperatures and for varying periods of incubation. The authors noted that this affinity at 37°C. (water bath temperature) was greater than at room temperature and this, in turn, greater than at icebox temperature. But the differences were small enough so that when the results of eleven experiments were averaged, room and 37°C. temperatures showed similar extractions for five, ten, fifteen and twenty-minute periods, while the icebox extraction was definitely lower. It was further noted that a ten-minute extraction period at room temperature in the presence of excessive amounts of hemolysin (25,000 units) was usually equivalent to

a one or two-hour extraction at 37°C. In some cases less extraction occurred at the higher temperature which the authors suggested might be due to a dissociation of the hemolysin-cell complex. Cromwell (1922) has confirmed these observations in an extensive study in which he noted that one rabbit hemolysin showed most complete absorption at 15° to 25°C. and less absorption at higher and lower temperatures; another rabbit antiserum showed greatest absorption of sheep cells at 40°C.

Other information has been obtained from the study of the effect of temperature on antibodies to erythrocytes. Stuart, et al., (1934) found that 24 of 25 normal human sera agglutinated sheep red blood cells (Paul-Bunnell test) of 0.02/ per cent final concentration in a serum dilution of from 1:160 to 1:1280 after 12 hours incubation at 5°C. Of the same group of sera only one showed a titer of more than 1:40 when the reactions were run at 37°C. for two hours, or when the cold reactions were incubated an additional two hours at 37°C. The agglutination of sheep cells by normal human sera at low temperatures could be reversed, therefore, in 23 of 24 instances merely by incubating at 37°C. Titers of from 1:320 to 1:5,120 were obtained with the sera of 14 infectious mononucleosis patients and these titers were independent of the temperature (5°C. or 37°C.) at which the agglutination tests were performed.

The conclusion that the combination of antigen and antibody are dissociated in the above instance would not be without precedence. Morgenroth and Rosenthal (1912) showed that although amboceptor markedly decreased in reactivity on being heated for 30 minutes at 56°C., some dissociation of the amboceptor-red cell complex occurred at the same time, making the amboceptor reavailable for cell sensitization. Similar heat dissociations in attempts to purify amboceptor have been reviewed by Muir (1931).

Wiener (1939) has reviewed effects of temperature in iso-agglutination phenomena. Kettel (1930) found that iso-agglutination titers decrease linearly with increasing temperature, the slope of the line depending upon the initial titer at 0°C., at which temperature highest titers were obtained. Titers obtained at 30°C. were about half as strong as those at 0° and titers obtained at 50°-60°C., approximately half as strong as those at 30°C. Similar titer-temperature relations hold for subgroup iso-hemagglutinins (Wiener, 1939).

Before turning to reports concerning the effect of temperature on precipitation tests, some recent complement-fixation studies might be considered in which guinea pig complement exhibits the property of combining with tissue extract antigen (in the absence of syphilitic serum) under conditions of icebox incubation and not at 37°C. Giordano

and Carlson (1939) reported that nonspecific reactions in the complement-fixation test for syphilis could be caused by a reacting substance in some guinea pig sera which was heat stable, nonreactive at 37°C., but reactive at icebox temperature. This property of fixation could not be noted, therefore, in the usual complement titration at 37°C., but only when the complement titration included a preliminary (three hour) incubation at icebox temperature in the presence of the antigen.

Kolmer and Lynch (1939) have confirmed this finding. They claimed that 2 to 10 per cent of normal guinea pig sera may contain the reactive factor. Of 132 guinea pigs, 9.1 per cent showed positive Kahn reactions, while 18 per cent to 32 per cent were Wassermann-positive depending on the mode of preliminary incubation. Heating these sera at 62°C. for 30 minutes reduced their serological activity, while heating at 56°C. for 20 minutes increased it.

Certain generalizations concerning precipitation (or flocculation) reactions have been made (Topley and Wilson, 1935; Eagle, 1930) which, in general, agree with the popular impression that broadly speaking, these reactions follow the Van't Hoff Law; namely, that the velocity of reaction is doubled with every ten-degree (centigrade) rise in temperature. Eagle has stated that the optimum temperature of flocculation in tests for syphilis is something less than

60°C. Topley and Wilson have stated (page 143): "An increase in temperature up to an optimum also increases the speed of flocculation, but it seems doubtful whether this effect is due wholly to an increased frequency of impact. With some reacting systems the higher water-bath temperatures (37°-55°C.) may be found to be unsuitable since the antigen-antibody compound may be partly soluble in this range."

Many exceptions, however, exist to these generalizations. Heidelberger, Kendall and Soo Hoo (1933) noted that the optimum temperature for the precipitation of an azoprotein antigen with its rabbit antiserum was anywhere between 0° and 37°C. On the other hand, Heidelberger and Kendall (1935a) noted that two horse antipneumococcus (type III) polysaccharide sera showed 25 percent more antibody nitrogen in the precipitate when the reaction was run at 0°C. (in the presence of an excess of antibody) than when run at 37°C. Follensby and Hooker (1939) suggested the possibility that the same degree of combination but not of precipitation occurred in the case of the 37°C. reaction. In the same article these authors also considered the reaction between diphtheria toxin and antitoxin. They concluded that a decrease in temperature from 40°C. to 5°C. has little effect on the degree of combination between the reactants but markedly increases the flocculation time.

Stahl and Pennell (1941) have determined by the methods of Heidelberger and his co-workers, the optimum conditions for the obtaining of antibody in the precipitation and agglutination reactions with brucella antisera. Using the B. abortus "endoantigen" preparation of Huddleson they found that over twice as much antibody N was precipitated from human abortus antisera by running the reaction for 48 hours at 4°C. as was obtained by incubating at 37°C. for 24 hours, or at 37°C. for two hours followed by 22 hours at 4°C. Under similar conditions, over twice as much antibody was precipitated from guinea pig abortus antisera at 37°C. in 24 hours as was precipitated in the cold by the same period of incubation.

However, in the case of the agglutination reaction the temperature-time relationships were almost diametrically opposite from the precipitation results. Three times as much human antibody N was present in the agglutinate at 37°C. in 48 hours as came down at 4°C. in 24 hours, while the same amount of guinea pig antibody N was present in the agglutinate under all time-temperature relations tried.

B. abortus, B. suis and B. melitensis antisera from cows and goats precipitated most heavily when incubated with homologous antigens at 37°C. for two hours followed by 22 hours at 4°C., however incubation at other temperatures gave similar results.

Certain studies which included observations of the effect of temperature on the product of antigen-antibody

interaction have been considered (Morgenroth and Rosenthal, 1912; Stuart, et al., 1934). Some other reports along these lines might be noted.

Jones (1928) observed that although antiserum to the hog-cholera bacillus lost its ability to agglutinate that organism on being heated at 75°C. for 30 minutes, it retained its combining ability since unheated antiserum added to this mixture had no effect on it. Such an experiment recalls the concepts of "Agglutinoids," "Precipitinoids," and "Complementoids;" substances which, according to Ehrlich and his co-workers (Zinsser, pps. 235, 265, 158; 1922) had been altered by heating, in a manner which did not affect their combining properties, but did inhibit their specific property of agglutinating, precipitating or bringing about cytolysis.

Recently, Rivers (1942) in reviewing some work of Smadel and Rivers stated that two antigenic components could be separated from the virus-free filtrates of the skin of vaccinia infected rabbits by the use of heat. One component (L) was found labile to heating at 56°C. for 30 minutes, i.e., "when heated at 56°C., L no longer is precipitated in the presence of its antiserum, however it inhibits L antibodies so that when unheated L is added to the mixture no precipitate occurs." The second component of the complex (S) was found to be unaltered by heating at 56°C.

Some reports dealing more directly with the effect of temperature on the product of antigen-antibody reaction are now considered.

Ottensooser (1923) found that the precipitate formed by the interaction of certain ratios of rabbit-anti-egg albumin serum and the corresponding antigen was dispersed when heated at 55°C. for ten minutes. The precipitate did not reform on cooling. Heidelberger and Kendall (1929) reported that precipitates formed by the reaction between Type III pneumococcus polysaccharide and its rabbit antibody, in certain proportions, dissolved on warming from 0° to 37°C., but reappeared on chilling. This phenomenon was not noted with rabbit antibody to an azoportein antigen (Heidelberger and Kendall, 1935b) or to chicken-egg albumin (Heidelberger and Kendall, 1935c). Duncan (1932) has found that a certain amount of the precipitate formed by the interaction of a yeast gum and its rabbit antiserum could be resolved by heating at 50°C. for two hours. The precipitate reappeared on cooling for 24-48 hours.

Turning to the effect of temperature on the flocculation test for syphilis, little work was done previous to 1940. Georgi (1919) claimed that low temperatures (icebox incubation) weakened or obliterated the Sachs-Georgi reaction with syphilitic sera. No attempt was made to determine any quantitative relationships.

Duran-Reynals (1940) observed that chicken sera flocculate saline extracts of tissues of many animals. This flocculation occurred optimally at low temperatures. As a result of his findings, Duran-Reynals suggested that "Alcoholic extracts of tissues (as well as saline extracts) are flocculated at low temperature by active chicken sera and with such sera Wassermann and Kahn reactions are positive, which suggests that the factor in chicken sera which flocculates tissue extracts is of the same nature as that responsible for the reactions with syphilitic sera."

This conclusion is open to serious doubt on the basis of the work of Georgi (1919) which suggests that syphilitic sera react very weakly in the flocculation test at low temperatures. Kahn, et al., (1941) have confirmed this finding, employing a different technique. It was further found by this group that Kahn antigen is flocculated optimally at ice-water bath temperatures by the sera of hogs, horses, rabbits and chickens, when these sera are first chilled to 1°C. and then mixed with the antigen at this temperature.

The effect of incubation of the completed Kahn test at different temperatures has received some attention. Older flocculation techniques employed in the serodiagnosis of syphilis (Meinicke, Sachs-Georgi, Sigma, Dold, Hecht[1]) routinely used various incubation periods at 37°C. Of the more

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[1] Techniques summarized by Kahn (1925).

modern flocculation procedures, Eagle and Hinton incubate at 37°C. Kahn (1923b) showed that incubation was not a necessary element in the flocculation test for syphilis. On studying the effect of various incubation periods at 37°C. on the completed Kahn test without the addition of saline, the latter author concluded (1925) that "the effect of incubation at 37°C. on the test is comparatively little."

Nagle and Lazarov (1930) incubated the completed Kahn test to which saline had been added at icebox (5°-7°C.) incubator (37°C.) and room (22°-28°C.) temperatures.

No quantitative study of the 4+ sera was made. Incubation of 965 specimens of different types overnight at room temperature resulted in very little change. A 1+ reaction became 4+, three 1+ reactions reverted to negative, one negative result became 2+ and another of this type became 1+. Six hundred forty-three negative results and 274 positive results were unchanged. Incubation of 521 tests at 37°C. showed other interesting characteristics. Sixty-nine 4+ reactions remained unchanged. Five 4+ reactions reverted to 2+ or 1+, and ten 4+ reactions became negative. It must be emphasized that the quantitative titers of these sera were not known. However, ten 3+ reactions (titer of 3 units) remained unchanged, while fourteen 3+ reactions became negative. Eight 2+ reactions showed negative results after incubation at 37°C., as did

twenty-one 1+ reactions. In no case did a 3+, 2+ or 1+ reaction become stronger on incubation at 37°C. One negative test, however, did show a 1+ reaction after such incubation. The results reported in the experimental section of this paper while not obtained on as extensive a scale, confirm and amplify these findings of Nagle and Lazarov. These two investigators further found that incubation of the completed test in the icebox had a tendency to give false positive reactions. This was based on the fact that 54 negative results showed varying degrees of positive reactions after the incubation period. The majority of the positive results (207) and negative results (432) were unchanged.

Muckenfuss and Ebel (1931) studied the effect of incubating the mixed reactants at varying temperatures before completing the tests. They concluded that incubation at low temperatures (icebox) weakensthe reaction, whereas incubation at 37°C. enhances the reaction. In relation to other results, it must be emphasized that this incubation occurred previous to the three-minute shaking period in the Kahn test.

This review of the effect of temperature on serological reactions will be summarized briefly by noting that the problem can be divided into at least two parts: (1) the effect of temperature on the reactants before they are brought together and (2) the effect of temperature on the reaction itself. High temperatures tend to reduce the reactivity of

antisera as well as dissociate certain formed antigen-antibody complexes. Certain reactions show an optimum degree of reactivity if the reaction is carried out at low temperatures, others show greatest reactivity at body temperature, and still other reactions are largely indifferent to temperature. It does not seem as though any all inclusive generalizations can be made with regard to the effect of temperature on immunological serum reactions as a whole.

4. Nonspecific Aspects in Tests for Syphilis

The development of our knowledge of the nonspecific reaction in tests for syphilis has hinged broadly on two experimental methods of approach. The first is concerned with the use of lipidal substances as antigen (obtained from mammalian organs and in no known manner related to the Spirochaeta pallida) and the second with the eliciting of serological reactions in nonsyphilitic sera by some physical or chemical means.

It would seem that if the antigen used was in no way related to the disease, its sensitivity might be altered to any desired degree, even to the extent of reacting with many nonluetic sera. Efforts to determine whether this is possible have been described by Kahn (1942). The variation in the types of lipids used in tests for syphilis, emphasizing this nonspecific aspect of the test, are reviewed below (in Part "a").

The possibility of eliciting positive reactions in nonsyphilitic sera has theoretical and practical significance - theoretical, since it would add evidence to the belief in the nonspecific nature of the test (unless it were assumed that the synthesis of the reactive substance in the serum of nonluetic individuals had been accomplished); practical, since it would further emphasize the possibility of nonsyphilitic reactions under certain conditions in practise. The first part of the experimental section deals with this problem

but in order to avoid a lengthy review of the literature at that point, the material has been considered here (in Part "b").

a. Antigens in Tests for Syphilis

The apparent lack of relationship between the antigens used in the test for syphilis and the Spirochaeta pallida was noted very early in the history of the serology of syphilis. Weygandt (1907), Marie and Levaditi (1907), Landsteiner, Müller and Pötzl (1907a, c), Weil (1907), and Michaelis (1907) showed that saline extracts of normal tissue could be used as the antigenic reagent in the Wassermann test. Landsteiner, Müller and Pötzl (1907b), Wassermann (1907), and Levaditi and Yamanouchi (1907) found that alcoholic extracts of normal tissue were fully as active as saline extracts of luetic tissue.

This nonspecificity of the alcoholic extract antigen was further emphasized in the employment of various other organic substances as antigen in the test. Wassermann (1907) gives credit to two of his assistants (Porges and Meier) for having noted "dass man mit Lecithin auf luetisches Serum eine im Wesen durchaus ähnliche Reaction zu erhalten vermag."

Porges and Meier (1908) later gave a full report of their results. They reported flocculation and complement-fixation results using a Kahlbaum lecithin preparation as their antigen. Complement-fixation test quantities used were 0.05 cc. of a 1 per cent saline suspension of the stock lecithin solution (about one-quarter its hemolytic amount.) Flocculation test amounts used were 0.2 cc. of a 1 per cent saline suspension of the same lecithin solution. This test was carried out by adding the antigen suspension to 1.0 cc. of a 1:5 dilution of serum. The results as a whole in 100 cases of all types of syphilis agreed remarkably well with the Wassermann reaction. The authors believed that this lecithin reagent could be used as antigen for routine clinical work.

These results were confirmed by Elias, et al. (1908) who ascribed the reaction to some colloidal property of serum distinct from any specific protective "anti" action and who reported optimum flocculation zones of lecithin suspensions in the presence of luetic sera. Levaditi and Yamanouchi (1907) claimed that solutions of sodium taurocholate or glycocholate could be used as antigens in the Wassermann test, but that a commercial lecithin preparation (Lecithol Riedel) was inactive. Sachs and Altmann (1908), using from 0.4 to 0.15 cc. of a 1 per cent sodium oleate solution as antigen in the Wassermann test, found that

this material gave no reactions with 11 nonsyphilitic control sera but showed good agreement with the routine Wassermann test in 13 cases of syphilis.

Sachs and Rondoni (1938) described a formula for the preparation of a "synthetic" Wassermann antigen. The ingredients were sodium oleate (Kahlbaum), ovo lecithin (Merck) and oleic acid (Kahlbaum). The procedure consisted of mixing and emulsifying 1.0 gram of dried sodium oleate in 5.0 cc. of water. This mixture was added to 95 cc. of absolute ethyl alcohol to give a 1 per cent soap solution. The material was filtered and to the clear filtrate was added 300 cc. of a 1 per cent solution of lecithin and sodium oleate in ethyl alcohol. The material was stirred, filtered and added to 500 cc. of alcohol. To use as "antigen," a 1:5 mixture was made by adding 4 parts of saline, a drop at a time, to one part of the alcoholic solution. This brought about a homogeneous and more reactive suspension than rapid addition of the saline. In their Wassermann system, 0.4 cc. of this antigen (one-half its anticomplimentary titer) was an effective test amount. Sachs and Rondoni claimed that results were specific and sensitive, although no data was given. This work has not been reported on further.

The enhancing of antigenic sensitivity in complement-fixation (and flocculation) tests by the addition of cholesterol (Browing, Cruickshank and M'Kenzie, 1910;

Sachs, 1911; Sachs and Georgi, 1918) has proved to be of great practical value but has further cast suspicion on the specificity of the test for syphilis. Of interest is the fact that cholesterol itself is apparently devoid of antigenic activity, as was shown by the aforementioned investigators.

Neymann and Gager (1917) in an attempt to determine the reasons for variation in the type of antigen obtained from different beef hearts, qualitatively analyzed the ether and alcohol extracts of beef heart and determined the antigenic activity of the different fractions obtained. Noguchi (1911) had previously classified a number of lipid "fractions" of tissue extracts on the basis of differential solubility; titrated and defined each in terms of "antigenic," "anticomplementary" and "hemolytic" amount in complement-fixation tests and claimed the superiority of the "acetone insoluble" fraction; i. e., alcoholic extractives of liver at 37° C., extracted with ether, precipitated by an excess of acetone and this precipitate taken up in alcohol. Essentially Noguchi employed a method for the preparation of crude lecithin.

The method of Neyman and Gager was to first extract the dried beef heart with ether and then with absolute alcohol, following the earlier procedure of Erlandsen (1907). The ether soluble fraction reduced to dryness and extracted with alcohol yielded an extract of some antigenic activity, believed by the authors to be largely due to a lecithin

compound. From the secondary extract of the tissue with absolute alcohol another antigenically active fraction was isolated which, on the basis of the isolation procedure fell into a class of phosphatides which Erlandsen (1907) had established as "diaminomonophosphatide." As a result of these studies the authors advocated a preliminary extraction of heart muscle with ether followed by an alcoholic extraction in order to obtain the most active and consistent antigens. Some slight variation of this procedure is used today by practically all serologists in the preparation of antigens for tests for syphilis.

Contemporarily attention has been redirected to the isolation of the substance (or substances) responsible for the antigenic activity of tissue extracts. Wellman and Lankelma (1941) describe a procedure for the isolation of lecithin fractions from tissue extracts. After testing the material they conclude that "a lecithin fraction purified through the formation of the cadmium salt not only retains its antigenic activity but also has undergone a measurable increase in concentration of the true 'antigenic substance.'" Brown and Kolmer (1941) adopted a different approach. They secured the flocculate from a mixture of pooled syphilitic sera and dried, weighed and analyzed it. The authors report that the antigenic portion of the flocculate contains mixed phosphatides of which the diaminomonophosphatide fraction is inactive. Two active fractions having solubility charac-

teristics of lecithins and cephalins were isolated but analyses showed them to be mixtures, each containing about 40 per cent of a nonnitrogenous, phosphorous containing substance. The authors conclude that "The available evidence indicates that the antigenic activity cannot be a property of either lecithin or cephalin but is due to an unknown substance absorbed in equal proportions by both."

Pangborn (1941) claimed a new procedure for the isolation of "lecithin" which yielded a substance the activity of which closely resembled that of the extract antigen in the Wassermann test. Later (1942), this author claimed the active compound to be a formerly unrecognized non-nitrogenous phospholipid (designated by her as cardiolipin) which on hydrolysis yielded fatty acids and a phosphorylated polysaccharide.

It would appear that the outstanding known facts regarding the chemical nature of tissue extract antigen are that the active constituents are soluble in lipid solvents and they are apparently related to phospholipids.

b. Eliciting Positive Serological Reactions in Nonsyphilitic Sera

Eliciting positive serological reactions in tests for syphilis with sera which are ordinarily negative with serodiagnostic tests has concerned many investigators. These workers have employed various physical or chemical

means to bring about such reactions. Landsteiner and Müller (1908) noted that globulin fractions of syphilitic and non-syphilitic human sera obtained by acidification with carbon dioxide of serum diluted with distilled water gave positive Wassermann reactions. This observation was confirmed by Forssman (1921) and Eagle (1932).

Craig (1911) found that certain bacteria (staphylococci, streptococci) growing in blood serum specimens could give rise not only to anticomplementary complement-fixation reactions but also to false positive reactions within six days after inoculation. Normal blood serum kept sterile did not show this tendency after nine months' standing. Craig's observation has been confirmed by Kendrick (1924) who found laboratory contamination (which occurred while Kahn-negative specimens were allowed to stand uncovered for three to ten days) caused 54 of 142 specimens to become anticomplementary for the Wassermann procedure and 10 to be unfit for the Kahn procedure. Of the remaining sera, five gave positive Wassermann reactions and three gave positive Kahn reactions. Pohlner (1938) has obtained similar results with B. subtilis contamination of serum and spinal fluids. His article contains references to a number of other workers who have obtained similar results.

Aminoff (1939) has studied the ability of different spore-forming aerobes to bring about false positive reactions in serum. B. cereus and B. mycoides were found to be

greater offenders than B. subtilis. Of 24 negative sera inoculated with a B. cereus suspension 20 showed positive serologic/reactions after incubation for 48 hours at 37°C. and 10 out of 11 sera inoculated with B. mycoides under similar conditions also showed reactions. Rytz (1939) inoculated 1.0 cc. amounts of 100 nonsyphilitic sera with 0.05 cc. amounts of a nutrient broth culture of B. coli and incubated at room temperature for 24 hours, then centrifugated and examined the supernatant fluid by his flocculation procedure. He reports five positive reactions in this group of sera. Sievers (1939) also reports upon seropositive reactions following inoculation of sera with microorganisms and reviews the literature on this subject.

Many other methods have been described for bringing about false positive reactions with sera. Hirschfeld and Klinger (1914) reported that excessive shaking of sera with saline or incubation of sera diluted with distilled water brought about varying degrees of positivity. Nathan (1918) confirmed the experiment with distilled water. He obtained two positive reactions with 25 negative sera which had been diluted with distilled water and incubated at 37°C. for 24 hours, and 11 positive reactions with 25 negative sera which had been diluted and incubated in the icebox for 24 hours.

Hirschfeld and Klinger (1914) also reported that "absorption" of negative sera with suspensions of agar, bacteria and kaolin brought about seroreactivity. Nathan (1920) confirmed these findings and also reported that acidification of sera followed by short incubation (one hour) and subsequent neutralization resulted in positive reactions. McMeans (1923) has also reported similar findings and reviewed the successful efforts of others (Hektoen, Sherwood) in bringing about artificial false positive complement-fixation reactions.

Eagle (1932) has not been able to confirm the reports of Hirschfeld and Klinger, of Nathan or of McMeans. He ascribes their results to an increase in the anti-complementary activity of the treated sera.

Forssman (1921) found that anesthetic ether mixed 1:1 with negative sera and allowed to incubate for from 5 to 24 hours caused the serum to assume reactivity in the Wassermann test. This did not occur when the sera were similarly treated and then heated for 30 minutes at 56°C. Hartley (1925) could not confirm Forssman's results and, in fact, found just the opposite; i.e., ether extraction (by the method of Hardy and Gardiner) destroyed the ability of even syphilitic sera to react.

Bachmann (1921) claimed that the addition of various amino acids to negative sera (glycine, leucine) resulted in positive Wassermann reactions. Bachmann credited

Mahlo with the observation of this fact in 1913. Fowweather and Gordon (1927) repeated these experiments and also injected glycine, leucine, "arginine base," arginine nitrate, and taurine into rabbits without eliciting positive results. They concluded that free amino acids were unrelated to positive Wassermann reactions.

Fractionation studies of the serum proteins have yielded interesting results. The early observations of Landsteiner and Müller (1908) concerning the Wassermann-positive reactivity of globulin fractions of syphilitic and nonsyphilitic human sera was confirmed by Forssman (1921) and by Eagle (1932), who used a flocculation procedure. The latter investigator claimed, however, that although globulins derived from nonluetic and luetic sources would react with aqueous suspensions of such diverse lipids as those derived from milk, egg lecithin, cholesterol, alcoholic extract of beef heart, sheep or human red blood cells "and presumably with any colloidal lipoid suspension," that luetic human sera would react only with suspensions of beef heart lipids.

Mackie (1923) using the CO_2 fractionation procedure (in which the 1:10 diluted chilled syphilitic serum saturated with CO_2 was found to be split into two fractions) assumed, on the basis of a limited number of experiments, that the insoluble fraction of the serum (euglobulin) was mainly responsible for the positive

complement-fixation reaction, while the soluble (pseudo-globulin plus albumin) fraction was the active principle in the flocculation reaction.

Mackie and Watson (1925) studied both nonluetic animal and luetic human serum fractions in the complement-fixation and flocculation procedures. The conclusions of these authors are not identical with those reported by Mackie two years before (1923). They reported that the euglobulin fraction showed a tendency to be Wassermann positive and the pseudoglobulin plus albumin fraction, flocculation positive. But they reported instances in which this tendency was not noted. They also reported that the globulin fractions derived from both syphilitic and nonsyphilitic seropositive sera reacted similarly.

Sherwood, Bond and Clark (1939) employing Na_2SO_4 fractionation found equal reactivity in flocculation tests of euglobulin and pseudoglobulin fractions of animal sera derived by this salting out procedure.

Mackie and Anderson (1937) attempted to induce nonspecific reactions by variation in the type of lipid used as antigen, by the heating of sera at temperatures of and above 56°C. and by a combination of these methods. These workers found that normal, heated (56°C. for 30 minutes) human sera, while nonreactive with beef heart lipids, showed flocculation results with cholesterolized B.diphtheriae lipids(4 of 6 different sera with extracts

of 4 different strains) but not with egg or vegetable lecithin suspensions. On heating these sera at 65°C. for 30 minutes, reactions occurred with egg lecithin. Different zones of reactivity with different lipids were noted when sera were heated at different temperatures. Of interest is the fact that the reaction complex representing syphilitic sera also characterized that of animal sera.

An earlier study of flocculation by normal sera with more emphasis on physical treatment of the antigen was that of Wolf and Rideal (1926). Using suspensions of gum benzoin "coated" with saponin as antigen, they found flocculation could be obtained with normal and syphilitic human sera (number not stated) although saline controls remained stable. In general, the luetic sera had a more extended zone of flocculation than did the normal sera, differentiation being most marked as the isoelectric point of serum globulin was approached by addition of acid to the serum.

The explanations of the findings by different authors on "induced" nonsyphilitic reactions is generally assumed to be based on the probability that such reactions are due to some labile, physico-chemical property of the serum and not to any specific antibody (Wells p. 211, 1929). This explanation by no means rules out the "normal antibody" hypothesis first expressed by Dohi (1911) with the statement that "....the antibodies (which react in the test for syphilis) may be a normal body substance, which are

produced in excess under the stimulus of syphilitic infection." Others (Mackie and Watson, 1926 ; Hinkleman, 1927; Kahn, 1940; Mohr, Moore and Eagle, 1941) have stated similar, if modified, ideas along these lines. It would seem that if the reactive substance were one that is present in normal sera, procedures might be evolved which would demonstrate its presence. Five recent papers bearing on this possibility are considered below.

Malloy and Kahn (1931) observed that when normal serum and standard or presumptive Kahn antigen were mixed there occurred a slow aggregation observed by means of darkfield illumination. In the case of standard antigen no aggregates were visible when the mixture was examined immediately. The cross sectional area of aggregates formed in 50 nonsyphilitic sera after one hour incubation at room temperature under a paraffin sealed coverslip varied from 0 to 500 square microns with an average of 60 μ^2; after six hours the variation listed was 0 to 1,500 μ^2. In contrast, 50 syphilitic sera similarly treated showed a cross sectional area for aggregates formed immediately as 420 (range from 20 to 1000 μ^2), after one hour as 1,730 (range 250-10,000 μ^2) and after six hours as 4,260 (range 500-25,000 μ^2). The authors concluded: "It is evident from these experiments that nonsyphilitic serums also show aggregates after contact with antigenic suspensions. It appears that the difference between nonsyphilitic and syphilitic serums is one

of degree rather than of kind - quantitative rather than qualitative. In syphilis, there seems to be a marked increase in the property possessed by serum to aggregate the lipoid particles of the antigenic suspension."

Schreus and Foerster (1934) described a procedure for the demonstration of small quantities of reactive material in treated seronegative syphilitic cases. When amounts of positive serum, so small as to be below the reacting threshold, were added to the seronegative specimen, there resulted a positive reaction. The authors believed this to be due to an additive (summation) effect.

Barnett, Jones and Kulchar (1935), using the Kline test, adapted the procedure of Schreus and Foerster to the demonstration of reactive material in normal serums. Using as "one unit" the least amount of reactive substance in a luetic serum that could be detected by their technique, the authors found that 27 of 42 normal sera showed 1.0 unit, 12 showed 0.9 units and 3 showed 0.6 units. The conclusion that "reagin is evidently present in the sera of non-syphilitic individuals - (and) the difference between syphilitic and nonsyphilitic sera seems to be a quantitative rather than a qualitative one --" agrees closely with the Malloy-Kahn conclusion.

Sherwood, Bond and Canuteson (1941) taking advantage of Kahn's early observation (1925) that as the ratio of

serum to antigen suspension was increased, the sensitivity of the test was increased, examined the sera of 1,018 college students with the Kahn test using serum-antigen ratios of from 100:1 to 1:5. Thirteen normal, healthy students showed reactions in the higher serum to antigen ratios.

Lund (1942) using a cholesterolized beef heart extract as antigen and the essential idea of Sherwood, et. al., (i. e., working in the range of excessive amounts of serum) succeeded in obtaining what appears to be an unlimited increase of sensitivity in tests for syphilis. Lund's procedure is based on the finding that if a small amount of antigen is added to a large amount of antiserum, incubation time allowed, and the reaction tube then centrifugated, highly sensitive results are obtained.

It is evident from the above review that positive serological reactions can be observed in nonsyphilitic sera under a comparatively large variety of conditions.

III. EXPERIMENTAL

1. Materials and Methods

Sera from chickens, cows, horses, pigs, rabbits and nonsyphilitic human beings were utilized in this study. These sources of sera were chosen largely because of their ready availability. Pig blood was obtained by arrangement with a slaughter house.[1] These animals are slaughtered by "sticking" the heart and opening a small flap of the skin over the chest. The first flow of blood was discarded. A chemically clean 50 cc. centrifuge tube was then filled with blood.

Chicken blood was obtained by arrangement with a commercial dealer.[2] Chickens were bled by cutting blood vessels of the throat. The first flow of blood was discarded and a centrifuge tube was then filled.

Horse sera were supplied through the courtesy of the Michigan State Health Department, Lansing. These sera, in sterile form, were from animals immunized to various microorgansims or bacterial toxins. Some of these sera were obtained from normal animals previous to inoculation.

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[1] Home Packing Company, Ann Arbor, Michigan.

[2] Otto Hack Inc., Ann Arbor, Michigan.

Cow blood was secured from the Ann Arbor slaughter house and through the courtesy of the Digestive Ferments Company of Detroit, Michigan. The Ann Arbor specimens were obtained from animals to be dressed as beef. Specimens were also sent from Detroit in sterile 50 cc. glass containers. These were collected on three occasions between 11:00 and 12:00 o'clock in the morning and shipped to Ann Arbor the same day where they arrived before 4:00 p. m.

Rabbit blood was obtained in all cases by heart puncture. These animals were of varying strains and were obtained from two dealers.[1] In a given number (32) of these animals, the date of birth was known.

The nonsyphilitic human sera were obtained from the Laboratory of Serology of the University of Michigan Hospital and were characterized by a complete lack of reactivity in the Kahn standard test and in the more sensitive presumptive test.

The routine procedure in the preparation of sera was as follows:

a) The clotted blood, after being brought to the laboratory, was placed in the icebox (4° - 7°C.).

b) A period of at least three hours was allowed for serum to separate by syneresis. The clot was then removed with wooden applicators and the serum centrifugated

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[1] Rockland Farms, New City, New York and Mr. A. Carus, University Hospital, Ann Arbor, Michigan.

at about 2000 r.p.m. in a size 2 International centrifuge. This centrifugation was carried out on the afternoon or evening preceding the day on which the sera were used experimentally. The clear sera were placed in the icebox.

c) The following morning, the sera were divided into two portions. One portion was placed in a water bath at 56°C. where it was allowed to remain for 30 minutes. The second portion was left unheated and allowed to reach room temperature while the first portion was being heated.

d) Following the 30 minute heating period, both heated and unheated serum specimens were examined for the presence of any precipitate, blood cells or other grossly observable foreign material. Any specimen showing extraneous matter was recentrifugated at high speed for three to five minutes. Rabbit sera heated at 56°C. for 30 minutes invariably showed a faint tendency to be less transparent than the unheated specimens. For this reason they were routinely recentrifugated after heating. In practically every case a slight, adherent, mucoid, greyish white precipitate appeared after centrifugation, leaving the serum as clear as the unheated specimens.

Generally, 10 cc. of rabbit blood yielded about 5 cc. of serum; 50 cc. of chicken blood 8 to 10 cc. of serum; 50 cc. of cow blood about 15-25 cc. of serum; 50 cc. of pig blood about 25-30 cc. serum.

Kahn antigen was used for both flocculation and complement-fixation tests. This antigen is prepared by four serial extractions of 100 gm. amounts of dried powdered beef heart (Difco) with ethyl ether in Erlenmeyer flasks. (The extraction time is 10 minutes and the amounts of ether used are 400 cc., 300 cc., 300 cc., and 300 cc., respectively.) The ether is filtered off after each extraction. Following the fourth extraction, the heart tissue is dried and extracted with 5 volumes / gram of 95 per cent alcohol at room temperature for three days. To each 100 cc. of the filtered alcoholic extract is added 600 mg. of cholesterol. The extract antigen was titrated and standardized according to the method of Kahn (1928). One lot of antigen (Lot 37) was used through the present studies.

For use in the flocculation test, 1.0 cc. of antigen was mixed with 1.3 cc. of saline. For use in the complement-fixation test, 9.99 cc. of saline was added to 0.01 cc. of the antigen. These ratios of antigen to saline represented the "titers" of the antigen.

Three basic serologic methods were employed in these experiments:

1) The standard Kahn test was the flocculation procedure used. This test is performed at room temperature (21° to 24°C., usually somewhat higher in summer). The same test was also performed at 37° and 1°C. These three temperatures were resorted to in most of the experiments.

The 1°C. temperature actually ranged between 0° and 1°C.

The technique at 37°C. was carried out as follows:

a) Kahn racks with test tubes were placed in a 37°C. water bath 15 minutes before performing the tests. The pipettes to be used were kept in a special waterproof compartment of the 37°C. water bath.

b) The serum to be used (or the dilution of serum decided upon) was split into two portions, one of which was then placed in the 37°C. water bath.

c) Standard Kahn antigen was mixed at room temperature in the usual manner with 0.9 per cent salt solution according to the titer. The resulting antigen suspension was then transferred to a small test tube and placed in the 37°C. water bath. The temperature of serum and antigen suspension reached the temperature of the water bath within 10 minutes as measured with ordinary mercury thermometers but a uniform period of 15 minutes incubation was allowed before employing these reagents.

d) The usual amounts of antigen suspension (0.05, 0.025, and 0.0125 cc.) were measured into the Kahn tubes by means of specially graduated pipettes. The serum (or serum dilution) in 0.15 cc. amounts was then pipetted into each tube. Pipettes graduated in 0.15 cc. amounts were used for this stage of the procedure. Following addition of the serum, the racks were shaken for about 10 seconds without being removed from the warm water bath.

e) The racks were allowed to stand in the water bath for from five to ten minutes and were then shaken for three minutes at room temperature in a standard shaking machine (275-285 oscillations per minute with a stroke of 4 cm.).

f) Saline (0.9 per cent salt solution), kept in the 37°C. water bath, was then added to each tube with a warmed 10 cc. pipette. To the first tube containing the 3:1 ratio of serum to antigen suspension, was added 1.0 cc., to the second tube containing a 6:1 ratio, 0.5 cc. and to the third tube containing a 12:1 ratio of serum to antigen suspension, 0.75 cc.

g) The racks were shaken by hand for a few seconds to insure mixing of the saline, and the flocculation results were then read.

h) A slit lamp was used in reading all results. The degree of flocculation was recorded as: ± (turbidity heavier than control tube but no visible flocculate), 1+ (few, small but clearly visible floccules in a usually dense menstrum), 2+ (small, numerous floccules in a cloudy mestrum), 3+ (numerous floccules in a fairly clear menstrum), 4+ (numerous large and small floccules in a relatively clear menstrum).

The technique at the cold temperature was carried out in a bath similar to that used for the 37°C. procedure with the exception that a cold water bath was employed containing chopped ice. Throughout this procedure the racks

were surrounded by floating ice. Mercury thermometers in this bath invariably recorded a temperature of 0°C., when the entire surface of the bath was covered with ice and a temperature of only 0.2° to 0.4°C. when there was a large amount of floating ice with some clear surface of water. Since the temperature did occasionally rise to as high as 0.7° to 0.8°C. because of time elapsed between filling the icebath and beginning an experiment, the range of temperature in this bath can be said to have varied between 0° and 1°C. In the text, the temperature of this bath is referred to as 1°C.

i) The steps in the technique as carried out in the 37°C. water bath were applied to the technique in the 1°C. water bath. The temperature of the serum and antigen suspension were brought to 1°C. and the serum was added to the antigen suspension and mixed at this temperature.

j) After shaking the racks and diluting with chilled saline the results were read in the slit lamp. Each tube, before being read, was dipped in a small bottle of chilled alcohol in order to remove the film of water condensation which formed while a series of tubes were being read.

Not more than twenty sera or dilutions of sera were tested at any one time at either warm or cold temperatures. Antigen controls were included in all experiments. When employing nonsyphilitic human or animal sera, a syphilitic serum was also employed as a control.

In the experiments to be described, the three minute shaking period was carried out at room temperature. The temperature drop during shaking in the 37°C. tests and the temperature rise in the 1°C. tests have not been carefully studied. Rough measurements with a mercury thermometer indicate that the temperature drop in the 37°C. tests may be about 4°C.; the rise in the 1°C. tests also about 4°C. On addition of warmed or chilled saline, temperatures again approach very closely to 37°C. and 1°C. Reading time for a series of 10 tests is about 1.5 minutes.

The Kahn-negative human sera used were occasionally tested in numbers exceeding twenty because of the availability of the sera, and the lack of necessity for previous treatment. These sera were obtained from the racks of completed tests of the Laboratory of Serology of the University Hospital. Only those sera which showed no reaction in either Kahn Standard or Presumptive tests and of which approximately 1.0 cc. of serum was available, were used.

2) The complement-fixation procedure was carried out in the usual manner and was based on the capacity of different serum-antigen ratios to "fix" designated amounts of complement. Guinea pig serum from at least three to four animals was used as complement. After collection, the serum was allowed to separate in the icebox overnight, centrifugated and stored in a frozen state between the coils in the icebox. The amboceptor - sheep cell suspension

mixture was prepared about every third day as follows:

a) Defibrinated blood, collected from a sheep maintained by the University Hospital was washed with saline until the supernatant fluid showed no visible red color after centrifugation (usually three washings of seven minutes each at 1000 r.p.m.)

b) A 5 per cent suspension was then prepared in saline and filtered through Whatman (number 1) filter paper.

c) Glycerinated amboceptor obtained from commercial sources was diluted 1:1000 and a volume equal in amount to the sheep cell suspension mixed with the cells.

Complement was then diluted with saline (1:10 to 1:30) and titrated in the usual manner by adding constant amounts of the amboceptor-cell mixture (0.2 cc.) to decreasing amounts of the complement.

The complement-fixation procedure with serum was a three tube test, as follows:

	FRONT ROW (Test)			BACK ROW (Control)		
	Tube 1	Tube 2	Tube 3	Tube 1	Tube 2	Tube 3
Serum	0.05cc.	0.05cc.	0.03cc	0.05cc.	0.05cc.	0.03cc.
1:100 Kahn Antigen	0.1cc.	0.1cc.	0.1cc	0	0	0
Units of complement	3	2	2	3	2	2
Drops of saline	1	2	2	2	3	3

A similar set up, in which saline was substituted for serum,

constituted the antigen control. One and two unit complement controls which contained no serum or antigen were also included as was an amboceptor-cell control.

The incubation temperature and time ("primary incubation") to permit fixation was generally carried out at: (a) 37°C. for 30 minutes. In other instances (b) room temperature for two hours or (c) icebox temperature (4° to 7°C.) for three hours were employed.

3) Combined flocculation-complement-fixation procedure. In this procedure a portion of the serum-antigen mixture in the completed Kahn test was used for complement-fixation studies. The procedure was carried out as follows:

a) After reading the flocculation results, 0.15 cc. amounts of the 12:1 ratio of serum to antigen suspension were pipetted into each of two tubes.

b) For the serum controls, 0.03 cc. (the amount of serum present in the 0.15 cc. above) quantities were employed.

c) To the first tube and a serum control were added two units of the complement and to the second tube and a serum control were added three units of complement.

d) One drop of saline from a 10 cc. pipette was then added to the three unit tubes and two drops to the two unit tubes in order to approximate the total volumes in each. Antigen controls containing 0.006 cc. of the antigen suspension (three times the test dose) and 0.012 cc. (six

times the test dose) were used. One unit and two unit complement controls plus saline were included as was an amboceptor-cell suspension plus saline control.

e) After the primary incubation of the serum, antigen, complement mixtures, the sheep cell suspension was added, the racks shaken, allowed to remain for 5 to 10 minutes and the secondary incubation carried out as 37°C. (Hemolysis is very slow at room temperature and does not occur at ice-box temperatures.)

Serum globulin and albumin fractions were also employed. These were prepared as follows:

a) To 2.0 cc. (or 1.5 cc.) of the undiluted serum was added an equal volume of saturated $(NH_4)_2SO_4$ solution. The sulfate was added slowly from a 2.0 cc. pipette, the suspension being stirred with a wooden applicator as it was being added.

b) The tubes were then centrifugated at about 2000 r.p.m.

c) The supernatant fluid was poured off[1] and the packed globulins allowed to drain.

d) The inner walls of the tubes were then washed two or three times with distilled water. The inverted

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[1] In not all cases was the supernatant material clear. Cloudiness would occur in about 2 or 3 of every 10 human, rabbit and pig sera. Horse, cow and chicken sera invariably showed well packed creamy white precipitates and a clear supernatant fluid. In cases where the supernatant material was not clear, it was treated as the other sera and a note was made of the fact.

tubes were allowed to drain, the lips dried with a towel and placed right side up.

e) To dissolve the globulins, a volume of saline equal to the original volume of serum was added slowly to the tube from a 2.0 cc. pipette while stirring with a wooden applicator. The final volume was about 1.5 times that of the original serum volume, due to the mass of the wet globulin.

f) Acid flocculation of the supernatant fluid from the globulin separation was resorted to in order to obtain the albumin. The volume of the supernatant fluid in the tube was marked with a glass marking pencil. Two drops of a methyl red solution were added and N/2 acetic acid added dropwise until a pink color developed ($_{p}H$ = approximately 4.2). This degree of acidity seemed to precipitate the albumins of all species tested equally well. In later experiments, the use of the indicator was abandoned and 0.6 cc. of the half normal acid routinely added to precipitate the protein fraction.

g) The tubes were centrifugated at about 2000 r.p.m. for 30 minutes, the supernatant material discarded, 0.5 cc. of saline added and mixed, then N/2 NaOH added until a weakly alkaline reaction was obtained with Fischer-Alk-Acid indicator paper. The volume was then made up with saline to the mark. The precipitates dissolved with little difficulty.

h) Globulin and albumin solutions were now centrifugated at about 2000 r.p.m. for 5 to 10 minutes in order to remove

extraneous material and foreign matter. They were then ready for use in tests parallel with those of sera.

Quantitative tests were then performed with the same dilutions at 37° and 1°C. In this way the serum dilutions acted as their own controls insofar as errors of dilution were concerned.

The quantitative Kahn test was also used in these experiments. In this test the serum is first diluted with saline and the diluted serum is then employed with standard antigen suspension.

The scheme of dilutions of the serum was as follows:

Dilution ratio	
undil.	
1:5	0.4 cc. serum plus 1.6 cc. saline.
1:10	1.4 cc. of 1:5 dilution plus 1.4 cc. saline.
1:20	0.8 cc. of 1:10 dilution plus 0.8 cc. saline.
1:30	0.4 cc. of 1:10 dilution plus 0.8 cc. saline.
1:40	0.2 cc. of 1:10 dilution plus 0.6 cc. saline.
1:50	0.2 cc. of 1:10 dilution plus 0.8 cc. saline.

The test was carried out by adding 0.15 cc. of each dilution to 0.0125 cc. of antigen suspension (i.e., 12:1 ratio). Numerical quantitative titers in the test are given in units. One unit is equivalent to a one plus reaction, 2 units to a two plus reaction, and so on. Titers of 4+ sera are determined by multiplying the last dilution of serum showing definite flocculation (2+ or more) by 4. An attempt was also made to finer approximation of the titer by interpolating any result showing a flocculate read as less than 2+ to have a titer between that tube and the

last positive tube, e.g., if the 1:10 dilution were positive and the 1:20 dilution doubtful, instead of the result being 40 units (10 x 4), it was 60 units (15 x 4). Practical experience with this method indicates that the error in the range of 1:5 (20 units) to 1:60 (240 units) is no more than one tube (1:10 dilution). Titers of 1 to 4 units were determined by averaging the standard Kahn result.

2. RESULTS

Part One. Sensitivity of Flocculation (Kahn) Reaction at 37°C., Room Temperature (21° - 24°C.), and 1°C. with Nonsyphilitic Sera and Globulin Fractions of the Sera.

Experiment 1. Effect on Sensitivity When Animal Sera Are Tested at 37°C., Room Temperature and 1°C.

The fact that the sera of normal animals give positive reactions with tests for syphilis has already been considered. In the present experiment, the effect on the sensitivity of the Kahn test at 37°C., room temperature and 1°C. was investigated with animal sera.

Preliminary serological determinations were made with horse serum and the results obtained are summarized in Table I. It is evident from the data of the table that of 17 horse sera tested, 10 gave positive reactions at 37°C., 13 gave reactions at room temperature and all 17 reacted at 1°C.

More extensive determinations were made with sera of chickens, cows, horses, pigs and rabbits. A total of 367 animal sera were employed. Each of these sera was examined with the Kahn test at 37°C., room temperature and 1°C. Sera which gave strong (4+) reactions at room temperature were diluted with saline to render them weakly (1+ to 3+) positive. The diluted sera were then tested at 37° and

TABLE I

FLOCCULATION REACTIONS WITH HORSE SERA IN THE KAHN TEST PERFORMED AT DIFFERENT TEMPERATURES

Indicating tendency toward increased flocculation at 1°C.

Serum Number[1]	Kahn Reaction at:		
	37°C.	Room Temperature	1°C.
	Tube 123	Tube 123	Tube 123
N9	---[2]	---	222
363	---	---	444
368	---	--±	444
N8	---	--±	444
959	---	3±-	444
592; 949	---	-24	444
T350	22±	444	444
P621	224	444	444
N821	444	444	444
N1 thru N7	444	444	444

[1] Serum numbers are those which marked samples of sterile horse sera received through the courtesy of the Michigan State Health Department, Lansing, Michigan.

[2] Designations of ±, 1, 2, 3 and 4 in this and in the succeeding tables indicate the degree of flocculation observed; no flocculation is indicated by the minus (-) sign.

1°C. to distinguish qualitative differences in reactivity at these temperatures. Quantitative Kahn tests were also performed at these temperatures. The results of these determinations are presented in Appendix A and illustrative cases are shown in Table II.

An examination of the appendix and table reveal that in every instance the sera, whether positive or negative with the Kahn test at room temperature, show less reactivity at 37° and greater reactivity at 1°C. The quantitative titers show a similar trend. It appears that no uniform relation exists between the titer at room temperature and the titer at the other two temperatures outside the fact that the titer rises as the reaction temperature is lowered. The quantitative results are statistically considered in forthcoming pages in Table XI in relation to the results obtained in parallel experiments with unheated sera.

The numbers of sera reacting at all temperatures taken from Appendix A have been tabulated in Table IIIA and IIIB. Table IIIA shows flocculation results obtained at room temperature with the various animal sera. Those sera which are described as "strongly positive" were diluted with saline so as to be made "weakly positive," tested at 37° and 1°C., and these results tabulated in Table IIIB.

TABLE II

ILLUSTRATIVE EXAMPLES OF FLOCCULATION REACTIONS AND QUANTITATIVE TITERS AT DIFFERENT TEMPERATURES WITH ANIMAL SERA

Serum Source and Number	Kahn Reaction	Dilution of Serum with 0.9% Saline	Reactions at: 37°C.		Room Temperature		1°C.	
	Tube 123		Tube 123	Quant. Titer	Tube 123	Quant. Titer	Tube 123	Quant. Titer
Chicken								
1	444	1:7	---	4	-±2	28	444	80
2	±44	Undil.	--3	1	144	3	444	40
3	-14	Undil.	--±	0	-14	2	444	40
4	---	Undil.	---	0	---	0	444	10
Cow								
1	444	1:10	--±	20	-±4	40	444	180
2	-44	1:2	--1	4	-±4	8	444	20
3	±24	Undil.	-±2	1	±24	2	444	4
4	---	Undil.	---	0	---	0	±34	2
Horse								
1	444	1:15	--3	60	-±4	60	±44	80
2	±44	1:1.5	---	0	-±2	6	444	20
3	-24	Undil.	---	0	-24	2	444	20
4	--±	Undil.	---	0	--±	0	444	4
Pig								
1	444	1:4	-22	16	±34	20	444	200
2	±44	1:2	---	4	--2	8	244	200
3	-±1	Undil.	---	0	-±1	0	444	40
4	---	Undil.	---	0	---	0	444	20
Rabbit								
1	444	1:3	---	4	-±4	12	144	60
2	±44	1:2	---	4	-±3	8	-44	10
3	--4	Undil.	---	0	--4	1	-24	2
4	---	Undil.	---	0	---	0	-44	3

The range of reactivity given by the animals tested at room temperature extended from 61 to 94 per cent (Table IIIA). These results are similar to those reported in the literature by different authors (Kemp, et al., 1940).

Although the majority of the animal sera studied showed positive flocculation reactions at room temperature (72 of 102 chicken sera, 57 of 62 cow sera, 38 of 42 horse sera, 96 of 102 pig sera, 30 of 49 rabbit sera), this reactivity was even more extended in the cold, approaching 100 per cent in the sera examined, as is seen in Table IIIB. This table reveals that all of the sera of three of the five animals studied (chickens, horses, pigs) showed flocculation reactions at 1°C. Sixty-one of 62 cows (98%) and 46 of 49 rabbits (94%) also showed reactions at the low temperature. It is to be noted that all of the sera listed in this table were either negative or weakly positive at room temperature. In all cases it was found that a positive reaction at room temperature is always associated with a positive reaction at 1°C., but the reactions at 37°C. depended to a large measure on the dilution of the sera. It has been a common observation that increased dilution of serum to a given point can weaken the room temperature results and render the 37°C., reaction negative, while maintaining the reaction at 1°C.

TABLE IIIA

FLOCCULATION (KAHN) RESULTS WITH ANIMAL SERA

Source of Serum	Number Tested	Kahn Reactions				
		Strongly Positive	Weakly Positive	Percent Positive	Negative	Percent Negative
Chickens	102	50	22	70.6	30	29.4
Cows	62	50	7	91.9	5	8.1
Horses	42	35	3	90.5	4	10.5
Pigs	102	86	10	94.1	6	5.9
Rabbits	49	12	18	61.4	19	38.6

TABLE IIIB

EXTENT TO WHICH KAHN-NEGATIVE OR WEAKLY POSITIVE ANIMAL SERA GIVE FLOCCULATION REACTIONS AT 37° AND 1°C.[1]

Source of Serum	Number Tested	Kahn Reactions at: 37°C. Number Positive[2]	37°C. Percent Positive	1°C. Number Positive	1°C. Percent Positive
Chickens	102	26	25.6	102	100
Cows	62	23	37.1	61	98.4
Horses	42	10	23.8	42	100
Pigs	102	33	32.4	102	100
Rabbits	49	10	20.4	46	94

[1]The sera listed in Table IIIA as strongly positive were diluted with salt solution to render them weakly positive.

[2]In no case did a serum negative in the standard Kahn test (at room temperature) show any reaction at 37°C.

Experiment 2. Effect on Sensitivity When Kahn-Negative Human Sera Are Tested at 37° and 1°C.

The flocculation results obtained with 208 Kahn-negative human sera tested at 37° and 1°C. are presented in Table IV. The term "Kahn-negative" is used to indicate sera which showed no reaction in the standard or presumptive Kahn tests in the Laboratory of Serology of the University of Michigan Hospital. These sera are therefore considered to be from presumably nonsyphilitic patients but are otherwise unselected. It is emphasized, therefore, that the human sera, unlike the animal sera studied, were characterized by complete seronegativity.

None of these sera showed any reaction when tested at 37°C. Seven sera (3.4%) showed some degree of flocculation at 1°C. in the 6:1 and/or 12:1 ratios of serum to antigen suspension. This tendency toward flocculation was not especially marked as is seen by the lack of a strong reaction (4+) in any serum examined. Kahn-negative sera which show 4+ flocculation in all three ratios of the test at 1°C. have been observed but none were present in this series. This experiment indicates that a group of hospitalized, presumably non-luetic individuals, who give negative Kahn reactions both at room temperature and at 37°C., possess potentialities for giving positive reactions at 1°C.

TABLE IV

FLOCCULATION REACTIONS AT DIFFERENT TEMPERATURES WITH KAHN-NEGATIVE HUMAN SERA

Indicating some tendency toward flocculation at 1°C.

Serum Number	Kahn Reaction at: 37°C.	Room Temperature	1°C.
	Tube 123	Tube 123	Tube 123
1-4	---	---	--+
5	---	---	--$\bar{1}$
6-7	---	---	-+1
8	---	---	-$\bar{1}$1
9	---	---	-+2
10	---	---	-$\bar{1}$2
11	---	---	-22
12-208	---	---	---

Percentage of positive reactions at 1°C. = 3.4

Experiment 3. Effect on Sensitivity When Unheated Animal Sera Are Tested at 37°C., Room Temperature and 1°C.

In the previous experiments, the serum specimens employed had been heated for 30 minutes at 56°C. before testing. In the present experiment the effect on the sensitivity of the Kahn test at 37°C., room temperature and 1°C. was investigated with unheated animal sera. The results of these determinations are presented in Appendix B and illustrative cases are shown in Table V. Sera which gave strong (4+) reactions at room temperature were treated as had been the heated sera; i.e., they were diluted with saline to render them weakly (1+ to 3+) positive at room temperature. The diluted sera were then tested at 37° and 1°C. to distinguish qualitative differences in reactivity at these temperatures.

An examination of Appendix B and Table V reveals a pattern of reactivity very similar to that seen with heated sera, with the exception of the results obtained with the unheated cow sera. Chicken's, horse's, pig's and rabbit's unheated sera reveal a capacity for flocculation reactions varying from an average of 4+ to negative at room temperature. The reactions are invariably more marked at 1° than at 37°C. Some cow sera, however, exhibit an unusual type of reaction. For example, cow serum 5 (Table V) shows a reaction of -24 in the Kahn test at room temperature. At

TABLE V

ILLUSTRATIVE EXAMPLES OF FLOCCULATION REACTIONS AND QUANTITATIVE TITERS AT DIFFERENT TEMPERATURES WITH UNHEATED ANIMAL SERA

Serum Source and Number	Kahn Reaction	Dilution of Serum with 0.9% Saline	Reactions at: 37°C		Room Temperature		1°C.	
	Tube 123		Tube 123	Quant. Titer	Tube 123	Quant. Titer	Tube 123	Quant. Titer
Chicken								
1	444	1:4	---	4	--4	16	-44	60
2	-44	Undil.	---	0	-44	3	444	80
3	-24	Undil.	---	0	-24	2	444	20
4	---	Undil.	---	0	---	0	444	10
Cow								
1	444	1:40	-±2	160	--2	160	-44	520
2	444	1:10	-34	40	-±2	40	-44	80
3	444	1:2.5	±44	10	-14	10	±±4	30
4	244	1:2.5	-44	10	--2	10	--1	160
5	-24	Undil.	-44	3	-24	2	---	20
6	±--	Undil.	444	4	±--	0	-±1	0
Horse								
1	444	1:4	---	4	-±2	16	444	100
2	-44	1:2.5	---	4	--2	10	444	40
3	21-	Undil.	---	0	21-	1	444	4
4	---	Undil.	---	0	---	0	444	80
Pig								
1	444	1:5	---	4	-±4	20	444	120
2	244	1:2	--3	8	-33	8	444	120
3	-±3	Undil.	---	0	-±3	1	444	60
4	---	Undil.	---	0	---	0	±44	3
Rabbit								
1	444	1:2.5	--±	4	-14	10	244	40
2	-44	1:2	---	4	--4	8	444	20
3	-24	Undil.	---	0	-24	2	444	4
4	---	Undil.	---	0	---	0	--1	0

37°C. the reaction is -44 and at 1°C. the reaction is negative (---). Apparently, therefore, the reaction is strongest at 37°, and weakest at 1°C., unlike the other animal sera tested. It has been shown that human syphilitic sera (heated) give stronger reactions at 37° than at icebox temperature (Georgi, 1919) or at 1°C. (Kahn, 1940b). It would seem then that certain unheated cow sera show a tendency to behave like heated human syphilitic sera in the flocculation test for syphilis.

Of interest is the fact that dilution of these unheated cow sera with saline usually reverses the flocculation trend at 37° and 1°C. Thus, serum 5 (Table V) which gave a stronger reaction at 37° than at 1°C. in an undiluted state showed, on dilution with saline, a lower quantitative titer at 37°C. (titer of 3) than at 1°C. (titer of 20). It should be pointed out also that this difference in reactivity of undiluted and diluted serum at 1°C. takes place under conditions in which approximately the same ratio of serum to antigen suspension is employed. Thus, in the first tube of the Kahn test the ratio of serum to antigen suspension is 3:1. In the first tube of the quantitative test, in which the serum dilution with saline is 1:5 and in which the diluted serum is employed with antigen suspension in a ratio of 12:1, the actual amount of serum in relation to the antigen suspension is 2.4:1. Again referring to cow serum 5 (Table V), it is evident that the serum in an

undiluted state gives a negative reaction in a 3:1 ratio of serum to antigen suspension at 1°C., while in a diluted state (1:5 dilution) the same serum gives a positive reaction in a closely similar ratio at 1°C.

Other considerations arise from an inspection of the unheated cow sera data in Table V. Reactions of the type shown by cow serum 6, characterized by greatest reactivity at 37°C. with no obvious reactions in the quantitative test at 1°C., occurred in ten instances. These are the sole exceptions noted to the generalization that heated and unheated sera of the nonsyphilitic animal species studied show optimum flocculation at 1°C. Of the ten sera, nine gave stronger reactions at 37° in an undiluted state and one in a dilution of 1:2 with saline. Sera diluted 1:5 or more all showed optimum reactions at 1°C. Sera diluted approximately 1:2.5 appear to be in a critical range usually exhibiting strongest reactivity at 37°C. In some cases stronger reactions were noted at both 37° and 1°C. than at room temperature (4, 10/14[1]; 5, 10/14; 2, 12/3; 5, 12/3; 7, 12/3; 2, 12/6. Appendix B).

A more extensive picture of the behavior of unheated cow sera (titer < 20) at 37°C., room temperature and 1°C. with qualitative and quantitative flocculation tests is

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[1] In this and succeeding references to certain sera, identification is made by giving the serum number first (e.g., 4) succeeded by the month and day of examination (e.g., 10/14).

presented in Table VI. Certain flocculation zones appearing on dilution of serum with saline are noted at different temperatures. At 1°C., the flocculation reactions with undiluted (unheated) serum are negative, becoming positive in a definite range of dilution and becoming negative again on higher dilution; the corresponding reactions at 37°C. merely become weaker on dilution. In certain instances, at low dilutions flocculation is more marked at 37°C. than at 1°C., while at high dilutions flocculation is more marked at 1°C. than at 37°C.

Seventeen weakly reacting sera exemplifying the zones of flocculation encountered in most unheated cow sera are shown in the table. With the exception of serum (1, 12/3), all the sera show heaviest Kahn reactions at 37°C. either in an undiluted state or in low dilution (less than 1:5). Serum (1, 12/3) was included as an example of a strongly reacting type which requires dilution of more than 1:5 with saline to be rendered weakly positive to meet the requirements of this experiment in testing for reactivity at 37° and at 1°C. At this high dilution, the zone of flocculation at 1°C. becomes evident.

Since the 12:1 ratio of the Kahn reaction is also the first tube of the quantitative Kahn test used in these experiments, the result obtained with it may also be described as the first tube (undiluted serum) result in the quantitative test. At 1°C., serum (1, 10/14) (Table VI) shows a reaction

TABLE VI

DIFFERENT ZONES OF FLOCCULATION EXHIBITED BY UNHEATED COW SERA AT 37° AND 1°C.

Serum Number; Date Examined	Kahn Reaction (Room Temperature)	Dilution of Serum with 0.9% Saline	Flocculation Reactions at:			Flocculation Zones at 37° and 1°C. under Conditions of Increasing Dilutions of Serum							
			37°C.	1°C.		Serial dilution of Serum with 0.9% Saline							
1941	Tube 123		Tube 123	Tube 123		undil.	1:5	1:10	1:20	1:30	1:40	1:50	1:60
1) 10/14	-44	1:2	-44	-11	37°	4	–	–	–	–	–	–	
					1°	0	3	4	2	1	–	–	
4) 10/14	444	1:2.5	-44	±14	37°	4	–	–	–	–	–	–	
					1°	4	3	–	–	–	–	–	
5) 10/14	-±1	Undil.	-44	-13	37°	4	–	–	–	–	–	–	
					1°	3	–	–	–	–	–	–	
6) 10/14	444	1:2.5	±44	± ±4	37°	4	–	–	–	–	–	–	
					1°	4	4	±	–	–	–	–	
4) 11/15	-24	Undil.	-44	---	37°	4	–	–	–	–	–	–	
					1°	–	4	–	–	–	–	–	
2) 11/20	244	1:2.5	-44	--1	37°	4	–	–	–	–	–	–	
					1°	1	4	4	4	4	2	–	
4) 11/20	---	Undil.	±44	---	37°	4	–	–	–	–	–	–	
					1°	–	4	4	–	–	–	–	
5) 11/20	±44	1:2.5	144	--1	37°	4	–	–	–	–	–	–	
					1°	1	4	±	–	–	–	–	
1) 12/3	±44	1:20	--4	444	37°	0	4	4	4	–	–	–	–
					1°	0	–	±	4	4	4	4	4
5) 12/6	--3	Undil.	-44	---	37°	4	–	–	–	–	–	–	
					1°	–	3	±	–	–	–	–	
7) 12/6	244	1:4	-44	--3	37°	4	4	–	–	–	–	–	
					1°	0	3	4	4	4	4	±	
1942													
1) 1/2	---	Undil.	--±	---	37°	±	–	–	–	–	–	–	
					1°	–	4	4	4	4	–	–	
2) 1/2	---	Undil.	244	---	37°	4	–	–	–	–	–	–	
					1°	–	1	4	4	4	±	–	
6) 1/2	---	Undil.	-44	---	37°	4	–	–	–	–	–	–	
					1°	–	–	4	±	–	–	–	
7) 1/2	-± ±	Undil.	±44	---	37°	4	–	–	–	–	–	–	
					1°	–	4	4	4	–	–	–	
8) 1/2	---	Undil.	-44	---	37°	4	–	–	–	–	–	–	
					1°	–	1	4	4	4	4	–	
1) 1/3	--1	Undil.	±44	---	37°	4	–	–	–	–	–	–	
					1°	–	1	2	4	4	–	–	
5) 1/3	-14	Undil.	-44	--1	37°	4	–	–	–	–	–	–	
					1°	1	±	4	4	4	4	4	

of 1+ in a dilution of 1:2, 3+ in a dilution of 1:5, 4+ in 1:10, 2+ in 1:20, 1+ in 1:30 and negative at 1:40. Evidently the optimum dilution of serum for reaction with antigen at this temperature, in a serum-antigen ratio of 12:1, is between 1:5 and 1:10, whereas this serum dilution is non-reactive at 37°C. Serum (1, 12/3) also shows the type of zone observed, i.e., no reaction at 1°C. in a dilution of 1:5, doubtful flocculation in 1:10 dilution and strong flocculation from dilutions of 1:20 to 1:60, while at 37°C. the reactions become progressively weaker with dilution. In the case of some sera, the flocculation zone at 1°C. appearing on dilution could not be elicited; these sera becoming completely negative on dilution. Serum (5, 10/14) is an example of this type.

Heating cow sera at 37°C. for 15 minutes. One of the possible sources of error in testing unheated cow sera at 37°C. with the technique used in these experiments is the subject of the experimental examples which are shown in Table VII. Since the unheated serum is incubated at 37°C. for 15 minutes before testing and since cow sera heated at 56°C. for 30 minutes show greater reactivity than do unheated sera, it is conceivable that the period of heating at 37°C. preceding testing at this temperature might increase the reactivity of the serum over that of the unheated serum. This increase would not be seen in unheated cow sera tested at 1°C. and might therefore account for the discrepant reactivity

TABLE VII

EFFECT ON FLOCCULATION OF HEATING COW SERA FOR 30 MINUTES AT 37°C. (INSTEAD OF 56°C.)

Serum Number and Date Examined	Kahn Reaction of: Unheated Serum	Kahn Reaction of: Serum heated at 37°C. for 30 Min.	Kahn Reaction of: Serum heated at 56°C. for 30 Min.	Serum Heated at 37°C. for 30 minutes. Flocculation Reactions at:	37°C.	1°C.
1942	Tube 123	Tube 123	Tube 123	Dilution of Serum with 0.9% Saline	Tube 123	Tube 123
1) 1/2	---	---	±24	Undil.	---	---
				1:10	---	±44
2) "	--1	-±3	444	Undil.	±44	±44
				1:2	-±4	-±-
				1:10	---	344
4) "	-12	-±2	444	Undil.	±44	±44
				1:2	-±4	-±-
				1:10	---	344
5) "	---	---	±44	Undil.	-44	---
				1:5	---	---
6) "	---	-±±	444	Undil.	-44	---
				1:10	---	-33
1) 1/3	--1	-±4	444	Undil.	±44	-±±
				1:10	---	±44
2) "	±44	±44	444	1:2	±44	-±±
				1:10	---	±44
4) "	-±1	---	-44	Undil.	-44	--±
				1:10	---	---

of unheated cow sera. The results observed would then include an uncontrolled variable. In order to determine this possibility, cow sera were tested unheated, after heating for 15 minutes at 37°C. and after the usual heating period of 30 minutes at 56°C. As is shown in the data of Table VII, sera heated for 15 minutes at 37°C. showed no appreciable difference in results obtained at room temperature from those obtained with unheated sera at room temperature. Moreover, the 37°C. heated sera exhibit reactivity similar to that shown by the unheated sera when tested at 37° and 1°C. (i. e., stronger reactions at 37°C. than at 1°C. in the undiluted state and zone reactions at 1°C.).

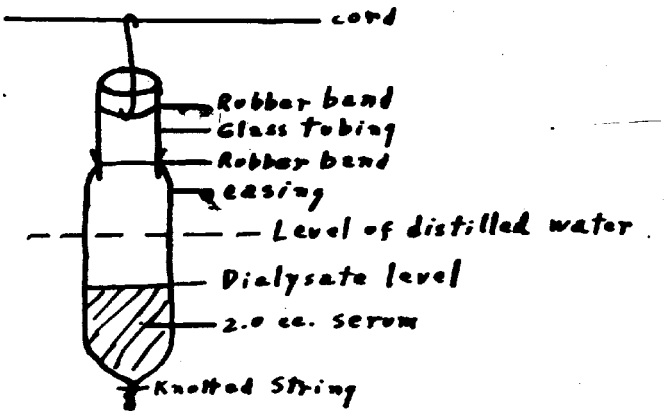
Dialysis of Unheated Cow Sera. A serum-dialysis experiment was performed with unheated cow sera in an attempt to determine whether the zone type of reaction was due to some non-protein factor of dialyzable nature.

> The dialysis of serum, in 2.0 cc. quantities, was carried out against running distilled water in a cellulose acetate casing.[1] Casing of approximately 5/8 inch diameter was used. The bags were prepared by twisting the casing repeatedly until a constriction about one inch in length appeared. A portion at the top and bottom of this constriction was then securely tied off with strong string and the constriction then cut between the two tied off portions. The casing was then cut about two inches above the knot. After wetting and opening the bag, the open portion was tied into a firepolished bit of glass-tubing, open at both ends, with a rubber band. Diagram I illustrates the appearance of the completed bag.

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[1] Sold by Visking Corporation, Chicago, Illinois.

DIAGRAM I



The bags were tested for leaks in the closed off portion by suspending in air and filling all the bags made at one time (usually 16 or 20) to the same point with distilled water. Since diameters were the same in all instances, it was assumed that the decrease in volume of each bag should be the same if the cut portions were securely closed. It was proposed to discard any bag which showed a lower water level than the other bags after standing on the bench overnight. However, uniform success, attending the preparation of the bags, none of which had to be discarded because of faulty construction.

Dialysis was carried out against running distilled water overnight. This time was sufficient in all cases to yield a negative chloride[1] and sulfate[2] test with the serum. The dialyzing bags were then fanned for about an hour to reduce the volume, the contents stirred up by "working" the bag, and then poured into a graduated test tube. The bag was washed out with successive 0.2 cc. amounts of distilled water to make the volume up

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[1]Qualitative test for chloride: a loopful of serum was added to 0.2 cc. distilled water which was acidified with a few drops of HNO_3. $AgNO_3$ solution was then added with a dropper.

[2]Qualitative test for sulfate: a loopful of serum was added to 0.2 cc. distilled water which was acidified with a few drops of HNO_3. $BaCl_2$ solution was then added with a dropper.

to 2.0 cc. (the starting amount of serum). Twenty milligrams of NaCl (Baker's A.R.) were poured into the mixture, whereupon the precipitate readily went into solution. Usually a slight amount of undissolved material remained necessitating a short period of centrifugation.

After testing the unheated sera at room temperature (results recorded in Table VIII as "Undialyzed") the specimens were split into two portions. One portion was dialyzed (results recorded in Table VIII as "Dialyzed") and the other ("Control") placed in glass test tubes and kept immersed in the dialyzing bath for the same length of time as the dialyzing sera.

It is seen from the results presented in Table VIII that dialysis had no effect on the zone type of reactivity peculiar to unheated cow sera.

Lability of unheated pig sera on standing at room temperature. In discussing the reactions of different unheated animal sera, it is apropos to insert here the observation of a possible source of error which would influence quantitative results quite markedly if it were found true for all species studied. The observation in question is that unheated pig sera giving negative Kahn reactions, begin to give positive reactions after standing at room temperature. Although in practically all experiments a uniform time elapsed between the taking of the blood and the testing of the serum, occasionally the time relationships in the routine procedures were varied.

TABLE VIII

EFFECT OF DIALYSIS OF UNHEATED COW SERA ON

REACTIVITY AT 37° AND 1°C.

Serum Number, Date Examined	Nature of Specimen	Kahn Reaction With Unheated Sera	Dilution with 0.9% Saline	Reactions at: 37°C.		Room Temperature		1°C.	
		Tube 123		Tube 123	Quant. Titer	Tube 123	Quant. Titer	Tube 123	Quant. Titer
1) 1/3	Undialyzed	-±3	Undil.	±44	3	-±3	1	---	120
	Dialyzed	--1	Undil.	-14	2	--1	0	--±	80
	Control	--2	Undil.	-34	2	--2	1	---	120
2) 1/3	Undialyzed	±44	1:2	±44	8	-14	8	-11	1
	Dialyzed	±24	1:2	±44	8	±33	8	±44	3
	Control	±44	1:2	±44	8	±24	8	±44	3
4) 1/3	Undialyzed	--2	Undil.	-44	3	--2	1	--±	0
	Dialyzed	---	Undil.	-±4	1	---	0	---	0
	Control	--1	Undil.	-44	3	--1	0	--±	0
5) 1/3	Undialyzed	-±4	Undil.	-44	3	-±4	1	--+	160
	Dialyzed	--3	Undil.	-24	2	--3	1	--1	140
	Control	-14	Undil.	-44	3	-14	1	---	200
2) 1/2	Undialyzed	-23	Undil.	144	3	-23	2	---	120
	Dialyzed	-±3	Undil.	±44	3	-±3	1	--2	120
	Control	-33	Undil.	±44	3	-33	2	---	160
3) 1/2	Undialyzed	444	1:2.5	-44	10	-±4	10	--+	4
	Dialyzed	444	1:2.5	-44	10	-±4	10	--1	4
	Control	444	1:2.5	-44	10	-14	10	--1	4

TABLE IX

ILLUSTRATIVE EXAMPLES OF THE TENDENCY OF UNHEATED PIG SERA TO SHOW ENHANCED REACTIVITY AFTER AGING AT ROOM TEMPERATURE

Serum Number, Date Examined	Intervals between First and Second Examination	Kahn Reaction	
		First Examination	Second Examination
		Tube 123	Tube 123
1. 11/3	5½ hours	---	444
2. "	"	---	-44
3. "	"	---	-44
4. "	"	---	-23
5. "	"	---	-±1
6. "	"	---	--1
7. "	"	---	-±4
8. "	"	---	444
9. "	"	---	±44

The data shown in Table IX are based on a chance observation noted during the performance of the experiments with pig sera. After five and one-half hours' standing at room temperature, nine negative pig sera showed varying degrees of reactivity. It should be noted that the horse sera examined in these experiments were received from Lansing in an unheated state. On the basis of the findings with pig sera, it is possible that the reactions obtained with horse sera might have been different if freshly separated sera had been employed.

Comparison of results obtained with heated and unheated animal sera. The unheated sera of the various animals

employed reacting at all temperatures have been tabulated in Tables XA and XB. These tables present summaries similar to those shown for heated sera in Tables IIIA and IIIB. Table XA shows flocculation results (Kahn) obtained at room temperature with the unheated sera. Those sera which were "strongly positive" were diluted with saline to render them "weakly positive," and tested at 37° and 1°C.; and the results are tabulated in Table XB.

As is true in the case of heated sera, unheated sera also approach 100 percent of positive reactions at 1°C. However, the number of reactions at room temperature and at 37°C. were not the same with unheated as with heated sera. Thus, an increase in the number of reactions obtained at room temperature with unheated sera over that of heated sera was noted in the case of chickens (15%) and of rabbits (31%). On the other hand, a decrease in the number of reactions obtained at room temperature with unheated sera was noted in the case of cows (33%) and of pigs (43%). In the case of horse sera, although individual specimens varied markedly in their reactions, as a whole they showed about equal activity in a heated and unheated state.

Table XB shows that with the exception of the cow sera, the reactions in the unheated group equalled or approached 100 percent at 1°C. If the reactions of cow sera following dilution are considered, however, (Appendix B) 52 of the 62 cow sera listed in Table XB (84%) showed reactions at 1°C.

TABLE X A

FLOCCULATION (KAHN) RESULTS WITH UNHEATED ANIMAL SERA

ource of erum	Number	Kahn Reactions: Strongly Positive	Weakly Positive	Percent Positive	Negative	Percent Negative
hickens	102	68	19	85.0	15	15.0
ows	62	25	11	58.1	26	41.9
orses	25	22	0	88.0	3	12.0
igs	102	17	35	51.0	50	49.0
abbits	41	18	20	92.5	3	7.5

TABLE X B

EXTENT TO WHICH KAHN-NEGATIVE OR WEAKLY POSITIVE UNHEATED ANIMAL SERA GIVE FLOCCULATION REACTIONS AT 37°C. AND 1°C[1]

Source of Serums	Number Tested	Kahn Reactions at: 37°C. Number Positive[2]	37°C. Percent Positive	1°C. Number Positive	1°C. Percent Positive
Chickens	102	26	25.6	102	100
Cows	62	51	82.5	24	38.8[3]
Horses	25	8	32.0	25	100
Pigs	102	7	6.2	101	99.0
Rabbits	41	6	14.6	40	97.6

1. The sera listed in Table XA as strongly positive were diluted with salt solution to render them weakly positive.

2. In no case did a serum negative in the standard Kahn test (at room temperature) show any reaction at 37°C.

3. Quantitative reactions not considered. See page 89 .

It is of interest to note that, although the number of room temperature reactions obtained with unheated chicken sera (Table XA) exceeded the number obtained with heated sera (Table IIIA) , there was no corresponding increase in the reactions with unheated sera at 37°C. Coincidentally, the same number of reactions at 37°C. were obtained with both heated and unheated sera (in 25.6 per cent of cases) although not with the same sera. It is of interest also that the unheated rabbit sera which showed an increased percentage of reactions in the unheated state at room temperature (92.5 per cent unheated as against 61.4 per cent heated), showed even fewer reactions at 37°C. in the unheated state than did the heated sera (14.6 per cent unheated as against 20.4 per cent heated). Unheated pig sera, on the other hand, showed fewer reactions at room temperature and at 37°C. than did the heated sera.

The data obtained with the Kahn test at the three temperatures with animal sera are amplified by the use of the quantitative test at these temperatures. Table XI lists the averages (arithmetic means) of the quantitative flocculation titers of the heated and unheated animal sera studied.

These titers were obtained by means of the quantitative procedure described on page 65 and are expressed in units. The averages of these titers at the three temperatures, for sera giving reactions less than 4 + as well

TABLE XI

AVERAGES OF QUANTITATIVE FLOCCULATION TITERS OF HEATED AND UNHEATED ANIMAL SERA TESTED AT DIFFERENT TEMPERATURES

Source of Sera	Number of Sera Tested	Sera Heated or Unheated	Mean Titer of All Sera Tested at: Room Temp.	37°C.	1°C.	Number of Sera Giving Kahn Reactions <4+ at Room Temp.	Mean Titer of Group at: Room Temp.	37°C.	1°C.	Number of Sera Giving Kahn Reactions of 4+ or >4+ at Room Temp.	Mean Titer of Group at: Room Temp.	37°C.	1°C.
Chicken	102*	Heated	5.7	2.7	55.3	55	0.7	0.3	26.6	47	11.5	5.5	88.8
	102*	Unheated	8.6	3.9	70.1	32	1.2	0.8	28.1	70	11.9	5.3	87.3
Cow	62*	Heated	30.1	20.5	77.2	11	1.0	0.6	28.5	51	36.5	24.9	88.1
	62*	Unheated	10.8	12.7	74.0	37	0.4	4.7	47.1	25	26.4	24.8	114.3
Horses	25*	Heated	10.2	8.9	45.5	6	1.0	0.2	25.5	19	13.2	11.5	52.3
	25*	Unheated	15.4	11.5	57.7	4	0.3	0	23.0	21	18.4	13.7	63.9
Pigs	102*	Heated	43.6	28.2	124.7	16	0.2	1.0	33.4	86	52.5	33.1	144.5
	102*	Unheated	3.6	1.5	98.2	84	0.5	0.1	90.6	18	20.0	8.9	139.5
Rabbits	38	Heated	2.2	1.1	9.9	31	0.9	0.1	7.6	7	8.5	5.2	26.1
	38	Unheated	3.9	1.7	12.7	25	1.6	0.1	6.9	13	8.2	4.8	24.2

* Same Sera used Heated and Unheated

as for sera giving reactions of 4+ or higher, are listed in Table XI. Averages for the total number of sera tested are also shown in the table. The quantitative trends follow those indicated by the Kahn test results; i.e., highest titers were obtained at 1°C., lowest titers at 37°C. One exception to this generalization is noted with unheated cow sera which had an average titer of 12.7 at 37°C. and of 10.8 at room temperature. The average titer of these sera at 1°C. was 74.

Of the animals studied, highest average titers were obtained with heated pig sera at 1°C. (124.7), lowest average titers with rabbit sera at 37°C. (one to two units in the heated and unheated state). Highest room temperature average titers were obtained with heated pig sera (43.6), lowest average titers with rabbit sera (0.9). The heated pig sera showed the highest average titers of the animal sera tested at 37°C. (28.2).

Experiment 4. Effect on Sensitivity When Kahn-Negative Human Sera Are Tested in an Unheated State at 37°C., Room Temperature and 1°C.

It was seen previously (Table IV) that heated Kahn-negative human sera show reactions in a small percentage of cases (3.4%) when tested at 1°C. and no reactions at 37°C. In the present experiment, unheated human sera were tested under similar conditions. Table XII gives Kahn results obtained with such sera tested at the three temperatures. Of 101 Kahn-negative human sera examined, reactions were noted at 1°C. only. Six sera (6%) showed flocculation at this temperature which seems to represent a definite increase over the number of reactions obtained with heated human sera. Three of the six unheated sera (3.0%) showed reactions of an average of 2+ in the 6:1 and 12:1 serum:antigen ratios. Based on the same ratios, only two of 208 heated sera (<1%) showed 2+ reactions at 1°C.

It should be emphasized, however, that the 101 unheated human sera and the 208 heated sera were all different specimens, whereas in the case of the animal sera tested, the heated and unheated specimens were from the same animals.

TABLE XII

FLOCCULATION REACTIONS AT DIFFERENT TEMPERATURES WITH UNHEATED KAHN-NEGATIVE HUMAN SERA

Serum Number	Reactions at 37°C.	Reactions at Room Temperature	Reactions at 1°C.
	Tube 123	Tube 123	Tube 123
1	0--	---	0-±
2-3	0--	---	0±+
4	0--	---	0-1
5-6	0--	---	0±1
7-8	0--	---	022
9	0--	---	013
10-101	0--	---	---

Percentage of positive reactions at 1°C. = 6.0

Experiment 5. Effect on Sensitivity When Kahn-Negative Human Sera Are Tested at 37°C., Room Temperature, and 1°C. with Sensitized Kahn Antigen

Although the reactivity of the animal sera studied approach 100 percent at 1°C., the same evidently does not apply to Kahn-negative human sera. It must be borne in mind, however, that while the animal species studied were characterized by seropositivity of varying degrees with the Kahn test at room temperature, the human sera were characterized by lack of reactivity at this temperature. Various procedures are available for increasing the sensitivity of serologic/reactions. A simple one is the use of the presumptive Kahn test which employs a "sensitized" antigen considerably more sensitive than standard Kahn antigen. The presumptive Kahn test was employed with heated and unheated human sera at 37°C., room temperature and 1°C. The results obtained are shown in Tables XIII and XIV, and are summarized in Table XV.

The pattern shown by the heated and unheated human sera at 1°C. was magnified by this use of sensitized Kahn antigen. Of the 109 heated sera tested (Table XIII), 14.9 per cent showed reactions at 1°C. with sensitized antigen, while 22.3 per cent of the unheated sera (Table XIV) showed these reactions. One unheated serum (33, Table XIV) showed some flocculation at 37°C. and equal reactivity at

TABLE XIII

FLOCCULATION REACTIONS AT DIFFERENT TEMPERATURES EMPLOYING KAHN-NEGATIVE HUMAN SERA WITH SENSITIZED KAHN ANTIGEN

Serum Number	Reactions at 37°C.	Reactions at Room Temperature	Reactions at 1°C.
	Tube 123	Tube 2	Tube 123
1-3	0--	-	0-+
4-7	0--	-	0+1
8	0--	-	011
9	0--	-	0+2
10	0--	-	020
11	0--	-	012
12	0--	-	021
13	0--	-	022
14	0--	-	032
15	0--	-	003
16	0--	-	023
17-18	0--	-	033
19	0--	-	044
20-109	0--	-	0--

TABLE XIV

FLOCCULATION REACTIONS AT DIFFERENT TEMPERATURES EMPLOYING UNHEATED KAHN-NEGATIVE HUMAN SERA WITH SENSITIZED KAHN ANTIGEN

Serum Number	Reactions at 37°C.	Reactions at Room Temperature	Reactions at 1°C.
	Tube 123	Tube 2	Tube 123
1-3	0--	-	0-±
4-7	0--	-	0±-
8-10	0--	-	0±+
11	0--	-	0-1
12-17	0--	-	0±1
18	0--	-	0-2
19-23	0--	-	0±2
24	0--	-	011
25	0--	-	0±3
26	0--	-	021
27	0--	-	012
28	0--	-	032
29	0--	-	033
30-32	0--	-	044
33	0±1	-	0±1
34-103	0--	-	0--

1°C. There were no other exceptions observed at 37°C. with heated or unheated sera.

This rise in reactivity at 1°C. with heated and unheated Kahn-negative human sera was thus greater with sensitized antigen than with standard antigen. In addition, many of the reactions obtained with sensitized antigen were quite marked, while most of the reactions with standard antigen were relatively weak. This increase in reactivity at 1°C. (14.9% with heated sera; 22.3% with unheated sera) would indicate that these sera could be made to yield even greater numbers of reactions with other changes in technique.

TABLE XV

EXTENT OF FLOCCULATION REACTIONS AT DIFFERENT TEMPERATURES WITH HEATED AND UNHEATED KAHN-NEGATIVE HUMAN SERA, EMPLOYING SENSITIZED KAHN ANTIGEN

Number of Sera Tested	Condition of Sera	Number of Reactions at: 37°C.	Room Temperature	1°C.
109	Heated	0	0	16 (14.9%)
103	Unheated	1 (.9%)	0	23 (22.3%)

Experiment 6. Effect on Sensitivity When Globulin Fractions of Kahn-Negative Human Sera Are Tested at 37°C., Room Temperature and 1°C.

A procedure for increasing sensitivity is the utilization of globulin fractions of serologically negative sera. The method for the separation of these protein fractions has been described on page 63. The globulin fractions were tested with standard and sensitized Kahn antigen. Illustrative examples of the results obtained with standard antigen are shown in Table XVI. It is noted that not only were reactions obtained at 1°C., but also at room temperature (in confirmation of Landsteiner and Müller, 1908; Forssman, 1921; Eagle, 1932), and at 37°C. The pattern for this reactivity followed that of the animal sera tested; namely, that the largest number of reactions were obtained at 1°C., fewer at room temperature and the least number at 37°C. In all cases where reactions were obtained at 37°C., stronger reactions were noted at 1°C. Similar, but more sensitive results were obtained by the use of globulin fractions with sensitized antigen as seen from Table XVII. It is evident from this table that not only are reactions observed with globulin fractions which originate from nonsyphilitic human sera, but that these reactions can reach as high as 94 per cent (Table XVII) of the fractions tested.

TABLE XVI

ILLUSTRATIVE EXAMPLES OF FLOCCULATION REACTIONS AT DIFFERENT TEMPERATURES IN THE KAHN TEST WITH GLOBULIN FRACTIONS OF KAHN-NEGATIVE HUMAN SERA

Serum Number, Date Examined	Reactions with Globulin Fractions at:		
	37°C.	Room Temperature	1°C.
1941	Tube 123	Tube 123	Tube 123
6) 2/14	---	---	---
24) 2/13	---	---	-±-
22) 2/13	---	---	--1
147) 2/17	---	---	-+1
138) 2/17	---	---	±12
94) 2/17	--±	---	±13
143) 2/17	---	---	-23
130) 2/17	---	---	±44
5) 2/13	--±	-±1	-44
136) 2/17	--±	---	244
3) 2/13	---	-13	244
82) 2/17	---	-23	244
108) 2/17	--±	-22	444
47) 2/13	±1±	±33	444
13) 2/13	±13	±24	444
96) 2/17	-44	±34	144
10) 2/13	444	344	444

TABLE XVII

EXTENT OF FLOCCULATION REACTIONS AT DIFFERENT TEMPERATURES IN THE KAHN STANDARD AND PRESUMPTIVE TESTS WITH THE GLOBULIN FRACTIONS OF KAHN-NEGATIVE HUMAN SERA

Number of Sera Tested	Antigen Employed	Number of Reactions with Globulin Fractions at: 37°C.	Room Temperature	1°C.
100	Standard	12 (12%)	20 (20%)	81 (81%)
114	Sensitized[1]	38 (27.0%)	76 (54.3%)	108 (94.0%)

[1]Specimens diluted 1:2 with saline in all cases.

It should be added that the actual percentage of positive globulin reactions may have been greater than that indicated in Table XVII due to a dilution factor in the preparation of the globulin solution, previously mentioned (page64). This dilution factor with human sera varied from 25 to 40 per cent.

The albumin fractions obtained after the separation of the globulins were also tested with the standard Kahn test at the three temperatures. The results obtained can be summarized by saying that they were negative or of very slight activity. Any slight reactions obtained were usually of equal intensity at all three temperatures.

Experiment 7. Effect on Sensitivity When Globulin Fractions of Animal Sera Are Tested at 37°C., Room Temperature and 1°C.

The globulin fractions of the animal sera used in these experiments were tested at the three temperatures. The results obtained with the fractions from heated sera are presented in Table XVIIIA; from unheated sera, in Table XVIIIB and more extensive illustrative examples with these globulins from heated and unheated sera, in Appendix C. The general plan of this experiment with globulins was essentially the same as with the sera considered in the previous experiments. Briefly, each globulin solution was subjected to the standard and quantitative tests at the three temperatures.

In analyzing the results with globulin fractions it will be well to bring to mind the results of Appendices A and B in which parallel data with the heated and unheated animal sera, from which the globulin fractions were derived, are presented. The results obtained with the globulin fractions of each of the animal species studied are discussed separately, in relation to the serum from which they were prepared.

An examination of Table XVIIIA reveals first that most globulin fractions of animal sera (heated) show a tendency to give weaker Kahn reactions than the sera from

ILLUSTRATIVE EXAMPLES OF FLOCCULATION REACTIONS AT DIFFERENT TEMPERATURES WITH GLOBULIN FRACTIONS OF HEATED ANIMAL SERA

Serum Source and Number	Kahn Reaction with Serum		Kahn Reaction with Globulin	Dilution of Globulin with 0.9% Saline	Reactions with Globulin Fractions at:					
					37°C.		Room Temperature		1°C.	
1941	Tube 123	Quant. Titer	Tube 123		Tube 123	Quant. Titer	Tube 123	Quant. Titer	Tube 123	Quant. Titer
Chicken										
7) 11/2	444	28	-22	undil	-34	2	-22	1	±11	20
1) 10/15	144	3	-12	undil.	-12	1	-12	1	±12	40
8) 10/18	--3	1	--±	undil.	---	0	--±	0	--2	20
7) 10/18	---	0	---	undil.	---	0	---	0	---	0
Cow										
2) 10/14	444	40	444	1:10	-±1	30	-±3	40	±24	80
2) 12/3	444	40	--1	1:15	--4	40	--2	60	-24	60
5) 12/6	144	8	-±4	undil.	--±	0	-±4	1	±44	40
3) 12/3	-±1	0	-44	undil.	±14	2	-44	3	244	40
Horse										
1) 12/11	444	60	444	1:10	---	20	--3	40	-±4	80
5) 12/16	144	10	--1	undil.	---	0	--1	0	-44	40
3) 12/11	-24	2	---	undil.	---	0	---	0	--4	1
2) 12/16	---	0	---	undil.	---	0	---	0	--4	1
Pig										
2) 10/4	444	48	±±4	1:5	---	4	--1	10	--4	20
7) 10/4	344	40	-32	1:5	---	4	--±	10	±44	20
1) 10/29	134	2	-±4	undil	--1	0	-±4	1	243	3
2) 10/1	---	0	±±-	undil	±2-	1	±±-	0	244	10
Rabbit										
7) 12/31	444	12	-±3	undil.	--2	1	-±3	1	±33	20
8) 12/31	±44	3	--2	undil.	--2	1	--2	1	-33	2
2a) 12/30	---	0	--2	undil.	---	0	--2	1	-22	1
6) 12/31	--±	0	-44	1:2	--2	8	-±4	8	±44	10

TABLE XVIII B

ILLUSTRATIVE EXAMPLES OF FLOCCULATION REACTIONS AT DIFFERENT TEMPERATURES WITH GLOBULIN FRACTIONS OF UNHEATED[1] ANIMAL SERA

Serum Source and Number	Kahn Reaction with Serum		Kahn Reaction with Globulin	Dilution of Globulin with 0.9% Saline	Reaction of Globulin Fraction at: 37°C.		Room Temperature		1°C.	
	Tube 123	Quant. Titer	Tube 123		Tube 123	Quant. Titer	Tube 123	Quant. Titer	Tube 123	Quant. Titer
Chicken										
7	444	40	-11	undil.	-44	3	-11	1	-±3	30
1	±44	3	-12	undil.	-12	1	-12	1	± ±2	30
8	±44	3	± ±2	undil.	±-1	0	± ±2	1	--±	10
7	-±2	1	---	undil.	---	0	---	0	---	0
Cow										
2	444	40	444	1:10	---	20	--1	40	±14	40
2	-44	10	--±	1:5	--4	40	--1	10	-44	40
5	--3	1	-±4	undil.	-24	2	-±4	1	±44	30
3	--1	0	±43	undil.	--4	1	±43	2	±44	40
Horse										
1	444	60	444	1:4	--±	4	-22	16	±44	60
5	-44	10	--±	undil.	---	0	--±	0	-44	40
3	444	8	--4	undil.	--±	0	--4	1	-44	20
2	--±	0	--±	undil.	---	0	--±	0	--3	1
Pig										
2	444	20	± ±4	1:5	---	4	--±	10	-24	20
7	-±3	1	-24	1:5	---	4	--1	10	±44	30
1	--±	0	-24	undil.	--2	1	-24	2	±44	3
2	---	0	-±±	undil.	±2-	1	-±±	0	±11	10

[1]The sera employed are unheated portions of those presented in Table XVIII A.

which the fractions were derived. Thus, the first serum listed in the table gives a strongly positive reaction (444), while the globulin fraction derived from it gives a very weakly positive reaction (-22). This tendency is evident with each of the animals studied. The same tendency is also noted with globulin fractions derived from unheated animal sera (Table XVIIIB). It was seen in the previous experiment that the globulin fractions of human Kahn-negative sera give a high percentage of positive serologic/al reactions. It would thus appear that the globulin fractions of animal sera do not follow the same serologic/al pattern.

In considering the reactions of the different animals, those obtained with chicken globulins are discussed first. Chicken serum (7, 11/2) showed a 4+ reaction in all three tubes of the Kahn test in both a heated and unheated state. On diluting the heated serum 1:7 and the unheated serum 1:8 with saline, room temperature reactions of weak nature (average of 1+ in three tubes) were noted. The sera at these dilutions showed no reaction at 37°C. and strongly positive (average of 4+) reactions at 1°C. with quantitative titers of 80 units.

The globulin fractions obtained from the heated and unheated sera reacted quite differently. The room temperature globulin reaction with both fractions showed an average of 2+ in the three tubes of the Kahn test. The 37°C. reactions with both fractions also averaged 2+, but the 1°C.

reaction in both cases averaged 1+.

When, however, the globulin fractions were diluted with saline, a different reaction pattern was observed. When the fractions were diluted from 1:5 to 1:40, the reactions at 37°C. and at room temperature were negative, while the reactions at 1°C. were positive in a 1:5 dilution with the fraction from heated serum and doubtful in a 1:10 dilution (positive in a 1:5 dilution) with the fraction from unheated serum. Thus, a zone reaction similar to that exhibited by low titer, unheated cow sera (in which in an undiluted state no reaction was obtained at 1°C., but on dilution a zone of flocculation was noted) was observed. It will be seen below that, on dialysis of the globulin from chicken sera, this zone reaction is eliminated, while, as was already noted (Table VIII), dialysis of the cow sera does not eliminate the reaction.

The other examples of the reactions of chicken globulins cited in Tables XVIIIA and B show a pattern of reactivity similar to that of the globulin fractions just considered. Some globulin fractions derived from weakly reacting sera showed the type of reactivity exemplified by serum (7, 10/18) in Tables XVIIIA and B. The serum was negative in the Kahn test but showed a 4+ reaction and 20 units at 1°C., whereas the globulin was negative throughout.

Cow globulins reacted alike both qualitatively and quantitatively whether derived from heated or unheated sera.

It will be recalled that heated cow sera gave stronger reactions than unheated sera and the globulin reactions approach those of the heated sera. To illustrate, cow serum (2, 10/14) had a Kahn titer of 40 units at room temperature and this titer was equalled by the globulin solution of both heated and unheated serum. The reactions at 37° and 1°C. followed the serum patterns. The globulin solution of cow serum (2, 10/13) shows, at room temperature, a more marked reaction on dilution with saline than in an undiluted state. This pattern is similar to the one noted at 1°C. with chicken globulin solutions.

Cow serum (3, 12/3) and its globulin fractions present another pattern of reactivity. The serum showed a weak room temperature reaction in the heated and unheated state. The globulin, however, reacted even more strongly at room temperature than had the serum. This type of reactivity, as has been previously shown (Table XVI, XVII), is a characteristic of seronegative human sera.

Horse globulins exhibited essentially the same types of reactivity as the globulins of cow sera. There were, however, no examples of enhanced reactivity on the part of the globulin fractions over that of the serum. The room temperature globulin reactions were usually weaker than the serum reaction. Also, the globulins derived from heated and unheated sera were usually of equal reactivity.

Pig globulins reacted much like horse and cow globulins; i.e., similar reactions were obtained from heated and unheated sera, and the pattern of reactivity at the three temperatures was similar to that observed with the sera. In several instances, the type of reaction exhibited by serum (2, 10/1) was noted; i.e., no reaction with serum at room temperature or at 37°C., and no reaction at room temperature with the globulin fraction, but weak flocculation at 37°C. and strong flocculation at 1°C. with this same globulin solution.

Rabbit globulin fractions were obtained from heated sera only and reacted in the same pattern at 37°C., room temperature and 1°C. as the sera from which they were derived. These globulin fractions showed the zone type of reaction at 1°C.; i.e., stronger reactions on dilution, in rare instances only.

Tables XIXA and XIXB list the percentages of positive and negative reactions obtained in the Kahn test at the three temperatures with the globulin fractions of the animal sera employed. Table XIXA gives the results obtained at room temperature, while Table XIXB gives the results obtained at 37° and 1°C. It must be kept in mind that, in a number of instances in which the reactions are negative at room temperature and at 1°C. in an undiluted state of the globulin, the reactions may be positive on dilution. This dilution factor in regard to the room temperature flocculation

TABLE XIX A

EXTENT OF FLOCCULATION (KAHN) REACTIONS WITH GLOBULIN FRACTIONS OF HEATED AND UNHEATED ANIMAL SERA

Source of Globulin Fraction	Number Tested	Globulin From Heated or Unheated Serum	Kahn Reactions: Strongly Positive	Weakly Positive	Percent Positive	Negative	Per Cent Negative
Chicken	102	Heated	1	14	14.7	87	85.3
	101	Unheated	1	17	17.9	83	82.2
Cow	62	Heated	31	19	80.5	12	19.5
	62	Unheated	28	25	85.5	9	14.5
Horse	25	Heated	8	9	68.0	8	32.0
	25	Unheated	10	6	64.0	9	36.0
Pig	102	Heated	57	19	74.5	26	25.5
	102	Unheated	58	18	74.5	26	25.5
Rabbit	33	Heated	3	16	57.5	14	42.5

TABLE XIX B

EXTENT TO WHICH KAHN-NEGATIVE OR WEAKLY POSITIVE ANIMAL GLOBULIN FRACTIONS GIVE FLOCCULATION REACTIONS AT 37° AND 1°C.[1]

Source of Globulin	Number Tested	Globulin from Heated or Unheated Serum	Flocculation (Kahn) Reactions at: 37°C. Number Positive	37°C. Percent Positive	1°C. Number Positive	1°C. Percent Positive
Chicken	102	Heated	18	17.7	57	56.0
	101	Unheated	15	14.9	56	55.5
Cow	62	Heated	40	64.5	60	96.8
	62	Unheated	31	50.0	60	96.8
Horse	25	Heated	7	28.0	23	92.0
	25	Unheated	4	16.0	23	92.0
Pig	102	Heated	33	32.4	99	97.1
	102	Unheated	30	29.5	92	90.2
Rabbit	33	Heated	9	27.2	26	79.8

[1] The Globulin fractions listed in Table XIX A as strongly positive were diluted with salt solution to render them weakly positive.

results is negligible insofar as strongly positive sera are concerned.

Chicken globulins showed a marked decrease in reactivity when compared to serum reactions (70.6 per cent of heated sera were Kahn-positive whereas only 14.7 per cent of globulins obtained from these sera were positive). Globulin reactions at 1°C. were only about half as many as occurred with serum. It is evident also from data in the tables that a similar lessening of reactivity was noted with the other animal globulins as compared with the sera.

Table XX presents flocculation zones shown by globulin fractions of heated and unheated animal sera at different temperatures. For example, chicken serum (1, 10/15) shows in a heated state a 144 Kahn reaction. The reaction of the globulin fraction is -12, as are the reactions at 37° and at 1°C. On dilution of the globulin fraction of 1:5 and 1:10, reactions are obtained at 1°C. but not at room temperature or at 37°C. The -12 reaction at 1°C. in an undiluted state and the positive reaction in a 1:5 and 1:10 dilution indicate inhibition of flocculation with the undiluted globulin.

The chicken specimens exhibit the "inhibition" phenomenon at 1°C. with both heated and unheated sera. Striking examples are those of chicken 1 (10/26) and 1 (11/7). In one case the reaction did not appear until the globulin was diluted 1:5; in another not until it was diluted 1:10.

TABLE XX

FLOCCULATION ZONES SHOWN BY GLOBULIN FRACTIONS OF HEATED AND UNHEATED ANIMAL SERA AT DIFFERENT TEMPERATURES

Indicating Some Inhibition of Flocculation with Undiluted Globulin

Serum Source, Number, Date Examined		Kahn Reaction of Serum	Reaction of Globulin Fractions in Kahn Test	Dilution of Globulin Fractions with 0.9% Saline	Reactions of Globulin Fractions at: 37°C.	1°C.	Temp. of Reaction	Reactions of Globulin Fractions in dilutions of: undil.	1:5	1:10	1:20	1:30	1:40
1941-42		Tube 123	Tube 123		Tube 123	Tube 123							
Chicken													
1) 10/15	H[1]	144	-12	undil.	-12	-12	37°C.	2	–	–	–		
							R.T.[2]	2	–	–	–		
							1°C.	2	4	2	–		
	Un.	-44	-12	undil.	-12	--2	37°C.	2	–	–	–		
							R.T.	2	–	–	–		
							1°C.	2	4	–	–		
6) 10/15	H	-14	--2	undil.	-22	--2	37°C.	2	–	–	–		
							R.T.	2	–	–	–		
							1°C.	2	4	–	–		
	Un.	-44	--2	undil.	--1-	--3	37°C.	1	–	–	–		
							R.T.	2	–	–	–		
							1°C.	3	4	2	–		
1) 10/17	H	421	---	undil.	-12	-±-	37°C.	2	–	–	–		
							R.T.	–	–	–	–		
							1°C.	–	2	1	–		
	Un.	±11	---	undil.	-21	-1±	37°C.	1	–	–	–		
							R.T.	–	–	–	–		
							1°C.	±	4	4	–		

([1]H = heated serum; Un. = unheated serum.
[2]R.T. = Room temperature)

6) 10/17	H	-44	--±	undil.	--±	--2	37°C.	±	–	–	–		
							R.T.	±	–	–	–		
							1°C.	2	4	4	–		
	Un.	144	--±	undil.	-±3	--2	37°C.	3	–	–	–		
							R.T.	±	–	–	–		
							1°C.	2	4	1	–		
1) 10/18	H	---	---	undil.	---	---	37°C.	–	4	–	–		
							R.T.	–	–	–	–		
							1°C.	–	±	4	–		
	Un.	-±4	---	undil	---	---	37°C.	–	–	–	–		
							R.T.						
							1°C.	–	4	1	1	–	
1) 10/26	H	±21	-13	undil.	--2	--±	37°C.	2	–	–	–		
							R.T.	3	–	–	–		
							1°C.	±	4	4	±	–	
	Un.	±44	-13	undil.	-±4	-±±	37°C.	4	–	–	–		
							R.T.	3	–	–	–		
							1°C.	±	4	4	2	2	–
1) 11/7	H	344	---	undil.	---	---	37°C.	–	–	–	–		
							R.T.	–	–	–	–		
							1°C.	–	–	4	4	–	
	Un.	444	---	undil.	---	---	37°C.	–	–	–	–		
							R.T.	–	–	–	–		
							1°C.	–	–	4	4	–	
Pig 5) 1-/4	H	444	--2	1:10	---	--4	37°C.	0	–	–	–		
							R.T.	2	4	4	–		
							1°C.	0	4	4	–		
	Un.	444	--4	1:10	--±	--4	37°C.	0	2	–	–		
							R.T.	4	4	3	–		
							1°C.	4	4	3	–		
2) 10/31	H	444	--4	undil.	--±	--4	37°C.	±	–	–			
							R.T.	4	–				
							1°C.	4	4	–			
	Un.	--4	--4	undil.	--±	--4	37°C.	±	–				
							R.T.	4	–				
							1°C.	4	4	–			

TABLE IX (CONT'D)

Serum Source, Number, Date Examined		Kahn Reaction of Serum	Reaction of Globulin Fractions in Kahn Test	Dilution of Globulin Fractions with 0.9% Saline	Reactions of Globulin Fractions at: 37°C.	1°C.	Temp. of Reaction	Reactions of Globulin Fractions in dilutions of: undil.	1:5	1:10	1:20	1:30	1:40
1941-42		Tube 123	Tube 123		Tube 123	Tube 123							
Pig													
4) 11-3	H	4 4 4	— — —	1:5	— — 4	— — 2	37°C.	0	4	—			
							R.T.	±	4	—			
							1°C.	0	4	1	—		
	Un.	— 2 3	— — —	1:5	— — 1	— — 4	37°C.	0	4	—			
							R.T.	—	4	—			
							1°C.	0	4	—			
1) 11/26	H	4 4 4	— — —	undil.	— — ±	4 4 4	37°C.	±	4	1	—		
							R.T.	—	4	2	—		
							1°C.	4	4	2	—		
	Un.	— — —	— — —	undil.	— — —	4 4 4	37°C.	—	4	—	—		
							R.T.	—	4	±	—		
							1°C.	4	4	±	—		
3) 11/26	H	± 2 2	— — —	undil.	— — —	— — —	37°C.	—	4	±	—		
							R.T.	—	4	—	—		
							1°C.	—	2	—	—		
	Un.	— ± 2	— — —	undil.	— — —	— — —	37°C.	—	4	±	—		
							R.T.	—	4	—	—		
							1°C.	—	4	—	—		
Horse													
1) 12/11	H	4 4 4	4 4 4	1:10	— — —	— ± 4	37°C.	0	4	—	—		
							R.T.	4	4	4	4		
							1°C.	4	4	4	4		
	Un.	4 4 4	4 4 4	1:4	— — ±	± 4 4	37°C.	0	±	—	—		
							R.T.	4	±	—	—		
							1°C.	0	4	4	±		
3) 12/11	H	— 2 4	— — —	undil.	— — —	— — 4	37°C.	—	—	—			
							R.T.	—	—	—			
							1°C.	4	±	—			
	Un.	4 4 4	— — 4	undil.	— — ±	— 4 4	37°C.	±	—	—			
							R.T.	4	—	—			
							1°C.	4	4	—			
4) 12/11	H	— — ±	— ± 4	undil.	— — —	3 4 4	37°C.	—	—	—			
							R.T.	4	—	—			
							1°C.	4	2	—			
	Un.	2 1 —	± 3 2	undil.	— — —	4 4 4	37°C.	—	—	—			
							R.T.	2	—	—			
							1°C.	4	±	—			
1) 12/16	H	4 4 4	± 4 4	1:2	— — —	— 4 4	37°C.	0	—	—			
							R.T.	4	—	—			
							1°C.	4	4	4	4	±	
	Un.	4 4 4	2 4 4	1:2	— — ±	± 4 4	37°C.	0	—	—			
							R.T.	4	—	—			
							1°C.	4	4	4	—	—	
Cow													
1) 10/14	H	— 4 4	1 4 4	1:2	— 2 4	4 4 4	37°C.	0	2	—			
							R.T.	0	4	4	±		
							1°C.	0	4	4	±		
	Un.	— 4 4	— ± 4	undil.	— 4 4	4 4 4	37°C.	4	1	—			
							R.T.	4	4	0	—		
							1°C.	4	4	—			
2) 11/15	H	± 2 4	— — —	undil.	— — —	— ± 3	37°C.	—	—	—			
							R.T.	—	—	—			
							1°C.	3	4	—			
	Un.	— — —	— — —	undil.	— — —	— — 2	37°C.	—	—				
							R.T.	—	0				
							1°C.	2	4	—			
5) 12-3	H	— 4 4	— — 1	undil.	— — —	— ± 2	37°C.	—	—				
							R.T.	1	—				
							1°C.	2	4	±	—		
	Un.	— ± 1	— — 2	undil.	— — —	± ± 4	37°C.	—	—				
							R.T.	—	—				
							1°C.	2	4	2	—		

TABLE XX (CONT'D)

Serum Source, Number, Date Examined		Kahn Reaction of Serum	Reaction of Globulin Fractions in Kahn Test	Dilution of Globulin Fractions with 0.9% Saline	Reactions of Globulin Fractions at: 37°C.	1°C.	Temp. of Reaction	Reactions of Globulin Fractions in dilutions of: undil.	1:5	1:10	1:20	1:30	1:40
1941-42		Tube 123	Tube 123		Tube 123	Tube 123							
Cow							37°C.	4	-	-			
5)1/2	H	±44	±±4	undil.	--4	144	R.T.	4	-	-			
							1°C.	4	4	±	-		
							37°C.	4	-	-			
	Un.	---	-14	undil.	--4	±44	R.T.	4	-	-			
							1°C	4	4	2	-		
Rabbit							37°C.	3	-	-			
7)12/31	H	444	-±3	undil.	--2	±33	21°C.	2	-	-			
							1°C.	3	4	-			
							37°C.	-	-				
1)12/30	H	--4	--±	undil.	---	--1	21°C.	±	-				
							1°C.	1	-				
							37°C.	-	-	-			
4)12/30	H	144	--3	undil.	---	±22	21°C.	3	-	-			
							1°C.	2	4	-			
							37°C.	-	-	-			
7)12/30	H	-14	--2	undil.	---	--1	21°C.	2	-	-			
							1°C.	1	-	-			
							37°C.	-	-	-			
9)12/30	H	-23	--1	undil.	-±2	±44	21°C.	1	-	-			
							1°C.	4	-	-			

The pig specimens exhibit the phenomenon at room temperature and at 1°C. Pig specimen (5, 10/4) shows a reaction of --2 at room temperature, yet reacts in dilutions of 1:5 and 1:10 at room temperature with globulins derived from heated and unheated sera.

Other examples of reactivity in diluted globulins at room temperature and at 1°C., in spite of their apparently weak reactivity in the Kahn test at room temperature, are seen in the other examples given in the table.

Rabbit sera did not show the effect of dilution in freeing the globulin from the inhibition of flocculation to any great extent. An exception is seen in the case of rabbit (7, 12/31) which showed a 2+ average reaction in the test at 1°C. but reacted in a 1:5 dilution (20 units). Serum (4, 12/30) is a similar example. Horse globulins did not show the inhibition of flocculation to any great extent at room temperature as is seen from the data in the tables. Some degree of inhibition of flocculation was observed at 1°C., however. Cow globulins follow the general reaction pattern of pig globulins.

Dialysis of $(NH_4)_2SO_4$ precipitated globulin fractions.

The apparent inhibition of flocculation reactions noted with undiluted globulin solutions suggested this dialysis experiment. It was desired to find if the removal of the residual $(NH_4)_2SO_4$ from the precipitated globulin fractions by dialysis would remove this inhibitory factor.

The dialysis experiments were carried out with pig, cow, and horse globulins. The heated and unheated sera from which the globulins were prepared were also examined at the three temperatures.

The method employed for the preparation of the specimens to be dialyzed was as follows:

> The tube containing the serum from which the globulin was to be precipitated was marked at the lower meniscus of the (2 cc.) serum content. Saturated $(NH_4)_2SO_4$ solution was added slowly with stirring and the precipitate centrifugated. This was done in duplicate. The supernatant fluid was poured into other tubes, acidified and again centrifugated to obtain the albumin. The amount of saline added to just bring the globulins into solution was determined by cautious addition of the saline from a 2 cc. pipette graduated in tenths of a cubic centimeter. The duplicate solutions were then pooled, mixed and divided into two equal portions. One portion was dialyzed overnight against running distilled water. The other was placed in the refrigerator overnight. The dialyzed fraction of globulin usually increased noticeably in volume and a precipitate appeared. The dialyzing bags were suspended in air and fanned for about one hour in order to reduce the volume to less than that of the original sample. The bag was then stirred up thoroughly and the contents poured into a 15 cc. graduated (tenths) test tube. The bag was washed out a few times with 0.2 cc. amounts of distilled water. NaCl was added in concentration of nine mg./cc.
>
> The albumin was similarly treated with the exception that NaOH was added to weak alkalinity (as determined by testing with alkacid paper) before salt was added. Albumins usually did not show a precipitate on dialyzing. The NaCl added to the albumin was calculated in terms of the entire volume which usually included 0.2 to 0.3 cc. of the N/2 NaOH. The albumin usually went into solution in about 1.0 cc. of liquid. After dialysis and drying, they invariably showed a volume less than 2.0 cc. This volume was then adjusted to 2.0 cc. (or the original volume of the serum from which they were derived) and 20 mg. of NaCl added.

The column in Table XXI headed "Dilution of Recovered Globulin" refers to the relationship between the final volume of the globulin solution tested and the original volume of serum. For example, a ratio of 1:1.5 in this column would indicate the final volume of globulin was 3.0 cc., whereas the original volume of serum was only 2.0 cc. If a globulin fraction is diluted with saline, the "Dilution of Recovered Globulin" is taken into account. That is, if this ratio is 1:1.5 and the fraction is diluted by the addition of an equal volume of saline, the "Final Dilution of Specimen" would be equal to 1:3 and not 1:2.

The results obtained are given in Table XXI. In the case of pig sera, dialysis of the globulin apparently removed the inhibitory effect on flocculation in most instances. For example, in the case of pig serum (2, 1/6), although both dialyzed and undialyzed globulins reacted to the extent of 4+ at room temperature, there was a quantitative difference in their reactivity at room temperature and 1°C., although not at 37°C.; i.e., the undialyzed fraction of globulin from heated serum showed a titer of 80 units at 1°C., whereas the same fraction dialyzed, showed a titer of 140 units. Pig serum (1, 1/6) exhibited the typical reaction pattern previously described for pig heated and unheated serum and for pig globulin. It will be noted that the globulin fractions were less reactive than the heated serum and more reactive than the unheated serum. Yet this could have been an artefact since the "Dilution of Recovered Globulin" of the undialyzed fraction of the globulin was 1:1.5 (i.e., total volume of globulin was 3 cc. after starting with 2.0 cc. of serum).

TABLE XXI

FLOCCULATION REACTIONS OF GLOBULIN FRACTIONS OF ANIMAL SERA BEFORE AND AFTER REMOVAL OF AMMONIUM SULFATE BY DIALYSIS

Serum Number; Nature of Specimen	Dilution of Recovered Globulin	Kahn Reaction	Final Dilution of Specimen	Flocculation Reactions at: 37°C.		Room Temp.		1°C.	
1942		Tube 123		Tube 123	Quant. Titer	Tube 123	Quant. Titer	Tube 123	Quant. Titer
Pig 1) 1/6									
Heated Serum		−44	1:2	----		---2	8	444	20
Unheated Serum		----	undil.	----	0	----	0	244	10
Undialyzed Globulin from Heated Serum	1:1.5	---3	1:1.5	---2	6	---3	6	−32	6
Undialyzed Globulin from Unheated Serum	1:1.5	---2	1:1.5	---2	6	---2	6	−22	6
Dialyzed Globulin from Heated Serum	1:1.5	−34	1:2	----		---4	8	444	10
Dialyzed Globulin from Unheated Serum	1:1.5	−44	1:2	----		---4	8	444	20
Pig 2) 1/6									
Heated Serum		444	1:10	---1	40	−14	40	444	180
Unheated Serum		−±3	undil.	----		−±3	1	444	120
Undialyzed Globulin from Heated Serum	1:1.3	444	1:5	---2	20	−34	20	443	80
Undialyzed Globulin from Unheated Serum	1:2	444	1:5	−11	20	−±3	20	444	80
Dialyzed Globulin from Heated Serum	1:2	444	1:10	---±	20	−±4	40	444	140
Dialyzed Globulin from Unheated Serum	1:2	444	1:10	----	20	---1	30	444	160
Pig 3) 1-6									
Heated Serum		444	1:12	----	20	±24	48	344	200
Unheated Serum		±44	1:2	----		−14	8	444	200
Undialized Globulin from Heated Serum	1:2	±±4	1:2	±14	8	±±4	8	±44	160
Undialized Globulin from Unheated Serum	1:2	±44	1:2	± ±1		±44	8	±44	180
Dialyzed Globulin from Heated Serum	1:2	344	1:7	----		−14	28	444	140
Dialyzed Globulin from Unheated Serum		±44	1:5	----		---2	20	444	200
Pig 5) 1-6									
Heated Serum		−±4	undil.	----		−±4	1	244	40
Unheated Serum		----	undil.	----		----		444	20
Undialized Globulin from Heated Serum	1:1.5	----	1:1.5	----		----		−±3	1
Undialized Globulin from Unheated Serum	1:1.2	----	1:1.2	−±±		----		± ±2	10
Dialyzed Globulin from Heated Serum		−24	undil.	−± ±		−24	2	444	30
Dialyzed Globulin from Unheated Serum		−14	undil.	−±1		−14	2	444	40
Pig 6) 1-6									
Heated Serum		444	1:2	---±	30	−±3	48	444	100
Unheated Serum		---±	undil.	----		---±		344	120
Undialyzed Globulin from Heated Serum	1:1.2	±44	1:2.5	−±−		−±3	10	±±4	40

Serum Number; Nature of Specimen	Dilution of Recovered Globulin	Kahn Reaction	Final Dilution of Specimen	Flocculation Reactions at: 37°C.		Room Temp.		1°C.	
1942		Tube 123		Tube 123	Quant. Titer	Tube 123	Quant. Titer	Tube 123	Quant. Titer
Pig 6) 1-6									
Undialyzed Globulin from Unheated Serum	1:1.2	4 4 4	1:2.5	— — —		± ± 4	10	± 4 4	30
Dialyzed Globulin from Heated Serum	1:1.5	± 4 4	1:7	— — —		— — 4	28	4 4 4	100
Dialyzed Globulin from Unheated Serum	1:1.5	4 4 4	1:7	— — —		— — ±	20	4 4 4	80
Cow 1) 1-9									
Heated Serum		± 4 4	1:4	— — —		— ± 3	16	— 4 4	80
Unheated Serum		— 4 4	1:2	— 4 4	8	— ± 1	8	— — —	40
Undialyzed Globulin from Heated Serum	1:1.2	— ± 4	1:2.4	— — 4	10	— ± 4	10	— 4 4	10
Undialyzed Globulin from Unheated Serum	1:1.3	— 4 4	1:2.6	— 4 4	10	— ± 4	10	— 4 4	10
Dialyzed Globulin from Heated Serum	1:1.3	± 4 4	1:1.3	± 4 4	4	± 4 4	4	4 4 4	20
Dialyzed Globulin from Unheated Serum		lost							
Cow 2) 1-9									
Heated Serum		4 4 4	1:13	— — —	30	± ± 3	52	— 2 4	120
Unheated Serum		4 4 4	1:8	— ± 3	30	— — 3	32	— 4 4	80
Undialyzed Globulin from Heated Serum		± 4 4	1:5	— ± 4	20	— ± 4	20	— 4 4	40
Undialyzed Globulin from Unheated Serum		± 4 4	1:5	— — 4	20	— ± 4	20	— 4 4	30
Dialyzed Globulin from Heated Serum	1:1.5	2 4 ±	1:1.5	4 4 ±	6	2 4 ±	6	4 4 4	60
Dialyzed Globulin from Unheated Serum	1:1.5	— — —	1:1.5	— — 2		— — —		4 4 4	60
Cow 3) 1-9									
Heated Serum		4 4 4	1:9	— — —	10	— ± 4	36	± 4 4	100
Unheated Serum		± 3 4	1:2	— 4 4	8	— 2 4	8	± 3 4	40
Undialyzed Globulin from Heated Serum	1:1.5	± 1 4	1:1.5	± 2 4	6	± 1 4	6	2 4 4	10
Undialyzed Globulin from Unheated Serum	1:1.6	± 1 4	1:1.6	— 1 4	6	± 1 4	6	± 4 4	3
Dialyzed Globulin from Heated Serum	1:1.5	± ± 2	1:1.5	— — —		± ± 2	6	4 4 4	30
Dialyzed Globulin from Unheated Serum	1:2	— ± ±	1:2	— — —		— ± ±		4 4 4	30
Cow 5) 1-9									
Heated Serum		4 4 4	1:13	— — ±	30	— ± 4	52	— 4 4	160
Unheated Serum		— ± 3	undil.	± 4 4	3	— ± 3	1	1 ± —	140
Undialyzed Globulin from Heated Serum	1:1.1	± 4 4	1:2.3	— ± 4	10	— ± 4	10	— 4 4	40
Undialyzed Globulin from Unheated Serum		± 4 4	1:3	— 4 4	20	— 1 4	12	± 4 4	40
Dialyzed Globulin from Heated Serum	1:1.5	— — —	1:1.5	— — —		— — —		4 4 4	100
Dialyzed Globulin from Unheated Serum	1:1.5	— — —	1:1.5	— — —		— — —		4 4 4	80

TABLE XXI (CONT'D)

Serum Number; Nature of Specimen	Dilution of Recovered Globulin	Kahn Reaction	Final Dilution of Specimen	Flocculation Reactions at: 37°C.		Room Temp.		1°C.	
1942		Tube 123		Tube 123	Quant. Titer	Tube 123	Quant. Titer	Tube 123	Quant. Titer
Cow 6) 1-9									
Heated Serum		444	1:1.5	---	40	-±2	60	±34	120
Unheated Serum		-44	1:2	-44	8	-±3	8	---	120
Undialyzed Globulin from Heated Serum	1:1.2	-44	1:2.4	--4	10	-±4	10	±44	40
Undialyzed Globulin from Unheated Serum	1:1.5	-34	1:2	--3	8	-±4	8	±44	60
Dialyzed Globulin from Heated Serum	1:1.5	---	1:1.5	---		---		444	40
Dialyzed Globulin from Unheated Serum	1:1.5	---	1:1.5	---		---		444	40
Horse 1) 12-16									
Heated Serum		444	1:7	---	4	-34	28	444	140
Unheated Serum		444	1:7	-±4	28	±44	30	444	160
Undialyzed Globulin from Heated Serum	1:1.5	444	1:4.5	--3	12	-±4	18	-44	30
Undialyzed Globulin from Unheated Serum	1:1.3	444	1:5	--2	12	--4	20	144	80
Dialyzed Globulin from Heated Serum	1:3	444	1:15	---	20	-±4	60	444	100
Dialyzed Globulin from Unheated Serum	1:2	444	1:15	-±1	40	--4	60	444	180
Horse 2) 12-16									
Heated Serum		444	1:5	---	4	-±4	20	444	80
Unheated Serum		444	1:5	--4	20	-44	20	444	80
Undialyzed Globulin from Heated Serum	1:1.7	--4	1:1.7	--±		--4	8	-43	10
Undialyzed Globulin from Unheated Serum	1:1.4	-44	1:2.5	--1		-±2	14	-44	20
Dialyzed Globulin from Heated Serum	1:3.5	-44	1:5	--4	20	-24	20	444	60
Dialyzed Globulin from Unheated Serum	1:2	444	1:8	---	10	-±4	40	444	40
Horse 3) 12-26									
Heated Serum		-44	1:2.5	---		--±	3	444	20
Unheated Serum		-44	1:2.5	---		--±	3	244	30
Undialyzed Globulin from Heated Serum	1:1.7	--±	1:1.7	---		--±		-1-	
Undialyzed Globulin from Unheated Serum	1:1.5	--±	1:1.5	--±		--±		---	
Dialyzed Globulin from Heated Serum	1:2.5	---	1:2.5	---		---		144	10
Dialyzed Globulin from Unheated Serum	1:2.5	---	1:2.5	---		---		444	20
Horse 4) 12-26									
Heated Serum		-±4	undil.	--3	1	-±4	1	444	40
Unheated Serum		±44	1:3	-±-		-±4	12	444	40

TABLE XXI (CONT'D)

Serum Number; Nature of Specimen	Dilution of Recovered Globulin	Kahn Reaction	Final Dilution of Specimen	Flocculation Reactions at: 37°C.		Room Temp.		1°C.	
1942		Tube 123		Tube 123	Quant. Titer	Tube 123	Quant. Titer	Tube 123	Quant. Titer
Horse 4) 12-26									
Undialyzed Globulin from Heated Serum	1:1.4	-±3	1:1.4	--3	1	-±3	1	±34	6
Undialyzed Globulin from Unheated Serum	1:1.2	-44	1:3	---		-±4	12	±44	30
Dialyzed Globulin from Heated Serum	1:3	-±4	1:3	---		-±4	12	444	40
Dialyzed Globulin from Unheated Serum	1:1.8	-14	1:3	---		--2	12	344	20
Horse 5) 12-26									
Heated Serum		-34	1:2.3	---		-12	10	444	20
Unheated Serum		±44	1:2.3	---		--3	10	444	40
Undialyzed Globulin from Heated Serum	1:1.3	-22	1:2.3	---		-22	10	-44	10
Undialyzed Globulin from Unheated Serum	1:1.2	---	1:1.2	---		---		±33	4
Dialyzed Globulin from Heated Serum	1:2.3	-±1	1:2.3	---		-±1		444	10
Dialyzed Globulin from Unheated Serum	1:2.3	-±1	1:2.3	---		-±1		444	40

Removal of the $(NH_4)_2SO_4$ seemed to remove an activitating influence on the fractions tested at 37°C. The undialyzed globulins showed reactions at 37°C., while the serum and dialyzed globulins did not. The 1°C. reaction of the sulphate-free globulin is like that of the serum, whereas the sulphate containing globulin fraction shows the inhibition effect. Pig serum (3, 1/6) although not of great reactivity at room temperature showed reaction in high dilution at 1°C. Although the dialyzed heated globulin showed much more reactivity at room temperature than the analogous undialyzed fraction, their 1°C. reactions were quantitatively equal. The other pig samples show similar patterns to these described ones.

Dialysis of cow globulins did not result in findings similar to those obtained with pig globulins. Cow serum (1, 1/9), for example, showed the reaction pattern described previously for cow sera, i.e., the heated serum more reactive than the unheated serum, with most marked activity in the cold; unheated serum exhibiting the zone type reaction with high titer at 1°C. The dialyzed heated globulin reacted much like the undialyzed globulin, however, with a quantitative titer far less than that of the heated serum. Cow serum (2, 1/9) shows the unusual picture of dialyzed fractions lower in reactivity at room temperature but higher in reactivity at 1°C. than undialyzed fractions. Still, these reactions did not reach the intensity of whole serum

reactions. Sera 3, 5 and 6 (1/9) present similar pictures.

Dialyzed horse globulins behaved almost exactly as the pig globulins had, as is seen from the data. Horse serum (1, 12/26) showed a standard Kahn reaction of 4+ in all fractions examined. Quantitatively, however, the dialyzed portions were much more reactive than the undialyzed fractions at all temperatures (unlike pig globulins which showed more reactivity at 37°C. in the undialyzed fractions). Serum (2, 12/26) shows a similar picture. Concerning serum (3, 12/26) it will be noted that although the standard Kahn reaction was -44, on diluting the serum to the value of "Dilution of Recovered Globulin" the same negative result was obtained. The undialyzed fraction was nonreactive in the cold, whereas the dialyzed portion reacted almost exactly like the whole serum.

The dialysis experiments, it is believed, explain in the case of horse and pig globulins why the apparently anomalous flocculation results were obtained with these specimens. The same experiments do not explain similarly anomalous results with cow sera. No experimentally based explanation can be offered for this.

Results obtained with albumin solutions of all[1] the animals tested were essentially negative at all temperatures and in dilutions of 1:5 at 37° and 1°C. These solutions were undialyzed and tested only with standard Kahn antigen.

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[1] A few of the samples were lost during centrifugation.

DISCUSSION

Whether or not the substance in syphilitic serum which reacts in tests for syphilis is an antibody to the Spirochaeta pallida remains unknown (Eagle, p. 295, 1935). A method of approach employed to determine whether the reactive substance is a specific one has been to attempt to induce reactivity in human sera derived from non-luetic sources. Results obtained have led to much controversy (pps. 43-48).

In practice, in spite of conflicting data (Malloy and Kahn, 1931; Schreus and Foerster, 1934; Barnett, Jones and Kulchar, 1935), it has been a working assumption that a qualitative difference between syphilitic and nonsyphilitic sera does exist which is detected by the test for syphilis. While this possibility remains, it would seem that the studies presented herein afford additional evidence that a test for syphilis can be so modified as to give increasing numbers of reactions with nonluetic sera (as well as with luetic sera); that "the difference between nonsyphilitic and syphilitic serums is one of degree rather than of kind -- quantitative rather than qualitative."

Heretofore, studies on nonsyphilitic serological reactions have generally been directed to human sera which

were negative in tests for syphilis. The attempts to render them positive have been considered (pps. 43-52). Animal sera have not been used, evidently because they already react in certain percentage in tests for syphilis. Up to the present time, no attempt has been made to determine the extent to which this reactivity of animal sera can be qualitatively and quantitatively decreased or increased. Experiments herein presented indicate that the serological reactivity of chicken, cow, horse, pig and rabbit sera can be lessened by carrying out the Kahn test at 37°C. and the same sera can be made to show reactions in numbers approaching 100% by carrying out the test at 1°C.

Problems associated with the reactivity in tests for syphilis of the globulins of animal sera have also been investigated. It has been previously shown that the reactivity of syphilitic sera reside in the globulin fraction (Eagle, p. 166, 1938) and that the albumin fraction is non-reactive. According to Sherwood, et al., (1938), the reactivity of nonsyphilitic animal sera is also present in the globulin; equally in water soluble and water insoluble fractions, although experimental data have not as yet been presented. As was observed from the data herein reported, the globulin fraction contained the serologically reactive portion of the animal sera except that

special means were often necessary to elicit this reactivity. Thus, the globulin of positively reacting cow sera frequently gave negative reactions in an undiluted state, but positive reactions on dilution with saline. Then again, the globulin of positively reacting pig sera, frequently gave negative reactions, while after dialysis, the reactivity was restored. It would appear that $(NH_4)_2SO_4$ exerts an inhibitory effect on serological reactivity of the globulin fraction of animal sera; an effect not exerted on the globulin fraction of syphilitic sera.

It is noteworthy also that, although heated and unheated animal sera showed considerable difference in serological reactivity, the globulins derived from heated and unheated sera reacted similarly. Evidently the treatment of the serum with $(NH_4)_2SO_4$ destroys the property responsible for the difference in the reactivity of heated and unheated sera. Nishio (1929) also observed that the globulin of syphilitic sera, whether heated or unheated, gave flocculation results in the Kahn test similar to that obtained with the heated sera, in spite of the difference in reactivity of the unfractioned heated and unheated sera.

Part Two. Degree of Fixation of Complement by Serum-Antigen Mixtures of a Flocculation (Kahn) Reaction Carried Out under Different Temperature Conditions.

Experiment 8. Fixation of Complement by the Serum-Antigen Mixture Obtained from the Completed Kahn Reaction Performed at 37°C.

Another aspect of the Kahn reaction with animal sera and nonsyphilitic human sera which was investigated involved a study of the question as to whether the negative flocculation results obtained at 37°C., with sera which showed flocculation at lower temperatures, was due to absence of union between serum (antibody) and antigen at this temperature, or in the presence of such union, to lack of aggregation of the formed complex, leading to an "invisible" (soluble[1]) flocculate. It has been assumed that if a serum-antigen mixture showing no precipitate at 37°C. did not fix complement, no union of the reactants had occurred; if this mixture did fix complement, union had occurred.

The assumption that fixation of complement may serve as an indicator of antigen-antibody union is based on the following: 1) the presence of floccules is evidence of a reaction between serum and antigen in the Kahn test and it

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[1]By the term "soluble" in reference to an antigen-antibody complex, the writer assumes a compound consisting of hydrophilic colloids, which are associated but retain an even dispersion throughout the containing menstrum and remain optically invisible.

has been shown that these floccules fix complement;[1] 2) it is generally accepted that complement fixation with human antisera is an indication of antigen-antibody union[2] (Wells, p. 196, 1929; Heidelberger, 1941), while absence of fixation is an indication of absence of such union.

Table XXII presents an outline of the combined flocculation-complement-fixation procedure used in these experiments. The procedure has been described in detail on page 60.

The results obtained in Experiment 8, shown in Table XXIII, indicate that serum-antigen mixtures which give negative Kahn reactions at 37°C. give correspondingly negative complement-fixation reactions at this temperature, while serum-antigen mixtures which give positive Kahn reactions at 37°C. give correspondingly positive complement-fixation reactions at this temperature. To consider a representative case: serum (36, 4/13) shows a reaction of --2 in the 3 tube test at room temperature in a 1:1.5 dilution. Reaction occurs at 1°C. (334) but not at 37°C.

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[1]Kahn (p. 29, 1928) has reported over 98% agreement between the flocculation reaction result and a complement-fixation test run on this completed reaction, in over 1,800 sera tested at the Michigan State Health Department laboratories.

[2]An exception of this finding has been noted by Stats and Bullowa (1942) in the case of sera from eight pneumonia patients who had been treated with sulfonamides. The type specific anticarbohydrate antibody which appeared in the blood serum of these patients did not fix complement in the presence of polysaccharide antigen.

TABLE XXII

OUTLINE OF PROCEDURE FOR COMBINED FLOCCULATION-COMPLEMENT FIXATION EXPERIMENTS

	Serum-Antigen Suspension from Tube 3 of Kahn Reaction, cc.			Antigen Suspension Control, cc.	Serum Control cc.
	0.15	0.15	0.15	0.002	0.03
Complement, cc. (2-4 units)	0.1	0.15	0.2	0.1	0.1
	Fixation Periods; either (1), (2) or (3)				
	(1) 30 minutes at 37°C. (2) 2 hours at Room Temperature (3) 3 hours at 5°C.				
Mixture of Sheep Red Blood Cells (5%) plus Hemolysin, cc.	0.2	0.2	0.2	0.2	0.2
	Incubation at either (1) or (2)				
	(1) 37°C. - until 5-10 minutes after all controls are hemolyzed. (2) Room Temperature - until controls are hemolyzed.				

Readings:
- \- (complete hemolysis
- ± (almost complete hemolysis
- 1 (about 75% "
- 2 (" 50% "
- 3 (" 25% "
- 4 (no hemolysis
- ac. (anticomplementary)

The 12:1 ratio tube of the 37°C. reaction does not fix two units of complement. In the control (syphilitic) sera, e.g., (33, 4/13), (139, 4/15), (92, 4/23) tested, good agreement existed between the flocculation and complement-fixation results.

The latter portion of Table XXIII shows results of a similar experiment with a super-sensitive antigen. This antigen was prepared from a beef heart powder which had undergone 15 days incubation at 37°C. Such incubation generally so modifies powdered heart muscle as to produce antigens of excessive sensitivity. The sensitivity of the antigen used in the present experiment was of such nature as to give positive reactions with most Kahn-negative sera when tested at 1°C. but not at 37°C. The serum-antigen mixtures which gave negative flocculation at 37°C. were examined with the complement fixation procedure. It is obvious from the results that no fixation occurred in any but the control syphilitic sera. It would seem safe to say that human sera which react in the Kahn test at room temperature but not at 37°C. are characterized by an inability to fix complement at this latter temperature in the combined flocculation complement-fixation procedure. This finding has been taken to indicate a lack of reaction between the reactant substances present.

TABLE XXIII

COMPLEMENT FIXATION REACTIONS AT 37°C. WITH SERUM-ANTIGEN MIXTURES GIVING NEGATIVE FLOCCULATION REACTIONS AT 37°C. AND POSITIVE FLOCCULATION REACTIONS AT 1°C.

Serum Number, Date Examined	Standard Kahn Reaction	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C.	Room Temp.	1°C.		Units of Complement Fixed by Serum-Antigen mixture in Tube 3 of 37°C. Flocculation Reaction. 1	2	3	4
1940	Tube 123		Tube 123	Tube 123	Tube 123					
32) 4/13	0-2	undil.	---	0-2	-22	T) SC) [1]	2 1	- -	- -	
33) " Control	044	1:3	-44	-24	---	T) SC)	4 2	4 -	2 -	
36) "	044	1:1.5	---	--2	344	T) SC)	4 4	- -	- -	
37) "	0--	undil.	---	0--	144	T) SC)	2 2	- -	- -	
40) "	044	1:3	---	-±+	±23	T) SC)	3 2	- -	- -	
139) 4/15 Control	444	1:20	±+4	±44	---	T) SC)	4 2	4 -	± -	
143) "	-±2	undil.	---	-±2	244	T) SC)	4 4	- -	- -	
144) "	030 [2]	undil.	---	030	244	T) SC)	3 3	4 4	± -	
42) 4/15 Control	---	undil.	0--	---	0--	T) SC)	± ±	- -	- -	
49) 4/15	0-0 [1]	undil.	---	0-0	234	T) SC)	1 1	- -	- -	
83) 4/22	---	undil.	---	---	344	T) SC)	- -	- -	- -	
84) " Control	---	undil.	---	---	---	T) SC)	- -	- -	- -	
98) " Control	222	1:50	444	144	21-	T) SC)	4 4	4 1	4 -	
88) 4/23	±34	1:1.3	--±	-±2	±12	T) SC)	3 3	- -	- -	
89) "	---	undil.	---	---	112	T) SC)	3 2	- -	- -	
92) " Control	444	1:5	-±4	-±3	444	T) SC)	4 1	3 -	- -	
87) 4/24	±44	1:2	---	--±	332	T) SC)	2 ±	- -	- -	
38) 4/25	444	1:20	---	-±1	-±3	T) SC)	3 2	- -	- -	
39) "	±23	undil.	--±	±23	343	T) SC)	1 2	± -	- -	
40) "	---	undil.	---	---	-±4	T) SC)	2 1	- -	- -	
30) 4/26 Control	---	undil.	---	---	---	T) SC)	- -	- -	- -	- -
87) 4/24 Repeat	±44	1:1.5	---	0-±	031	T) SC	4 4	- -	- -	- -
38) 4/25 Repeat	444	1:5	---	-±2	444	T) SC)	± ±	- -	- -	- -
39) 4/25	±23	undil.	0--	±23	034	T) SC)	4 4	- -	- -	- -
91) 4/25 Control	±44	1:1.5	±14	0±2	--±	T) SC)	4 3	4 -	± -	- -

TABLE XVIII (cont'd)

Serum Number, Date Examined 1940	Standard Kahn Reaction Tube	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube	Room Temp. Tube	1°C. Tube		Units of Complement fixed by Serum-Antigen mixture in Tube 3 of 37°C. Flocculation Reaction. 1	2	3	4
119) 4/30	----	undil.	----	----	----	T)	±	-	-	-
						SC)	±	-	-	-
120) "	4444	1:3	-34	±±1	--±	T)	4	4	±	-
Control						SC)	3	-	-	-
123) "	±34	1:2	---	-±1	444	T)	4	±	-	-
						SC)	4	-	-	-
127) "	-±3	undil.	-±±	-±3	-±3	T)	4	3	-	-
						SC)	2	±	-	-
129) 4/30	4444	1:20	----	-±2	±444	T)	±	-	-	-
						SC)	±	-	-	-
130 "	4444	1:10	---	211	4444	T)	-	-	-	-
						SC)	-	-	-	-
131) "	4444	1:5	±444	-23	±±1	T)	4	4	4	1
Control						SC)	4	±	-	-
132) "	4444	1:3	----	-11	124	T)	4	±	-	-
						SC)	3	-	-	-
83) 5/14	4444	1:15	±444	-±1	-±1	T)	4	4	4	±
Control						SC)	-	-	-	-
134) "	----	undil.	----	----	4444	T)	-	-	-	-
						SC)	-	-	-	-
45) 5/15	±34	undil.	----	±34	4444	T)	4	-	-	-
						SC)	4	-	-	-
90) 5/16	4444	1:60	4444	4444	----	T)	4	4	4	4
Control						SC)	±	-	-	-
42) 5/16	±34	undil.	00-	±34	004	T)	2	-	-	-
						SC)	1	-	-	-
24) 5/20	---	undil.	0--	---	041	T)	-	-	-	
						SC)	-	-	-	
27) "	----	undil.	----	----	244	T)	±	-	-	
						SC)	±	-	-	
25) 5/18	244	1:1.5	±24	-44	----	T)	4	4	±	
Control						SC)	3	-	-	
83) 6/21	4444	1:2	±444	±34	----	T)	4	4	±	-
Control						SC)	3	-	-	-
84) "	0-0[2]	undil.	-±±	0-0	±1±	T)	3	2	-	-
						SC)	2	-	-	-
86) "	----	undil.	----	----	144	T)	2	-	-	-
						SC)	3	-	-	-
121) 6/24	----	undil.	----	----	344	T)	±	-	-	-
						SC)	-	-	-	-
39) 6/26	±13	undil.	----	±13	144	T)	2	-	-	-
						SC)	3	-	-	-
30) 6/27	444	1:7	-±4	--4	244	T)	4	3	-	-
						SC)	-	-	-	-
47) "	444	1:1--	-±3	--2	---	T)	4	2	-	-
Control						SC)	-	-	-	-
95) 6/27	0-0[2]	undil.	----	0-0	±23	T)	2	-	-	-
						SC)	2	-	-	-
96) "	----	undil.	----	----	----	T)	-	-	-	-
Control						SC)	-	-	-	-
105) "	±34	undil.	244	±34	--±	T)	4	4	3	-
Control						SC)	3	-	-	-
43) 6/28	±34	1:2	----	-±2	±33	T)	±	-	-	-
						SC)	1	-	-	-
81) "	444	1:3	±44	±34	----	T)	4	4	±	-
Control						SC)	1	-	-	-
66) 6/29	±44	1:2	--3	-±4	-24	T)	4	1	-	-
						SC)	1	-	-	-

TABLE XXIII (CONT'D)

Results With A "Nonspecific" Antigen

4/5/1941	Tube 123		Tube 123	Tube 123					
2) 4-5	---	undil.	0--	012	T)	–	–	–	–
					SC)	–	–	–	–
3) "	---	"	0--	022	T)	1	–	–	–
					SC)	1	–	–	–
7) "	---	"	0--	022	T)	–	–	–	–
					SC)	–	–	–	–
8) "	---	"	0--	044	T)	±	–	–	–
					SC)	±	–	–	–
11) "	---	"	0--	042	T)	±	–	–	–
					SC)	–	–	–	–
14) "	---	"	0--	044	T)	±	–	–	–
					SC)	1	–	–	–
16) "	---	"	0--	022	T)	±	–	–	–
					SC)	±	–	–	–
17) "	---	"	0--	044	T)	±	–	–	–
					SC)	±	–	–	–
18) "	---	"	0--	0±1	T)	–	–	–	–
					SC)	–	–	–	–
19) "	---	"	0--	044	T)	–	–	–	–
					SC)	–	–	–	–
20) "	---	"	0--	032	T)	–	–	–	–
					SC)	–	–	–	–
24) Control	--2	"	044	014	T)	4	4	4	–
					SC)	4	4	4	–
120) 4-4 Control	±34	"	044	044	T)	4	4	±	–
					SC)	3	–	–	–

[1] T = test; SC = serum control – in this and succeeding tables.

[2] Presumptive Kahn Test

Experiment 9A. Dispersability of Floccules in the Completed Kahn Reaction with Human Sera by Incubation at 37° or at 56°C.

It was seen in Experiment 8 that lack of flocculation in the serum-antigen mixture of the Kahn reaction carried out at 37°C. is associated with the inability of the serum-antigen mixture to fix complement. It has been noted that the floccules in a positive serum-antigen mixture tend to disappear when incubated at certain temperatures. The question arose whether the heat-dissolved floccules would fix complement.

It was first necessary to note to what extent solution of the floccules in Kahn-positive reactions was obtained by incubating the serum-antigen mixtures at 37° or 56°C.

It has long been known that, on incubation of the completed Kahn reaction at temperatures higher than room temperature, some flocculates tend to disperse while others increased in size. The reports of Kahn (1925), Nagle and Lazarov (1930) and of Muckenfuss and Ebel (1931) have been considered (pps. 35-36).

In the experiments to be discussed herewith a definite relationship has been observed between the dispersability of the floccules following incubation at 37° or 56°C. and the quantitative titer of the serum. Floccules from sera with a quantitative titer of less than four units tended to

become readily dispersed; those with a titer of four to twenty units showed about equal propensities to disperse or to remain undispersed, while those sera with titers of more than twenty units almost invariably showed no dispersion of the floccules.

Results of the effect of incubating completed Kahn reactions at 37° and at 56°C. are shown in Table XXIV. The general plan of the experiment was to carry out Kahn tests with syphilitic sera and incubate the completed reactions at 37° and 56°C. at various intervals up to 24 hours. With one group of sera readings were made after 5, 10, 15, 30, 45, 60 minutes and after 2, 5 and 24 hours incubation. In the case of the majority of the sera readings were made after 15,30, 60 minutes and 5 and 24 hours. The results indicate that floccules incubated at 56°C. tend to disperse more markedly than when incubated at 37°C. The results also indicate that in certain serum-antigen mixtures, there is a tendency toward reaggregation of the floccules upon 24 hours' incubation.

A summary of the incubation results at 56°C. is given in Table XXV.

DISTRIBILITY OF FLOCCULES IN KAHN REACTIONS WITH SERUM AND INCUBATING SERUM-ANTIGEN MIXTURES AT 37°C. AND 56°C.

Serum Number, Date Obtained 1940	Standard Kahn Reaction	Tube Titer	Quant.		Flocculation Reading after Incubating for: 5	10	15	30	45	60	1	2	3	4	24
22) 5/23	4444	120	37°C.		4444	4444	4444	4444	4444	4444	4444	4444	4444	2444	132
			56°C.		4444	4444	4444	4444	4444	4444	4444	4444	4444	2444	132
37) "	3444	4	37°C.		+3	1	1	1	1	1	1	1	1	1	2
			56°C.												
39) "	1444	3	37°C.												
			56°C.												
41) "	2444	3	37°C.												
			56°C.												
42) "	2444	3	37°C.		+1	1	+	+							
			56°C.				+	+							
43) "	1444	3	37°C.		3	3	2	2	2	2	2	2	2	2	2
			56°C.												
44) "	4444	4	37°C.		4	4	4	4	3	4	4	3	3	2	
			56°C.												
50) " (Control)	1941	0	37°C.												
			56°C.												
27) 9/18	4444	4	37°C.				1444	1444		2444				4444	
			56°C.												
28) "	4444	80	37°C.				4444	4444		4444				4444	4444
			56°C.				+444	+444		11					4444
29) "	+34	2	37°C.				4	4		4				4	+14
			56°C.												14
32) "	3444	4	37°C.				1444	1444		1444				1444	4444
			56°C.				24								21
34) "	4444	4	37°C.				144	144		144				144	144
			56°C.												++
5) "	4444	4	37°C.				4444	4444		4444				4444	4444
			56°C.												
30) " (Control)	1941	0	37°C.												
			56°C.												
45) 4/3	4444	4	37°C.				144	144		144				+444	4444
			56°C.				144	144		+					
46) 4/3	4444	40	37°C.				4444	4444		4444				4444	4444
			56°C.				4444	4444						444	4444
47) "	+444	3	37°C.				14	14		+4				2	+24
			56°C.												
48) "	4444	20	37°C.				4444	4444		4444				4444	4444
			56°C.												
49) "	4444	4	37°C.												+14
			56°C.				1	+		+					
53) "	4444	4	37°C.				4	4		4				2	4
			56°C.												+
54) "	3444	4	37°C.				4	4		2				+	+44
			56°C.												+2
27) " (Control)	1941	0	37°C.												
			56°C.												
25) 8/21	24	2	37°C.				1								13
			56°C.												
29) "	4444	4	37°C.				4444	4444		4444				4444	4444
			56°C.				+444	34		24				13	244
53) "	144	3	37°C.												
			56°C.												+
56) "	+444	3	37°C.												
			56°C.												
59) "	4444	4	37°C.				4	4		2				1	14
			56°C.												+
51) " (Control)	1941	0	37°C.												
			56°C.												

TABLE XXV

Differences in Dispersability of Flocculates
In Kahn Reacting Sera (Human) of Different Potency

Type of Sera	Number Tested	After 30-Min. Incubation at 56°C.	
		Number showing Flocculates	Number not showing Flocculates
Positive Sera Quantitative Titer of 20+	39	36	3
Positive Sera Quantitative Titer of 4 to <20	42	16	26
Positive Sera Quantitative Titer of<4	54	4	50
Negative Sera	16	0	16

Although the number of tests made were not large, the trend is definite. Of 39 reactions with a titer of 20 units or more, the flocculates of only three reactions were dispersed at 56°C., while of 54 reactions of titer less than four units, as many as 50 flocculates were dispersed.

Experiment 9B. Fixation of Complement by Human Serum-Antigen Floccules Dispersed at 56°C.

Human serum-antigen mixtures (Kahn) containing floccules were incubated for 30 minutes at 56°C. After ascertaining that there were no visible trace of floccules in the mixtures, a portion of these mixtures was employed in the complement fixation test, as previously described (p. 60). The results are presented in Table XXVI.

To examine a single serum listed in the table (87, 4/24); the original Kahn reaction was -44. On incubation at 56°C., the floccules were dispersed and the reaction appeared negative. Still, complement fixation was obtained in spite of the absence of flocculation. In most instances in which four plus flocculation becomes negative on incubation, complemen- fixation of two or more units is noted. Several examples are present of positive flocculation results which were dispersed to negative and which did not fix complement.

The experiment thus indicates that certain Kahn floceules can be so dispersed as to render them invisible to the eye[1] and still fix complement.

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[1] A number of these negatively appearing reactions were observed under the microscope (low power) where they showed the appearance of true negatives.

TABLE XIVI

FIXATION OF COMPLEMENT BY FLOCCULES (ZAHN) STIMULATED BY HEATING HOMOLOGOUS EXUDATES AT 56-9.

Serum Number, Date Examined 1950	Standard Kahn Reaction Tube .123	Quant.-Titer	Reaction after 24 hrs. at 56-9 for 30 Min. Tube 123	Date of Sensitization	Duration of Sensitization by Serum-Antigen Mixture at 56-9. for 30 Min. 1	2	3	4
30) 4/26 Control	———	0	———	80	—	—	—	+
87) 4/24	—++	3	———	80	++	+	—	—
38) 4/25 Control	++++	20	—++	80	+	+	+	2
39) 4/25	—23	2	———	80	++	+	—	—
89) 4/25 Control	++++	20	++++	80	++	+	3	—
91) 4/25	—++	3	———	80	3	3	—	—
119) 4/30 Control	———	0	———	80	22	—	—	—
120) 4/30 Control	++++	4	—22	80	++	+	+	+
122) 4/30 Control	3++	4	——3	80	++	+	2	—
123) "	—3+	2	———	80	+3	+	—	—
124) " Control	++++	20	—++	80	++	+	+	2
127) "	—+3	1	———	80				
37) 5/13	+3+	2	——	80	+2	2	—	—
95) "	——+	0	———	80	22	+	—	—
96) "	———	0	———	80	++	—	—	—
106) "	+++	3	—+ +	80	+2	3	—	—
105) 5/13	+++	3	———	80	+3	+	—	—
42) 5/14 Control	++++	20	+ +++	80	++	+	2	—
29) 5/17	—+2	1	———	80	++	3	—	
30) " Control	++++	20	—++	80	++	+	+	
31) 5/17	—+3	1	———	80	++	2	+	
32) " Control	———	0	———	80	++	—	—	
92) " Control	0——	0	0——	80	++	++	—	—
93) " Control	0++	1200	0++	80	++	++	+	+
94) " Control	0++	560	0++	80	++	2+	+	+
95) "	0+2	1	0——	80	++	23	+	—
86) "	0—+	0	0——	80	++	22	—	—
26) 5/20	—+3	1	———	80	++	2	—	—
28) " Control	———	0	———	80	++	+	—	—
29) "	—+2	1	———	80	++	3	—	—
31) "	+++	2	———	80	++	2	—	—
40) "	+13	1	———	80	++	2	—	—
24) 5/16	3++	4	———	80	++	2	2	—
25) "	++	3	———	80	++	2	+	—
27) "	++	1	———	80	++	2	+	—

TABLE XXVI (CONT'D)

Serum Number, Date Examined	Standard Kahn Reaction		Reaction after Heating at 56°C. for 30 Min.		Units of Complement Fixed By Serum-Antigen Mixture of Tube 3 Heated at 56°C. for 30 Min. 1	2	3	4
28) 5/18	±44	3	—	T)	4	4	±	—
				SC)	3	—	—	—
49) 5/21 Control	444	20	244	T)	4	4	3	±
				SC)	4	—	—	—
50) "	±44	3	—±	T)	4	4	±	—
				SC)	4	—	—	—
84) "	[illegible]	0	—[1]	T)	—	—	—	—
				SC)	—	—	—	—
95) "	344	4	—	T)	4	4	±	—
				SC)	2	—	—	—
130) "	444	4	—1	T)	4	4	1	—
				SC)	4	—	—	—
111) 6/4	-±4	1	—	T)	4	4	—	—
				SC)	±	—	—	—
112) "	-±3	1	—	T)	4	3	—	—
				SC)	4	—	—	—
24) 6/17	442	40	444	T)	4	4	4	2
				SC)	4	—	—	—
33) "	—	0	—[1]	T)	2	—	—	—
				SC)	2	—	—	—
49) 6/18	±44	3	—	T)	4	4	1	—
				SC)	±	—	—	—
50) "	444	20	±44	T)	4	4	4	1
				SC)	1	—	—	—
51) "	444	4	—4	T)	4	4	4	±
				SC)	—	—	—	—
88) "	±44	3	±—±	T)	4	4	±	—
				SC)	±	—	—	—
89) "	-±2	1	—	T)	4	±	—	—
				SC)	—	—	—	—
99) 6/19	444	80	444	T)	4	4	4	4
				SC)	4	4	—	—
97) "	-±2	1	—	T)	4	2	—	—
				SC)	—	—	—	—
88) "	144	3	—±—	T)	4	4	—	—
				SC)	±	—	—	—
43) 6/19	444	40	244	T)	4	4	4	1
				SC)	2	—	—	—
46) "	444	20	144	T)	4	4	4	4
				SC)	4	—	—	—

Experiment 10A. Dispersability of Floccules in the Completed Kahn Reaction with Animal Sera by Incubation at 37° or at 56°C.

The problems considered in Experiments 9A and 9B with human sera were investigated with animal sera, except that in addition to employing heated (30 minutes at 56°C.) sera, unheated sera were also employed. Table XXVII presents illustrative results obtained when completed Kahn tests with heated and unheated animal sera are incubated at 37° or 56°C. Such incubation has broadly the same dispersion effect on floccules obtained with animal sera as that obtained with human sera. However, differences in the dispersability of floccules obtained with different animal sera were noted.

Floccules obtained with cow sera tend to become dispersed more readily if derived from unheated serum than if derived from heated serum. Generally, the higher the quantitative titer the less the dispersability of the floccules, while the lower the titer, the greater the dispersability. Thus, in the case of heated cow sera, there is a marked tendency toward dispersion when titers are below 4 and a moderate tendency when the titers are 4 or above. In the case of unheated cow sera, there is a marked tendency toward dispersion even when the titers are high. The degree of dispersion is consistently greater when incubation is carried out at 56°C. than when carried out at 37°C.

TABLE XXVII

DISPERSIBILITY OF FLOCCULES IN KAHN REACTIONS WITH HEATED AND UNHEATED ANIMAL SERA ON INCUBATING SERUM-ANTIGEN MIXTURES AT 37°C. AND 56°C.

Serum Source, Number, Date Examined	Kahn Reaction With Heated Serum		Kahn Reaction With Unheated Serum		Flocculation Reaction Reading After Incubation of: Heated Serum Reaction at 37°C. for 30 Min.	Unheated Serum Reaction at 37°C. for 30 Min.	Heated Serum Reaction at 56°C. for 30 Min.	Unheated Serum Reaction at 56°C. for 30 Min.
1940	Tube 123	Quant. Titer	Tube 123	Quant. Titer	Tube 123	Tube 123	Tube 123	Tube 123
Cow								
1) 10/14	-44	8	-44	8	-14	-23	--2	----
2) "	444	40	444	40	444	444	---	---
3) "	444	40	444	40	444	444	---	---
4) "	444	10	444	10	444	444	±44	±1±
5) "	-44	8	-±1	0	--2	±44	---	---
1) 11/15	---	0	---	0	---	---	---	---
2) "	±24	2	---	0	--4	-±-	---	---
3) "	444	40	---	0	444	---	---	---
4) "	444	10	-24	2	444	-44	-44	---
5) "	444	20	-±-	0	444	-±2	-±-	---
5) 11/20	444	60	±44	10	444	444	444	---
6) "	-±4	1	---	0	--4	--2	---	---
4) 12/31	444	160	444	160	444	444	442	±--
5) "	-44	3	-±1	0	±22	-±1	---	---
3) 12/6	±23	2	--±	0	-11	--±	---	---
2) 1/2	444	32	---	0	444	-±4	---	---
3) "	444	16	±42	8	444	-±4	---	---
Chicken								
1) 10/15	144	3	±44	3	---	±44	---	---
2) "	344	4	---	0	---	---	---	---
1) 10/17	421	12	±11	1	---	---	---	---
3) "	---	0	---	0	---	---	---	---
6) "	-44	8	144	8	---	---	---	---
2) 10/26	-14	2	-44	3	---	-±4	---	---
3) "	±44	10	444	16	---	---	---	444
4) "	-41	12	244	16	---	---	444	444
5) "	±4±	10	444	16	---	-±2	44±	444
6) "	±44	10	444	20	---	--1	--±	444
1) 11/2	-44	12	444	12	---	-44	---	---
2) "	-44	12	444	12	---	± ±4	---	---
3) "	--±	0	±44	3	---	---	---	---
6) "	±44	12	444	16	---	--1	---	---
7) "	444	28	444	32	---	-44	---	---
8) "	- ±4	1	344	12	---	---	---	---
9) "	--±	0	-24	2	---	-±4	---	---
1) 11/7	344	12	444	20	---	-34	---	---
2) "	-44	12	444	16	---	---	---	---
3) "	±44	8	444	16	--3	-44	---	±44
6) "	144	20	444	20	--4	-44	---	-44
5) 11/16	-44	3	444	8	---	--4	---	±44
Horse								
1) 12/11	444	60	444	60	444	444	---	---
2) "	444	28	444	28	--4	444	---	---
3) "	-24	2	444	4	---	---	---	---
4) "	--±	0	21-	1	---	---	---	---
5) "	-44	3	444	8	---	---	---	---
6) "	±44	3	444	10	---	---	---	---
1) 12/12	3±-	1	---	0	---	---	---	---
2) "	±24	2	---	0	---	---	---	---
3) "	444	16	444	16	---	---	---	---
4) "	444	10	444	14	---	---	---	---
5) "	-44	10	444	12	---	---	---	---
6) "	444	16	444	16	---	---	---	---
1) "	444	48	444	48	---	---	---	---
2) "	---	0	--±	0	---	---	---	---
3) "	444	40	444	40	---	---	---	---
4) "	444	12	444	12	---	---	---	---
5) "	144	10	-44	4	---	---	---	---
6) "	144	10	444	10	---	---	---	---
Pig								
1) 9/26	-±1	0	---	0	---	---	---	---
2) "	444	20	--±	0	--3	---	---	---
4) "	444	36	± ±1	0	-44	---	-±4	---
1) 10/1	444	28	--2	1	444	--±	-44	---
3) "	444	200	-±3	1	444	-±1	±44	---
4) "	444	28	--±	0	444	---	-±4	---
7) "	444	40	-±2	1	±44	---	--3	---
8) "	---	0	---	0	---	---	---	---
2) 10/4	444	48	444	20	-44	-±4	-44	-44
3) "	444	80	244	8	-44	--4	-44	--4
5) "	444	100	444	20	444	444	-44	-±4
6) "	444	60	-±4	1	-44	--±	-± ±	---
7) "	344	32	-±3	1	---	---	---	---

TABLE XXVII (CONT'D)

Serum Source, Number, Date Examined	Kahn Reaction With Heated Serum		Kahn Reaction With Unheated Serum		Flocculation Reaction Reading After Incubation of: Heated Serum Reaction at 37°C. for 30 Min.	Unheated Serum Reaction at 37°C. for 30 Min.	Heated Serum Reaction at 56°C. for 30 Min.	Unheated Serum Reaction at 56°C. for 30 Min.
1940	Tube 123	Quant. Titer	Tube 123	Quant. Titer	Tube 123	Tube 123	Tube 123	Tube 123
Pig								
9) 10/4	444	80	144	12	-44	-±4	--±	---
4) 10/10	443	10	-24	2	-24	--1	--±	---
5) "	444	10	---	0	±44	---	±44	---
6) "	444	10	---	0	-24	---	---	---
9) "	444	240	44±	120	444	443	444	44±
7) 11/27	444	48	244	8	-44	-44	-44	--3
1) 11/26	444	40	---	0	44±	---	-±4	---
2) "	132	2	---	0	---	---	---	---
3) "	±22	1	-±2	1	---	---	---	---
5) "	444	24	--1	0	44-	---	-±-	---
Rabbit								
1) 12/30	--4	1	-14	1	---	-±3	---	---
2) "	--3	1	-14	1	---	---	---	---
3) "	±44	3	±44	8	---	---	---	---
4) "	144	10	444	10	-14	-±4	---	---
5) "	-14	2	-44	10	---	---	---	---
6) "	±44	10	244	10	-44	---	---	---
7) "	-14	2	--4	1	---	---	---	---
8) "	±24	2	±44	8	---	---	---	---
9) "	-34	8	-44	8	---	---	---	---
10) "	--1	0	-24	2	---	---	---	---
11) "	--±	0	-14	2	---	---	---	---
12) "	-±4	1	-44	3	-±1	---	---	---
1a "	-±4	1	-44	3	---	--4	---	---
3a "	-14	2	-24	2	--4	--2	---	---

Floccules obtained with chicken sera tend to become dispersed more readily if derived from heated serum than if derived from unheated serum. As in the case of cow sera, the floccules formed by high-titer sera showed less tendency to disperse on incubation than did floccules formed by low-titer sera.

Floccules obtained with heated or unheated horse sera showed a high lability to heat and were readily dispersed in all cases by incubating at 56°C. Only a few horse sera, showing titers of 30 or over, formed floccules which were not dispersed at 37°C.

The behavior, on incubation, of floccules obtained with pig and rabbit sera was much like that observed with cow sera. Only heated rabbit sera were examined and no serum with a quantitative titer of more than 20 units was encountered. As might have been predicted in this case, all floccules formed were dispersed on incubating at 56°C.

Table XXVIII summarizes the results of the complete experiment on the dispersability of floccules obtained with animal sera following incubation at 37° and 56°C. The positive reacting sera are divided into three groups on the basis of the quantitative titers; namely, those with titers of 20 units or more, those with titers of 4 to 20 units and those with titers of less than 4 units. Of 33 heated cow sera showing a titer of 20 or higher, the floccules were little

TABLE XXVIII

DIFFERENCES IN DISPERSABILITY OF FLOCCULES IN KAHN REACTING SERA (ANIMAL) OF DIFFERENT POTENCY

Type of Serum	Number Showing Flocculation Reactions at: Room Temp: Heated Serum	Unheated Serum	Number of Reactions after Incubation at: 37°C. for 30 minutes Heated Serum	Unheated Serum	56°C. for 30 minutes Heated Serum	Unheated Serum
Cow						
Positive Sera Quantitative Titer of 20+	33	11	32	10	22	0
Positive Sera Quantitative Titer of 4 to <20	16	14	16	11	10	1
Positive Sera Quantitative Titer of <4	10	13	6	7	0	2
Negative Sera	3	24	1	3	0	0
Chicken						
Positive Sera Quantitative Titer of 20+	6	8	2	6	2	2
Positive Sera Quantitative Titer of 4 to <20	39	54	5	22	5	19
Positive Sera Quantitative Titer of <4	31	25	0	5	1	7
Negative Sera	26	14	0	0	0	0
Horse						
Positive Sera Quantitative Titer of 20+	6	7	2	3	0	0
Positive Sera Quantitative Titer of 4 to <20	11	13	1	0	0	0

TABLE XXVIII (Cont'd)

Type of Serum	Number Showing Flocculation Reactions at: Room Temp.		Number of Reactions after Incubation at: 37°C. for 30 minutes		56°C. for 30 minutes	
	Heated Serum	Unheated Serum	Heated Serum	Unheated Serum	Heated Serum	Unheated Serum
Horse						
Positive Sera Quantitative Titer of < 4	5	2	0	0"	0	0
Negative Sera	3	3	0	0	0	0
Pig						
Positive Sera Quantitative Titer of 20+	63	4	55	4	41	3
Positive Sera Quantitative Titer of 4 to <20	22	8	14	6	6	2
Positive Sera Quantitative Titer of < 4	10	36	3	5	2	4
Negative Sera	6	53	0	0	0	0
Rabbit						
Positive Sera Quantitative Titer of 4 to <20	8	17	2	3	0	0
Positive Sera Quantitative Titer of < 4	14	15	1	2	0	0
Negative Sera	11	1	0	0	0	0

affected by incubation at 37°C. for 30 minutes, and were dispersed in 11 sera by incubation at 56°C. for 30 minutes. Of 11 unheated sera of similar titer, the floccules were little affected by incubation at 37°C. but were all dispersed by incubation at 56°C. In the case of sera showing titers lower than 20, the same general picture is noted except that, as a whole, the floccules are apparently more labile and show a greater tendency toward dispersion.

It is seen that one of 3 negatively reacting heated cow sera showed floccules after 30 minutes incubation at 37°C. (although not after 30 minutes incubation at 56°C.) and 3 of 24 negatively reacting unheated cow sera showed similar activity. These are the only examples of heated or unheated human or animal sera which were negative in the Kahn test at room temperature and showed flocculation after incubation.

Among the chicken serum floccules, in which a dispersion tendency opposite that seen with cow serum floccules was noted, (i.e., greater dispersability of floccules obtained with heated serum than with unheated serum), two of six heated sera with titers of 20 units or more, formed floccules which resisted 37°C. incubation whereas 6 of 8 unheated serum floccules remained after this incubation period.

Among the horse sera, all floccules formed by 22 positive sera, including heated and unheated sera with titers of 20 or more units, were dispersed by heating at 56°C. for 30 minutes.

Pig serum floccules proved to be the most resistant to incubation of the animals tested, 41 of 63 heated serum reactions being undispersed after 56°C. incubation and 3 of 4 unheated serum reactions behaving similarly.

The order of lability of the heated serum floccules to incubation, of the animals tested might be classified as follows:

horse > rabbit > chicken > cow > pig > man.

Experiment 10B. Fixation of Complement by Animal Serum-Antigen Floccules Dispersed at 56°C.

Before considering complement fixation results with these dispersed floccules from animal sera, a consideration of the ability of animal sera to fix complement was necessary.

It is notable that sharp discrepancy exists in the literature between fixation and flocculation results obtained with sera of different animals. Kemp, et al. (1940) have summarized the literature on this subject. Complement-fixation tests on chickens in the hands of five investigators and totaling 441 animals yielded only one positive result. In flocculation tests, however, Kemp obtained 42 positive Kahn reactions with 107 sera; Greene obtained 5 reactions with 9 sera in the Kline test; Martelli obtained 200 positives with 200 sera in the Meinicke and Sach-Georgi tests; and so on. Similarly cattle show much fewer fixation than flocculation reactions. Horse, sheep and pig sera are in an indeterminate class showing approximately equal numbers of fixation and flocculation reactions. Rabbits also show less reactivity in fixation than in flocculation tests.

Table XXIX summarizes the complement-fixation results obtained with heated chicken, cow, horse, pig, and rabbit sera. Of 102 chicken sera tested, none were found to fix complement (with the exception of 6 partial fixations of 2 units) under any of the conditions of primary indubation

TABLE XXIX

EXTENT OF FIXATION OF COMPLEMENT BY ANIMAL SERA UNDER DIFFERENT CONDITIONS

Serum Source, Number Tested	Temperature and Period of Fixation	Number of Sera Showing:			
		No Fixation	Fixation of 2 units of Complement	Fixation of > 2 units of Complement	Anti-complementary Results
Chicken					
53	37°C. for 30 minutes	47	6	0	0
27	Room Temp. for 2 hours	21	0	0	6
22	5-7°C. for 3 hours	20	0	0	2
Cow[1]					
48	37°C. for 30 minutes	44	3	1	0
14	Room Temp. for 2 hours	7	3	4	0
6	5-7°C. for 3 hours	2	1	3	0
Horse[1]					
25	37°C. for 30 minutes	11	5	9	0
6	Room Temp. for 2 hours	1	2	2	1
14	5-7°C. for 3 hours	3	2	9	0
Pig					
48	37°C. for 30 minutes	37	10	1	0
34	Room Temp. for 2 hours	34	0	0	0
20	5-7°C. for 3 hours	17	3	0	0
Rabbit					
33	37°C. for 30 minutes	7	14	12	0

[1]Some of these specimens were tested with more than one fixation temperature.

employed. This same group of sera, it was shown (Table III), yielded 72 positive Kahn reactions. Cow sera showed a tendency for increased fixation as the primary incubation temperature was lowered (4 of 48 positive on 37°C. fixation, 7 of 14 positive on room temperature fixation, 4 of 6 positive on 5-7°C. fixation). Some horse sera fixed complement under all the conditions of primary incubation employed. Pig sera showed 11 positives in 48 sera with primary incubation at 37°C. and no positives with 34 sera with primary incubation at room temperature. Only 3 of 20 pig sera fixed any complement at 5-7°C. On the other hand, rabbits showed fixation results with 26 of 33 sera with a primary incubation at 37°C., while only 22 of the same sera showed flocculation reactions.

It would seem that unlike human luetic sera, animal sera showed a marked difference with their behavior in flocculation and complement-fixation tests.

Another point considered before determining the extent to which heat-dispersed floccules formed in the flocculation (Kahn) test would fix complement, was the extent to which the undispersed floccules obtained in the Kahn test with animal sera would fix complement. Accordingly, animal sera were examined with the Kahn test and with complement fixation tests carried out with the serum-antigen mixtures containing floccules, and also with those which were free from floccules, for control purposes. The results are summarized in Table XXX.

TABLE XXX

FIXATION OF COMPLEMENT BY SERUM-ANTIGEN MIXTURES OF POSITIVE FLOCCULATION (KAHN) REACTIONS OBTAINED WITH ANIMAL SERA

Serum Source, Number Tested[1]	Temperature and Period of Fixation	Number of Sera Showing:[2]		
		No Fixation	Fixation of 2 units of Complement	Fixation of > 2 units of Complement
Chicken				
40	37°C. for 30 minutes	40	0	0
13	Room Temp. for 2 hours	10	3	0
11	5-7°C. for	11	0	0
Cow[3]				
49	37°C. for 30 minutes	45	4	0
20	Room Temp. for 2 hours	13	7	0
6	5-7°C. for 3 hours	2	4	0
Horses[3]				
23	37°C. for 30 minutes	9	12	2
5	Room Temp. for 2 hours	0	3	2
13	5-7°C. for 3 hours	5	4	4
Pig				
48	37°C. for 30 minutes	34	13	1
28	Room Temp. for 2 hours	25	3	0
19	5-7°C. for 3 hours	16	2	1

TABLE XXX (CONT'D)

Serum Source, Number Tested[1]	Temperature and Period of Fixation	Number of Sera Showing:[2]		
		No Fixation	Fixation of 2 units of Complement	Fixation of > 2 units of Complement
Rabbit[4] 20	37°C. for 30 minutes	6	14	0

[1]Only those sera which showed flocculation in tube 3 of the Standard Kahn test are considered.

[2]Reactions anticomplementary with 2 units of complement not counted.

[3]Some of these specimens were tested with more than one fixation temperature.

[4]Preinoculation specimens from animals used in Spirochete inoculation experiment.

It is evident that the flocculate obtained with chicken serum showed no greater disposition to fix complement than did the serum itself. Fixation periods at 37°C., room temperature and in the icebox met with uniform negativity. In only three instances was partial fixation of complement obtained after primary incubation at room temperature for 2 hours. Not much better results were obtained with cow floccules. In 4 of 49 positive Kahn reactions partial fixation of 2 units of complement was obtained when 37°C. was the primary incubation temperature employed; in 7 of 20 positives complete or partial fixation was obtained when room temperature was used for primary incubation and in 4 of 6 positives fixation was obtained when incubation was carried out at 5°-7°C.

The floccules obtained with horse sera gave more positive fixation results than those obtained with sera from cows and chickens. In 14 of 23 Kahn positive horse sera fixation of complement was obtained when incubation was carried out at 37°C.; in 5 positive sera, fixation was obtained when incubation was carried out at room temperature, and in 8 of 13 positive sera when icebox fixation was employed.

When combining the various primary incubation methods employed, it is noted that in only 27 of 41 instances did the floccules in horse sera fix complement. A total of 25 horse sera were tested, but some of the specimens were tested with more than one fixation temperature.

Pigs showed higher agreement between flocculation and complement-fixation results when the primary incubation was carried out at 37°C. Yet, in only 14 of 48 positive Kahn reactions was fixation of complement obtained under these conditions. Rabbit sera tested for the degree of fixation of complement at 37°C. showed 14 instances of fixation in 20 Kahn-positive sera.

The data in Table XXX indicate therefore that floccules obtained in the Kahn test with animal sera do not always fix complement. It cannot be said that a given primary incubation period will give optimum fixation results with animal sera, since only in the case of cow and horse sera was a tendency toward more marked fixation at one temperature than at another noted.

While these studies were in progress, Sherwood, et. al., (1941) stated that: "The floccules formed on the addition of beef serum to Kahn antigen failed to fix complement, unlike the similar floccules obtained with syphilitic serum. However, the floccules formed by horse and rabbit serum did fix complement---." These authors have as yet not published their data.

Another aspect to be considered before discussing the fixation of complement by heat-dispersed floccules, was whether fixation of complement may be obtained by serum-antigen mixtures with animal sera from the 37°C. Kahn reac-

tion in which no flocculation is noted, but in which the floccules obtained at room temperature did fix complement. The data are given in Table XXXI. It is evident from this table that in no instance did these floccule-free mixtures fix complement.

TABLE XXXI

COMPLEMENT-FIXATION BY SERUM-ANTIGEN MIXTURES WITH ANIMAL SERA FROM THE 37°C. KAHN REACTION IN WHICH NO FLOCCULATION IS NOTED BUT IN WHICH THE FLOCCULES OBTAINED AT ROOM TEMPERATURE DID FIX COMPLEMENT

Source of Serum	Number Tested	Number Showing Fixation of Complement by Serum-Antigen Mixture Which at 37°C. Showed No Flocculation
Chicken[1]	0	0
Cow	3	0
Horse	14	0
Pig	14	0
Rabbit[2]	14	0

[1] Floccules formed at Room Temperature did not fix complement.

[2] Preinoculation specimens from animals used in Spirochete inoculation experiment.

We may now consider to what extent heat-dispersed floccules possess the property to fix complement. Table XXXII summarizes the results of the experiments with animal

sera in which the floccules obtained with the Kahn test were first dispersed by heating for 30 minutes at 56°C. and then tested for complement fixation. Chicken sera (54) showed no fixation. Cow sera (29) showed fixation in four instances. Horse sera (38) showed fixation in nine instances. Pig sera (43) showed fixation in three instances, and no fixation was obtained with the dispersed floccules of rabbit (22) sera. As might have been predicted from previous considerations, it appears that there is relatively little tendency toward fixation by floccules dispersed as a result of 30 minutes heating at 56°C.

It was of interest to determine if those floccules which were not dispersed by heating at 56°C., possessed the capacity to fix complement. As is evident from the data in Table XXXIII, this capacity is only slightly greater than that shown by the heat-dispersed floccules.

TABLE XXXII

FIXATION OF COMPLEMENT BY ANIMAL SERUM - ANTIGEN MIXTURES IN WHICH THE FLOCCULES (KAHN) WERE DISPERSED BY INCUBATION AT 56°C.

Serum Source, Number Tested	Temperature and Period of Fixation	No Fixation	Fixation of 2 units of Complement	Fixation of >2 units of Complement
Chicken				
39	37°C. for 30 minutes	39	0	0
6	Room Temp. for 2 hours	6	0	0
9	5-7°C. for 3 hours	9	0	0
Cow				
17	37°C. for 30 minutes	16	1	0
10	Room Temp. for 2 hours	8	1	1
2	5-7°C. for 30 minutes	1	1	0
Horse				
22	37°C. for 30 minutes	22	0	0
4	Room Temp. for 2 hours	0	4	0
12	5-7°C. for 3 hours	7	2	3
Pig				
31	37°C. for 30 minutes	29	2	0
11	Room Temp. for 2 hours	10	1	0
4	5-7°C. for 3 hours	4	0	0
Rabbit				
22	37°C. for 30 minutes	22	0	0

[1] Anticomplementary Results not Considered in Tabulation.

TABLE XXXIII

FIXATION OF COMPLEMENT OF ANIMAL SERUM - ANTIGEN MIXTURES IN WHICH THE FLOCCULES (KAHN) WERE LITTLE AFFECTED BY INCUBATION

Serum Source, Number Tested	Temperature and Period of Fixation	Number of Sera Showing:[1]		
		No Fixation	Fixation of 2 units of Complement	Fixation of >2 units of Complement
Chicken				
4	37°C. for 30 minutes	4	0	0
0	Room Temp. for 2 hours	0	0	0
1	5-7°C. for 3 hours	1	0	0
Cow[1]				
29	37°C. for 30 minutes	26	3	0
3	Room Temp. for 2 hours	0	2	1
5	5-7°C. for 3 hours	2	3	0
Horse	(All Reactions were Dispersed on Incubating at 56°C. for 30 minutes).			
Pig				
17	37°C. for 30 minutes	16	1	0
18	Room Temp. for 2 hours	17	1	0
16	5-7°C. for 3 hours	16	0	0
Rabbits[2]	(All Reactions were Dispersed on Incubating at 56°C. for 30 minutes).			

[1] Anticomplementary results not considered in tabulation

[2] Pre-inoculation specimens from animals used in spirochete inoculation experiment.

DISCUSSION

The problem of soluble antigen-antibody complexes has received consideration in connection with other immunological studies. Gay (1905) in discussing observations of Sachs related to "anticomplements of normal sera" claimed that they "are simply specific serum precipitates (non-visible) capable of fixing alexin."

The Wassermann reaction has been considered an example of an ordinarily invisible union of reactants which can fix complement. Jacobsthal (1911), for example, observed aggregates of particles when Wassermann-positive mixtures were subjected to darkfield illumination. "There are no large visible or sedimenting clumps because the antigen is used in too small quantities to give optically visible aggregates." (Eagle, 1930).

Hartley (1925) found that diphtheria antitoxin minus its ether soluble constituents did not show flocculation when mixed with toxin although it did seem to combine with and neutralize the toxin, since guinea pigs injected with the mixtures showed no toxic effects. Similarly, Horsfall and Goodner (1935) found that removal of lipids from Type I antipneumococcus horse and rabbit serum caused a loss or marked reduction of visible agglutination or precipitation. Topley and Wilson (p. 182, 1936), include "Simple haptens," in a classification

of antigens, as those "possessing immunological specificity, combining with the homologous antibody and so preventing precipitation, but neither forming a precipitate *in vitro*, nor stimulating antibody production *in vivo*."

Heidelberger and his associates have investigated the problem from the point of view that certain specific antigen-antibody mixtures in certain ratios may be "soluble," *i. e.*, non-precipitating. Heidelberger (1939) has reviewed previous speculation and experimentation of various workers along this line. Using an azoprotein-antibody system, Heidelberger and Kendall (1935a) believed they proved the existence of a "soluble" union in the ratio's containing small amounts of antibody in relation to the amounts of antigen present, *i. e.*, 1:1 (actual ratios given are 1.08 and 1.11 to 1). Proof rested on finding that further addition antibody precipitated *in toto* antigen and antibody in the supernatant fluid (ratio's reaching 10:1). The authors state: "It would seem that the entire amount of dissolved antigen-antibody compound present in the aliquot appears in the precipitate formed with fresh antibody in excess--." Similar relationships were later found by these authors (1935b) to hold in the case of the reaction between crystalline chicken egg albumin and its rabbit antiserum.

Heidelberger, Treffers, and Mayer (1940) characterized as "univalent," the antibody from a rabbit and a horse which was considered "low grade" or "incomplete" because of its

inability to alone precipitate the antigen, though on addition of a strongly precipitating antibody, this univalent antibody was shown to have been in a state of "soluble" union with the antigen.

Pappenheimer (1940) also demonstrated the non-precipitating reaction between chicken egg albumin and the same horse anti-albumin serum. On mixing the reactants no precipitate was observed after usual incubation. However, on addition of a strongly precipitating rabbit anti-egg albumin serum, no reaction occurred which was interpreted as meaning that the antigen was no longer available; that it had combined in a "completely soluble antigen-antibody system" with the originally added horse antiserum.

More recently, Rivers (1942) in reviewing some recent work of Smadel and Rivers stated that fresh virus-free filtrates from vaccinia-infected skin of rabbits contain an antigenic, soluble, specific substance (designated L) which is labile to the effect of heat, i.e., "when heated at 56°C. L no longer is precipitated in the presence of its antiserum; however, it inhibits L antibodies so that when unheated L is added to the mixture no precipitate occurs." The explanation of this finding would seem to necessitate the existence of some soluble complex of L and its antiserum.

Kleczkowski (1941) has shown that bushy stunt virus antisera from the rabbit loses its precipitating power for this antigen on being heated at 75°C. for 10 minutes. Yet the ability of this heated material to combine in a "soluble" state with the virus seems preserved since the addition of unheated antiserum does not result in precipitation.

This brief review of "soluble" antigen-antibody reactions is presented because of the question whether the lack of flocculation in mixtures of serum and lipid antigen at certain temperatures is due to a "soluble" union. It was seen in the experimental section that certain non-syphilitic human sera, when mixed with lipid antigen showed flocculation at 1°C., but no flocculation at 37°C. Whether or not this lack of flocculation at 37°C. was due to a "soluble" factor was investigated by means of complement fixation. It was believed that by adding complement to this serum-lipid antigen mixture, fixation would take place if actual union had occurred between the serum and antigen and no fixation would take place if no union had occurred. The fact that no fixation had taken place would thus indicate that in this instance no "soluble" compound existed.

In connection with "soluble" antigen-antibody mixtures, it is of interest to note that syphilitic serum-antigen floccules "dissolved" by incubation at 56°C. were still able to fix complement. This finding would indicate that the disappearance of serum-antigen aggregates does not necessarily indicate

the splitting of the serum-antigen complex.

This method is not applicable to animal sera showing flocculation although indistinguishable from the flocculation shown by leutic sera. Their behavior is not like leutic sera, i.e., the formed floccules (Kahn reaction) do not consistently fix complement. The floccules of luetic sera almost invariably fix complement (Kahn, 1928), while the floccules obtained with animal sera rarely fix complement (pps. 152-6). The non-fixation of complement by floccules of animal sera might lead one to suspect a basic difference between flocculation reactions with these sera and reactions between antigen and antibody in the classical sense. However, Zinsser and Parker (1923) observed that a saline soluble antigen obtained from pneumoccocci, reacted with specific horse antiserum (two different pooled lots) but did not fix complement although 4 rabbit antisera did fix complement under the same conditions.

Goodner and Horsfall (1936) have since confirmed this observation and found that specific horse antisera for a capsular polysaccharide of H. influenzae and a somatic carbohydrate of the Pneumococcus will not fix complement although reaction does occur. Pneumococcus protein added to immune horse serum did result in reaction and fixation of complement.

Heidelberger and Mayer (1942) found that horse anti-pneumococcus Type VIII serum did not fix either guinea pig or human complement although reacting with homologous antigen. It would seem to be established, therefore, that certain specific antigen-antibody reactions may occur which do not fix guinea pig complement. The fact, therefore, that animal serum floccules obtained with a test for syphilis rarely fix complement cannot be used to distinguish this reaction from reactions obtained with immune sera.

Part Three. Flocculation Reactions at 37°C., Room Temperature and 1°C. Given by Rabbits in Relation to their Age. Experiment 11.

The third experimental part of this thesis is concerned with the flocculation reactions of sera obtained with a group of rabbits which were tested at intervals of from 14 to 20 days beginning with the time when they were 7 to 8 weeks old, until maturity. An opportunity was afforded to make this study as a result of the next experiment, in Part IV, in which serological studies were made on rabbits before and after inoculation with Spirochaeta pallida. It was desired to secure the rabbits at an early age in an attempt to control their environment as completely as possible as they matured.[1] Also, it was deemed necessary to study the serological behavior of these animals as they grew to maturity in order to learn as much as possible concerning this behavior preceding inoculation.

To our knowledge, no controlled study has as yet been made of the relationship between age and the appearance of serological activity in tests for syphilis in normal animals, though incidental observations have been noted. Wendt (1925) has found that while many cattle sera showed (icebox fixation)

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[1] Some evidence has been noted which points to a relationship between certain environmental conditions and the positive serology of normal rabbits.

Wassermann positive reactions, calf sera were usually negative. Mackie and Watson (1926) observed that, while the sera of adult cattle, sheep and rabbits, with few exceptions, were positive in the (37°C. fixation) Wassermann or Sachs-Georgi reactions, the sera of calves and lambs and the pooled sera of young rabbits were usually negative. Positive reactions were noted by these authors in the pooled rabbit sera taken at about 60 days of age. Sherwood, Bond and Clark (1941) and Kemp, Fitzgerald and Shepherd (1941) have more recently reaffirmed these observations and claimed to have obtained similar results for beef, dog and sheep sera.

Most of the rabbits used in the experiments to be reported were obtained from a commercial source[1] with the stipulation that the day of birth of each animal be known. Newborn litters had their birthdays tatooed on their ears with India ink and were mailed when approximately 40-60 days old. The weights of the several strains of rabbits on arrival varied from approximately one to two pounds.

Thirty-two young rabbits were employed. The ages of these animals varied from 6-8 weeks at the beginning of the experiment. Twenty-five animals (9 females and 17 males) survived throughout the experiment. At approximately 4 months of age, all except 4 (2 males and 2 females) of these

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[1]Rockland Farms, New York City, New York.

animals were inoculated with the Spirochaeta pallida.

The animals were bled by heart puncture at 14 to 20 day intervals and serological studies at 37°C. room temperature and at 1°C. with heated (30 minutes at 56°C.) and unheated sera made as previously described for other animals (page 56).

Table XXXIV presents the Kahn reactions given by 9 of the rabbits at different intervals. The reactions given by the other animals, fall into patterns similar to those illustrated in this table as can be seen by observing the preinoculation Kahn reactions for all the rabbits in Appendix D.

It will be noted from the table and the appendix that all the 32 animals were negative with the standard Kahn Test when originally examined at the ages of 6 to 8 weeks. At this first examination three rabbits showed positive flocculation reactions with heated serum at 1°C. and seven animals showed this reaction with the unheated serum at the same temperature. This early response of these rabbits at low temperature did not seem to presage any future high titer as is seen in the succeeding results.

Most animals began to become Kahn-positive at an age of about 80 to 100 days. When the Kahn tests were performed with unheated sera, the animals began to give reactions at an earlier age (approximately 60-80 days). Reactions were

TABLE XXXIV

INCREASE IN POTENCY OF SEROLOGIC REACTIONS IN RABBITS WITH AGE

Age in Days	Kahn Reaction at Room Temperature				Kahn Reaction at 1°C.[1]			
	Heated Serum		Unheated Serum		Heated Serum		Unheated Serum	
	Tube 123	Quant. Titer	Tube 123	Quant. Titer	Tube 123	Quant. Titer	Tube 123	Quant. Titer
				Rabbit 497 ♀				
52	———		———		———		1+4	
56	———	0	———	0	———	0	———	1
82	———	0	———	1	———	0	1+4	1
104	———	0	———	1	———	0	1+4	1
131	———	1	———	2	———	2	1+4	3
				Rabbit 481 ♀				
51	———		———		———		———	
56	———	0	———	0	———	0	———	0
81	———	0	———	1	———	2	1+4	4
103	———	0	———	1	———	1	1+4	3
130	———	0	———	1	———	2	1+4	3
158	———	0	———	1	———	2	1+4	3
170	———	0	———	2	———	1	1+4	3
				Rabbit 148 ♀				
56	———		———		———		———	
86	———	0	———	0	———	0	———	0
86	———	0	———	1	———	2	1+4	3
108	———	0	———	1	———	1	1+4	3
135	———	0	———	1	———	1	1+4	3
199	———	0	———	1	———	1	1+4	3
219	———	0	———	2	———	2	1+4	4
				Rabbit 482 ♀				
51	———		———		———		———	
56	———	0	———	0	———	0	———	0
81	———	0	———	1	———	0	1+4	1
103	1+4+4	1	1+4+4	1	1+4+4	1	1+4+4	2
130	1+4+4	1	1+4+4	1	3+4+4	4	1+4+4	2
				Rabbit 491 ♂				
52	———		———		———		1+4	
56	———	0	———	0	———	0	1+4	0
82	———	0	1+4	0	1+4	1	2+4	2
104	———	0	1+4	0	1+4	1	1+4	2
131	———	0	1+4	0	1+4	1	1+4	2
145	———	0	1+4	0	1+4	1	1+4	2
157	———	0	1+4	0	1+4	1	1+4	2
169	———	0	1+4	0	1+4	1	1+4	2
193	———	0	1+4	0	1+4	1	1+4	2
213	———	0	———	0	1+4	2	1+4	2
				Rabbit 498 ♂				
50	———		1+4		3+4		1+4	
62	3+4	2	1+4	2	1+4	4	1+4	4
80	1+4	2	1+4	2	1+4	4	1+4	4
102	1+4	2	3+4	2	1+4	4	1+4	4
129	1+4	3	1+4	3	1+4	2	1+4	3
				Rabbit 487 ♂				
43	———		———		———		———	
52	———	0	———	0	———	0	2+4	0
68	1+4	0	1+4	1	1+4	0	1+4	1
111	1+4	0	1+4	1	1+4	0	1+4	1
				Rabbit U493 ♂				
2	———		———		———		———	
56	———	0	———	0	———	0	———	0
82	1+4	0	1+4	0	1+4	0	1+4	1
104	1+4	0	1+4	0	1+4	0	1+4	1
131	1+4	0	1+4	1	1+4	0	1+4	1
				Rabbit 478 ♂				
6	———		———		———		———	
56	———	0	———	0	———	0	———	0
86	———	0	1+4	0	1+4	0	1+4	1
108	1+4	0	1+4	1	1+4	0	1+4	1
135	1+4	0	1+4	2	1+4	0	1+4	1

[1] Tests were also carried out at 37°C., but the results were essentially negative.

elicited earliest (60 days) and were most marked when the tests were performed with unheated sera at 1°C. Fluctuation of the standard Kahn reaction in the case of rabbits 481 and 484 was seen as they matured. In the case of rabbit 481, although the reaction appeared early (in 63 days), it showed no persistent behavior. The unheated sera and the reactions at 1°C., on the other hand, tended to be consistent and stationary at the level of reactivity first developed.

Seven animals[1] showed the reaction typified by Rabbit 491, i. e., a persistently negative Kahn reaction in the standard test; weak fluctuating reactions with heated sera at 1°C.; and consistent flocculation with unheated sera both at room temperature and in the cold. The other animals showed the pattern of reactivity illustrated by Rabbits 148, 498, and 487, in which the Kahn reactions, negative at first, became weakly positive, then gradually stronger, with heightened activity in the cold especially in an unheated state.

To summarize, the Kahn reactions showed a definite tendency to become stronger with age; at 1°C., the reactions were consistently stronger than at room temperature; also, unheated sera gave stronger reactions than did heated sera.

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[1] Numbers 488, 491, 476, 493, 492, 479, 499.

DISCUSSION

It has long been known that a relationship exists between age and immunity. The fact that many diseases occur most commonly in childhood is generally explained by a lower degree of immunity to these diseases in childhood than in adult life. That the immune response is directly related to age, is seen for example in the classical studies with the Schick and Dick tests in establishing susceptibility curves. Hirszfeld (Halber et al., 1927) believes that certain so-called "normal" antibodies, (e.g., isohemagglutinins, hemolysins) are inherited genetically and that these antibodies become functionally mature at some specific time in the individual's development; a characteristic which is referred to as "serological maturity." Baumgartner (1934) has reviewed the literature on the direct relationship of age to immunological reactions through 1933 and has given further evidence of this relationship by immunization experiments on young and old rabbits, to substantiate the Hirszfeld theory. Kahn (1936) has demonstrated that the localizing power of the cutaneous tissues for protein antigens possessed by immunized adult rabbits is much greater than that possessed by immunized young rabbits. More recent reviews are those of Cannon (1940) and of Perla and Marmorston (1941).

Although there exists ample evidence to indicate that the "antibody" responsible for the complement-fixation and flocculation reactions in syphilis is *per se* without immunological significance in the sense of being spirochæticidal—or even spirochæteistatic (though lately Turner (1939) has indicated that protective antibodies of varying activity may appear in the blood of syphilitic rabbits), in other respects this "antibody" behaves like other antibodies. The syphilitic antibody is affected by pH, salt concentration, temperature, alcohol, etc., as are specific antibodies; the antibody reactions in syphilis show "prozone" or "inhibition" phenomena similar to those shown by other antibody reactions. We have seen, however, that some of these criteria do not apply to non-specific serological reactions with animal sera (*i.e.*, temperature factors are different and little relationship exists between flocculation and complement fixation reactions). However, the majority of the criteria do hold and the experiments herein presented indicate that one more might be added; namely, the increase of serological activity with age.

Part Four. Flocculation Reactions at 37°C., Room Temperature and 1°C., Given by Rabbits Before and After Inoculation with Spirochaeta pallida. Experiment 12.

From the preceding experiments it was seen that a characteristic of sero-positive (nonsyphilitic) animal sera, including rabbit sera, is to give stronger reactions when the Kahn test is performed at 1°C., and weaker reactions when performed at 37°C. A characteristic of syphilitic sera, however, is to give weaker reactions at low temperatures and stronger reactions at 37°C. (Kahn, et. al., 1941). The question suggested itself as to what effect inoculation with Spirochaeta pallida in rabbits would have on the serological reactions of these animals when the Kahn tests are performed at these temperatures. Since normal rabbits often give positive serological reactions and since they can be rendered syphilitic by inoculation with the Spirochaeta pallida, they obviously represent a suitable laboratory animal for the study of the effect of temperature on the serological reactions before and after inoculation.

Thirty-nine rabbits were used in this study. The serological reactions of these animals preceding their inoculation had been followed for from one to three months. All the animals were mature and weighed at least 5 pounds when inoculated.

The method of innoculation with Spirochaeta pallida was as follows:

The infected testes of two syphilitic rabbits were aseptically removed from the scrotal sac, stripped of adventitious membranes and epididymis, washed off with sterile saline solution then dropped into a sterile glass mortar where they were first minced with sterile scissors and then ground with sand. Warm saline (40cc.) was gradually added during this grinding period. The tissue and sand were allowed to settle and the supernatant material used as inoculum. Six wet preparations of the material were made on glass slides and 6 or 7 fields on each slide were searched for spirochetes in the darkfield microscope. The overall average for 4 slides and 26 fields was 2 organisms per field.

In all, 29 animals were inoculated; of these 22 were males and 7 females. Seven rabbits (5 males and 2 females) were kept as noninoculated controls. Male rabbits were inoculated intratesticularly into either one or both testes with 0.5 cc. to 1.0 cc. of the inoculum. Testes were swabbed with iodine (2%) and alcohol(70%) before inoculating. Female rabbits were inoculated mid-dorsally, in shaved areas, with 0.1 to 0.2 cc. amounts of material injected intracutaneously into 4 or 5 areas with size 24 hypodermic needles.

After inoculation, the animals were restored to their cages, and treated in all respects as they had previously been treated. The intervals of bleeding for serological studies were the same as those prior to inoculation; namely 14 to 20 days. The rabbits were under observation for two and one-half months post-inoculation, during which time they were bled approximately five times and the sera examined as previously.

Appendix D presents the pre- and post-inoculation record of the serological findings. Each serological examination included: the Kahn reaction at 37°C., room temperature and at 1°C. with heated and unheated sera; the quantitative reaction; the complement fixation reaction; the combined flocculation complement-fixation reaction. A summary of the data in the Appendix will be considered below. At present it is aimed to limit the discussion to flocculation results obtained at the three temperatures. These are summarized in Table XXXV. The results of three serological examinations are presented in the table: the last examination before inoculation of the rabbits and two examinations after the inoculation. The latter two were so chosen as to bring out the early and late serological effects of the inoculation.

On examining Table XXXV, it is seen that there are listed 18 inoculated rabbits (12 males and 6 females) and three noninoculated controls. The serological response

TABLE XXXV

EFFECT OF INOCULATION WITH SPIROCHAETA PALLIDA ON KAHN REACTION IN RABBITS

(Abstract of Appendix D)

Rabbit Number	Serum Heated or Unheated	Preinoculation Reactions[1]				Reactions Postinoculation (In Days)				Reactions Postinoculation (In Days)			
		Dilution of Serum with 0.9% Saline	Kahn Reactions at: 37°C. Tube 123	Room Temp. Tube 123	1°C. Tube 123	Dilution of Serum with 0.9% Saline	Kahn Reactions at: 37°C. Tube 123	Room Temp. Tube 123	1°C. Tube 123	Dilution of Serum with 0.9% Saline	Kahn Reactions at: 37°C. Tube 123	Room Temp. Tube 123	1°C. Tube 123
							(nineteen days)				(Seventy-five days)		
486♀	H	undil.	−±2	−14	±44	undil.	---	−+1	−+1	1:3	±44	±14	±+4
	Un.	undil.	---	−44	−44	undil.	---	−24	444	1:3	--3	−±4	344
							(nineteen days)				(seventy-five days)		
489♀	H	undil.	---	−14	144	undil.	---	--+	±44	undil.	±44	−24	−++
	Un.	undil.	---	--4	±44	undil.	---	±44	−44	undil.	--±	--4	444
							(thirty-one days)				(seventy-five days)		
150♀	H	undil.	−±3	±24	±44	1:2	±14	−±4	±34	1:3	±44	−±4	±+4
	Un.	1:2	---	±14	444	undil.	--2	--3	±44	undil.	---	−24	444
							(nineteen days)				(thirty-one days)		
U497♀	H	1:2	---	−±4	444	1:2	−1±	−±3	−24	1:3	±44	−14	−±4
	Un.	1:2	---	−14	444	1:2	--1	−±4	−44	1:2	±+4	−±4	±44
							(thirty-one days)				(seventy-five days)		
497♀	H	undil.	---	--4	−24	undil.	---	---	−±4	undil.	---	---	−±3
	Un.	undil.	---	−14	−44	undil.	--1	−±4	−44	undil.	---	--±	±44
							(thirty-one days)				(seventy-five days)		
482♀	H	1:2	--1	−±4	344	undil.	---	---	--+	undil.	---	---	−±4
	Un.	1:2	--±	−14	244	undil.	---	−±4	144	undil.	---	---	−44
							(fifty-days)				(seventy-five days)		
U495♂	H	undil.	--2	−±4	144	undil.	---	---	−±2	undil.	−44	−±4	−13
	Un.	1:2	---	−±2	244	undil.	---	--±	−14	undil.	---	±--	144
							(thirty-one days)				(seventy-five days)		
494♂	H	undil.	---	−±4	±44	undil.	−±3	−±4	−24	1:7.5	−44	±34	±12
	Un.	1:2	---	−44	244	undil.	---	−44	−44	1:3	---	−34	244
							(thirty-one days)				(seventy-five days)		
493♂	H.	undil.	---	−±1	−44	1:2.5	---	−±4	−24	1:17	±44	−±4	±+4
	Un.	undil.	---	−±4	444	1:2	−12	−±3	±44	1:18	−13	−34	±44
							(fifty-five days)				(seventy-five days)		
499♂	H.	undil.	---	--+	−±4	1:6	−14	−14	−44	1:7	±44	−±4	±12
	Un.	undil.	---	−±4	±44	1:3	−44	−44	444	1:7	---	−±4	−±4
							(thirty-one days)				(fifty-five days)		
498♂	H.	undil.	---	−44	444	undil.	---	--2	−44	1:60	±44	−±4	---
	Un.	1:2	--2	−±4	444	undil.	−±1	--4	444	1:25	−44	−44	144
							(thirty-one days)				(fifty-five days)		
U493♂	H.	undil.	---	−±4	±44	undil.	---	---	--±	1:20	−±4	−±2	--1
	Un.	1:2	---	−±4	444	1:2	---	−±4	−44	1:4	−44	−44	−44
							(fifty-six days)				(seventy-five days)		
26♂	H.	1:2	---	−14	−44	1:20	−±4	−±4	−±4	1:15	±44	±24	−±4
	Un.	1:2	±±4	−14	444	1:12	−±4	−24	443	1:14	--±	−24	−44
							(thirty-one days)				(seventy-five days)		
32♂	H.	undil.	---	−±3	244	1:2	--1	--4	±44	1:16	±24	−±4	−±4
	Un.	undil.	---	±14	444	1:2	---	−22	−44	1:7	--±	−14	244
							(thirty-one days)				(seventy-five days)		
33♂	H.	1:2.5	---	−14	±44	undil.	---	−±3	−14	1:8	±44	±±4	−±4
	Un.	1:2.5	---	±±4	±44	1:2	--±	−±4	−44	1:3	---	−24	344
							(twenty days)				(sixty-four days)		
M 3♂	H.	1:2	−±2	−24	−14	1:3	−±4	--4	±±3	1:15	±44	−24	−±3
	Un.	1:2	−±2	−14	−44	1:3	--4	--4	−44	1:12	±44	−14	--±
							(twenty days)				(ninety days)		
M22♂	H.	undil.	−±2	−14	−44	undil.	-34	−14	±24	1:8	±±4	−±4	---
	Un.	undil.	−±1	−14	−44	undil.	--±	−14	−44	1:7	±44	−±4	--3

[1] Blood specimens taken 9 days before inoculation. Other results in Appendix D.

TABLE XXXV (CONT'D)

Rabbit Number	Serum Heated Or Un-heated	Preinoculation Reactions[1] Dilution of Serum with 0.9% Saline	Kahn Reactions at: 37°C. Tube 123	Room Temp. Tube 123	1°C. Tube 123	Reactions Postinoculation (In Days) Dilution of Serum with 0.9% Saline	Kahn Reactions at: 37°C. Tube 123	Room Temp. Tube 123	1°C. Tube 123	Reactions Postinoculation (In Days) Dilution of Serum with 0.9% Saline	Kahn Reactions at: 37°C. Tube 123	Room Temp. Tube 123	1°C. Tube 123
							(twenty days)				(ninety days)		
N 11♂	H	undil.	---	---	---	undil.	-24	±24	±14	1:25	±44	-±4	-±2
	Un.	undil.	---	---	---	undil.	--1	-±4	±44	1:15	-44	-14	--±
						CONTROL SPECIMENS[2]							
			(12/30/41)[3]				(1/14/42)				(3/3/42)		
34♂	H	1:2	---	-±4	444	undil.	---	-±4	144	1:2	-±1	-±4	-44
	Un.	1:2	---	-14	444	undil.	-14	±34	444	undil.	---	-±2	±44
			(12/29/41)				(1/14/42)				(2/8/42)		
481♀	H	undil.	---	--3	-24	undil.	---	-±2	-±4	undil.	---	---	-±4
	Un.	undil.	---	-14	244	undil.	---	-±4	244	undil.	---	-24	244
			(12/30/41)				(1/14/42)				(3/13/42)		
25♂	H	1:3	---	-±4	144	1:3	-±4	±24	±44	1:4	-±4	-14	-44
	Un.	1:3	---	-14	±44	1:2	---	-±4	444	1:2.5	--4	-24	144

[2]Results with 3 of 7 control Rabbits are shown as examples. Other results in Appendix D.

[3]Date examined. Only approximate ages of these animals were known.

preinoculation in all the rabbits with one exception is as follows: Weak or negative Kahn reactions at 37°C.; moderately strong reactions at room temperature and relatively marked reactions at 1°C. In the case of rabbit N11, the reactions were negative at the three temperatures.

With regard to the flocculation results post-inoculation, it is noted that the Kahn reactions at the three temperatures show a reverse tendency; namely, strong reactions at 37°C., relatively weaker at room temperature and weakest at 1°C. This tendency is especially exemplified in the latter columns of the table in which the late post-inoculation data is presented. This reversal is noted almost entirely with sera that have been heated for 30 minutes at 56°C. before examination. In the middle columns a number of reactions are listed with a view of demonstrating the transition to the reversed serological reactions.

To illustrate the serological reversal of the reactions at the three temperatures, the results given by rabbit 486♀, the first listed in the table, might be noted.

The last preinoculation reaction with the heated serum was -±2 at 37°C., -14 at room temperature and ±44 at 1°C. The 19-day postinoculation examination revealed a trend similar to that seen in the preinoculation examinations. Reactions obtained 75 days post-inoculation were notably different than the previous results. The room temperature

reactions had become 444. Since strongly positive sera (444) generally show no differentiation in the flocculation reactions at the different temperatures, it was necessary to render the sera "weak" by dilution with salt solution before examining at 37° and 1°C. When the serum was diluted 1:3 with salt solution and then tested, it was found that the reaction at 37°C. was ±44 and at 1°C., ±±4. It is thus seen that while the reaction was stronger at 1° than at 37°C. before inoculation, it was stronger at 37° than at 1°C. after inoculation.

This same trend of reactivity was seen with all the other heated sera from inoculated animals listed in Table XXXV with the exception of sera obtained from two female rabbits (497♀ and 482♀). These did not show the reversal in serological reactivity characterizing the other inoculated animals and their serological reactions were not altered during the course of the experiment. These animals however never showed signs of having taken the infection, i. e., all the other animals, both male and female, developed lesions at the site of inoculation and among the other 4 female rabbits at least two chancres developed on the back of each, becoming confluent in two cases. It thus seems possible that these two rabbits were not infected.

Among the 12 inoculated male rabbits listed in the table, all except one (U495♂) showed sharp rises in the

quantitative Kahn titer within a period of 75 days following inoculation. This rise varied from 30 to over 200 units. In every case testicular chancres appeared in these animals. Relatively little rise in the quantitative titer occurred among the 4 female rabbits listed in the table which developed chancres. Of these, rabbits 486♀ and 150♀ showed a rise in titer of 12 units, rabbit U497♀ showed a rise of 4 units and rabbit 489♀ showed no rise in titer.

It might be suspected that the change in the flocculation temperature optima would not be an abrupt change. Transitional types of reactions were noted and are seen in the table in the case of the early post-inoculation results of rabbits 150♀ , 499♂ , 26♂ , N3♂ , N22♂ and N11♂ . This transition period wherein the serum displays equal reactivity at all temperatures, occurred after the quantitative titer rose in these animals.

The reversal of the pattern of serological reactivity before and after inoculation referred to above occurred with heated sera. Unheated sera, almost uniformly retained their original property of reacting more strongly at 1° than at 37°C. However, these rabbits (N3♂ , N22♂ , N11♂) did show stronger reactions at 37° than at 1°C. with unheated sera.

It should be stated that although 22 males and 7 females were inoculated, only 12 males and 6 females have survived the experiment. The death of 2 males and one female was attributable to incorrect technique during heart

puncture in the post-inoculation period. The deaths of the other 8 males was due to some common factor. All these animals showed similar gross autopsy changes characterized by a thick, caseous, nonodorous material which completely lined the inner wall of the peritoneum. Some of this material was inoculated on blood agar plates and into Hibler's broth. No growth was found in 24 or 48 hours or after one week. The media was discarded and no further attempt made to identify the cause of death in the rabbits.

The serological reactions of the control animals were characterized by a relative degree of stability. In the three examples listed in Table XXXV (34 ♂ , 25 ♀ , 481 ♂) it will be noted that the original serological reactions observed were unchanged over a period of two and one-half months.

The flocculation reactions shown in Appendix D give the complete results for each rabbit used in the experiment.

Complement-fixation results (noted in Appendix D) with the previously described "combined" technique showed interesting results in the 75 day post-inoculation group in particular. Excluding the two female rabbits which were inoculated but remained asymptomatic, the entire group of syphilitic rabbits with the exception of two animals (U497 ♀ , 33 ♂) showed fixation of complement when the combined flocculation-complement fixation technique was employed. On the other hand, none of the control rabbits gave reactions with the combined technique.

The results of additional complement-fixation experiments are presented in the appendix. These results deal with the relationship between flocculation and complement fixation reactions and with the question whether flocculates fix complement. These findings, however, are not essentially different from those previously presented (pp.150-160).

DISCUSSION

The data presented in this section would suggest that syphilitic rabbits, will at some time following inoculation, show an optimum flocculation temperature in the Kahn test different from that shown by noninfected rabbits. Whereas normal rabbits show optimum serological reactivity at 1°C., infected rabbits show optimum reactivity at 37°C. This change in reactivity begins to become evident in a month or two following inoculation. That different animals (cows, chickens, horses, pigs, rabbits) show optimum serological reactivity at 1°C. has been emphasized in these studies. Kahn (1940) reported that optimum serological reactivity in human syphilis is noted at 37°C. It would thus appear that syphilitic rabbits and syphilitic human beings have a common flocculation temperature optimum (37°C.) while nonsyphilitic animals have a different flocculation temperature optimum (1°C.)

It is of interest to note that shifting of the optimum serological reactivity from 1°C. to 37°C. following infection of rabbits with Spirochaeta pallida is apparently due to a relative change in the quantitative titers at these temperatures. Thus a rabbit (26♂) before inoculation may show at 1°C. a titer of 10 units and at 37°C. a titer of zero. After inoculation the same rabbit may show at 1°C. a titer of 40 units, and at 37°C. a titer of 60 units.

It should be mentioned that the unheated sera of the inoculated rabbits did not show the shift in the optimum flocculation temperature shown by the heated (30 minutes at 56°C.) sera.

Concerning the two female rabbits (497♀ and 482♀) the sera of which showed no reversal in optimum flocculation temperature with the Kahn test, the possibility was already mentioned that these animals may not have become infected. Lymph node transfer as a test of infection has not been resorted to. The possibility of a symptomless infection such as is known in other rodents (mice, rats, guinea pigs) cannot be excluded.

IV. SUMMARY

A study has been made of some aspects of the Kahn reaction with animal sera and nonsyphilitic human sera:

1. It was found that the sera of chickens (102), cows (62), horses (42), pigs (102), and rabbits (49), previously heated at 56°C. for 30 minutes gave positive Kahn reactions in a certain percentage of cases, the range of reactivity extending from 60 per cent to 90 per cent. This finding confirms the results of pervious authors (Kemp, et al., 1940).

2. A technique was employed to investigate the serological reactivity of animal sera at different temperatures; 37° and 1°C. were used. It was found that by this procedure the reactions occurred in considerably greater numbers at 1°C. than at 37°C. Thus, chicken sera gave 100 per cent of positive reactions at 1°C. and 37 per cent at 37°C.; horse sera, 100 per cent reactions at 1°C. and 23 per cent at 37°C.; pig sera, 100 per cent reactions at 1°C. and 32 per cent reactions at 37°C.; rabbit sera, 94 per cent reactions at 1°C. and 20 per cent at 37°C. These findings apply to sera that had been heated for 30 minutes at 56°C.

3. When the unheated animal sera were tested the reactivity was not the same as that shown by heated sera. Unheated cow and pig sera generally gave reactions which were weaker than those given by heated sera. Un-

heated chicken and rabbit sera generally gave reactions stronger than those given by heated sera. In the case of horse sera, the reactions in an unheated and heated state varied in either direction, being in some instances stronger and in others weaker in an unheated state.

4. Unheated animal sera tested at 37° and 1°C. showed the same pattern of reactivity as the heated sera, with the exception of some (10) weakly reacting cow sera which gave stronger reactions at 37°C. than at 1°C.

5. Unheated cow sera showed a tendency toward zone reactivity at 1°C., giving flocculation reactions in certain dilutions of serum with saline, but weak or no flocculation reactions in an undiluted state. This tendency was removed by heating the sera for 30 minutes at 56°C.; it was not removed by dialysis of the serum.

6. Globulin fractions of heated and unheated animal sera tended to react alike despite differences in reactivity of the whole serum.

7. Globulin fractions derived from positive animal sera showed instances of zone reactivity similar to that shown by unheated cow sera. This reactivity could in most instances be removed by dialyzing the residual $(NH_4)_2SO_4$ from the globulin precipitate.

8. Kahn-negative human sera may be induced to yield positive results in progressively increasing numbers by:

a. carrying out the test with heated serum at 1°C., (3.4 per cent positive reactions).

b. carrying out the test with unheated serum at 1°C., (6.0 per cent positive reactions).

c. carrying out the test with heated serum and sensitized antigen at 1°C., (14.9 per cent positive reactions).

d. carrying out the test with unheated serum and sensitized antigen at 1°C., (22.3 per cent positive reactions).

e. carrying out the test with the globulin fraction of heated sera at 1°C. using standard Kahn antigen. (81.0 per cent positive reactions).

f. carrying out the test with the globulin fraction of heated sera at 1°C. using sensitized Kahn reaction. (94.0 per cent positive reactions).

9. Albumin fractions of positively reacting animal and human sera and albumin fractions of negatively reacting animal sera showed either no serological reactivity or insignificant reactivity at room temperature, 37°C. or at 1°C.

10. Mixtures of human sera and lipid antigen (Kahn) that show no flocculation at 37°C. (although positive at 1°C. indicating union of reactants at this temperature) do not fix complement, indicating non-union of reactants at 37°C.

11. Incubation at 56°C. of mixtures of serum and antigen (Kahn) showing flocculation, results in dissolving the floccules, the extent of this disappearance being

inversely related to the titer. The floccules in animal sera are more labile in this respect than the floccules in syphilitic human sera.

12. The heat-dissolved floccules from syphilitic sera are capable of fixing complement, suggesting that the reactants are in a state of "soluble union."

13. Floccules in chicken, cow, horse, and pig sera showed marked discrepancy in their capability to fix complement. Employing different conditions of primary incubation, these floccules in most instances did not fix complement. Floccules of rabbit sera showed a higher tendency to fix complement than those of other animal sera. Of 20 rabbits giving positive reactions, the floccules in 14 instances (70%) fixed complement (as compared to over 98 per cent of complement fixation of floccules in Kahn-positive human syphilitic sera). With few exceptions animal serum floccules previously dispersed by incubating at 56°C. for 30 minutes did not fix complement.

14. Rabbits 50 to 60 days old showed no serological reactivity (Kahn). When the sera of these animals were tested at 37° and at 1°C. no reactivity was noted at 37°C., while some sera (3 out of 32) showed reactivity at 1°C. As the rabbits grew older their serological reactivity increased. This increase was more notable at 1°C. than at

room temperature. The reactions at 37°C. were essentially negative.

15. The inoculation of rabbits with Spirochaeta pallida resulted in a general increase in serological reactivity at room temperature, 37°C. and at 1°C., the increase being greatest at 37°C. The pattern of serological reactivity at the three temperatures, in these animals, was thus reversed as compared with that shown by normal rabbits. While the normal animals continued to show their most marked serological reactivity at 1°C., the inoculated animals showed most marked serological reactivity at 37°C. This reversal began to manifest itself within three months after inoculation.

BIBLIOGRAPHY

Aminoff, M.: Über die Fähigkeit der Staubbakterien bei Sera W R - Positiv Reactionen Nervorzurufen. Acta path. et microbiol. Scandinav. 16: 376-385, 1931.

Bachmann, W.: Beitrag zu den Beziehung zwischen Organabbauprodukten und Wassermannscher Reaktion. Ztschr. f. Immunitätsforsch. u. exper. Therap. 33: 233-246, 1921.

Barnett, C. W., Jones, R. B., and Kulchar, G. V.: Measurement of Reagin in Non-Syphilitic Sera. Proc. Soc. Exper. Biol. and Med. 33: 214-218, 1935.

Baumgartner, L.: The Relationship of Age to Immunological Reactions. Yale J. Biol. and Med. 6: 403-434, 1934.

Beck, A.: The Role of the Spirochaete in the Wassermann Reaction. J. Hyg. 39: 298-310, 1939.

Berstein, A.: False-Positive Wassermann Reactions in Infectious Mononucleosis. Am. J. M. Sc. 196: 79-83, 1938.

Bier, O., and Trapp, E.: Dissociation of the Aggregates obtained on Adding Beef Heart Lipoid to Wassermann-Positive Sera. J. Immunol. 40: 465-470, 1941.

Blum, K.: Ueber die Wassermannsche Reaktion im Serum normaler und syphilitisches Kaninchen. Ztschr. f. Immunitätsforsch. u. exper. Therap. 40: 195-214, 1924.

Bordet, J.: Les Leucocytes et les Propriétés Actives du Sérum Chez las Vaccinés. Ann. Inst. Pasteur 9: 462-506, 1895.

_____, and Gengou, O.: Sur L'Existence de Substances sensibilisatrices dans la plupart des Sérums Antimicrobiens. Ann. Inst. Pasteur 15: 289-302, 1901.

Breazeale, E. L., Reusser, T. R., and Breazeale, J. F.: Flocculation tests for Syphilis (plant sap reactions). Southwestern Med. 25: 256-257, 1941.

Brown, H., and Kolmer, J. A.: Studies on the Chemical Constitution of the Antigenic Substance in Alcoholic Tissue Extracts concerned in the Serum Diagnosis of Syphilis. J. Biol. Chem. 137: 525-533, 1941.

BIBLIOGRAPHY (Continued)

Browning, C. H., Cruickshank, J., and M'Kenzie, I.: Constituents Concerned in the Wassermann Syphilis Reaction, with Special Reference to Lecithin and Cholesterin. J. Path. and Bact. 14: 484-502, 1910.

_____, _____, and _____: Recent Methods in the Diagnosis and Treatment of Syphilis. Constable and Company; London: 1924.

Bruck, C.: Serodiagnostik. Pps. 345-505 of "Beiträge zur Pathologie und Therapie der Syphilis" by A. Neisser. J. Springer; Berlin: 1911.

_____: Serodiagnose der Syphilis. J. Springer; Berlin: 1924.

_____: Geschichte der Medizin. 25 Jahr Serodiagnostik der Syphilis. Deutsche Med. Wchnschr. 57: 812-813, 1931.

Buchner, H.: Untersuchung über die bakterienfeindlichen Wirkung des Blutes und Blutserums. I. Vorbemerkungen. Ztschr. f. Hyg. u. Infectionskr. 10: 84-101, 1890.

II. _____, and Voit, F.: Ibid, 10: 101-120, 1890.

III. _____, and Sittman, G.: Welchen Bestantheilen des Blutes ist die bakterientödtende Wirkung zuschreiben. Ibid, 10: 121-139, 1890

IV. _____, and Orthenberger M.: Versuche über die Natur der bakterientödtenden Substanz im Serum. Ibid, 10: 149-173, 1890.

Cannon, P. R.: Defense Mechanisms in Infectious and Related Diseases. Ann. Rev. Physiol. 2: 387-410, 1940.

Cappelli, E.: La "Pallidareazione" di Gaehtgens sui sieri labbrosi; Contributo allo studio dell' essenga della R. Wassermann. Gior. di batteriol. e immunol. 22: 425-436, 1939.

Chargin, L., and Rein, C. R.: The Kahn Verification Test. An Appraisal of the Test Based on Clinical and Serological Evidence. Arch. Dermat. and Syph. 44: 1031-1047, 1941.

BIBLIOGRAPHY (Continued)

Chung, H. L. and Chang, F. C.: Relapsing Fever. Chinese M. J. 55: 6-33, 1939.

Craig, C. F.: The Relation of Certain Bacteria to Non-Specific Reactions with the Complement Fixation Test for Lues. J. Exper. Med. 13: 521-530, 1911.

Crawford, G. M., and Ray, L. F.: Serologic Discrepancies in Syphilis. J. A. M. A. 113: 1715-1720, 1939.

Cromwell, H. W.: A Study of the Hemolytic Antibody - Antigen Combination. J. Immunol. 7: 461-496, 1922.

D'Allessandro, G., and Sofia, F.: Beitrag zur Kenntnis der Witebskyschen Bestätigungsreaktion bei Syphilis. Ztschr. f. Immunitätsforsch. u. exper. Therap. 83: 478-489, 1934.

Detre, L.: Ueber den nachweis von spezifischen Syphilis-antisubstanzen und deren Antigenen bei Luetikern. Wien. klin. Wchnschr. 19: 619-620, 1906.

Dohi, S.: Experimentelle Studien über das Wesen der Wassermann - Neisser - Bruckschen Reaktion bei Syphilis. Pps. 514-565 of "Beiträge zur Pathologie und Therapie der Syphilis" by A. Neisser. J. Springer; Berlin: 1911.

Dreyer, G., and Ainley-Walker, E. W.: Neues zur Theorie der Wassermannschen Reaktion. Biochem. Ztschr. 54: 11-15, 1913.

Duncan, J. T.: The Use of Equivalent Proportions of Antigen and Serum in Absorption of Precipitin. Brit. J. Exper. Path. 13: 489-497, 1932.

Duran-Reynals, F.: The Flocculation of Tissue Extracts by Normal and Immune Sera of Fowls and of Other Animals. Yale J. Biol. and Med. 12: 361-398, 1940.

Eagle, H.: Studies in the Serology of Syphilis. II. The Physical Basis of the Wassermann Reaction. J. Exper. Med. 52: 739-745, 1930.

_____: Studies in the Serology of Syphilis. VII. On the Supposed Artificial Induction of a Positive Wassermann Reaction in Originally Negative Human Sera. J. Lab. and Clin. Med. 17: 778-786, 1932.

BIBLIOGRAPHY (Continued)

_____: Laboratory Diagnosis of Syphilis. The C. V. Mosby Company; St. Louis: 1937.

_____: Eagle Flocculation and Wassermann Technics, in "Technics of Serodiagnostic Tests for Syphilis"; Supplement No. 11 to Ven. Dis. Inform., p. 2, 1940.

_____, and Hogan, R.: On the Presence in Syphilitic Serum of Antibodies to Spirochaetes, Their Relation to so-called Wassermann Reagin, and Their Significance for the Serodiagnosis of Syphilis. J. Exper. Med. 71: 215-230, 1940.

_____, _____, Mays, J. R. S., and Burney, L. E.: The Reactivity of the Serum of Malarial Patients with Spirochetal Suspensions. Am. J. Syph., Gonor., and Ven. Dis. 25: 406-411, 1941.

Ecker, E. E., and Sasano, K.: A Rapid Method for Preparing Antigens from Normal Heart Muscle. J. Infect. Dis. 25:174-177, 1919.

Elias, H., Neubauer, E., Porges, O. and Salomon, H.: Theoretisches über die Serumreaktion auf Syphilis. Wien. klin. Wchnschr. 21: 748-752, 1908.

Erlandsen, A.: Untersuchungen uber die lecithinartigen Substanzen des Myocardiums und der quergestreiften Muskeln. Ztschr. f. physiol. Chem. 51: 71-155, 1907.

Fodor, J.: Die Fähigkeit des Blutes Bakterien zu vernichten. Deutsche med. Wchnschr. No. 34: 745, 747, 1887

Follensby, E. M., and Hooker, S. B.: The Effect of Temperature upon Combination and Aggregation and Upon Equilibrium in the Reaction Between Antigen and Antibody. J. Immunol. 37: 367-382, 1939.

Forssman, J.: Zur Chemie der Wassermann Reaktion. Biochem. Ztschr. 121: 180-214, 1921.

Fowweather, F. S., and Gordon, J.: The Relation Between Amino Acids and the Wassermann Reaction. Brit. J. Exper. Path. 8: 93-100, 1927.

Furth, J.: Antigenic Character of Heated Protein. J. Immunol. 10: 777-789, 1925.

BIBLIOGRAPHY (Continued)

Gaehtgens, W.: Weitere Erfahrungen über das wässerige karbolisierte Pallida antigen fur den serologischen Luesnachweis. Zentralbl. f. Bakt. (Originale) 118: 26-35, 1930.

Gay, F. P.: The Fixation of Alexines by Specific Precipitates. Zentralbl. f. Bakt. (Originale) 39: 603-610, 1905.

Georgi, W.: Studien über Serum-Ausflockung bei Syphilis. Biochem. Ztschr. 93: 16-33, 1919.

Giordana, A. S., and Carlson, B.: Occurrence of a Non-Specific Substance in Guinea Pig Serum Fixed by Antigen in the Wassermann Test. Am. J. Clin. Path. 9: 130-135, 1939.

Gold, A.: Personal Communication.

Goodner, K., and Horsfall, F. L.: The Complement Fixation Reaction with Pneumococcus Capsular Polysaccharide. J. Exper. Med. 64: 201-216, 1936.

Greene, R. A., and Breazeale, E. L.: A Quantitative Study of the Reaction of Heated and Unheated Cow Sera to the Hinton, Kline, and Mazzini Tests. Am. J. Syph., Gonor., and Ven. Dis. 25: 85-88, 1941.

Griffith, F., and Scott, W. M.: Ministry of Health Reports on Public Health and Medical Subjects. I. London, 1920. (Cited by Mackie and Watson, 1926.)

Haag, F. E., and Leven, W.: Ueber die Anwendung neuerer Flockungsreaktionen zur Feststellung der Kaninchen-syphilis. Ztschr. f. Immunitätsforsch. u. exper. Therap. 77: 25-37, 1932.

Halber, W., Hirzfeld, H. and Mayzner, M.: Beiträge zur Konstitutionsserologie. II. Mitteilung Untersuchungen über die Antikorperentstehung bei Kindern im Zusammenhang mit dem Alter. Ztschr. f. Immunitäts-forsch. u. exper. Therap. 53: 391-418, 1927.

Hardesty, W. L., and Jurischk, A.: The Influence of Temperature on the Time Required for Inactivation in the Kahn Reaction. Am. J. Syph. 16: 110-112, 1932.

Hartley, P.: The Effect of Physical and Chemical Agencies on the Properties of Antigen and Antibodies. "A System of Bacteriology in Relation to Medicine," Medical Research Council, His Majesty's Stationery Office. 6: 224-265, 1931.

BIBLIOGRAPHY (Continued)

_____: Observations on the Role of the Ether-Soluble Constituents of Serum in Certain Serological Reactions. Brit. J. Exper. Path. 6: 180-196, 1925.

Heidelberger, M.: Quantitative Absolute Methods in the Study of Antigen-Antibody Reactions. Bact. Rev. 3: 49-95, 1939

_____: Quantitative Chemical Studies on Complement or Alexin. I. A Method. J. Exper. Med. 73: 681-694, 1941.

_____, and Kendall, F. E.: A Quantitative Study of the Precipitin Reaction between Type III Pneumococcus Polysaccharide and Purified Homologous Antibody. J. Exper. Med. 50: 809-823, 1929.

_____, and _____: The Precipitin Reaction between Type III Pneumococcus Polysaccharide and Homologous Antibody. II. Conditions for Quantitative Precipitation of Antibody in Horse Sera. J. Exper. Med. 61: 559-562, 1935. 1935a.

_____, and _____: A Quantitative Theory of the Precipitin Reaction. II. A Study of an Azoprotein-Antibody System. J. Exper. Med. 62: 467-483, 1935. 1935b.

_____, and _____: A Quantitative Theory of the Precipitin Reaction. III. The Reaction Between Crystalline Egg Albumin and its Homologous Antibody. J. Exper. Med. 62: 697-720, 1935. 1935c.

_____, _____, and Soo Hoo, C. M.: Quantitative Studies on the Precipitin Reaction. Antibody Production in Rabbits Injected with an Azo Protein. J. Exper. Med. 58: 137-151, 1933.

_____, Treffers, H. P., and Mayer, M.: A Quantitative Theory of the Precipitin Reaction. VII. The Egg Albumin-Antibody Reaction in Antisera from the Rabbit and Horse. J. Exper. Med. 71: 271-282, 1940.

_____, and Mayer, M.: Quantitative Chemical Studies on Complement or Alexin. IV. Addition of Human Complement to Specific Precipitates. J. Exper. Med. 75: 285-295, 1942.

Hinkleman, A. J.: The Biochemistry of the Wassermann Reaction. Am. J. Syph. 11: 594-608, 1927.

BIBLIOGRAPHY (Continued)

Hinton, W. A.: The Hinton and Davies-Hinton Tests for Syphilis, in "Technics of Serodiagnostic Tests for Syphilis;" Supplement No. 11 to Ven. Dis. Inform., p. 22, 1940.

Hirschfeld, L., and Klinger, R.: Ueber das Wesen der Inaktivierung und der Komplementbindung. Ztschr. f. Immunitätsforsch. u. exper. Therap. 21: 40-76, 1914.

Holtzer, R. R., and Ssuschkowa, E. G.: Zur Frage über das Wesen der Wassermann-Reaktion. Ztschr. f. Immunitätsforsch. u. exper. Therap. 68: 81-98, 1930.

Horsfall, F. L., and Goodner, K.: Lipoids and Immunological Reactions. I. The Relation of Phospholipids to the Type-Specific Reactions of Antipneumococcus Horse and Rabbit Sera. J. Exper. Med. 62: 485-503, 1935

Hunter, C. A.: A Rapid Method for Preparing Antigens for the Wassermann Reaction. J. Lab. and Clin. Med. 21: 417-420, 1936.

Jacobsthal, E.: Notiz zur Theorie und Praxis der Wassermannschen Reaktion. München. med. Wchnschr. 57: 689-690, 1910.

_____: Versuche zu einer optischen Serodiagnose der Syphilis. Ztschr. f. Immunitätsforsch. u. exper. Therap. 8: 107-128, 1911.

Jones, F. S.: The Effect of Heat on Antibodies. J. Exper. Med. 46: 291-301, 1927.

_____: The Effect on Subsequent Agglutination of the Exposure of Bacteria to Heated Antiserum. J. Exper. Med. 47: 245-254, 1928.

Kahn, R. L.: Observations on Kahn Precipitation Reaction for Syphilis. Am. J. Syph. 7: 389-403, 1923. 1923a.

_____: Rapid Precipitation Phase of the Kahn Test for Syphilis. J. A. M. A. 81: 88-90, 1923. 1923b.

_____: Serum Diagnosis of Syphilis by Precipitation. Williams and Wilkins Company; Baltimore: 1925.

_____: The Kahn Test - A Practical Guide. Williams and Wilkins Company; Baltimore: 1928.

BIBLIOGRAPHY (Continued)

_____: Tissue Immunity. C. C. Thomas; Springfield: 1936.

_____: A Serologic Verification Test in the Diagnosis of Latent Syphilis. Arch. Dermat. and Syph. 41: 817-830; 1940. 1940a.

_____: The Serology of Syphilis. J. Lab. and Clin. Med. 26: 139-155, 1940. 1940b.

_____: Serology in Syphilis Control, Principles of Sensitivity and Specificity. Williams and Wilkins Company; Baltimore: 1942.

_____, and Lyon, D. S.: Studies on Complement Fixation. IV. On the Affinity of Sheep Corpuscles for Anti-sheep hemolysin. J. Infect. Dis. 29: 651-662, 1921.

_____, McDermott, E. B., and Marcus, S.: Effect of Temperature on Kahn Reaction. I. With Serologically Positive Sera of Lower Animals. II. With Serologically Positive Sera of Human Syphilis. III. With Serologically Positive Sera in the Absence of Syphilis. IV. With Serologically Negative Sera in the Absence of Syphilis. Am. J. Syph., Gonor., and Ven Dis. 25: 151-156, 157-161, 162-172, 173-178, 1940.

Kemp, J. E., Chesney, A. M., and Poole, A. K.: Studies in Experimental Syphilis. V. The Wassermann Test in Normal and Syphilitic Rabbits. Bull. Johns Hopkins Hosp. 39: 132-148, 1926.

_____, Fitzgerald, E. M., and Shepherd, M.: The Occurrence of Positive Serologic Tests for Syphilis in Animals Other Than Man. Am. J. Syph., Gonor., and Ven. Dis. 24: 537-562, 1940.

Kendrick, P.: Effect of Contamination and Age of Serum on Kahn Precipitation Test. Am. J. Pub. Health 14: 673-676, 1924.

Kettel, K.: Undersøgelser over Kuldehaemagglutininer i Menneskeserum. Levin and Munksgaard; Copenhagen: 1930. (Cited by Wiener, 1939.)

Kitchen, S. F., Webb, E. L., and Kupper, W. H.: The Influence of Malarial Infections on the Wassermann and Kahn Reactions. J. A. M. A. 112: 1443-1449, 1939.

Kleczkowski, A.: Effect of Heat on Flocculating Antibodies of Rabbit Antisera. Brit. J. Exper. Path. 22: 192-208, 1941.

BIBLIOGRAPHY (Continued)

Kline, B. S.: Microscopic Slide Precipitation Tests for the Diagnosis and Exclusion of Syphilis, in "Technics of Serodiagnostic Tests for Syphilis;" Supplement No. 11 to Ven. Dis. Inform., p. 45, 1940.

Klopstock, A.: Die methoden zur Serodiagnostik der Syphilis. Urban und Schwarzenberg; Berlin: 1933.

Kolmer, J. A.: Studies in the Standardization of the Wassermann Reaction. XXX. A New Complement Fixation Test for Syphilis Based upon the Results of Studies in the Standardization of Technique. Am. J. Syph. 6: 82-110, 1922.

_____: Serum Diagnosis by Complement Fixation. Lea and Febiger; Philadelphia: 1928.

_____: The Kolmer Complement Fixation Test for Syphilis, in "Technics of Serodiagnostic Tests for Syphilis;" Supplement No. 11 to Ven. Dis. Inform., p. 56, 1940.

_____, and Lynch, E. R.: Guinea Pig Serum in Relation to Prezone and Non-Specific Wassermann Reactions. Am. J. Clin. Path. 9: 136-150, 1939.

_____, Kast, C. C., and Lynch, E. R.: Studies on the Role of Spirocheta Pallida in the Wassermann Reaction. I. Complement Fixation in Syphilis, Leprosy, and Malaria with Spirochetal Antigens. Am. J. Syph., Gonor., and Ven. Dis. 25: 300-318, 1941. 1941a.

II. The Relation of Spirochetal Antibodies to the Wassermann Reagin. Ibid, 25: 412-434, 1941. 1941b.

Krag, P., and Lonberg, A.: The Occurrence of Strong Unspecific Wassermann-Kahn Reaction. Acta dermat.-venereol. 19: 612-621, 1938.

Kraus, R., and Volk, R.: Weitere Studien über Immunität bei Syphilis und bei der Vakzenation gegen Variola. Wien. klin. Wchnschr. 19: 620-622, 1906.

Kroó, A., Schulze, F. O., and Zander, I.: Untersuchungen über die Immunitätsvorgänge bei Syphilis. II. Die Syphilitische Blutveränderung. Klin. Wchnschr. 8: 783-785, 1929.

BIBLIOGRAPHY (Continued)

Landsteiner, K.: Specificity of Serological Reactions. C. C. Thomas; Springfield: 1936.

_____, Müller, R., and Pötzl, O.: Offizielles Protokoll der k-k. Gesellschaft der Aertze in Wien. Sitzung von 19, April 1907. Wien. klin. Wchnschr. 20: 514, 1907. 1907a.

_____, _____, and _____: Zur Frage der Komplementbindungsreaktionen bei Syphilis. Wien. klin. Wchnschr. 20: 1565-1567, 1907. 1907b.

_____, _____, and _____: Ueber Komplementbindungsreaktionen mit dem Serum von Dourinetieren. Wien. klin. Wchnschr. 20: 1421-1422, 1907. 1907c.

_____, and _____: Meeting of the Wiener dermatologische Gesellschaft. Wien. klin. Wchnschr. 21: 1076-1077, 1908.

Lane, J. F.: False Positive Kahn Reactions in Measles. Hosp. News, 6: 19-24, 1939.

League of Nations Health Organization: Report of the Technical Laboratory Conference on the Serodiagnosis of Syphilis at Copenhagen; Paris: 1923.

League of Nations Health Organization: Report of the Second Laboratory Conference on the Serodiagnosis of Syphilis at Copenhagen; Geneva: 1928.

League of Nations Health Organization: Report of the Laboratory Conference on the Serodiagnosis of Syphilis at Montevideo; Geneva: 1931.

Evaluation of Serodiagnostic Tests for Syphilis in the United States, [illegible]. Supplement No. 1 to Ven. Dis. Inform. 1935.

Evaluation of Serodiagnostic Tests for Syphilis in the United States, 1941. To be published; (Preliminary publication in Ven. Dis. Inform. 22: 161-194, 1942.)

Levaditi, C., and Yamanouchi, T.: Le Sero-Diagnostic de la Syphilis. Compt. Rend. Société de Biol. 63: 740-742, 1907.

Lund, H.: The Titration of Traces of Reagin. Am. J. Syph., Gonor., and Ven. Dis. 26: 1-15, 1942.

BIBLIOGRAPHY (Continued)

Lynch, F. W., Boynton, R. E., and Kimball, A. C.: False Positive Serologic Reactions for Syphilis Due to Smallpox Vaccinations (Vaccinia). J. A. M. A. 117: 591-595, 1941.

Mackie, T. J.: The Serum Constituents Responsible for the Sachs-Georgi and Wassermann Reactions. J. Hyg. 21: 386-391, 1923.

_____, and Watson, H. F.: On the Immunologic Nature of the Principle in Serum Responsible for the Wassermann Reaction, with Reference also to the Flocculation Reaction of Sachs and Georgi. J. Hyg. 25: 176-208, 1926.

_____, and Anderson, C. G.: The Precipitation Reactions of Normal Serum and Lipoid Suspension. J. Path. and Bact. 44: 603-631, 1937.

Malloy, A. M. and Kahn, R. L.: The Ultramicroscopic Precipitation Reaction in Syphilis. J. Infect. Dis. 48: 243-254, 1931.

Marie, A., and Levaditi, C.: Les "Anticorps Syphilitiques" in le Liquide Cephalorachidien des Paralytiques Generaux. Ann. Inst. Pasteur. 21: 138-155, 1907.

McMeans, J. W.: Environmental Conditions Influencing Complement Fixation. J. Immunol. 8: 433-447, 1923.

Michaelis, L.: Die Wassermann'sche Syphilisreaktion. Berl. klin. Wchnschr. 44: 1103-1107, 1907.

Mohr, C. F., Moore, J. E., and Eagle, H.: Biologic False Positive Serologic Reactions in Tests for Syphilis. I. Occurrence in Normal Persons. Arch. Int. Med. 68: 898-912, 1941.

Moore, J. E., and Eagle, H.: The Confusing Multiplicity of Serologic Tests for Syphilis. J. A. M. A. 117: 243-247, 1941.

Morgenroth, J., and Rosenthal, F.: Amboceptoren und Receptoren. Biochem. Ztschr. 36: 190-208, 1911.

Muckenfuss, R. S., and Ebel, E.: The Influence of Temperature on the Kahn Test. Am. J. Syph. 15: 391-395, 1931.

Muir, R.: Hemolytic Action. "A System of Bacteriology in Relation to Medicine", Medical Research Council. His Majesty's Stationery Office. 6: 295-315, 1931.

BIBLIOGRAPHY (Continued)

Nagle, N., and Lazarov, J.: Incubation of the Completed Kahn Test. Am. J. Pub. Health. 20: 1216-1218, 1930.

Nathan, E.: Experimentelle Untersuchungen über das Wesen der Wassermannschen Reaktion. Ztschr. f. Immunitätsforsch. u. exper. Therap. 27: 219-286, 1918.

Neymann, C. A., and Gager, L. T.: A New Method for Making Wassermann Antigen from Normal Heart Tissue. J. Immunol. 2: 573-583, 1917.

Nishio, M.: Effect of Heating Syphilitic Serum and its Protein Fractions on Precipitation Reaction. J. Infect. Dis. 45: 148-155, 1929.

Nuttall, G.: Experimente über die bakterienfeindlichen Einfluss des thierischen Körpers. Ztschr. f. Hyg. u. Infectionskr. 4: 353-394, 1888.

Ottensooser, F.: Ueber die Zusammensetzung des Spezifischen Ovalbuminpräzipitats. Kolloidztschr. 33: 176-178, 1923.

Pangborn, M. C.: A New Serologically Active Phospholipid from Beef Heart. Proc. Soc. Exper. Biol. and Med. 48: 484-486, 1941.

_____: Isolation and Purification of a Serologically Active Phospholipid from Beef Heart. J. Biol. Chem. 143: 247-256, 1942.

Pappenheimer, A. M.: Anti-Egg Albumen Antibody in the Horse. J. Exper. Med. 71: 263-269, 1940.

Perla, D., and Marmorston, J.: Natural Resistance and Clinical Medicine. Little, Brown and Company; Boston: 1941.

Pfeiffer, R., and Issaeff.: Ueber die specifische Bedeutung der Choleraimmunität. Ztschr. f. Hyg. u. Infectionskr. 17: 355-400, 1894.

Pohlner, A.: Unspezifische Wassermann-Reaktion im Blute und Liquor bedingt durch Bacillus subtilis. Dermat. Wchnschr. 106: 701-704, 1938.

BIBLIOGRAPHY (Continued)

Porges, O., and Meier, G.: Ueber die Rolle der Lipoide bei der Wassermannschen Syphilis Reaktion. Berl. klin. Wchnschr. 45: 731-736, 1908.

Rivers, T. M.: Immunity in Virus Infection. Science. 95: 107-112, 1942.

Rothbart, H. B.: The Variability of the Kahn Reaction in Children. J. Pediat. 11: 484-489, 1937.

Rytz, F.: Specificity in the Serodiagnosis of Syphilis. A Differential Method. Am. J. Clin. Path. 9: 512-515, 1939.

_____: Specificity in the Serodiagnosis of Syphilis. Am. J. Clin. Path. 12: 166-173, 1942.

Sachs, H.: Ueber den Einfluss des Cholesterins auf die Verwendborkeit der Organextrakte zur Wasserman'schen Syphilisreaktion. Berl. klin. Wchnschr. 48: 2066-2067, 1911.

_____: 25 Jahr Wassermannsche Reaktion. Klin. Wchnschr. 10: 894-897, 1931.

_____, and Altmann, K.: Ueber die Wirkung des oleinsauren natrons bei der Wassermann'schen Reaktion auf Syphilis. Berl. klin. Wchnschr. 45:494-497, 1908.

_____, and Georgi, W.: Zur Serodiagnostik der Syphilis mittels Ausflockung durch cholesterinierte Extracte. Med. Klinik. 14: 805-809, 1918.

_____, and _____: Ein Vorschlag zur Serodiagnostik der Syphilis beim Kaninchen. Klin. Wchnschr. 2: 880-881, 1923.

_____, and Rondoni, P.: Beitrage zur Theorie und Praxis der Wassermannschen Syphilisreaktion. Ztschr. f. Immunitätsforsch. u. exper. Therap. 1: 132-151, 1908.

Schreus, Th. A., and Foerster, R.: Spezifische Sensibilisierung von serologischen Reaktionen. I. Mitteilung. Grundlage und Methodik der spezifisch sensibilisierten Wassermann-Reaktion (WSR^s). Ztschr. f. Immunitätsforsch. u. exper. Therap. 82: 53-59, 1934.

BIBLIOGRAPHY (Continued)

Sherwood, M. P., Bond, G. C., Clark, M. F.: Biological and Chemical Studies of the Serological Tests Used in the Diagnosis of Syphilis, (Abstract). J. Bact. 38: 231, 1939.

_____, _____, and _____: Results Obtained with the Kolmer, Kahn, Kline, and Eagle Tests on Animal Sera. Am. J. Syph., Gonor., and Ven. Dis. 25: 93-95, 1941.

_____, _____, and Canuteson, R.I.: On the Possible Presence of a Reagin-Like Factor in Normal Human Serum. Am. J. Syph., Gonor., and Ven. Dis. 25: 179-185, 1941.

Sievers, O.: Der Einfluss von Bakterien auf Wassermann Negative Sera. Acta path. et microbiol. Scandinav. 16: 365-375, 1939.

Stahl, W. H., and Pennell, R. B.: Conditions for Maximum Precipitation and Agglutination of Antibody in Various Brucella Antiserums. Michigan Technical Bulletin 177, Michigan State College Agricultural Experiment Station, (pps. 20-22). 1941.

Stats, D., and Bullowa, J. G. M.: Failure of the Human Convalescent Type-Specific Antipneumococcic Antibody to Fix Complement. J. Immunol. 43: 41-48, 1942.

Strauss, J. H.: Modification of the Eagle Flocculation Test for Syphilis. Am. J. Syph., Gonor., and Ven. Dis. 21: 406-407, 1937.

Stuart, C. A., Burgess, A., Lawson, H. A., and Wellman, H. E.: Some Cytologic and Serologic Aspects of Infectious Mononucleosis. Arch. Int. Med. 54: 199-214, 1934.

Topley, W. W. C., and Wilson, G. S.: The Principles of Bacteriology and Immunity. Second Edition. William Wood and Company; Baltimore: 1936.

Turner, T. B.: Protective Antibodies in the Serum of Syphilitic Rabbits. J. Exper. Med. 69: 867-890, 1939.

Vogelsang, Th. M.: Sero-Diagnostic de la Syphilis. J. W. Eides Boktrykkeri; Bergen: 1940.

BIBLIOGRAPHY (Continued)

Wakerlin, G. E., and Carroll, P. H.: The Blood Wassermann Reaction in Experimental Rabbit Syphilis. Arch. Dermat. and Syph. 12: 670-678, 1925.

Wassermann, A.: Ueber die Entwicklung und den gegenwärtigen Stand der Serodiagnostik gegenüber Syphilis. Berl. klin. Wchnschr. 44: 1599-1602, 1907.

_____: Zur Geschichte der Serodiagnostik der Syphilis. Berl. klin. Wchnschr. 58: 888-890, 1921.

_____, and Bruck, C.: Ist die Komplementbindung beim Entstehen spezifischer Niederschläge eine mit der Präzipitierung zusammenhängende Erscheinung oder Ambozeptorenwirkung? Med. Klinik. No. 55: 1409-1411, 1905.

_____, and Citron, H.: Weitere Mitteilungen über die Zerlegung des Wassermannaggregates und ihre Anwendungsfähigkeit zur Bestätigung der Positiven Wassermannschen Reaktion. I. Teil von A. v. Wassermann. II. Teil. Methodik für die Gewinnung der Wassermann-Substanz, H. Citron. Klin. Wchnschr. 1: 1101-1103, 1922.

_____, Neisser, A., and Bruck, C.: Eine serodiagnostische Reaktion bei Syphilis. Deutsche med. Wchnschr. No. 32: 745-746, 1906.

_____, _____, _____, and Schucht, A.: Weitere Mitteilungen über den Nachweis spezifischluetischer Substanzen durch Komplementverankerung. Ztschr. f. Hyg. u. Infectionskr. 55: 451-477, 1906.

Wellman, J. W., and Lankelma, H. P.: Purification of the Antigen used in Diagnostic Tests for Syphilis by Means of the Cadmium Salt. Ven. Dis. Inform. 21: 12-14, 1941.

Weil, E.: Ueber den Lues-Antikorpernachweis im Blute von Luetischen. Wien. klin. Wchnschr. 20: 527-530, 1907.

Wells, H. G.: The Chemical Aspects of Immunity, Second Edition. Am. Chem. Soc. Monograph Series, Chem. Catalog Company; New York: 1929.

Wendt, H.: Ueber Erfahrungen mit der v. Wassermannschen Tuberkulosereaktion und der Lezithinflockungsprobe bei Rindern und Kalbern. Zentralbl. f. Bakt. (Originale) 94: 26-35, 1925.

BIBLIOGRAPHY (Continued)

Weygandt: Syphilitische Antistoffe in der Cerebrospinalflüssigkeit bei Tabes dorsalis. Deutsche med. Wchnschr. 33: 1239, 1907.

Wicks, E.: Standardized Antigen in the Serodiagnosis of Syphilis. Doctoral Dissertation, Horace H. Rackham School of Graduate Studies of the University of Michigan. 1937.

Wiener, A. S.: Blood Groups and Blood Transfusion. C. C. Thomas, Springfield: 1939.

Witebsky, E.: Die Bedeutung der Wiedergewinnung von Antikorpern aus ihrer Antigenverbindung für Serologische Probleme. Zentralbl. f. Bakt. (Originale) 122: 70-73, 1931.

_____: Eine Bestätigungsreaktion zur Serodiagnostik der Syphilis. Ztschr. f. Immunitätsforsch. u. exper. Therap. 80: 323-335, 1933.

Wolf, C. G. L., and Rideal, E. K.: Precipitation Phenomena and the Wassermann Reaction. J. Hyg. 25: 366-384, 1926.

Zinsser, H.: Infection and Resistance. Second Edition. Macmillan Company; New York: 1922.

_____, and Parker, J. T.: Observations on a Substance in Immune Horse Serum which Interferes with Alexin Fixation. J. Immunol. 8: 151-161, 1923.

APPENDIX A

FLOCCULATION REACTIONS AND QUANTITATIVE TITERS AT DIFFERENT TEMPERATURES WITH CHICKEN, COW, HORSE, PIG AND RABBIT SERA

Serum Source, Number, Date Examined 1941	Standard Kahn Reaction Tube 123	Dilution of Serum with 0.9% Saline	Reactions at: 37°C. Tube 123	37°C. Quant. Titer	Room Temperature Tube 123	Room Temperature Quant. Titer	1°C. Tube 123	1°C. Quant. Titer
Chicken								
1 (10-15)	144	Undil.	--3	1	144	3	444	40
2 "	344	Undil.	--4	1	344	4	444	40
3 "	±44	Undil.	--2	1	±44	3	444	100
4 "	±14	Undil.	--4	1	±14	2	444	80
5 "	244	Undil.	--4	1	244	3	444	20
6 "	-14	Undil.	--±	0	-14	2	444	40
1 (10-17)	421	1:3	---	4	3±-	12	444	40
2 "	±44	1:3	---	4	-±2	12	444	120
3 "	---	Undil.	---	0	---	0	444	10
4 "	---	Undil.	---	0	---	0	444	10
5 "	---	Undil.	---	0	---	0	444	4
6 "	-44	1:2	--3	8	-34	8	444	80
1 (10-18)	---	Undil.	---	0	---	0	444	20
2 "	---	Undil.	---	0	---	0	444	10
3 "	---	Undil.	---	0	---	0	-±2	1
4 "	---	Undil.	---	0	---	0	444	4
5 "	---	Undil.	---	0	---	0	244	3
6 "	---	Undil.	---	0	---	0	144	3
7 "	---	Undil.	---	0	---	0	444	20
8 "	--3	Undil.	---	0	--3	1	444	20
9 "	---	Undil.	---	0	---	0	--3	1
1 (10-26)	±21	1:2	---	4	-±3	8	444	100
2 "	-14	1:1.5	---	4	-±3	6	444	40
3 "	±44	1:2.5	---	4	-±4	10	444	40
4 "	-41	1:3	---	4	-±3	12	444	100
5 "	±4±	1:2.5	---	4	-±4	10	444	120
6 "	±44	1:5	---	4	--±	10	444	80
1 (11-2)	-44	1:3	---	4	-±3	12	444	60
2 "	-44	1:3	-±4	12	-24	12	444	140
3 "	--±	Undil.	---	0	--±	0	444	20
4 "	-±1	Undil.	---	0	-±1	0	444	80
5 "	--1	Undil.	---	0	--1	0	444	4
6 "	±44	1:3	-±1	6	-±4	12	444	80
7 "	444	1:7	---	4	-±2	28	444	80
8 "	-±4	Undil.	-±-	0	-±4	1	444	20
9 "	--±	Undil.	---	0	--±	0	444	4
1 (11-7)	344	1:3	---	4	-24	12	444	40
2 "	-44	1:3	---	4	-14	12	444	80
3 "	±44	1:2	--1	4	-14	8	444	120
4 "	-±4	Undil.	---	0	-±4	1	444	40
5 "	-44	1:4	---	4	-±4	16	444	60
6 "	144	1:5	--±	10	-±4	20	444	100
1 (11-16)	-1-	Undil.	---	0	-1-	0	444	60
2 "	---	Undil.	---	0	---	0	444	60
3 "	---	Undil.	---	0	---	0	444	60
4 "	-44	1:1.5	---	4	-34	6	444	80
5 "	-44	1:1.5	---	4	-±2	6	444	80
6 "	---	Undil.	---	0	---	0	444	60
1 (12-6)	±44	1:2	-±4	8	-24	8	444	120
2 "	±44	1:2	---	4	-44	8	-44	10
3 "	±44	1:2	---	4	-14	8	444	40
4 "	-44	1:2	---	4	-14	0	444	200
5 "	444	1:10	---	10	-13	40	444	100
6 "	444	1:6	---	4	-24	24	444	40
7 "	444	1:3	-±4	12	-±4	12	444	40
8 "	±44	1:2	---	4	-24	8	444	120
1 (12-13)	444	1:3	---	4	--2	12	444	2
2 "	--±	Undil.	--1	0	--±	0	444	4
3 "	---	Undil.	---	0	---	0	444	80
4 "	-±4	Undil.	--2	1	-±4	1	444	200
5 "	244	1:2.5	---	4	--4	10	444	140
6 "	444	1:5	---	4	-±4	20	444	40
7 "	-±4	Undil.	--1	0	-±4	1	224	3
8 "	---	Undil.	---	0	---	0	±44	3
1 (12-14)	---	Undil.	---	0	---	0	-24	2
2 "	---	Undil.	---	0	---	0	444	[illegible]
3 "	-±4	Undil.	--±	0	-±4	1	444	20
4 "	---	Undil.	---	0	---	0	44±	3
5 "	--±	Undil.	---	0	--±	0	444	80
6 "	--±	Undil.	---	0	--±	0	444	20
7 "	--4	Undil.	---	0	--4	1	444	40
8 "	244	1:2.5	---	4	-±3	10	444	80
1 (12-15)	244	1:2	--4	8	-24	8	444	20
2 "	444	1:3	--4	12	--4	12	444	20
3 "	--4	Undil.	--2	1	--4	1	444	60
4 "	±44	1:2	---	4	--1	4	444	40
5 "	-44	1:3	---	4	-±4	6	444	[illegible]
6 "	-±4	Undil.	-±3	1	-±4	1	444	[illegible]
1 (1-4)	32±	Undil.	3--	1	32±	2	444	160
2 "	-24	Undil.	--4	1	-24	2	444	80
3 "	-44	1:2	---	4	-±4	8	444	[illegible]
4 "	±44	1:2	---	4	-14	8	444	120
5 "	-24	Undil.	--4	1	-24	2	444	[illegible]
6 "	--4	Undil.	--4	1	[illegible]	1	[illegible]	[illegible]
7 "	-±4	Undil.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
8 "	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1 (1-5)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2 "	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
3 "	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

APPENDIX A (CONT'D)

Serum Source, Number, Date Examined 1941	Standard Kahn Reaction Tube 123	Dilution of Serum with 0.9% Saline	Reactions at: 37°C. Tube 123	37°C. Quant. Titer	Room Temperature Tube 123	Room Temperature Quant. Titer	1°C. Tube 123	1°C. Quant. Titer
(Chicken)								
1 (1-11)	--±	Undil.	---	0	--±	0	--4	1
2 "	-24	Undil.	--4	1	-24	2	444	20
3 "	--4	Undil.	--2	1	--4	1	444	20
4 "	-±4	Undil.	--3	1	-±4	1	444	20
5 "	-44	1:2.5	--2	10	--4	10	444	80
6 "	±44	1:2	--±	4	-13	8	444	40
7 "	444	1:3	--4	12	-34	12	444	40
8 "	344	1:3	--4	12	--4	12	444	60
Cow								
1 (10-14)	-44	1:2	--1	4	-±4	8	444	4
2 "	444	1:10	-±4	40	-±3	40	-±4	120
3 "	444	1:10	-±±	30	-±4	40	444	180
4 "	444	1:2.5	±24	10	-34	10	444	80
5 "	-44	1:2	---	4	--2	8	444	120
6 "	444	1:2.5	--3	10	-24	10	±44	40
1 (11-15)	---	Undil.	---	0	---	0	±34	2
2 "	±24	Undil.	-±2	1	±24	2	244	3
3 "	444	1:10	-±±	30	--3	40	-44	100
4 "	444	1:2.5	-13	10	-24	10	344	40
5 "	444	1:5	--±	10	--4	20	-44	40
6 "	-44	1:2	--±	4	-13	8	-44	20
1 (11-19)	-±2	Undil.	---	0	-±2	1	-±4	1
2 "	444	1:12	---	20	--4	48	-44	180
3 "	-±2	Undil.	--±	0	-±2	1	--2	1
4 "	-±2	Undil.	--2	1	-±2	1	-24	40
5 "	444	1:15	---	40	±33	60	-33	60
6 "	-±4	Undil.	--2	1	-±4	1	-44	3
1 (12-2)	444	1:25	---	40	--4	100	±44	300
2 "	444	1:10	---	4	--2	40	-44	240
3 "	-±1	Undil.	---	0	-±1	0	±11	40
4 "	444	1:40	---	90	--3	120	-44	440
5 "	-44	1:+.5	---	4	--2	6	±22	140
6 "	--±	Undil.	---	0	--±	0	---	0
7 "	444	1:20	---	40	--4	80	-44	180
8 "	444	1:5	---	4	-±4	20	-44	80
1 (12-6)	444	1:5	--4	20	±14	20	±44	200
2 "	444	1:3	--1	6	±±4	12	444	40
3 "	±23	Undil.	--3	1	±23	2	±44	3
4 "	444	1:10	--±	40	-14	40	134	220
5 "	144	1:2	--4	8	-14	8	244	20
6 "	--2	Undil.	---	0	--2	1	-22	1
7 "	444	1:6	--±	20	-±4	24	444	240+
8 "	±44	1:+.5	---	4	-34	6	244	20
1 (1-2)	±24	Undil.	-44	3	-24	2	444	220
2 "	444	1:8	--3	32	-±3	40	±44	120
3 "	444	1:4	-±4	20	-14	20	144	40
4 "	444	1:3	-12	12	--4	12	444	120
5 "	±44	1:2	--±	4	-±4	8	444	40
6 "	444	1:8	-±1	20	-±4	32	444	40
7 "	444	1:12	--3	48	-±4	48	±44	80
8 "	444	1:12	---	20	--1	24	-44	160
1 (1-3)	444	1:6	-13	24	-24	24	±44	140
2 "	444	1:12	-±4	48	-±4	48	444	80
3 "	444	1:25	--3	100	-±4	100	444	120
4 "	-44	1:2	--4	8	-±4	8	444	20
5 "	444	1:4	---	4	--3	12	±44	110
6 "	444	1:8	--3	40	-24	40	444	120
7 "	±44	1:13	---	20	-±1	52	±24	80
8 "	444	1:10	-±2	40	--4	40	±44	140
1 (1-8)	±44	1:4	---	4	-±2	8	-14	40
2 "	444	1:22	---	40	-±3	88	444	200
3 "	444	1:22	--3	88	-14	88	444	160
4 "	444	1:7	---	20	-±4	28	±44	40
5 "	444	1:4	---	4	-±4	16	±44	40
6 "	444	1:2.5	-±3	10	-14	10	244	80
1 (1-9)	±44	1:4	---	4	-±3	12	-14	80
2 "	444	1:13	---	30	±±3	52	-24	120
3 "	444	1:9	---	10	-±4	36	±44	100
4 "	444	1:30	---	60	-±2	120	-±4	160
5 "	444	1:13	--±	30	-±4	52	-44	160
6 "	444	1:15	---	40	-±2	60	±34	120
Horse								
1 (12-11)	444	1:15	--3	60	-±4	60	±44	80
2 "	444	1:7	-±2	40	±14	40	244	60
3 "	-24	Undil.	---	0	-24	2	444	20
4 "	--±	Undil.	---	0	--±	0	444	4
5 "	-44	1+.5	---	0	-44	6	444	40
6 "	±44	1+.5	---	0	-±2	6	444	20
1 (12-12)	3±-	Undil.	---	0	3±-	1	444	80
2 "	±24	Undil.	---	0	±24	2	444	4
3 "	444	1:4	---	4	-±2	8	444	100
4 "	444	1:2.5	---	4	-±4	10	444	40
5 "	-44	1:2	---	4	-±3	8	444	40
6 "	444	1:4	---	4	±22	16	444	40
1 (12-16)	444	1:12	--4	48	-34	48	444	120
2 "	---	Undil.	---	0	---	0	444	10
3 "	444	1:10	---	20	-±3	40	234	40
4 "	444	1:3	--4	4	--4	12	444	20
5 "	144	1:2.5	---	4	--2	10	444	[illegible]
6 "	144	1:2.5	--3	10	-±4	10	444	[illegible]
1 (12-17)	444	1:2	---	4	-±4	8	444	[illegible]
2 "	444	1:2.5	---	4	--±	5	444	[illegible]
1 (12-26)	444	1:7	---	4	-34	28	444	[illegible]
2 "	444	1:5	---	4	-±4	20	444	[illegible]
3 "	-44	1:2.5	---	4	--±	5	444	[illegible]
4 "	[illegible]	Undil.	--3	1	-±4	1	444	[illegible]
5 "	-34	1:2.5	[illegible]	[illegible]	-12	[illegible]	[illegible]	[illegible]

APPENDIX A (CONT'D)

Reactions at Room Temperature

Test No.	Time, Hrs.	Temp. °C	Pressure, psig	Feed, Moles	Product, Moles	Conversion, Mole %	Selectivity, Mole %
CT-2-46	0	25	0	0.0	0.0	0.0	0.0
	1	25	0	0.0	0.0	0.0	0.0
	2	25	0	0.0	0.0	0.0	0.0
	3	25	0	0.0	0.0	0.0	0.0
	4	25	0	0.0	0.0	0.0	0.0
	5	25	0	0.0	0.0	0.0	0.0
	6	25	0	0.0	0.0	0.0	0.0
	7	25	0	0.0	0.0	0.0	0.0
	8	25	0	0.0	0.0	0.0	0.0
	9	25	0	0.0	0.0	0.0	0.0
	10	25	0	0.0	0.0	0.0	0.0
	11	25	0	0.0	0.0	0.0	0.0
	12	25	0	0.0	0.0	0.0	0.0
	13	25	0	0.0	0.0	0.0	0.0
	14	25	0	0.0	0.0	0.0	0.0
	15	25	0	0.0	0.0	0.0	0.0
	16	25	0	0.0	0.0	0.0	0.0
	17	25	0	0.0	0.0	0.0	0.0
	18	25	0	0.0	0.0	0.0	0.0
	19	25	0	0.0	0.0	0.0	0.0
	20	25	0	0.0	0.0	0.0	0.0
	21	25	0	0.0	0.0	0.0	0.0
	22	25	0	0.0	0.0	0.0	0.0
	23	25	0	0.0	0.0	0.0	0.0
	24	25	0	0.0	0.0	0.0	0.0
CT-2-47	0	25	0	0.0	0.0	0.0	0.0
	1	25	0	0.0	0.0	0.0	0.0
	2	25	0	0.0	0.0	0.0	0.0
	3	25	0	0.0	0.0	0.0	0.0
	4	25	0	0.0	0.0	0.0	0.0
	5	25	0	0.0	0.0	0.0	0.0
	6	25	0	0.0	0.0	0.0	0.0
	7	25	0	0.0	0.0	0.0	0.0
	8	25	0	0.0	0.0	0.0	0.0
	9	25	0	0.0	0.0	0.0	0.0
	10	25	0	0.0	0.0	0.0	0.0
	11	25	0	0.0	0.0	0.0	0.0
	12	25	0	0.0	0.0	0.0	0.0
	13	25	0	0.0	0.0	0.0	0.0
	14	25	0	0.0	0.0	0.0	0.0
	15	25	0	0.0	0.0	0.0	0.0
	16	25	0	0.0	0.0	0.0	0.0
	17	25	0	0.0	0.0	0.0	0.0
	18	25	0	0.0	0.0	0.0	0.0
	19	25	0	0.0	0.0	0.0	0.0
	20	25	0	0.0	0.0	0.0	0.0
	21	25	0	0.0	0.0	0.0	0.0
	22	25	0	0.0	0.0	0.0	0.0
	23	25	0	0.0	0.0	0.0	0.0
	24	25	0	0.0	0.0	0.0	0.0
CT-2-48	0	25	0	0.0	0.0	0.0	0.0
	1	25	0	0.0	0.0	0.0	0.0
	2	25	0	0.0	0.0	0.0	0.0
	3	25	0	0.0	0.0	0.0	0.0
	4	25	0	0.0	0.0	0.0	0.0
	5	25	0	0.0	0.0	0.0	0.0
	6	25	0	0.0	0.0	0.0	0.0
	7	25	0	0.0	0.0	0.0	0.0
	8	25	0	0.0	0.0	0.0	0.0
	9	25	0	0.0	0.0	0.0	0.0
	10	25	0	0.0	0.0	0.0	0.0
	11	25	0	0.0	0.0	0.0	0.0
	12	25	0	0.0	0.0	0.0	0.0
	13	25	0	0.0	0.0	0.0	0.0
	14	25	0	0.0	0.0	0.0	0.0
	15	25	0	0.0	0.0	0.0	0.0
	16	25	0	0.0	0.0	0.0	0.0
	17	25	0	0.0	0.0	0.0	0.0
	18	25	0	0.0	0.0	0.0	0.0
	19	25	0	0.0	0.0	0.0	0.0
	20	25	0	0.0	0.0	0.0	0.0
	21	25	0	0.0	0.0	0.0	0.0
	22	25	0	0.0	0.0	0.0	0.0
	23	25	0	0.0	0.0	0.0	0.0
	24	25	0	0.0	0.0	0.0	0.0
CT-2-49	0	25	0	0.0	0.0	0.0	0.0
	1	25	0	0.0	0.0	0.0	0.0
	2	25	0	0.0	0.0	0.0	0.0
	3	25	0	0.0	0.0	0.0	0.0
	4	25	0	0.0	0.0	0.0	0.0
	5	25	0	0.0	0.0	0.0	0.0
	6	25	0	0.0	0.0	0.0	0.0
	7	25	0	0.0	0.0	0.0	0.0
	8	25	0	0.0	0.0	0.0	0.0
	9	25	0	0.0	0.0	0.0	0.0
	10	25	0	0.0	0.0	0.0	0.0
	11	25	0	0.0	0.0	0.0	0.0
	12	25	0	0.0	0.0	0.0	0.0
	13	25	0	0.0	0.0	0.0	0.0
	14	25	0	0.0	0.0	0.0	0.0
	15	25	0	0.0	0.0	0.0	0.0
	16	25	0	0.0	0.0	0.0	0.0
	17	25	0	0.0	0.0	0.0	0.0
	18	25	0	0.0	0.0	0.0	0.0
	19	25	0	0.0	0.0	0.0	0.0
	20	25	0	0.0	0.0	0.0	0.0
	21	25	0	0.0	0.0	0.0	0.0
	22	25	0	0.0	0.0	0.0	0.0
	23	25	0	0.0	0.0	0.0	0.0
	24	25	0	0.0	0.0	0.0	0.0
CT-2-50	0	25	0	0.0	0.0	0.0	0.0
	1	25	0	0.0	0.0	0.0	0.0
	2	25	0	0.0	0.0	0.0	0.0
	3	25	0	0.0	0.0	0.0	0.0
	4	25	0	0.0	0.0	0.0	0.0
	5	25	0	0.0	0.0	0.0	0.0
	6	25	0	0.0	0.0	0.0	0.0
	7	25	0	0.0	0.0	0.0	0.0
	8	25	0	0.0	0.0	0.0	0.0
	9	25	0	0.0	0.0	0.0	0.0
	10	25	0	0.0	0.0	0.0	0.0
	11	25	0	0.0	0.0	0.0	0.0
	12	25	0	0.0	0.0	0.0	0.0
	13	25	0	0.0	0.0	0.0	0.0
	14	25	0	0.0	0.0	0.0	0.0
	15	25	0	0.0	0.0	0.0	0.0
	16	25	0	0.0	0.0	0.0	0.0
	17	25	0	0.0	0.0	0.0	0.0
	18	25	0	0.0	0.0	0.0	0.0
	19	25	0	0.0	0.0	0.0	0.0
	20	25	0	0.0	0.0	0.0	0.0
	21	25	0	0.0	0.0	0.0	0.0
	22	25	0	0.0	0.0	0.0	0.0
	23	25	0	0.0	0.0	0.0	0.0
	24	25	0	0.0	0.0	0.0	0.0

Reactions at Room Temperature

Test No.	Time, Hrs.	Temp. °C	Pressure, psig	Feed, Moles	Product, Moles	Conversion, Mole %	Selectivity, Mole %
CT-2-51	0	25	0	0.0	0.0	0.0	0.0
	1	25	0	0.0	0.0	0.0	0.0
	2	25	0	0.0	0.0	0.0	0.0
	3	25	0	0.0	0.0	0.0	0.0
	4	25	0	0.0	0.0	0.0	0.0
	5	25	0	0.0	0.0	0.0	0.0
	6	25	0	0.0	0.0	0.0	0.0
	7	25	0	0.0	0.0	0.0	0.0
	8	25	0	0.0	0.0	0.0	0.0
	9	25	0	0.0	0.0	0.0	0.0
	10	25	0	0.0	0.0	0.0	0.0
	11	25	0	0.0	0.0	0.0	0.0
	12	25	0	0.0	0.0	0.0	0.0
	13	25	0	0.0	0.0	0.0	0.0
	14	25	0	0.0	0.0	0.0	0.0
	15	25	0	0.0	0.0	0.0	0.0
	16	25	0	0.0	0.0	0.0	0.0
	17	25	0	0.0	0.0	0.0	0.0
	18	25	0	0.0	0.0	0.0	0.0
	19	25	0	0.0	0.0	0.0	0.0
	20	25	0	0.0	0.0	0.0	0.0
	21	25	0	0.0	0.0	0.0	0.0
	22	25	0	0.0	0.0	0.0	0.0
	23	25	0	0.0	0.0	0.0	0.0
	24	25	0	0.0	0.0	0.0	0.0
CT-2-52	0	25	0	0.0	0.0	0.0	0.0
	1	25	0	0.0	0.0	0.0	0.0
	2	25	0	0.0	0.0	0.0	0.0
	3	25	0	0.0	0.0	0.0	0.0
	4	25	0	0.0	0.0	0.0	0.0
	5	25	0	0.0	0.0	0.0	0.0
	6	25	0	0.0	0.0	0.0	0.0
	7	25	0	0.0	0.0	0.0	0.0
	8	25	0	0.0	0.0	0.0	0.0
	9	25	0	0.0	0.0	0.0	0.0
	10	25	0	0.0	0.0	0.0	0.0
	11	25	0	0.0	0.0	0.0	0.0
	12	25	0	0.0	0.0	0.0	0.0
	13	25	0	0.0	0.0	0.0	0.0
	14	25	0	0.0	0.0	0.0	0.0
	15	25	0	0.0	0.0	0.0	0.0
	16	25	0	0.0	0.0	0.0	0.0
	17	25	0	0.0	0.0	0.0	0.0
	18	25	0	0.0	0.0	0.0	0.0
	19	25	0	0.0	0.0	0.0	0.0
	20	25	0	0.0	0.0	0.0	0.0
	21	25	0	0.0	0.0	0.0	0.0
	22	25	0	0.0	0.0	0.0	0.0
	23	25	0	0.0	0.0	0.0	0.0
	24	25	0	0.0	0.0	0.0	0.0
CT-2-53	0	25	0	0.0	0.0	0.0	0.0
	1	25	0	0.0	0.0	0.0	0.0
	2	25	0	0.0	0.0	0.0	0.0
	3	25	0	0.0	0.0	0.0	0.0
	4	25	0	0.0	0.0	0.0	0.0
	5	25	0	0.0	0.0	0.0	0.0
	6	25	0	0.0	0.0	0.0	0.0
	7	25	0	0.0	0.0	0.0	0.0
	8	25	0	0.0	0.0	0.0	0.0
	9	25	0	0.0	0.0	0.0	0.0
	10	25	0	0.0	0.0	0.0	0.0
	11	25	0	0.0	0.0	0.0	0.0
	12	25	0	0.0	0.0	0.0	0.0
	13	25	0	0.0	0.0	0.0	0.0
	14	25	0	0.0	0.0	0.0	0.0
	15	25	0	0.0	0.0	0.0	0.0
	16	25	0	0.0	0.0	0.0	0.0
	17	25	0	0.0	0.0	0.0	0.0
	18	25	0	0.0	0.0	0.0	0.0
	19	25	0	0.0	0.0	0.0	0.0
	20	25	0	0.0	0.0	0.0	0.0
	21	25	0	0.0	0.0	0.0	0.0
	22	25	0	0.0	0.0	0.0	0.0
	23	25	0	0.0	0.0	0.0	0.0
	24	25	0	0.0	0.0	0.0	0.0
CT-2-54	0	25	0	0.0	0.0	0.0	0.0
	1	25	0	0.0	0.0	0.0	0.0
	2	25	0	0.0	0.0	0.0	0.0
	3	25	0	0.0	0.0	0.0	0.0
	4	25	0	0.0	0.0	0.0	0.0
	5	25	0	0.0	0.0	0.0	0.0
	6	25	0	0.0	0.0	0.0	0.0
	7	25	0	0.0	0.0	0.0	0.0
	8	25	0	0.0	0.0	0.0	0.0
	9	25	0	0.0	0.0	0.0	0.0
	10	25	0	0.0	0.0	0.0	0.0
	11	25	0	0.0	0.0	0.0	0.0
	12	25	0	0.0	0.0	0.0	0.0
	13	25	0	0.0	0.0	0.0	0.0
	14	25	0	0.0	0.0	0.0	0.0
	15	25	0	0.0	0.0	0.0	0.0
	16	25	0	0.0	0.0	0.0	0.0
	17	25	0	0.0	0.0	0.0	0.0
	18	25	0	0.0	0.0	0.0	0.0
	19	25	0	0.0	0.0	0.0	0.0
	20	25	0	0.0	0.0	0.0	0.0
	21	25	0	0.0	0.0	0.0	0.0
	22	25	0	0.0	0.0	0.0	0.0
	23	25	0	0.0	0.0	0.0	0.0
	24	25	0	0.0	0.0	0.0	0.0

APPENDIX B

FLOCCULATION REACTIONS AND QUANTITATIVE TITERS AT DIVERGENT TEMPERATURES WITH UNHEATED AND CHLOROFORM-TREATED, HOMOLOGOUS RABBIT SERA

Serum number: [illegible]
Date examined: 1941

Tube standard reaction
Tube 24 hour reaction
Tube 48 hour reaction

37°C. Tube
Room temperature Tube

4°C. Tube

Chicken (10—15)
(10—17)
(10—18)
(10—20)
(11—22)
(11—7)
(11—16)
(12—6)
(12—13)
(12—14)
(12—15)
(1—2)
(1—9)
(1—3)

Enzyme Standard Dilution
Coupling: Reaction Setting
Reagent: Reaction Setting
1941

Code	Tube 123	Tube 123	37°C. Thaw Shake	Room Temperature Thaw Shake	37°C. Thaw Shake	Room Temperature Thaw Shake
A (10-14)						
(11-15)						
(12-20)						
(13)						
(22)						
(23)						
(1-2)						
B (13)						
(23)						
(6)						
Hose (11-14)						
(12-15)						
(16)						
(17-26)						
C (1-2)						

APPENDIX B (CONT'D)

Serum source: Standard Kahn Reaction
Number tested: 100
Examined 1941 Petroleum ether-5% Saline

Reactions at: 37°C. Tube Quant. Ether — Room Temperature Tube Quant. Ether

Tube	1	2	3	4	5	6	7	8	9	10	11	12
(1)	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
(2)	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
(3)	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
(4)	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
(5)	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
(6)	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
(7)	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
(8)	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
(9)	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
(10)	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
(11)	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
(12)	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

Results of Examination — Tabulated as presented

APPENDIX C

FLOCCULATION REACTIONS AT DIFFERENT TEMPERATURES WITH GLOBULIN FRACTIONS OF HEATED (H) AND UNHEATED (U) CHICKEN, COW, HORSE, PIG AND RABBIT SERA

Serum Source and Number	(H) or (U)	Kahn Reaction with Serum	Kahn Reaction with Globulin Fraction	Dilution of Globulin Fraction with 0.9% Saline	Reactions with Globulin Fractions at: 37°C.		Room Temperature		1°C.	
		Tube 123	Tube 123		Tube 123	Quant. Titer	Tube 123	Quant. Titer	Tube 123	Quant. Titer
Chicken										
1 (10-15)	H	144	-12	Undil.	-12	1	-12	1	±12	40
	U	±44	-12	Undil.	-12	1	-12	1	±±2	30
2 (10-15)	H	344	-11	Undil.	--±	0	-11	1	-1±	0
	U	---	-22	Undil.	-±1	0	-12	1	-11	1
3 (10-15)	H	±44	--1	Undil.	--2	1	--1	0	±22	40
	U	---	-12	Undil.	-±3	1	-12	1	±23	30
6 (10-15)	H	-14	--2	Undil.	-22	1	--2	1	--2	30
	U	±44	--2	Undil.	--1	0	--2	1	--3	40
1 (10-17)	H	421	---	Undil.	-12	1	---	0	-±-	30
	U	±11	---	Undil.	-21	1	---	0	-1±	40
3 (10-17)	H	---	--3	Undil.	±±3	1	--3	1	±-2	60
	U	---	-13	Undil.	124	2	-13	1	±11	60
4 (10-17)	H	---	---	Undil.	±11	1	---	0	-±-	0
	U	±14	---	Undil.	-11	1	---	0	-±2	10
6 (10-17)	H	-44	--±	Undil.	--±	0	--±	0	--2	40
	U	144	--±	Undil.	-±3	1	--±	0	--3	30
1 (10-18)	H	---	---	Undil.	---	20	---	0	---	40
	U	-±4	---	Undil.	---	0	---	0	---	60
3 (10-18)	H	---	---	Undil.	---	0	---	0	---	0
	U	---	--±	Undil.	---	0	--±	0	---	20
4 (10-18)	H	---	---	Undil.	---	0	---	0	---	0
	U	---	---	Undil.	---	0	---	0	---	40
7 (10-18)	H	---	---	Undil.	---	0	---	0	---	0
	U	---	---	Undil.	---	0	---	0	---	0
1 (10-26)	H	±21	-13	Undil.	--2	1	-13	1	--±	60
	U	±44	-13	Undil.	-±4	1	-13	1	-±±	120
2 (10-26)	H	-14	--1	Undil.	---	0	--1	0	--±	40
	U	-44	---	Undil.	---	0	---	0	-±±	40
5 (10-26)	H	±4±	---	Undil.	-±3	1	---	0	-±+	40
	U	444	---	Undil.	--±	0	---	0	--±	40
1 (11-2)	H	-44	---	Undil.	---	0	---	0	-12	20
	U	444	---	Undil.	---	0	---	0	±±2	10
5 (11-2)	H	--1	---	Undil.	--±	0	---	0	---	0
	U	-±3	---	Undil.	--±	0	---	0	---	0
7 (11-2)	H	444	-22	Undil.	-34	2	-22	1	±11	20
	U	444	-11	Undil.	-44	2	-11	1	-±3	30
8 (11-2)	H	-±4	-±+	Undil.	---	0	-±+	0	-±+	20
	U	344	-1±	Undil.	---	0	-1±	0	±±-	0
1 (11-7)	H	344	---	Undil.	---	0	---		---	80
	U	444	---	Undil.	---	0	---		---	80
2 (11-7)	H	-44	---	Undil.	--1	0	---		--±	80
	U	444	---	Undil.	--±	0	---		---	120
3 (11-7)	H	±44	---	Undil.	---	0	---		---	40
	U	444	---	Undil.	-±1	0	---		---	40
4 (11-7)	H	-±4	---	Undil.	---	0	---		---	20
	U	-44	---	Undil.	---	0	---		---	40
6 (11-7)	H	144	---	Undil.	---	20	---		---	120
	U	444	---	Undil.	---	20	---		---	100

Serum Source and Number	(H) or (U)	Kahn Reaction with Serum Tube 123	Kahn Reaction with Globulin Fraction Tube 123	Dilution of Globulin Fraction with 0.9% Saline	Reactions with Globulin Fractions at 37°C. Tube 123	37°C. Quant. Titer	Room Temperature Tube 123	Room Temperature Quant. Titer	1°C. Tube 123	1°C. Quant. Titer
(Chicken)										
1 (12-6)	H	±44	−±2	Undil.	−12	1	−±2		−22	40
	U	±44	−−2	Undil.	−−±	0	−−2		−21	30
3 (12-6)	H	±44	−±3	Undil.	−±3	1	−±3	1	−22	1
	U	−44	±±3	Undil.	−13	1	±±3	1	−21	10
6 (12-13)	H	444	---	Undil.	−−±	0	---	0	±12	120
	U	444	---	Undil.	−−±	0	---	0	±11	120
Cow										
1 (10-14)	H	−44	144	1:2	−24	20	±24	8	444	60
	U	−44	−±4	Undil.	−44	10	−±4	1	444	20
2 (10-14)	H	444	444	1:10	−±1	30	−±3	40	±24	80
	U	444	444	1:10	---	20	−−1	30	±14	40
3 (10-14)	H	444	444	1:10	−24	40	±14	40	444	100
	U	444	±44	1:10	−±−	20	±14	40	444	120
4 (10-14)	H	444	444	1:3.5	244	30	±44	14	444	120
	U	444	444	1:3.5	244	30	±44	14	444	60
5 (10-14)	H	−44	±44	1:2	−±4	8	−±4	8	444	4
	U	−±1	−±4	Undil.	---	0	−±4	1	444	4
1 (11-15)	H	---	---	Undil.	---	0	---	0	±23	10
	U	---	---	Undil.	---	0	---	0	±14	20
2 (11-15)	H	±24	---	Undil.	---	0	---	0	−±3	20
	U	---	---	Undil.	---	0	---	0	−−2	20
3 (11-15)	H	444	±44	Undil.	−44	3	±44	3	444	40
	U	---	±44	Undil.	−44	3	±44	3	444	40
4 (11-15)	H	444	−44	Undil.	−44	3	−44	3	−44	60
	U	−24	−44	Undil.	−24	2	−44	3	−44	40
6 (11-15)	H	−44	−−±	Undil.	−−2	1	−−±	0	−44	20
	U	---	−−±	Undil.	−−2	1	−−±	0	−44	30
1 (11-20)	H	−±2	−−2	Undil.	---	0	−−2	1	±44	3
	U	1±±	−−3	Undil.	---	0	−−3	1	±44	3
2 (11-20)	H	444	−−4	Undil.	−−3	1	−−4	1	±44	240+
	U	244	−−4	Undil.	---	0	−−4	1	444	240+
4 (11-20)	H	−±2	−±4	Undil.	---	0	−±4	1	±44	20
	U	---	−±4	Undil.	−−4	1	−±4	1	±44	20
6 (11-20)	H	−±4	−−2	Undil.	−−2	1	−−2	1	±44	3
	U	---	---	Undil.	−±−	0	---	0	444	4
1 (12-3)	H	444	444	1:12	−−2	80	−±3	48	−−4	160
	U	±44	444	1:12	−−4	80	−−4	48	−−4	120
2 (12-3)	H	444	−−1	1:15	−−4	40	−−2	60	−24	40
	U	−44	−−±	1:5	−−4	40	−−1	10	−44	40
3 (12-3)	H	−±1	−44	Undil.	±14	2	−44	3	244	40
	U	−−1	±43	Undil.	−−4	1	±43	2	±44	40
4 (12-3)	H	444	144	1:5	−±4	20	−±3	20	244	80
	U	444	144	1:5	−±4	20	−±3	20	±44	120
6 (12-3)	H	−−±	---	Undil.	---	0	---	0	---	0
	U	---	---	Undil.	---	0	---	0	---	0
1 (12-6)	H	444	±44	1:2	−±4	8	−±4	8	444	160
	U	144	−44	1:2	−14	8	−±4	8	444	100
2 (12-6)	H	444	---	Undil.	−−2	1	---	0	244	80
	U	244	---	Undil.	---	0	---	0	444	40
3 (12-6)	H	±23	−±4	Undil.	−−3	1	−±4	1	±34	2
	U	−−±	−−4	Undil.	---	0	−−4	1	−±4	1

Serum Source and Number	(H) or (U)	Kahn Reaction with Serum Tube 123	Kahn Reaction with Globulin Fraction Tube 123	Dilution of Globulin Fraction with 0.9% Saline	Reactions with Globulin Fractions at: 37°C. Tube 123	37°C. Quant. Titer	Room Temperature Tube 123	Room Temperature Quant. Titer	1°C. Tube 123	1°C. Quant. Titer
(Cow)										
1 (1-2)	H	±24	-++	Undil.	--1	0	-±+	0	±44	80
	U	---	-±3	Undil.	-±±	0	-±3	1	±44	100
2 (1-2)	H	444	±+1	Undil.	-±4	1	±+1	0	444	10
	U	---	-±2	Undil.	--±	0	-±2	1	444	4
1 (1-3)	H	444	±+1	Undil.	--2	1	±+1	0	444	40
	U	--1	-±3	Undil.	--±	0	-±3	1	444	60
2 (1-3)	H	444	±-+	Undil.	±11	1	±-+	0	444	10
	U	±44	-±1	Undil.	-±±	0	-±1	0	444	20
3 (1-3)	H	444	444	1:10	--3	40	-24	40	444	100
	U	±44	444	1:10	-44	40	-44	40	444	80
Horse										
1 (12-11)	H	444	444	1:10	---	20	--3	40	-±4	80
	U	444	444	1:4	--±	4	-22	16	±44	60
2 (12-11)	H	444	±44	1:2.5	---	0	-±4	10	-44	30
	U	444	444	1:2.5	--4	1	-±4	10	244	40
3 (12-11)	H	-24	---	Undil.	---	0	---	0	--4	10
	U	444	--4	Undil.	--±	0	--4	1	-44	20
4 (12-11)	H	--±	-±4	Undil.	---	0	-±4	1	344	20
	U	21-	±32	Undil.	---	0	±32	2	444	10
5 (12-11)	H	-44	--4	Undil.	--4	1	--4	1	-44	30
	U	444	-+4	Undil.	--4	1	-±4	1	-44	40
6 (12-11)	H	±44	--±	Undil.	---	0	--±	0	-44	3
	U	444	---	Undil.	---	0	---	0	-24	2
1 (12-12)	H	3±-	---	Undil.	---	0	---	0	---	0
	U	---	---	Undil.	---	0	---	0	--±	0
2 (12-12)	H	±24	---	Undil.	---	0	---	0	±14	2
	U	---	---	Undil.	---	0	---	0	±22	1
3 (12-12)	H	444	±44							
	U	444	±44	1:2	---		--2	4	444	40
				1:2	---		-±1	4	444	20
4 (12-12)	H	444	--±	Undil.	---	0	--±	0	134	3
	U	444	---	Undil.	---	0	---	0	-±4	1
5 (12-12)	H	-44	±44	1:2	---		-±3	8	±14	8
	U	444	±+4	1:2	---		--1	4	-±4	8
6 (12-12)	H	444	±34	Undil.	--2	1	±34	2	444	20
	U	444	±12	Undil.	---	0	±12	1	444	10
1 (12-16)	H	444	±44	1:2	---		-13	8	-44	100
	U	444	244	1:2	--±		--4	8	±44	40
2 (12-16)	H	---	---	Undil.	---	0	---	0	--4	1
	U	--±	--±	Undil.	---	0	--±	0	--3	1
3 (12-16)	H	444	-44	1:2	---		--4	8	±+4	40
	U	444	-44	1:2	---		-±4	8	±24	30
4 (12-16)	H	444	±44	1:2	---		-±4	8	-44	20
	U	444	-44	1:2	---		-±4	8	-24	20
5 (12-16)	H	144	--1	Undil.	---	0	--1	0	-44	40
	U	-44	--±	Undil.	---	0	--±	0	-44	40
6 (12-16)	H	144	--2	Undil.	---	0	--2	1	244	20
	U	444	--±	Undil.	---	0	--±	0	--4	1
1 (12-17)	H	444	±+4	Undil.	--4	1	±+4	1	±44	20
	U	444	-±4	Undil.	--4	1	-±4	1	±44	20

Serum Source and Number	(H) or (U)	Kahn Reaction with Serum Tube 123	Kahn Reaction with Globulin Fraction Tube 123	Dilution of Globulin Fraction with 0.9% Saline	Reactions with Globulin Fractions at: 37°C. Tube 123	37°C. Quant. Titer	Room Temperature Tube 123	Room Temperature Quant. Titer	1°C. Tube 123	1°C. Quant. Titer
(Horse)										
5 (12-11)	H	-44	--4	Undil.	--4	1	--4	1	-44	30
	U	444	-±4	Undil.	--4	1	-±4	1	-44	40
6 (12-11)	H	±44	--±	Undil.	---	0	--±	0	-44	3
	U	444	---	Undil.	---	0	---	0	-24	2
1 (12-12)	H	3±-	---	Undil.	---	0	---	0	---	0
	U	---	---	Undil.	---	0	---	0	--±	0
2 (12-12)	H	±24	---	Undil.	---	0	---	0	±14	2
	U	---	---	Undil.	---	0	---	0	±22	1
3 (12-12)	H	444	±44	1:2	---		--2	4	444	40
	U	444	±44	1:2	---		-±1	4	444	20
4 (12-12	H	444	--±	Undil.	---	0	--±	0	134	3
	U	444	---	Undil.	---	0	---	0	-±4	1
5 (12-12)	H	-44	±44	1:2	---		-±3	8	±14	8
	U	444	±±4	1:2	---		--1	4	-±4	8
6 (12-12)	H	444	±34	Undil.	--2	1	±34	2	444	20
	U	444	±12	Undil.	---	0	±12	1	444	10
1 (12-16)	H	444	±44	1:2	---		-13	8	-44	100
	U	444	244	1:2	--±		--4	8	±44	40
2 (12-16)	H	---	---	Undil.	---	0	---	0	--4	1
	U	--±	--±	Undil.	---	0	--±	0	--3	1
3 (12-16)	H	444	-44	1:2	---		--4	8	±±4	40
	U	444	-44	1:2	---		-±4	8	±24	30
4 (12-16)	H	444	±44	1:2	---		-±4	8	-44	20
	U	444	-44	1:2	---		-±4	8	-24	20
5 (12-16)	H	144	--1	Undil.	---	0	--1	0	-44	40
	U	-44	--±	Undil.	---	0	--±	0	-44	40
6 (12-16)	H	144	--2	Undil.	---	0	--2	1	244	20
	U	444	--±	Undil.	---	0	--±	0	--4	1
1 (12-17)	H	444	±±4	Undil.	--4	1	±±4	1	±44	20
	U	444	-±4	Undil.	--4	1	-±4	1	±44	20
2 (12-17)	H	444	--4	Undil.	--4	1	--4	1	±44	3
	U	444	--4	Undil.	--4	1	--4	1	444	4
Pig										
1 (10-1)	H	444	±44	1:12	--4	48	-±4	48	±44	160
	U	--2	±44	1:12	--4	48	-±4	48	±14	120
2 (10-1)	H	---	±±-	Undil.	±2-	1	±±-	0	244	10
	U	---	-±±	Undil.	±2±	1	-±±	0	±11	1
3 (10-1)	H	444	-±4	1:25	--2	100	-±4	100	±24	160
	U	-±3	-±4	1:25	--2	100	-±4	100	±14	140
5 (10-1)	H	21-	12±	Undil.	-±-	0	12±	1	444	60
	U	---	222	Undil.	---	0	222	2	444	100
7 (10-1)	H	444	±±4	1:10	--4	40	-±4	40	±44	180
	U	-±2	-±4	1:10	--4	40	-±4	40	±44	160
1 (9-26)	H	-±1	-±4	Undil.	--3	1	-14	2	444	60
	U	---	-±4	Undil.	-±2	1	-±4	1	444	30
2 (9-26)	H	444	-44	1:2.5	-44	10	-14	10	444	80
	U	--±	-44	1:2.5	-24	10	±34	10	444	120
1 (10-4)	H	444	--4	1:5	---		--2	20	-34	40
	U	---	--2	1:5	---		--±		--4	40
2 (10-4)	H	444	±±4	1:5	---		--1		--4	20
	U	444	±±4	1:5	---		--±		-24	20

Serum Source and Number	(H) or (U)	Kahn Reaction with Serum	Kahn Reaction with Globulin Fraction	Dilution of Globulin Fraction with 0.9% Saline	Reactions with Globulin Fractions at: 37°C.		Room Temperature		1°C.	
		Tube 123	Tube 123		Tube 123	Quant. Titer	Tube 123	Quant. Titer	Tube 123	Quant. Titer
(Pig)										
4 (10-4)	H	-44	-±3	Undil.	--2	1	-±3	1	±34	2
	U	---	--4	Undil.	--4	1	--4	1	±12	10
1 (10-10)	H	333	-44	1:2.5	--±		++3	10	±44	20
	U	---	-24	1:2.5	--2	10	-±3	10	444	20
2 (10-10)	H	444	244	1:2	++3	8	±44	8	444	40
	U	444	144	1:2	±14	8	±24	8	444	40
3 (10-10)	H	143	343	1:2	--1		++2	8	444	40
	U	---	±31	1:2	--±		-±3	8	444	40
6 (10-10)	H	444	---	Undil.	--±	0	---	0	±24	30
	U	---	---	Undil.	--±	0	---	0	±44	20
9 (10-10)	H	444	444	1:40	±14	160	-24	160	±44	160
	U	44±	444	1:30	++4	120	±14	120	±24	200
1 (10-29)	H	134	-±4	Undil.	--1	0	-±4	1	243	3
	U	--±	-24	Undil.	--2	1	-24	2	±44	3
2 (10-29)	H	444	±44	1:2	--±		-±4	8	±44	160
	U	---	±24	1:2	--±		--2	8	±44	200
5 (10-29)	H	444	±14	1:2	---		--1	4	±24	8
	U	-±3	±44	1:2	---		++4	8	++4	8
6 (10-29)	H	444	444	1:20	---	40	-±4	80	±44	220
	U	323	444	1:20	---	40	-±4	80	±44	260
1 (10-31)	H	±44	-33	1:2	---		--2	8	333	20
	U	---	±13	1:2	---		-±2	8	223	20
3 (10-31)	H	444	++4	Undil.	--4	1	++4	1	-14	20
	U	-±1	++4	Undil.	---	0	++4	1	-±4	20
5 (10-31)	H	444	--4	Undil.	---	0	--4	1	±44	20
	U	221	--4	Undil.	---	0	--4	1	±44	20
5 (11-3)	H	444	---	1:2.5	--2	10	---		-±4	10
	U	---	---	1:2.5	--±		---		--2	10
6 (11-3)	H	444	---	Undil.	---	0	---	0	--4	1
	U	---	---	Undil.	---	0	---	0	--±	0
2 (11-26)	H	132	---	Undil.	---	0	---	0	2±1	1
	U	---	---	Undil.	---	0	---	0	---	0
6 (11-26)	H	±44	-±4	Undil.	-±4	1	-±4	1	±33	20
	U	---	-±3	Undil.	--4	1	-±3	1	±32	20
Rabbit										
1 (12-30)	H	--4	--±	Undil.	--	0	--±	0	--1	0
2 "	H	--3	--1	Undil.	---	0	--1	0	-13	1
3 "	H	±44	---	Undil.	---	0	---	0	++4	1
4 "	H	144	--3	Undil.	---	0	--3	1	±22	20
5 "	H	-14	-14	Undil.	-±2	1	-14	2	±44	3
6 "	H	±44	--4	Undil.	-±4	1	--4	1	±24	10
7 "	H	-14	--2	Undil.	---	0	--2	1	--1	0
8 "	H	±24	--2	Undil.	---	0	--2	1	±14	2
9 "	H	-34	--1	Undil.	-±2	1	--1	0	±44	3
10 "	H	--1	---	Undil.	---	0	---	0	-±-	0
11 "	H	--±	--1	Undil.	---	0	--1	0	++3	1

Serum Source and Number	(H) or (U)	Kahn Reaction with Serum	Kahn Reaction with Globulin Fraction	Dilution of Globulin Fraction with 0.9% Saline	Reactions with Globulin Fractions at: 37°C.		Room Temperature		1°C.	
		Tube 123	Tube 123		Tube 123	Quant. Titer	Tube 123	Quant. Titer	Tube 123	Quant. Titer
(Rabbit)										
12 (12-30)	H	-±4	--1	Undil.	---	0	--1	0	-12	1
1 (12-31)	H	--2	---	Undil.	--±	0	---	0	±44	3
2 "	H	---	--1	Undil.	--1	0	--1	0	-±1	0
3 "	H	--±	---	Undil.	---	0	---	0	-±±	0
4 "	H	-±1	-34	1:2	--±		--3	8	344	8
5 "	H	-±4	-±2	Undil.	---	0	-±2	1	-44	3
6 "	H	--±	-44	1:2	--2	8	-±4	8	±44	10
7 "	H	444	-±3	Undil.	--2	1	-±3	1	±33	20
8 "	H	±44	--2	Undil.	--2	1	--2	1	-33	2
9 "	H	-14	---	Undil.	---	0	---	0	-44	3
10 "	H	---	--±	Undil.	-± ±	0	--±	0	-±3	1
11 "	H	-±1	---	Undil.	± ±-	0	---	0	-±1	0

APPENDIX D

Record of flocculation reactions (at different temperatures) and of Complement-Fixation reactions given by rabbits before and after inoculation with Spirochaeta pallida. The footnote for the serologic record of Rabbit 486 ♀ , (the first record shown), applies to the other rabbits examined as well.

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 486♀ Inoculated with *Spirochaeta pallida*

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Kahn Reactions (Room Temperature) Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	37°C. Quant. Titer	1°C. Tube 123	1°C. Quant. Titer	Complement-Fixation Reactions	Reaction I			Reaction II		Reaction III	
1941										S)[2] A) C)	a d 3	a d 2	b d 2	c e 2	c e 3	c e 2	c e 3
					Preinoculation Reactions												
10-11	57	H[1]	---		Undil.	---		---		T.)[3]	0	ac.	ac.	ac.	ac.	0	0
		U	---		Undil.	---		--2	1	SC.)							
10-23	69	H	--2	1	Undil.	---		144	3	T.)	4	ac.	4	4	3	–	–
		U	--4	1	Undil.	---		344	20	SC.)	–		2	–	–	–	–
11-10	87	H	244	8	1:2	--±		+14	8	T.)	4	0	4	4	±	±	–
		U	-44	8	1:2	---		±44	10	SC.)	–		–	–	–	–	–
12-2	109	H	--+		Undil.	---		-+4	1	T.)	3	0	3	ac.	–	1	–
		U	-44	3	1:2	---		244	8	SC.)	1		2		–	1	0
12-29	136	H	-14	2	Undil.	-+2		±44	3	T.)	1		4	2	–	–	–
		U	-44	3	1:2	---	1	±44	8	SC.)	–		–	–	–	–	–
	Days Post inoculation				1/7/42; Rabbit inoculated intradermally on back with 0.5 cc. of testicular suspension of *Spirochaeta pallida*												
1942																	
1-14	7	H	--+		Undil.	---		-+3	1	T.)	–	ac.	–	–	–	–	–
		U	-+3	1	Undil.	---		-44	3	SC.)							
1-26	19	H	-+1		Undil.	---		-+1		T.)	ac.	ac.	ac.	ac.	ac.	–	–
		U	-24	2	Undil.	---		444	4	SC.)							
2-7	31	H	444	20	1:5	-34	20	--2	20	T.)	4	4	4	4	2	4	+
		U	244	12	1:3	-44	12	-34	20	SC.)	–	±	–	–	–	–	–
3-3	55	H	444	48	1:12	±44	48	++1	40	T.)	3	0	–	1	–	1	–
		U	--4	1	Undil.	-14	2	±44	20	SC.)	–		–	–	–	–	–
3-23	75	H	444	12	1:3	±44	20	++4	20	T.)	4	4	4	4	±	3	–
		U	±44	12	1:3	--3	12	344	20	SC.)	–	–	–	–	–	–	–

1. H = Serum heated (30 minutes at 56°C.). U = Serum unheated.
2. S) = cc. of Serum; a = 0.05; b = 0.03; c = 0.03.
   A) = cc. of Antigen; d = 0.0001 (0.1 of 1:100 Wassermann suspension); e = 0.003 (flocculation suspension).
   C) = Units of complement.
3. T = Test and SC. = Serum control; ac = anticomplementary

Record of Flocculation Reactions (at Different Temperatures) and of

Complement-Fixation Reactions Given by Rabbit 489 # Inoculated with Spirochaeta pallida.

Date of Examination	Age of Rabbit in Days	Kahn Reactions (Room Temperature)		Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C.		1°C.		Complement-Fixation Reactions	Reaction I		Reaction II		Reaction III	
1941		Tube 123	Quant. Titer		Tube 123	Quant. Titer	Tube 123	Quant. Titer	S) 2 A) C)	a d 3	a d 2	b d 2	c e 2	c e 3	c e 2	c e 3
					Preinoculation Reactions											
10-11	60	H[1] U	---		Undil.	---		---	T.) 3 SC.)	0	ac.	ac.	ac.	ac.	0	0
10-23	72	H	-+3	1	Undil.	---		+24	T.)	4	ac.	4	4	4	–	–
		U	-13	1	Undil.	---		-44	SC.)	–		–	2	±	–	–
11-10	90	H U	004		Undil.	00-		004	T.) SC.)	0	0	0	4 –	0	0	0
12-2	112	H	-+4	1	Undil.	--+		-44	T.)	4	ac.	4	ac.	2	ac.	–
		U	-+4	1	Undil.	--4	1	444	SC.)	±		1		1		–
12-29	139	H	-14	2	Undil.	---		144	T.)	–	4	2	3	–	–	–
		U	--4	1	Undil.	---		+44	SC.)	–	–	–	–	–	–	–
1942	Days Post-inoculation				1/7/42; Rabbit inoculated intradermally on back with 1.0 cc. of testicular suspension of Spirochaeta pallida.											
1-14	7	H	---		Undil.	---		-14	T.)	–	2	–			–	–
		U	--+		Undil.	---		+44	SC.)	–	+	–	ac.	–	–	–
1-26	19	H	--+		Undil.	---		-+2	T.)	ac.	ac.	ac.	ac.	ac.	–	–
		U	+44	8	1:2	---		-44	SC.)						–	–
2-7	31	H	---		Undil.	---		-+4	T.)	3	4	2	ac.	+	ac.	–
		U	--4	1	Undil.	---		+44	SC.)	–	2	–		–		–
3-3	55	H	444	12	1:3	+24	20	--+	T.)	2	0	4	0	–	2	+
		U	--+		Undil.	-+1		+14	SC.)	–		–	–	–	–	–
3-23	75	H	-24	2	Undil.	+44	3	-++	T.)	3	4	1	3	–	3	–
		U	--4	1	Undil.	--+		444	SC.)	–	–	–	–	–	–	–

Record of Flocculation Reactions (at Different Temperatures) and Complement-Fixation Reactions Given by Rabbit 150 ♀ Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Kahn Reactions (Room Temperature) Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	37°C. Quant. Titer	1°C. Tube 123	1°C. Quant. Titer	Complement-Fixation Reactions	Reaction I			Reaction II		Reaction III	
1941											a d 3	a d 2	b d 2	c e 2	c e 3	c e 2	c e 3
Preinoculation Reactions																	
10-11	56	H[1]	---		Undil.	---		---		T.[3]	0	ac.	ac.	ac.	ac.	–	–
		U	---		Undil.	---		---		SC.						–	–
10-23	68	H	--+		Undil.	---		-+4	1	T.	4	4	4	4	4	–	–
		U	--2		Undil.	---		+44	3	SC.	–	–	–	–	–	–	–
11-10	86	H	+44	8	1:2	---		+44	20	T.	4	4	4	4	+	–	–
		U	444	12	1:3	---		+44	20	SC.	–	2	+	–	–	–	–
12-2	108	H	-+3	1	Undil.	---		-44	3	T.	4	ac.	ac.	ac.	1	ac.	–
		U	-+4	1	Undil.	--3	1	444	4	SC.	+				–		–
12-29	135	H	+24	2	Undil.	-+3	1	+44	10	T.	4		4	2	+	–	–
		U	+44	8	1:2	---		444	10	SC.	–	o	–	–	–	–	–
	Days Post-inoculation				1/7/42; Rabbit inoculated intradermally on back with testicular suspension of Spirochaeta pallida.												
1942																	
1-14	7	H	--+		Undil.	---		-+3	1	T.	–	3	–	ac.	–	ac.	–
		U	-+4	1	Undil.	---		+44	3	SC.	–	1	–		–		–
1-26	19	H	-+1		Undil.	---		-+4	10	T.	ac.	ac.	ac.	ac.	ac.	–	–
		U	+44	8	1:2	---		-44	20	SC.						–	–
2-7	31	H	---		Undil.	---		+34	10	T.	–	2	+	2	–	+	–
		U	-24	2	Undil.	--+		444	10	SC.	–	–	–	1	–	+	–
3-3	55	H	-44	8	1:2	+14	2	+34	2	T.	–	o	1	–	–	1	–
		U	--3	1	Undil.	--2	1	+44	3	SC.	–		–	–	–	–	–
3-23	75	H	144	12	1:3	+44	12	++4	12	T.	3	4	1	4	+	3	–
		U	-24	2	Undil.	---		444	10	SC.	–	1	–	–	–	–	–

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit U497; Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Kahn Reactions (Room Temperature) Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	37°C. Quant. Titer	1°C. Tube 123	1°C. Quant. Titer	Complement-Fixation Reactions	Reaction I			Reaction II		Reaction III	
1941										S)[2] A) C)	a d 3	a d 2	b d 2	c e 2	c e 3	c e 2	c e 3
					Preinoculation Reactions												
10-11	56	H[1]	---		Undil.	---		---		T.) SC.)[3]	0	ac.	ac.	ac.	ac.	–	–
		U	---		Undil.	---		---									
10-23	68	H	-24	2	1:1.5	---		124	2	T.)	1	4	4	4	4	–	–
		U	-44	3	1:1.5	---		444	20	SC.)	–	2	–	–	–	–	–
11-10	86	H	±44	8	1:2	---		±44	20	T.)	4	4	4	2	–	–	–
		U	444	12	1:3	---		—44	20	SC.)	–	–	–	–	–	–	–
12-2	108	H	-±4	1	Undil.	---		-44	3	T.)	4				3		–
		U	-44	8	1:2	---		244	8	SC.)	–	ac.	ac.	ac.	–	ac.	–
12-29	135	H	-34	8	1:2	---		444	20	T.)	4	4	4	3	–	–	–
		U	-44	8	1:2	---		444	20	SC.)	–	–	–	–	–	–	–
	Days Post-inoculation				1/7/42; Rabbit inoculated intradermally on back with testicular suspension of Spirochaeta pallida.												
1942																	
1-14	7	H	-±1		Undil.	---		±24	2	T.)	–	2	–	3	–		–
		U	-±1		Undil.	---		±44	3	SC.)	–	2	–	1	–	ac.	–
1-26	19	H	±44	8	1:2	-1±		-24	2	T.)	ac.	ac.	ac.	ac.	ac.	±	–
		U	-44	8	1:2	—-1		-44	3	SC.)						–	–
2-7	31	H	444	12	1:3	-±4	12	±44	12	T.)	4	4	4	4	3	3	±
		U	244	8	1:2	-±4	8	±44	10	SC.)	–	–	–	±	–	–	–
3-3	55	H	344	12	1:3	-24	12	±12	12	T.)	–	o	±	±	–	±	–
		U	-24	2	Undil.	-14	2	244	20	SC.)	–			–	–	–	–
3-23	75	H	±44	10	1:2.5	-34	10	-±3	10	T.)	±	4	3	3	–	±	–
		U	--4	1	Undil.	-±4	1	444	20	SC.)	–	–	–	–	–	–	–

Record of Flocculation Reactions (at Different Temperatures) and of

Complement-Fixation Reactions Given by Rabbit 497 ♀ Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Kahn Reactions (Room Temperature) Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	37°C. Quant. Titer	1°C. Tube 123	1°C. Quant. Titer	Complement-Fixation Reactions	Reaction I		Reaction II		Reaction III	
1941										S)[2] A) C)	a d 3	a d 2	b d 2	c e 2	c e 3	e e 2	e e 3
					Preinoculation Reactions												
10-11	52	H[1]	---		Undil.	---		---		T.)[3]	0	ac.	ac.	ac.	ac.	0	0
		U	---		Undil.	---		--3	1	SC.)							
10-23	64	H	---		Undil.	---		---	1	T.)	3	ac.	3	ac.	4	ac.	—
		U	--4	1	Undil.	---		-±4		SC.)	—		±		2		—
11-10	82	H	---		Undil.	---		-±3	1	T.)	—	2	—	—	—	—	—
		U	-±4	1	Undil.	---		-44	2	SC.)	—	±	—	—	—	—	—
12-2	104	H	--4	1	Undil.	--±		-24	2	T.)	4	ac.	ac.	ac.	4	ac.	—
		U	-14	2	Undil.	--3	1	-44	3	SC.)	—				±		—
12-29	131	H	--4	1	Undil.	---		-24	2	T.)	3	4	1	2	—	—	—
		U	-14	2	Undil.	---		-44	3	SC.)	—	—	—	—	—	—	—
	Days Post-inoculation				1/7/42; Rabbit inoculated intradermally on back with 0.5 cc. of testicular suspension of Spirochaeta pallida.												
1942																	
1-14	7	H	---		Undil.	---		-±4	3	T.)	1	0	4	ac.	—	ac.	—
		U	---		UNDIL.	---		-44		SC.)	—		±		—		—
1-26	19	H	--±		Undil.	---		-±1		T.)	ac.	ac.	ac.	ac.	ac.	—	—
		U	--4	1	Undil.	---		±44	3	SC.)						—	—
2-7	31	H	---		Undil.	---		-±1		T.)	±	ac.	±	4	1	1	—
		U	-±4	1	Undil.	--1		-44	3	SC.)	—		±	2	—	1	—
3-3	55	H	---		Undil.	--±		-±3	1	T.)	—	o	—	—	—	—	—
		U	--3	1	Undil.	--1		-44	3	SC.)	—		±	—	—	—	—
3-23	75	H	---		Undil.	---		-±3	1	T.)	—	—	±	—	—	—	—
		U	--4	1	Undil.	---		±44	3	SC.)	—	—	—	—	—	—	—

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 482 ♀ Inoculated with *Spirochaeta pallida*

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Kahn Reactions (Room Temperature) Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	37°C. Quant. Titer	1°C. Tube 123	1°C. Quant. Titer	Complement-Fixation Reactions S)[2] A) C)	Reaction I a d 3	Reaction I a d 2	Reaction I b d 2	Reaction II c e 2	Reaction II c e 3	Reaction III c e 2	Reaction III c e 3
1941																	
					Preinoculation Reactions												
10-11	51	H[1]	---		Undil.	---		---		T.)[3]	0	ac.	ac.	ac.	ac.	0	0
		U	---		Undil.	---		---		SC.)							
10-23	63	H	---		Undil.	---		---		T.)	-	ac.	3	-	-	-	-
		U	---		Undil.	---		-±2	1	SC.)	-		-	-	±	-	-
11-10	81	H	---		Undil.	---		---		T.)	-	0	±	-	-	-	-
		U	-±4	1	Undil.	---		±44	3	SC.)	-		-	-	-	-	-
12-2	103	H	444	12	1:3	---		444	40	T.)	ac.	ac.	ac.	ac.	ac.	ac.	-
		U	444	12	1:3	--2	12	444	20	SC.)							-
12-29	130	H	144	10	1:2.5	--1		344	40	T.)	±	0	4	3	-	-	-
		U	444	10	1:2.5	--±		444	40	SC.)	-		-	-	-	-	-
					1/7/42; Rabbit inoculated intradermally on back with 0.5 cc. of testicular suspension of *Spirochaeta pallida*												
1942	Days Post-inoculation																
1-14	7	H	---		Undil.	---		---		T.)	-	-	-	-	-	-	-
		U	---		Undil.	---		-±4	1	SC.)	-	-	-	-	-	-	-
1-26	19	H	---		Undil.	---		---		T.)	ac.	ac.	ac.	ac.	ac.	-	-
		U	-±2	1	Undil.	---		-44	3	SC.)							
2-7	31	H	---		Undil.	---		---		T.)	-	-	-	1	-	±	-
		U	-±4	1	Undil.	---		144	3	SC.)	-	-	-	1	-	-	-
3-3	55	H	--±		Undil.	-±1		--+		T.)	-	0	-	-	-	-	-
		U	--2	1	Undil.	---		±44	3	SC.)	-		-	-	-	-	-
3-23	75	H	---		Undil.	--±		-±4	1	T.)	-	-	-	-	-	-	-
		U	--3	1	Undil.	---		-44	3	SC.)	-	-	-	-	-	-	-

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit U495 ♂ Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days	Kahn Reactions (Room Temperature)		Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C.		1°C.		Complement-Fixation Reactions	Reaction I			Reaction II		Reaction III	
1941		Tube 123	Quant. Titer		Tube 123	Quant. Titer	Tube 123	Quant. Titer	S)[2] A) C)	a d 3	a d 2	b d 2	c e 2	c e 3	c e 2	c e 3
					Preinoculation Reactions											
10-11	52	H[1]	---		Undil.	---		---	T.)[3]	0	ac.	ac.	ac.	ac.	0	0
		U	---		Undil.	---		±23 2	SC.)							
10-23	64	H	---		Undil.	---		-+	T.)	ac.	ac.	ac.	±	-	0	-
		U	---		Undil.	---		±34 2	SC.)				±	-		-
11-10	82	H	-+2	1	Undil.	---		444 4	T.)	1	4	4	1	-	-	-
		U	-14	2	Undil.	--±		244 3	SC.)	-	-	-	-	-	-	-
12-2	104	H	-±3	1	Undil.	--±		±44 3	T.)	0	4	1	ac.	4	ac.	-
		U	-24	2	Undil.	---		144 3	SC.)		-	-		-		-
12-29	131	H	-±4	1	Undil.	--2	1	144 3	T.)	±	4	2	3	-	-	-
		U	-44	2	1:2	---		244 8	SC.)	-	-	-	-	-	-	-
1942	Days Post-inoculation				1/7/42; Rabbit inoculated with 1.0 cc. of testicular suspension of Spirochaeta pallida into both testes.											
1-14	7	H	---		Undil.	---		---	T.)	-	3	±	-	-	-	-
		U	--±		Undil.	---		-±3 1	SC.)	-	-	-	-	-	-	-
1-26	19	H	--±		Undil.	---		---	T.)	ac.	ac.	ac.	ac.	ac.	-	-
		U	--±		Undil.	---		-±4 1	SC.)							
2-7	31	H	---		Undil.	---		---	T.)	-	-	-	-	-	-	-
		U	---		Undil.	---		-14 2	SC.)	-	-	-	-	-	-	-
3-3	55	H	---		Undil.	---		-+2 1	T.)	-	Q	-	-	-	-	-
		U	--±		Undil.	--±		-14 2	SC.)	-		-	-	-	-	-
3-23	75	H	-±4	1	Undil.	-44	3	-13 2	T.)	-	2	-	-	-	2	-
		U	±--		Undil.	---		144 3	SC.)	-	-	-	-	-	-	-

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 494 ♂ Inoculated with *Spirochaeta pallida*

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Kahn Reactions (Room Temperature) Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	37°C. Quant. Titer	1°C. Tube 123	1°C. Quant. Titer	Complement-Fixation Reactions	Reaction I			Reaction II		Reaction III	
1941										S)[2] A) C)	a d 3	a d 2	b d 2	c e 2	c e 3	c e 2	c e 3
					Preinoculation Reactions												
10-11	56	H[1]	---		Undil.	---		---		T.)[3]	o	ac.	ac.	ac.	ac.	o	o
		U	---		Undil.	---		---		SC.)							
10-23	68	H	-±4		Undil.	---		444	20	T.)	4	ac.	3	ac.	–	o	–
		U	-34		Undil.	--±		444	20	SC.)	1		2		–		–
11-10	86	H	-±2		Undil.	-±2	1	±44	20	T.)	–	4	2	1	–	–	–
		U	-44		1:1.5	--2	1	444	20	SC.)	–	–	–	–	–	–	–
12-2	108	H	-±2		Undil.	---		±44	10	T.)	o	4	3	4	1	ac.	–
		U	-±4		Undil.	--2	1	±44	10	SC.)		–	–	1	–		–
12-29	135	H	-±4		Undil.	---		±44	20	T.)	±	4	2	3	±	–	–
		U	-44		1:2	---		244	20	SC.)	–	–	–	–	–	–	–
	Days Post-inoculation				1/7/42; Rabbit inoculated with 1.0 cc. of testicular suspension of *Spirochaeta pallida* into both tests.												
1942																	
1-14	7	H	-±3		Undil.	---		±34	2	T.)	ac.	ac.	ac.	ac.	±	ac.	–
		U	---		Undil.	---		±24	2	SC.)					–		–
1-26	19	H	--±		Undil.	--1		-±2	1	T.)	ac.	ac.	ac.	ac.	ac.	–	–
		U	-44	8	1:2	---		-44	10	SC.)						–	–
2-7	31	H	-±4	1	Undil.	-±3	1	-24	10	T.)	3	ac.	ac.	ac.	±	ac.	±
		U	-44	8	1:2	---		-44	20	SC.)	–				±		–
3-3	55	H	444	12	1:3	±44	12	-±1	10	T.)	4	o	4	±	–	1	–
		U	-±3	1	Undil.	--3	1	±44	3	SC.)	–		–	–	–	–	–
3-23	75	H	444	30	1:7.5	-44	40	±12	40	T.)	4	4	4	4	2	4	±
		U	443	12	1:3	---	4	244	40	SC.)	–	–	–	–	–	–	–

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 493 ♂ Inoculated with *Spirochaeta pallida*

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Kahn Reactions (Room Temperature) Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	37°C. Quant. Titer	1°C. Tube 123	1°C. Quant. Titer	Complement-Fixation Reactions S)[2] A) C)	Reaction I			Reaction II		Reaction III	
1941											a d 3	a d 2	b d 2	c e 2	c e 3	c e 2	c e 3
					Preinoculation Reactions												
10-11	50	H[1]	---		Undil.	---		---		T.)[3]	0	0	0	ac.	ac.	0	0
		U	---		Undil.	---		--1		SC.)							
10-25	64	H	---		Undil.	---		--2	1	T.)	ac.	ac.	ac.	±	–	–	–
		U	–±3	1	Undil.	---		123	2	SC.)				±	–	–	–
11-12	84	H	–12	1	Undil.	--1		±44	20	T.)	–	o	–	–	–	–	–
		U	444	8	1:2	–±4		444	20	SC.)	–		–	–	–	–	–
12-3	103	H	--2	1	Undil.	---		±44	20	T.)	o	4	o	ac.	±	ac.	–
		U	–±4	1	Undil.	--±		244	20	SC.)		–			–		–
12-29	129	H	–±1		Undil.	---		-44	10	T.)	±	4	±	–	–	–	–
		U	–±4	1	Undil.	---		444	20	SC.)	–	–	–	–	–	–	–
	Days Post-inoculation				1/7/42; Rabbit inoculated with 1.5 cc. testicular suspension of *Spirochaeta pallida* into right testes.												
1942																	
1-14	7	H	---		Undil.	---		±24	2	T.)	–	4	±	ac.	–	–	–
		U	–±1		Undil.	---		±44	3	SC.)	–	±	–		–	–	–
1-26	19	H	–±4	1	Undil.	-14	2	-24	2	T.)	ac.	ac.	ac.	o	o	–	–
		U	-14	2	Undil.	---		±44	20	SC.)						–	–
2-7	31	H	±44	10	1:2.5	---		-24	20	T.)	3	4	4	4	2	–	–
		U	-44	8	1:2	-12	8	±44	20	SC.)	–	–	–	–	–	–	–
3-3	55	H	444	140	1:35	-24	220	–±4	140	T.)	4	o	4	4	–	4	±
		U	444	100	1:25	-14	100	444	220	SC.)	–		–	–	–	–	–
3-23	75	H	444	60	1:17	±44	80	±±4	80	T.)	4	4	4	4	3	4	1
		U	444	80	1:18	-13	80	±44	80	SC.)	–	–	–	–	–	–	–

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 499 ♂ Inoculated with *Spirochaeta pallida*

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Kahn Reactions (Room Temperature) Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	37°C. Quant. Titer	1°C. Tube 123	1°C. Quant. Titer	Complement-Fixation Reactions	Reaction I		Reaction II		Reaction III	
1941										S)[2] A) C)	a d 3	a d 2	b d 2	c ● 2	c ● 3	c ● 2	c ● 3
					Preinoculation Reactions												
10-11	56	H[1]	---		Undil.	---		---		T.)[3]	o	ac.	ac.	ac.	ac.	o	o
		U	---		Undil.	---		---		SC.)							
10-23	68	H	-12	1	Undil.	---		-24	2	T.)	4	ac.	ac.	ac.	–	o	o
		U	-24	2	Undil.	---		144	3	SC.)	±				±		
11-10	86	H	---		Undil.	---		-±3	1	T.)	–	o	–	–	–	–	–
		U	---		Undil.	---		---		SC.)	–		–	–	–	–	–
12-2	108	H	---		Undil.	---		±24	2	T.)	o	4	±	4	4	–	–
		U	--3	1	Undil.	---		±14	2	SC.)		–	–	–	–	–	–
12-29	135	H	--±		Undil.	---		-±4	1	T.)	±	4	1	±	–	–	–
		U	-±4	1	Undil.	---		±44	3	SC.)	–	–	–	–	–	–	–
					1/7/42; Rabbit inoculated with 1.5 cc. testicular suspension of *Spirochaeta pallida* into both testes.												
1942	Days Post-inoculation																
1-14	7	H	---		Undil.	---		-±2	1	T.)	–	ac.	–	–	–	–	–
		U	---		Undil.	---		±44	3	SC.)	–		–	–	–	–	–
1-26	19	H	-14	2	Undil.	-±2	1	±44	20	T.)	ac.	ac.	ac.	o	o	–	–
		U	-13	1	Undil.	--1		344	40	SC.)						–	–
2-7	31	H	--3	1	Undil.	---		±44	20	T.)	–	3	±	2	–	–	–
		U	-±4	1	Undil.	-32	2	444	20	SC.)	–	–	–	–	–	–	–
3-3	55	H	444	24	1:6	-14	24	-44	24	T.)	4	o	4	±	–	1	–
		U	444	12	1:3	-44	12	444	40	SC.)	–		–	–	–	–	–
3-23	75	H	444	28	1:7	±44	30	±12	30	T.)	4	4	4	4	1	4	±
		U	44-	28	1:7	---	20	-±4	30	SC.)	–	–	–	–	–	–	–

Complement-Fixation Reactions Given by Rabbit 498 ♂ Inoculated with *Spirochaeta pallida*

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Kahn Reactions (Room Temperature) Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	37°C. Quant. Titer	1°C. Tube 123	1°C. Quant. Titer		Complement-Fixation Reactions: Reaction I		Reaction II		Reaction III	
1941										S)[2] A) C)	a d 3	a d 2	b d 2	c • 2	c • 3	c • 2	c • 3
						Preinoculation Reactions											
10-11	50	H[1]	---		Undil.	---		--3	1	T.)[3]	o	o	o	ac.	ac.	ac.	ac.
		U	-±1		Undil.	---		±44	3	SC.)							
10-23	64	H	±34	2	Undil.	---		444	12	T.)	4	ac.	ac.	ac.	3	0	0
		U	144	3	1:1.5	---		444	20	SC.)	±				1		
11-10	84	H	±14	2	Undil.	---		444	4	T.)	-	ac.	1	±	-	-	-
		U	-44	3	1:2	--±		344	4	SC.)	-		-	-	-	-	-
12-2	103	H	±14	2	Undil.	---		444	20	T.)	o	4	2	ac.	3	ac.	-
		U	±24	2	Undil.	---		444	20	SC.)		-	-		-		-
12-29	127	H	-44	8	1:2	---		444	20	T.)	2	ac.	-	ac.	-	ac.	-
		U	144	8	1:2	--2		444	30	SC.)	-		-		-		-
	Days Post-inoculation				1/7/42; Rabbit inoculated with 1.0 cc. of testicular suspension of *Spirochaeta pallida* into both testes.												
1942																	
1-14	7	H	-±2	1	Undil.	---		±44	10	T.)	-	ac.	ac.	ac.	-	±	-
		U	---		Undil.	---		144	3	SC.)	-				-	±	-
1-26	19	H	-13	1	Undil.	-±1		344	4	T.)	ac.	ac.	ac.	o	o	-	-
		U	-±±		Undil.	---		±21	1	SC.)						-	-
2-7	31	H	--2	1	Undil.	---		-44	20	T.)	-	4	-	ac.	-	ac.	-
		U	--4	1	Undil.	-±1		444	20	SC.)	-	-	-		-		-
3-3	55	H	444	240	1:60	±44	240	---	200	T.)	4	o	4	4	4	4	4
		U	444	100	1:25	-44	120	144	120	SC.)	-		-	-	-	-	-
3-23	75	H	444	80	1:18	±44	80	---	40	T.)	4	4	4	4	4	3	1
		U	442	40	1:15	---	40	-34	80	SC.)	-	±	-	-	-	-	-

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit U493 ♂ Inoculated with *Spirochaeta pallida*

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Kahn Reactions (Room Temperature) Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	37°C. Quant. Titer	1°C. Tube 123	1°C. Quant. Titer	Complement-Fixation Reactions S) A) C)[2]	Reaction I		Reaction II		Reaction III	
1941																
						Preinoculation Reactions										
10-11	52	H[1]	---		Undil.	---		---		T.)[3]	0	ac.	ac.	ac.	ac.	0
		U	---		Undil.	---		---		SC.)						
10-23	64	H	---		Undil.	---		---		T.)	ac.	ac.	ac.	ac.	ac.	0
		U	---		Undil.	---		--3		SC.)						
11-10	82	H	2--	1	Undil.	---		-44	3	T.)	-	ac.	3	-	-	-
		U	444	60	1:15	-+3	60	444	80	SC.)	-		-	-	-	-
12-2	104	H	-+4	1	Undil.	---		244	20	T.)	0	4	4	ac.	3	ac.
		U	+44	8	1:2	---		444	30	SC.)		-	-		-	-
12-29	131	H	-+4	1	Undil.	---		+44	20	T.)	1	4	2	-	-	+
		U	+44	8	1:2	---		444	20	SC.)	-	-	-	-	-	-
						1/7/42; Rabbit inoculated with 1.0 cc. of testicular suspension of *Spirochaeta pallida* into left testes.											
1942	Days Post-inoculation															
1-14	7	H	---		Undil.	---		---		T.)	-	+	-	-	-	-
		U	---		Undil.	---		-44	3	SC.)	-	-	-	-	-	-
1-26	19	H	--+		Undil.	--1		+13	1	T.)	ac.	ac.	ac.	ac.	ac.	-
		U	444	12	1:3	---		-24	12	SC.)						-
2-7	31	H	---		Undil.	---		--+		T.)	+	ac.	ac.	ac.	-	ac.
		U	-44		1:2	---		-44	20	SC.)	+				-	-
3-3	55	H	444	80	1:20	-+4	80	--1	60	T.)	4	0	4	-	-	+
		U	442	20	1:4	-44	20	-44	20	SC.)	-		-	-	-	-
3-23	75	H	444	60	1:15	-44	60	-+3	40	T.)	4	4	4	4	2	3	+
		U	442	32	1:8	---	4	-24	40	SC.)	-	-	-	-	-	-	-

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 26 ♂ Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Kahn Reactions (Room Temperature) Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	37°C. Quant. Titer	1°C. Tube 123	1°C. Quant. Titer	Complement-Fixation Reactions	Reaction I		Reaction II		Reaction III	
1941										S)[2] A) C)	a d 3	a d 2	b d 2	c e 2	c e 3	c e 2	c e 3
						Preinoculation Reactions											
11-14		H[1]	244	8	1:2	---	4	144	20	T.)[3]	4	ac.	4	4	1	-	-
		U	244	8	1:2	---	4	±44	10	SC.)	-		-	-	-	-	-
11-27		H	-44	4	1:1.5	---		±34	20	T.)	-	3	1	2	-	-	-
		U	-44	4	1:1.5	---		444	20	SC.)	-	-	-	-	-	-	-
12-7		H	±44	4	1:1.5	---		±44	20	T.)	-	3	2	4	-	-	-
		U	±44	4	1:1.5	---		±44	20	SC.)	-	-	-	-	-	-	-
12-30		H	±44	8	1:2	---		-44	10	T.)	4	ac.	±	4	-	ac.	-
		U	244	8	1:2	-±4		444	30	SC.)	-		-	±	-		-
1942	Days Post-inoculation					1/7/42; Rabbit inoculated with 1.0 cc. of testicular suspension of Spirochaeta pallida into left testes.											
1-14	7	H	--1		Undil.	---		±44	3	T.)	-	±	-	-	-	0	0
		U	---		Undil.	---		±44	3	SC.)	-	-	-	-	-		
1-26	19	H	--1		Undil.	---		±14	2	T.)	ac.	ac.	ac.	0	0	-	-
		U	-±2	1	Undil.	---		-13	1	SC.)						-	-
2-8	31	H	444	16	1:4	-±4	20	-44	30	T.)	3	ac.	2	4	-	3	±
		U	--±		Undil.	244	10	444	40	SC.)	-		-	2	-	-	-
3-3	55	H	444	80	1:20	-±4	80	-±4	80	T.)	0	0	4	-	-	±	-
		U	444	48	1:12	-±4	48	443	80	SC.)			-	-	-	-	-
3-23	75	H	444	60	1:15	±44	60	-±4	40	T.)	4	4	4	4	2	2	±
		U	443	60	1:15	--±	40	-44	80	SC.)	-	-	-	-	-	-	-

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 32 ♂ Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Quant. Titer	1°C. Tube 123	Quant. Titer		Complement-Fixation Reactions: Reaction I		Reaction II			Reaction III	
										S[2]	a	a	b	c	c	c	c
										A	d	d	d	e	e	e	e
1941										C	3	2	2	2	3	2	3
					Preinoculation Reactions												
11-28		H[1]	-14	2	Undil.	---		±44	20	T.[3]	±	4	±	2	-	-	-
		U	-±4	1	Undil.	---		444	20	SC.	-	-	-	-	-	-	-
12-7		H	-14	2	Undil.	---		±44	20	T.	-	4	1	3	-	-	-
		U	±14	2	Undil.	---		244	20	SC.	-	-	-	-	-	-	-
12-30		H	-±3	1	Undil.	---		244	20	T.	-	3	-	-	-	-	-
		U	±14	2	Undil.	--±		444	20	SC.	-	-	-	-	-	-	-
1942	Days Post-inoculation				1/7/42; Rabbit inoculated with 0.5 cc. testicular suspension of Spirochaeta pallida intradermally on back and 0.5 cc. into left testes.												
1-14	7	H	-23	2	Undil.	-22	1	344	4	T.	-	ac.	-	3	-	2	-
		U	-±4	1	Undil.	-±3	1	±44	3	SC.	-		-	-	-	-	-
1-27	20	H	244	10	1:2.5	--2	10	±44	30	T.	ac.	ac.	ac.	0	0	3	-
		U	±44	8	1:2	--±	4	-44	20	SC.						-	-
2-7	31	H	-44	8	1:2	--1	4	±44	20	T.	-	4	2	ac.	1	-	-
		U	-44	8	1:2	---	4	-44	20	SC.	-	-	-		-	-	-
3-2	55	H	444	80	1:20	-±4	80	-±1	60	T.	4	0	2	4	-	3	-
		U	444	24	1:6	±44	40	244	40	SC.	-	-	-	-	-	-	-
3-22	75	H	444	64	1:16	±24	64	-±4	40	T.	4	4	4	4	4	4	±
		U	444	28	1:7	--±	20	244	40	SC.	-	-	-	-	-	-	-

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 33 ♂ Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Quant. Titer	1°C. Tube 123	Quant. Titer		Complement-Fixation Reactions: Reaction I a d 3	Reaction I a d 2	Reaction II b d 2	Reaction II c e 2	Reaction II c e 3	Reaction III c e 2	Reaction III c e 3
1941																		
					Preinoculation Reactions													
11-28		H[1]	144	4	1:1.5	---		-44	4	T.[3]	-	2	1	±	-	-	-
		U	-34	2	Undil.	---		244	3	SC.	-	-	-	-	-	-	-
12-7		H	±44	4	1:1.5	---		±44	4	T.	-	3	±	2	-	-	-
		U	±44	4	1:1.5	---		144	4	SC.	-	-	-	-	-	-	-
12-30		H	±44	4	1:2.5	---		±44	10	T.	4	4	4	3	-	±	-
		U	244	10	1:2.5	---		±44	10	SC.	-	-	-	±	-	-	-
1942	Days Post-inoculation				1/7/42; Rabbit inoculated with 1.0 cc. testicular suspension of Spirochaeta pallida into left testes.													
1-14	7	H	-13	1	Undil.	--1		±44	3	T.	-	-	-	±	-	-	-
		U	--±		Undil.	--±		144	3	SC.	-	-	-	-	-	-	-
1-27	20	H	-±1		Undil.	--±		-±2	1	T.	ac.	ac.	ac.	0	-	-	-
		U	-44	8	1:2	---		-44	8	SC.					-	-	-
2-7	31	H	---		Undil.	---		-±4	1	T.	-	-	-	-	-	-	-
		U	-44	8	1:2	--±		-44	20	SC.	-	-	-	-	-	-	-
3-2	55	H	444	24	1:6	-14	24	---	4	T.	±	0	3	1	-	1	-
		U	--3	1	Undil.	--2	1	±44	3	SC.	-		-	-	-	-	-
3-22	75	H	444	32	1:8	±44	40	-±4	20	T.	4	4	4	4	3	1	-
		U	344	12	1:3	---	4	344	20	SC.	-	-	-	-	-	-	-

[2] S = Serum, A = Antigen, C = Complement

[3] T. = Tube, SC. = Quantitative Serum Control

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit N3 ♂ Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Kahn Reactions (Room Temperature) Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	37°C. Quant. Titer	1°C. Tube 123	1°C. Quant. Titer	Complement-Fixation Reactions [2]	Reaction I		Reaction II			Reaction III	
1941										S) A) C)	a d 3	a d 2	b d 2	c e 2	c e 3	c e 2	c e 3
						Preinoculation Reactions											
11-4		H[1]	144	8	1:2	-±2	8	-14	8	T.) [3]	4	4	4	4	±	±	-
		U	344	8	1:2	--2	8	-44	8	SC.)	-	1	-	-	-	-	-
	Days Post-inoculation					11/4/41; Rabbit inoculated with testicular suspension of Spirochaeta pallida into both testes.											
11-27	23	H	444	12	1:3	-±4	20	±+3	20	T.)	4	4	4	4	±	4	±
		U	444	12	1:3	--4	12	-44	20	SC.)	-	-	-	-	-	-	-
12-7	34	H	444	100	1:25	-14	100	--±	80	T.)	4	4	4	4	4	4	1
		U	444	48	1:12	-44	80	-44	80	SC.)	-	-	-	-	-	-	-
12-29	56	H	444	60	1:12	-44	60	--±	30	T.)	4	4	4	4	4	4	4
		U	444	48	1:12	-24	48	-±1	40	SC.)	-	-	-	-	-	-	-
1942																	
1-14	72	H	444	60	1:15	±44	80	-±3	40	T.)	4	4	4	4	4	4	2
		U	444	48	1:12	±44	40	--±	40	SC.)	-	-	-	-	-	-	-
1-26	84	H	444	100	1:25	±24	120	--4	80	T.)	ac.	ac.	ac.	0	0	4	±
		U	444	40	1:10	-34	40	-±4	40	SC.)						-	-
2-8	97	H	444	80	1:20	±44	80	--4	80	T.)	4	4	4	4	1	4	2
		U	44-	40	1:10	±44	60	--4	40	SC.)	-	-	-	-	-	-	-
3-3	110	H	444	40	1:10	-±4	40	--1	20	T.)	4	0	4	-	-	-	-
		U	444	12	1:3.5	±44	30	-±4	20	SC.)	-		-	-	-	-	-
3-23	130	H	144	12	1:3	244	12	--4	4	T.)	±	4	1	3	-	2	-
		U	344	12	1:3	---	4	444	20	SC.)	-	-	-	-	-	±	-

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit N22 ♂ Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Kahn Reactions (Room Temperature) Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	37°C. Quant. Titer	1°C. Tube 123	1°C. Quant. Titer	Complement-Fixation Reactions	Reaction I S)[2]	Reaction I A)	Reaction I C)	Reaction II c	Reaction II e	Reaction III c	Reaction III e
1941																	
						Preinoculation Reactions											
10-3		H[1]	-14	2	Undil.	±2	1	-44	3	T.)[3]	0	4	3	4	±	2	-
		U	-14	2	Undil.	±1		-44	3	SC.)		-	-	-	-	-	-
	Days Post-inoculation				10/6/41; Rabbit inoculated with lymph node suspension obtained from a rabbit with old syphilis.												
10-22	19	H	-14	2	Undil.	-34	2	±44	3	T.)	0	ac.	ac.	ac.	ac.	ac.	ac.
		U	-14	2	Undil.	--±		-44	3	SC.)							
11-27	55	H	444	12	1:3	-44	12	±14	20	T.)	4	4	4	4	±	4	-
		U	444	12	1:3	--4	20	-44	20	SC.)		-	-	-	-	-	-
12-7	65	H	444	24	1:6	-±4	24	--±	10	T.)	4	4	4	4	4	4	±
		U	444	24	1:6	-±4	24	--3	20	SC.)		-	-	-	-	-	-
12-30	88	H	444	32	1:8	±±4	32	---	20	T.)	4	4	4	4	4	4	4
		U	444	28	1:7	±44	40	--3	28	SC.)		-	-	-	-	-	-
1942																	
1-14	103	H	444	40	1:10	±44	40	-±4	40	T.)	4	4	4	4	3	4	4
		U	444	32	1:8	±34	32	-±4	30	SC.)		-	-	-	-	-	-
2-8	128	H	444	280	1:70	-24	360	-±3	280	T.)	4	4	4	4	4	4	1
		U	442	200	1:50	-14	220	---	140	SC.)		2	±	3	-	-	-
3-3	150	H	344	12	1:3,	-14	20	--±	4	T.)	±	0	2	1	-	2	-
		U	-44	10	1:2.5	-±4	10	---	4	SC.)	-		-	-	-	-	-
3-23	170	H	±44	10	1:2.5	-14	10	-±2	10	T.)	2	4	1	3	±	2	-
		U	±44	12	1:3	---	4	±24	20	SC.)	-	-	-	-	-	-	-

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit N11 ♂ Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Quant. Titer	1°C. Tube 123	Quant. Titer	Complement-Fixation Reactions	Reaction I			Reaction II		Reaction III	
1941										S) 2 A) C)	a d 3	a d 2	a d 2	c e 2	c e 3	c e 2	c e 3
					Preinoculation Reactions												
10-3		H[1]	---		Undil.	---		---		T.) 3 SC.) 0	-	-	-	-	-	-	-
		U	---		Undil.	---		---			-	-	-	-	-	-	-
	Days Post-inoculation				10/6/41; Rabbit inoculated with lymph node suspension obtained from a rabbit with old syphilis.												
10-22	19	H	±24	2	Undil.	-24	2	±24	10	T.) 3 SC.)	4	4		ac.	4	ac.	2
		U	-±4	1	Undil.	--1		±44	20		-	±	±		1		-
11-27	55	H	444	30	1:8	-±4	40	--±	20	T.) 4 SC.)	4	4		4	-	4	±
		U	444	20	1:5	-44	40	-44	30		-	-	-	-	-	-	-
12-7	65	H	444	80	1:20	-14	100	--±	80	T.) 4 SC.)	4	4		4	4	4	±
		U	444	48	1:12	-14	48	-±4	48		-	-	-	-	-	-	-
12-30	88	H	444	120	1:25	±44	120	-±2	80	T.) 4 SC.)	4	4		4	4	4	4
		U	444	60	1:15	-44	60	--±	40		-	-	-	-	-	-	-

Died following cardiac puncture.

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit U478 ♀ Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days	Kahn Reactions (Room Temperature)		Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C.		1°C.		Complement-Fixation Reactions	Reaction I			Reaction II		Reaction III	
1941			Tube 123	Quant. Titer		Tube 123	Quant.	Titer	S)[2] A) C)	a d 3	a d 2	b d 2	c e 2	c e 3	c e 2	c e 3
					Preinoculation Reactions											
10-11	56	H[1]	---		Undil.	---		---	T.[3]	0	4	4	ac.	-	0	0
		U	Insufficient serum						SC.		2	2		-		
10-23	68	H	244	3	1:4	-++		-44	40	T.	4	4	4	4	4	3	-
		U	144	3	1:4	-+-		±44	40	SC.	-	±	±	-	-	-	-
11-10	86	H	-44	3	1:1.5	---		144	20	T.	3	4	3	2	-	-	-
		U	-44	3	1:1.5	---		444	20	SC.	-	±	-	-	-	-	-
12-2	108	H	±44	3	Undil.	---		444	20	T.	±	ac.	4	ac.	2	-	-
		U	±24	2	Undil.	-±2	1	444	20	SC.	-		-		-	-	-
12-29	135	H	±44	3	1:2	--2	1	444	20	T.	-	0	4	3	-	-	-
		U	244	3	1:2	-±3	1	444	20	SC.	-		-	-	-	-	-
	Days Post-inoculation				1/7/42; Rabbit inoculated intradermally on back with 1.0 cc. of testicular suspension of Spirochaeta pallida.											
1942																
1-14	7	H	±12	1	Undil.	---		444	20	T.	-	3	±	ac.	-	-	-
		U	-44	8	1:2	---		444	30	SC.	-	1	±		-	-	-
1-26	19	H	±44	2	Undil.	---		±44	20	T.	ac.	ac.	ac.	ac.	ac.	-	-
		U	-44	8	1:2	---		-44	10	SC.						-	-

Animal found dead 2-4-42. Autopsy: on gross examination, the abdominal organs appeared normal; inoculated areas faintly pink; fluid and clots in thoracic cavity.

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 478 ♂ Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Quant. Titer	1°C. Tube 123	Quant. Titer	Complement-Fixation Reactions[2]	Reaction I			Reaction II		Reaction III	
1941										S) A) C)	a d 3	a d 2	a d 2	c e 2	c e 3	c e 2	c e 3
					Preinoculation Reactions												
10-11	56	H[1]	---		Undil.	---		---		T.)[3]	0	0	0	ac.	ac.	0	0
		U	---		Undil.	---		-+3	1	SC.)							
10-25	70	H	---		Undil.	---		---		T.)	ac.	ac.	ac.	ac.	±	0	±
		U	---		Undil.	---		--2	1	SC.)					-		-
11-12	88	H	---		Undil.	---		+24	2	T.)	-	0	1	-	-	-	-
		U	-24		Undil.	-+3		144	3	SC.)	-		-	-	-	-	-
12-3	109	H	-+4		Undil.	--+		-44	3	T.)	0	1	-	4	2	-	-
		U	-24		Undil.	++2		+44	3	SC.)		-	-	1	-	-	-
12-29	135	H	-14		Undil.	---		144	3	T.)	±	ac.	2	ac.	-	-	-
		U	-24		Undil.	---		444	3	SC.)	-		-		-	-	-
1942	Days Post-inoculation				1/7/42; Rabbit inoculated with 1.0 cc. of testicular suspension of Spirochaeta pallida into left testes.												
1-14	7	H	++1		Undil.	---		++3	1	T.)	-	-	-	-	-	-	-
		U	--+		Undil.	---		---		SC.)	-	-	-	-	-	-	-
1-26	19	H	0--		Undil.	---		--+		T.)	ac.	ac.	ac.	0	0	-	-
		U	--1		Undil.	---		-+4	1	SC.)						-	-

Died 1/27/42. Clots in thorax. Gross picture on autopsy similar to that in Rabbit 488.

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 479 ♂ Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Quant. Titer	1°C. Tube 123	Quant. Titer	Complement-Fixation Reactions[2]	Reaction I			Reaction II		Reaction III	
										S) A) C)	a d 3	a d 2	a d 2	c e 2	c e 3	c e 2	c e 3
1941																	
						Preinoculation Reactions											
10-25	57	H[1]	---		Undil.	---		---		T.)[3]							
		U	---		Undil.	---		---		SC.)	0	ac.	ac.	ac.	ac.	0	0
11-12	78	H	---		Undil.	---		±3	1	T.)	-		-	-	-	-	-
		U	---		Undil.	---		-+		SC.)	-	0	-	-	-	-	-
12-3	97	H	---		Undil.	---		-44	3	T.)		3	±		1	1	-
		U	-±4	1	Undil.	---		-44	3	SC.)	0	±	-	ac.	1	1	-
12-29	122	H	---		Undil.	---		-44	3	T.)	±	3	±	-	-	-	-
		U	--4	1	Undil.	---		-44	3	SC.)	-	-	-	-	-	-	-
	Days Post-inoculation				1/7/42; Rabbit inoculated with 1.5 cc. testicular suspension of Spirochaeta pallida into both testes.												
1942																	
1-14	7	H	---		Undil.	---		---		T.)	-	-	-	1	-	-	-
		U	---		Undil.	---		---		SC.)	-	-	-	-	-	-	-

Died 1/24/42. Gross picture on autopsy similar to that in Rabbit 488.

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 487 ♂ Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Quant. Titer	1°C. Tube 123	Quant. Titer	Complement-Fixation Reactions	Reaction I	Reaction II	Reaction III
1941										S)[2] A) C)	a d 3	a d 2 a d 2	c e 2 c e 3	c e 2 c e 3
					Preinoculation Reactions								
10-11	43	H[1]	---		Undil.	---		---		T.)[3] SC.)	0	ac. ac.	ac. ac.	0 0
		U	---		Undil.	---		---					
10-23	55	H	---		Undil.	---		---		T.) SC.)	ac.	ac. ac.	ac. -	0 -
		U	---		Undil.	---		---					
11-10	73	H	0--		Undil.	---		±44	3	T.) SC.)	- -	0 3 -	ac. -	2 - 1 -
		U	--3	1	Undil.	---		-24	2				
12-2	95	H	-±4	1	Undil.	--1		±44	3	T.) SC.)	0	4 4 - -	ac. 4 -	2 - 2 -
		U	-44	3	1:1.5	-±2		±44	3				
12-29	117	H	-±4	1	Undil.	--1		-44	3	T.) SC.)	3 -	ac. ac.	ac. ac.	ac. ac.
		U	-44	3	1:2	---		+34	8				
1942	Days Post-inoculation				1/7/42; Rabbit inoculated with 0.8 cc. testicular suspension of Spirochaeta pallida into both testes.								
1-14	7	H	---		Undil.	---		-±2	1	T.) SC.)	- -	ac. ac.	ac. -	± - ± -
		U	---		Undil.	---		-24	2				

Died 1/17/42. Gross picture on autopsy similar to that in Rabbit 488.

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 484 ♂ Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Quant. Titer	1°C. Tube 123	Quant. Titer	Complement-Fixation Reactions: Reaction I	Reaction II				Reaction III	
1941										S)[2] A) C)	a d 3	a d 2	a d 2	c e 2	c e 3	c e 2	c e 3
					Preinoculation Reactions												
10-11	54	H[1]	---		Undil.	---		---		T.)[3] SC.)	0	ac.	ac.	ac.	ac.	0	0
		U	---		Undil.	---		--1									
10-25	68	H	±44	8	1:2	---		±44	8	T.) SC.)	ac.	ac.	ac.	ac.	4 ±	0	0
		U	-44	8	1:2	---		±14	8								
11-12	88	H	00±		Undil.	00-		004		T.) SC.)	0	0	0	0	0	0	0
		U	Insufficient serum														
12-3	107	H	---		Undil.	---		-14	2	T.) SC.)	0	3 -	± -	3 -	± -	- -	- -
		U	--3	1	Undil.	---		-±4	1								
12-29	131	H	-24	2	Undil.	--3	1	-44	3	T.) SC.)	0	4 -	2 -	3 -	- -	- -	- -
		U	-44	8	1:2	---		-44	8								

1/7/42; Rabbit inoculated with 0.5 cc. testicular suspension of Spirochaeta pallida into both testes.

Died 1/11/42; Gross picture on autopsy similar to that in Rabbit 488.

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 488 ♂ Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Quant. Titer	1°C. Tube 123	Quant. Titer	Complement-Fixation Reactions	Reaction I			Reaction II		Reaction III	
1941										S)[2] A) C)	a d 3	a d 2	a d 2	c e 2	c e 3	c e 2	c e 3
						Preinoculation Reactions											
10-11	56	H[1]	---		Undil.	---		--+ 123	2	T.) SC.) [3]	0	ac.	ac.	ac.	ac.	0	0
		U	---		Undil.	---											
10-23	68	H	---		Undil.	---		-44	3	T.)	ac.	ac.	ac.	ac.	-	0	-
		U	---		Undil.	---		-44	3	SC.)					-		-
11-10	86	H	---		Undil.	Insufficient				T.)	0	0	2	-	-	0	0
		U	--3		Undil.					SC.)			-	-	-		
12-2	108	H	--+		Undil.	---		-44	3	T.)	-	-	-	-	-	-	-
		U	-+4		Undil.	---		444	4	SC.)	-	-	-	-	-	-	-
12-29	135	H	--1		Undil.	---		144	3	T.)	-	1	-	-	-	-	-
		U	-24		Undil.	---		444	4	SC.)	-	-	-	-	-	-	-
1942	Days post-inoculation				1/7/42; Rabbit inoculated with 0.5 cc. of testicular suspension of Spirochaeta pallida into both testes.												
1-14	151	H	---		Undil.	---		---		T.)	-	-	-	-	-	-	-
		U	---		Undil.	---		--+		SC.)	-	-	-	-	-	-	-

Died 1/17/42. Autopsy: Thorax contained some fluid. Abdominal viscera appeared grossly normal; all visceral organs and intestine, as well as peritoneal wall, covered with a thick, caseous, non-odorous material consisting almost exclusively of white blood cells.

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 492 ♂ Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Quant. Titer	1°C. Tube 123	Quant. Titer	Complement-Fixation Reactions[2]	Reaction I			Reaction II		Reaction III	
1941										S) A) C)	a d 3	a d 2	a d 2	c e 2	c e 3	c e 2	c e 3
						Preinoculation Reactions											
10-25	56	H[1]	---		Undil.	---		---		T.)[3]	-	-	-	-	-	-	-
		U	---		Undil.	---		---		SC.)	-	-	-	-	-	-	-
11-12	77	H	00-		Undil.	00-		0--		T.)	0	0	-	-	-	-	-
		U	---		Undil.	---		---		SC.)			-	-	-	-	-
12-3	96	H	---		Undil.	---		---		T.)	0	-	-	ac.	-	ac.	-
		U	---		Undil.	---		---		SC.)		-	-		-		-
12-29	122	H	---		Undil.	---		---		T.)	-	-	-	-	-	-	-
		U	---		Undil.	---		±1		SC.)	-	-	-	-	-	-	-

1/7/42; Rabbit inoculated with 0.5 cc. testicular suspension of Spirochaeta pallida into both testes.

Died 1/11/42. Gross picture on autopsy similar to that in Rabbit 488.

Record of Flocculation Reactions (at Different Temperatures) and of

Complement-Fixation Reactions Given by Rabbit 27♂ Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Quant. Titer	1°C. Tube 123	Quant. Titer	Complement-Fixation Reactions	Reaction I			Reaction II		Reaction III	
1941										S)[2] A) C)	a d 3	a d 2	a d 2	c e 2	c e 3	c e 2	c e 3
						Preinoculation Reactions											
11-4		H[1]	-14	2	Undil.	---		-14	2	T.)[3]	1		2	3	±	-	-
		U	--4	1	Undil.	---		-24	2	SC.)	-	ac.	-	-	-	-	-
11-27		H	-±3	1	Undil.	---		-14	2	T.)	-	2	1	-	-	-	-
		U	--4	1	Undil.	---		244	3	SC.)	-	-	-	-	-	-	-
12-7		H	-24	2	Undil.	---		-44	3	T.)	-	1	±	±	—	-	-
		U	-±4	1	Undil.	---		±44	10	SC.)	-	-	-	-	-	-	-
12-30		H	-14	2	Undil.	---		±24	2	T.)	-	1	-	±	-	-	-
		U	-14	2	Undil.	---		±44	3	SC.)	-	-	-	-	-	-	-
1942	Days Post-inoculation				1/7/42; Rabbit inoculated with 1.5 cc. of testicular suspension of Spirochaeta pallida into both testes.												
1-14	7	H	---		Undil.	---		--±		T.)	-	2	-	-	-	-	-
		U	--±		Undil.	---		--±		SC.)	-	-	-	-	-	-	-

Died 1/24/42. Gross picture on autopsy similar to that in Rabbit 488.

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 28 ♂ Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Quant. Titer	1°C. Tube 123	Quant. Titer	Complement-Fixation Reactions	Reaction I			Reaction II		Reaction III	
1941										S)[2] A) C)	a d 3	a d 2	a d 2	c e 2	c e 3	c e 2	c e 3
					Preinoculation Reactions												
11-4		H[1]	--±		Undil.	---		--±		T.)[3]	3	ac.	3	4	±	-	-
		U	--4	1	Undil.	---		-±4	1	SC.)	-		±	-	-	-	-
11-28		H	---		Undil.	---		--±		T.)	-	ac.	2	-	-	-	-
		U	--1		Undil.	---		-44	3	SC.)	-		-	-	-	-	-
12-7		H	---		Undil.	---		--1		T.)	-	3	-	-	-	-	-
		U	-±2	1	Undil.	---		-24	2	SC.)	-	-	-	-	-	-	-
12-30		H	---		Undil.	---		--2	1	T.)	±	ac.	1	ac.	±	ac.	-
		U	--3	1	Undil.	---		-34	2	SC.)	-		-		-		-
			1/7/42; Rabbit inoculated with 1.0 cc. of testicular suspension of Spirochaeta pallida into both testes.														
1942																	
1-14		H	---		Undil.	---		---		T.)	-	-	-	-	-	-	-
		U	---		Undil.	---		---		SC.)	-	-	-	-	-	-	-

Died 1/17/42. Gross picture on autopsy (1/18/42) similar to that in Rabbit 488.

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 35 ♂ Inoculated with Spirochaeta pallida

Date of Examination	Age of Rabbit in Days		Kahn Reactions' (Room Temperature) Tube 123	Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Quant. Titer	1°C. Tube 123	Quant. Titer	Complement-Fixation Reactions	Reaction I			Reaction II		Reaction III	
1941										S)[2]	a	a	a	c	c	c	c
										A)	d	d	d	e	e	e	e
										C)	3	2	2	2	3	2	3
						Preinoculation Reactions											
12-17		H[1]	-12	1	Undil.	--±		244	3	T.)[3]	3	4	4	4	4	-	-
		U	-23	2	Undil.	--2	1	344	4	SC.)	-	-	-	-	-	-	-
12-29		H	-+3	1	Undil.	---		444	10	T.)	±	4	4	-	-	-	-
		U	-34	2	Undil.	--3	1	444	10	SC.)	-	-	-	-	-	-	-
1942	Days Post-inoculation				1/7/42; Rabbit inoculated with 1.0 cc. testicular suspension of Spirochaeta pallida into left testes.												
1-14	7	H	---		Undil.	---		-+4	1	T.)	-	3	-	3	-	-	-
		U	--±		Undil.	---		-+4	1	SC.)	-	±	-	-	-	-	-

Died 1/20/42. Gross picture on autopsy similar to that in Rabbit 488.

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 34 ♂, A Noninoculated Control

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Kahn Reactions (Room Temperature) Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	37°C. Quant. Titer	1°C. Tube 123	1°C. Quant. Titer	Complement-Fixation Reactions	Reaction I			Reaction II		Reaction III	
										S)[2] A) C)	a d 3	a d 2	a d 2	c e 2	c e 3	c e 2	c e 3
1941																	
						Preinoculation Reactions											
12-17		H[1]	134	8	1:2	---	4	344	20	T.)[3]	4				4		-
		U	±34	8	1:2	---	4	244	20	SC.)	1	ac.	ac.	ac.	±	ac.	-
12-29		H	±44	8	1:2	---	4	444	20	T.)	4	4	4	3	-	-	-
		U	±44	8	1:2	---	4	444	20	SC.)	-	-	-	-	-	-	-
1942																	
1-14		H	-±4	1	Undil.	---		144	30	T.)	±	4	4	1	-	-	-
		U	±34	2	Undil.	-±4	1	444	40	SC.)	-	-	-	-	-	-	-
1-24		H	244	10	1:2.5	-11	4	-44	20	T.)						±	-
		U	-24	2	Undil.	--±		344	10	SC.)	ac.	ac.	ac.	0	0	-	-
2-7		H	-44	8	1:2	---	4	244	30	T.)	-	3	1	4	-	-	-
		U	-34	2	Undil.	--4	1	444	40	SC.)	-	-	-	1	-	-	-
3-3		H	±44	8	1:2	-±1	4	-44	40	T.)	-		-	-	-	-	-
		U	-±2	1	Undil.	---		±44	20	SC.)	-	0	-	-	-	-	-
3-23		H	±44	8	1:2	---	4	±44	40	T.)	-	3	±	4	±	-	-
		U	-33	8	1:2	---	4	444	40	SC.)	-	-	-	-	-	-	-

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 481 ♀, A Noninoculated Control

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Quant. Titer	1°C. Tube 123	Quant. Titer	Complement-Fixation Reactions: Reaction I (a d 3)	Reaction I (a d 2)	Reaction I (a d 2)	Reaction II (c e 2)	Reaction II (c e 3)	Reaction III (c e 2)	Reaction III (c e 3)
1941										S)[2] A) C)						
						Preinoculation Reactions										
10-11	51	H[1]	---		Undil.	---		---		T.)[3] SC.) 0	ac.	ac.	2 2	- -	0	0
		U	---		Undil.	---		--±								
10-23	63	H	-14	2	Undil.	-13	1	-44	3	T.) 4	4	4	4	4	3	-
		U	-14	2	Undil.	-21	1	444	4	SC.) -	1	2	-	-	-	-
11-10	81	H	--1		Undil.	---		-±4	1	T.) 0	0	4	-	-	-	-
		U	-14	1	Undil.	---		-44	3	SC.)		-	-	-	-	-
12-2	103	H	--±		Undil.	---		-±4	1	T.) 2	ac.	3	ac.	±	2	-
		U	-±4	1	Undil.	---		444	4	SC.) -		1		-	2	-
12-29	130	H	--3	1	Undil.	---		-24	2	T.) ±	2	-	±	-	-	-
		U	-14	2	Undil.	---		244	3	SC.) -	-	-	-	-	-	-
1942																
1-14	146	H	---		Undil.	---		--1		T.) -	ac.	-	ac.	-	ac.	-
		U	---		Undil.	---		-14	2	SC.) -		-		-		-
1-26	158	H	-±2	1	Undil.	---		-±4	1	T.)					-	-
		U	-14	2	Undil.	--±		244	3	SC.) ac.	ac.	ac.	ac.	ac.	-	-
2-7	170	H	---		Undil.	---		-±4	1	T.) -	ac.	3	ac.	-	2	-
		U	-24	2	Undil.	---		244	3	SC.) -		±		-	1	-
3-3	194	H	--2	1	Undil.	---		-±4	1	T.) -	0	-	-	-	-	-
		U	--4	1	Undil.	---		-34	2	SC.) -		-	-	-	-	-
3-23	214	H	---		Undil.	---		-±4	1	T.) -	-	-	-	-	-	-
		U	---		Undil.	---		444	4	SC.) -	-	-	-	-	-	-

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 25 ♂, A Noninoculated Control

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Kahn Reactions (Room Temperature) Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	37°C. Quant. Titer	1°C. Tube 123	1°C. Quant. Titer		Complement-Fixation Reactions: Reaction I			Reaction II		Reaction III	
1941										S)[2] A) C)	a d 3	a d 2	a d 2	c e 2	c e 3	c e 2	c e 3
						Preinoculation Reactions											
11-4		H[1]	444	12	1:3	-14	12	444	80	T.)[3]	4	4	4	4	4	4	4
		U	444	12	1:3	-14	12	444	60	SC.)	-	2	-	-	-	-	-
11-27		H	444	10	1:2.5	---	4	-44	40	T.)	-	3	2	3	-	-	-
		U	444	10	1:2.5	--±	4	-44	40	SC.)	-	-	-	-	-	-	-
12-7		H	444	12	1:3	---	4	±44	40	T.)	-	4	1	4	-	-	-
		U	444	12	1:3	---	4	±44	40	SC.)	-	-	-	-	-	-	-
12-30		H	444	12	1:3	---	4	144	60	T.)	-	4	2	4	±	-	-
		U	444	12	1:3	---	4	±44	60	SC.)	-	-	-	-	-	-	-
1942																	
1-14		H	444	12	1:3	-±4	12	-24	12	T.)	-	ac.	-	4	±	3	-
		U	±44	8	1:2	---	4	444	30	SC.)	-		-	-	-	-	-
1-26		H	444	12	1:3	-14	20	-44	30	T.)	ac.	ac.	ac.	0	0	4	-
		U	±44	8	1:2	--3	8	444	60	SC.)						-	-
2-8		H	144	20	1:4	-±4	20	±44	40	T.)	-	1	2	4	-	4	-
		U	-24	2	Undil.	-14	2	±44	40	SC.)	-	-	-	-	-	-	-
3-3		H	444	16	1:4	-±4	16	-44	30	T.)	-	0	3	-	-	±	-
		U	244	10	1:2.5	--4	10	144	20	SC.)	-		-	-	-	-	-
3-23		H	344	12	1:3	--±	4	±44	30	T.)	-	±	-	4	±	-	0
		U	±44	10	1:2.5	-13	10	444	40	SC.)	-	-	-	-	-	-	

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 476 ♂, A Noninoculated Control

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Kahn Reactions (Room Temperature) Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	37°C. Quant. Titer	1°C. Tube 123	1°C. Quant. Titer		Complement-Fixation Reactions: Reaction I	Reaction II		Reaction II		Reaction III	
1941										S)[2] A) C)	a d 3	a d 2	a d 2	c e 2	c e 3	c e 2	c e 3
					Preinoculation Reactions												
10-11	56	H[1]	---		Undil.	---		-14	2	T.)[3]	ac.	ac.	ac.	ac.	ac.	0	0
		U	-±1		Undil.	---		144	3	SC.)							
10-23	68	H	---		Undil.	---		-14	2	T.)	ac.	ac.	ac.	ac.	-	0	-
		U	---		Undil.	---		±42	2	SC.)					±		-
11-10	86	H	---		Undil.	---		±24	2	T.)	±	ac.	ac.	ac.	-	ac.	-
		U	-±2	1	Undil.	---		±44	10	SC.)	-				-		-
12-2	108	H	---		Undil.	---		±14	2	T.)	0	3	2	ac.	3	ac.	-
		U	--3	1	Undil.	---		-14	2	SC.)		-	-		±		-
12-29	135	H	---		Undil.	---		±34	2	T.)	-	1	-	-	-	-	-
		U	-±2	1	Undil.	---		±44	3	SC.)	-	-	-	-	-	-	-
1942																	
1-14	151	H	---		Undil.	---		±33	2	T.)	-	2	-	ac.	-	-	-
		U	-±1		Undil.	---		444	4	SC.)	-	1	-		-	-	-
1-26	163	H	---		Undil.	---		-±4	1	T.)	ac.	ac.	ac.	ac.	ac.	-	-
		U	-±1		Undil.	---		444	40	SC.)						-	-
2-7	175	H	---		Undil.	---		-±1		T.)	-	2	-	1	-	±	-
		U	-±4	1	Undil.	---		-44	3	SC.)	-	-	-	1	-	±	-
3-3	199	H	-±1		Undil.	-±-		-44	3	T.)	-	0	-	-	-	-	-
		U	--±		Undil.	---		-44	3	SC.)	-		-	-	-	-	-
3-23	219	H	-±2	1	Undil.	---		±44	3	T.)	-	-	-	-	-	-	-
		U	---		Undil.	---		444	20	SC.)	-	-	-	-	-	-	-

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 491 ♂, A Noninoculated Control

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Quant. Titer	1°C. Tube 123	Quant. Titer	Complement-Fixation Reactions	Reaction I			Reaction II		Reaction III	
1941										S)[2]	a	a	a	c	c	c	c
										A)	d	d	d	e	e	e	e
										C)	3	2	2	2	3	2	3
						Preinoculation Reactions											
10-11	52	H[1]	---		Undil.	---		---		T.)[3]							
		U	---		Undil.	---		--1		SC.)	0	ac.	ac.	ac.	ac.	0	0
10-23	64	H	---		Undil.	---		-11	1	T.)	1				±	0	-
		U	--1		Undil.	---		±44	3	SC.)	±	ac.	ac.	ac.	±	0	-
11-10	82	H	--1		Undil.	---		-±3	1	T.)			±	-	-	-	-
		U	±34	2	Undil.	Insufficient serum				SC.)	0	0	-	-	-	-	-
12-2	104	H	---		Undil.	---		--±		T.)	-	±	-	-	-	-	-
		U	-±4	1	Undil.	---		-24	2	SC.)	-	-	-	-	-	-	-
12-29	131	H	--±		Undil.	---		-±3		T.)	-	±	-	-	-	-	-
		U	-14	2	Undil.	---		-44	3	SC.)	-	-	-	-	-	-	-
1942																	
1-14	145	H	---		Undil.	---		±--		T.)	-	2	-	-	-	-	-
		U	--4	1	Undil.	---		±44	3	SC.)	-	-	-	-	-	-	-
1-26	157	H	--±		Undil.	---		--±		T.)						-	-
		U	-24	2	Undil.	--2	1	±44	3	SC.)	ac.	ac.	ac.	ac.	ac.	-	-
2-7	169	H	---		Undil.	---		-±+		T.)	-	ac.	3	2	-	1	-
		U	-±4	1	Undil.	--3	1	444	4	SC.)	-		-	2	-	±	-
3-3	193	H	---		Undil.	---		-±4	1	T.)	0	0	-	-	-	-	-
		U	--±		Undil.	---		244	3	SC.)			-	-	-	-	-
3-23	213	H	---		Undil.	---		-44	20	T.)	-	-	-	-	-	-	-
		U	--3	1	Undil.	---		444	20	SC.)	-	-	-	-	-	-	-

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 148 ♀, A Noninoculated Control

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Quant. Titer	1°C. Tube 123	Quant. Titer	Complement-Fixation Reactions: S)[2] A) C)	Reaction I a d 3	Reaction I a d 2	Reaction I a d 2	Reaction II c e 2	Reaction II c e 3	Reaction III c e 2	Reaction III c e 3
1941																	
						Preinoculation Reactions											
10-11	56	H[1]	---		Undil.	---		---		T.)[3] SC.)	0	ac.	ac.	4 2	4 2	0	0
		U	---		Undil.	---		---									
10-23	68	H	--±		Undil.	---		-23		T.) SC.)	- -	ac.	2 -	- -	- -	- -	- -
		U	--4	1	Undil.	---		±44									
11-10	86	H	-±1		Undil.	---		--4		T.) SC.)	0	0	4 -	± -	- -	- -	- -
		U	144	3	Undil.	---		244									
12-2	108	H	134	3	1:2	---		444	30	T.) SC.)	4 ±	ac.	ac.	ac.	3 2	- -	- -
		U	244	3	1:2	---		444	40								
12-29	135	H	±44	3	1:2	---		±44	30	T.) SC.)	4 -	4 -	4 -	3 -	± -	- -	- -
		U	±44	3	1:2	---		244	40								
1942																	
1-14	151	H	---		Undil.	---		-±4	1	T.) SC.)	- -	2 ±	- -	ac.	- -	ac.	- -
		U	---		Undil.	---		-44	3								
1-26	163	H	-±1		Undil.	---		±24	2	T.) SC.)	ac.	ac.	ac.	ac.	ac.	- -	- -
		U	±24	2	Undil.	---		244	3								
2-7	175	H	---		Undil.	---		-24	2	T.) SC.)	- -	- -	- -	± ±	- -	- -	- -
		U	-±4	1	Undil.	---		-44	3								
3-3	199	H	-±1		Undil.	-±1		±34	2	T.) SC.)	- -	0 -	- -	- -	- -	- -	- -
		U	-±3	1	Undil.	---		-44	10								
3-23	219	H	-14	2	Undil.	-±1		444	20	T.) SC.)	- -	1 -	- -	- -	- -	± -	- -
		U			Undil.	---		444	20								

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 31 ♂, A Noninoculated Control

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Kahn Reactions (Room Temperature) Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Flocculation Reactions at: 37°C. Quant. Titer	Flocculation Reactions at: 1°C. Tube 123	Flocculation Reactions at: 1°C. Quant. Titer	Complement-Fixation Reactions	Reaction I			Reaction II		Reaction III	
										S)[2] A) C)	a d 3	a d 2	a d 2	c e 2	c e 3	c e 2	c e 3
1941																	
						Preinoculation Reactions											
11-4		H[1]	---		Undil.	---		---		T.)[3]	ac.	0	ac.	ac.	ac.	ac.	ac.
		U	-14	2	Undil.	---		±34	2	SC.)							
11-28		H	---		Undil.	---		---		T.)	-	ac.	-	-	-	-	-
		U	-±4	1	Undil.	--±		444	4	SC.)	-		-	-	-	-	-
12-6		H	-±1		Undil.	---		-±3	1	T.)	-	4	1	-	-	-	-
		U	-±4	1	Undil.	---		244	3	SC.)	-	-	-	-	-	-	-
12-30		H	-±1		Undil.	---		-±4	1	T.)	-	4	±	-	-	-	-
		U	--4	1	Undil.	---		±44	3	SC.)	-	±	-	-	-	-	-
1942																	
1-14		H	---		Undil.	---		---		T.)	-	3	2	ac.	-	ac.	-
		U	-44	8	1:2	---		144	8	SC.)	-	±	-		-		-
1-26		H	--3	1	Undil.	--±		-±4	1	T.)	ac.	ac.	ac.	0	0	±	-
		U	±44	8	1:2	---		-44	20	SC.)						-	-
2-7		H	--±		Undil.	-±1		-24	2	T.)	ac.	ac.	ac.	ac.	-	ac.	-
		U	-24	2	Undil.	-14		444	4	SC.)					-		-
3-3		H	-±1		Undil.	--±		-24	2	T.)	-	0	-	-	-	-	-
		U	-±2	1	Undil.	--1		±44	3	SC.)	-		-	-	-	-	-
3-23		H	--2	1	Undil.	---		±44	3	T.)	-	1	-	±	-	-	-
		U	-±4	1	Undil.	---		±44	3	SC.)	-	-	-	-	-	-	-

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 495 ♂

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Quant. Titer	1°C. Tube 123	Quant. Titer	Complement-Fixation Reactions[2]	Reaction I			Reaction II		Reaction III	
1941										S) A) C)	a d 3	a d 2	a d 2	c e 2	c e 3	e e 2	c e 3
					Preinoculation Reactions												
10-11	52	H[1]	---		Undil.	---		---		T.)[3] SC.)	0	ac.	ac.	ac.	ac.	0	0
		U	---		Undil.	---		134	3								
10-23	64	H	±44	8	1:2	---		-14	10	T.) SC.)	ac.	ac.	ac.	ac.	ac.	0	0
		U	-24	2	Undil.	--1		444	20								
11-10	82	H	-14	2	Undil.	-±4	1	±44	10	T.)	4	0	4	2	-	3	-
		U	-44	3	1:1.5	---		±44	20	SC.)	-		±	-	-	-	-

Died 11/18/41. No autopsy.

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 485 ♂

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Quant. Titer	1°C. Tube 123	Quant. Titer	Complement-Fixation Reactions	Reaction I			Reaction II		Reaction III	
1941										S)[2] A) C)	a d 3	a d 2	a d 2	c e 2	c e 3	c e 2	c e 3
						Preinoculation Reactions											
10-11	52	H[1]	---		Undil.	---		---		T.) SC.)[3]	0	0	0	ac.	ac.	0	0
		U	Insufficient for examination														
10-25	66	H	---		Undil.	---		±14	3	T.)	ac.	ac.	ac.	ac.	ac.	0	1
		U	---		Undil.	---		±44	3	SC.)							1
11-12	86	H	---		Undil.	---		144	3	T.)	-	-	-	-	-	-	-
		U	-±2		Undil.	---		±44	3	SC.)	-	-	-	-	-	-	-

Died after cardiac puncture. No autopsy.

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 149 ♂

Date of Examination	Age of Rabbit in Days	Kahn Reactions (Room Temperature)	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Flocculation Reactions at: 37°C. Quant. Titer	Flocculation Reactions at: 1°C. Tube 123	Flocculation Reactions at: 1°C. Quant. Titer	Complement-Fixation Reactions	Reaction I	Reaction II	Reaction III
1941		Tube 123	Quant. Titer	Tube 123	Quant. Titer	Tube 123	Quant. Titer	S) A) C) [2]	a d 3 / a d 2 / a d 2	c e 2 / c e 3	c e 2 / c e 3
					Preinoculation Reactions						
10-11	56	H [1]	---	Undil.	---	---		T.) SC.) [3]	0	ac.ac.	ac. ac.	0 0
		U	Insufficient Serum								
10-23	68	H	---	Undil.	---	---		T.) SC.)	ac.	ac.ac.	ac. ac.	0 0
		U	---	Undil.	---	--3					

Died following cardiac puncture. Autopsy showed large clots surrounding heart.

Record of Flocculation Reactions (at Different Temperatures) and of Complement-Fixation Reactions Given by Rabbit 500 ♂

1941															
10-11	56	H	---		Undil.	---		144	3	T.) SC.)	0	ac.ac.	ac. ac.	0	0
		U	-12	1	Undil.	---		444	4						
10-23	68	H	+24	2	Undil.	-12	1	344	4	T.) SC.)	4 ±	ac.ac.	ac. 4 1	0	0
		U	-24	2	Undil.	-1±		144	3						

Died 10/25/41. No autopsy.

Record of Flocculation Reactions (at Different Temperatures) and of

Complement-Fixation Reactions Given by Rabbit U483 ♀, A Noninoculated Control

Date of Examination	Age of Rabbit in Days		Kahn Reactions (Room Temperature) Tube 123	Quant. Titer	Dilution of Serum with 0.9% Saline	Flocculation Reactions at: 37°C. Tube 123	Quant. Titer	1°C. Tube 123	Quant. Titer		Complement-Fixation Reactions Reaction I			Reaction II		Reaction III	
1941										S)[2] A) C)	a d 3	a d 2	a d 2	c e 2	c e 3	c e 2	c e 3
					Preinoculation Reactions												
10-11	61	H[1]	---		Undil.	---		±24	2	T.)[3]	0	ac.	ac.	ac.	-	ac.	-
		U	---		Undil.	---		444	4	SC.)					-		-
			Died 10/14/41. No autopsy.														
			; by Rabbit U489 ♀														
10-11	56	H	---		Undil.	---		-13	1	T.)	0	ac.	ac.	ac.	ac.	ac.	ac.
		U	---		Undil.	---		444	4	SC.)							
			Died 10/12/41. No autopsy.														
			; by Rabbit 483 ♀														
10-11	56	H	---		Undil.	---		---		T.)	0	ac.	ac.	ac.	ac.	0	0
		U	---		Undil.	---		-13	1	SC.)							
			Died 10/13/41. No autopsy.														

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