IMMOBILIZATION OF TREPONEMA PALLIDUM IN VITRO BY ANTIBODY PRODUCED IN SYPHILITIC INFECTION*

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It is well established that animals and human beings infected with *Treponema pallidum* become resistant to reinfection with the same organism, but the mechanism of this immunity is poorly understood. One of the serious handicaps to studies on this problem has been the lack of *in vitro* methods for the detection of antibody to *T. pallidum*.

Early reports of an *in vitro* spirocheticidal action of serum and spinal fluid from patients in the late stages of syphilitic infection (1–3) were based on crude qualitative tests and did not offer experimental data adequate to establish the claims made. Such findings were not confirmed by others (4, 5) and recent experiments in this laboratory (10) did not yield convincing evidence of an *in vitro* effect.

The presence of spirocheticidal antibody in serum from syphilitic individuals has been demonstrated by animal "protection" tests (6–10), but the costly and cumbersome nature of these tests, as well as their qualitative character, renders them unsuitable for systematic investigation of the rôle and mechanism of specific humoral immunity in syphilis.

Since it is now agreed that *T. pallidum* has not as yet been cultivated and is therefore not available in adequate amounts for the usual *in vitro* immunological studies, various cultivatable, non-pathogenic spirochetes have been used in agglutination and complement-fixation tests (11–25) with sera from syphilitic animals and human beings. However, a considerable proportion of presumably normal sera has been found to react with these antigens, though usually in low titre. This reactivity with normal sera, as well as the lack of a clear understanding of the relationship of these non-virulent spirochetes to pathogenic *T. pallidum*, renders their use as antigens in the study of humoral immunity in syphilis of dubious value.

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While standard serological tests for syphilis, carried out with lipoidal antigens from beef heart or other mammalian tissues, serve to detect the presence of syphilitic infection with a relatively high degree of specificity, they do not provide an index of immunity to the disease. This is apparent from the decline in the titre of Wassermann antibody, or "reagin," with progression of the disease into the latent stage, although it is during this stage that animals exhibit a high resistance to reinfection (26, 27). Furthermore, it is questionable whether the appearance of reagin represents a specific immunological response to an antigenic constituent of *T. pallidum* (28–35). Thus, studies on immunity to syphilis have been restricted to *in vivo* experiments involving either active or passive "protection" tests.

As a result of the development in this laboratory of techniques which permit the extraction of virulent *T. pallida* from rabbit testicular syphilomas in a relatively tissue-free state and the maintenance of these organisms *in vitro* in a highly active state for several days (36), it has become possible to demonstrate the presence of an antibody in sera from syphilitic animals and human beings, which immobilizes virulent *T. pallida in vitro*.

The present report is concerned with: (*a*) the demonstration that this immobilizing activity is due to specific antibody acting in conjunction with complement, and that immobilized treponemes are non-infectious and presumably dead; (*b*) a study of factors which affect the measurement of the immobilizing antibody in sera from syphilitic individuals; (*c*) the occurrence of this antibody in sera from animals and human beings in various stages of syphilitic infection; and (*d*) the differentiation of the immobilizing antibody from the Wassermann antibody or reagin.

Materials and Techniques

Preparation of Treponeme Suspensions.—The general procedures for the collection and maintenance of virulent *T. pallidum in vitro*, are described and discussed in detail in a preceding paper from this laboratory (36).

In brief, rabbits to be used as source animals are inoculated intratesticularly with 0.5 ml. of a suspension of *T. pallida* freshly isolated from rabbit testicular syphilomas. These suspensions are prepared by Waring blendor emulsification of 2 testicular syphilomas with approximately 15 ml. of a 5 per cent solution of crystalline bovine albumin in saline, and contain about 5 to 10 × 10^7 treponemes per ml. The animals are caged in an air-conditioned room (16–18°C.) and are examined daily for the development of syphilitic orchitis. After 7 to 14 days a recognizable orchitis becomes manifest, during the first 48 hours of which the animal is exsanguinated and the testes are removed. Following removal, the testes are immediately cut into thin slices with a specially constructed plastic instrument[1] fitted with 10 razor blades, and

[1] Constructed by Acme Metal Products, Inc., Baltimore. The instrument measures 5 × 9 × 2 cm. and consists of 2 separate parts. The base has one deep transverse depression into which the rabbit testes can be placed securely, and 10 narrow longitudinal grooves. The upper

then washed with chilled 0.85 per cent saline to remove loose tissue particles. The slices are placed in 34 ml. of basal medium[2] under an atmosphere of 5 per cent carbon dioxide and 95

part is fitted with 10 Durham Duplex razor blades, held in parallel approximately 5 mm. apart. After the testes are placed in the transverse depression in the base the upper part may be placed on the base with the blades fitting into the longitudinal grooves and the testis cut into 8 to 10 slices of approximately 5 mm. width each by a downward and backward slicing movement.

[2] The constituents of the basal medium are made up individually and mixed in proper proportion just prior to use. The method of preparation and amounts used to make 85 ml. of medium are listed below. In previous experiments, these amounts were put into 100 ml. of medium to yield concentrations optimal for survival (36). In order to achieve the same concentrations in the present immobilization test mixtures, the same amounts were dissolved in 85 ml. since the constituents of the basal medium are diluted by the factor 85/100 when 1.7 ml. of medium containing treponemes is mixed with 0.2 ml. of serum and 0.1 ml. of guinea pig complement.

	Final concentration in test mixture
Crystalline bovine albumin	0.00028 M
5 gm. are dissolved in about 95 ml. of 0.85 per cent NaCl; approximately 1 M NaOH added until pH is 7.0; saline added to make 100 ml.; sterilized by "ultrafine" filtration (Corning U. F.); stored at −20°C. Use 40 ml. per 85 ml. of medium.	
Phosphate buffer:	
$Na_2HPO_4 \cdot 12\ H_2O$	0.010 M
KH_2PO_4	0.0038 M
Stock solution: 40 ml. 0.10 M $Na_2HPO_4 \cdot 12\ H_2O$ plus 10 ml. 0.15 M KH_2PO_4 (mixture pH 7.1 by glass electrode measurement); sterilized by U. F. filter; stored at 5°C. Use 12.5 ml. per 85 ml. of medium.	
Sodium thioglycollate	0.0016 M
1.50 per cent in distilled H_2O; sterilized by U. F. filter; stored in glass-stoppered bottle at 5°C.; made fresh every 2 weeks. Use 1.20 ml. per 85 ml. of medium.	
1 (+) cysteine·HCl	0.001 M
0.630 per cent in 0.85 per cent NaCl; sterilized by U. F. filter; stored at −20°C. Use 2.5 ml. per 85 ml. of medium.	
Glutathione	0.001 M
1.23 per cent in 0.85 per cent NaCl; sterilized by U. F. filter; stored at −20°C. Use 2.5 ml. per 85 ml. of medium.	
Sodium pyruvate	0.001 M
0.69 ml. pyruvic acid plus 80 ml. 0.85 per cent NaCl plus approximately 1 M NaOH to bring to pH 7.0 (about 10 ml. required); made to 100 ml. with 0.85 per cent NaCl; sterilized by U. F. filter; stored in brown bottles at −20°C.; made fresh weekly. Use 1.0 ml. per 85 ml. of medium.	
Vitamin mixture:	
Thiamin·HCl	1,000 μg./liter
Niacin	1,000 μg./liter
d-calcium pantothenate	500 μg./liter
Pyridoxine	500 μg./liter
Riboflavin	500 μg./liter

(Footnote continued on following page)

per cent nitrogen, and the treponemes extracted from the tissue with gentle rocking for approximately 2 hours at 35°C.[3] The extracted treponemes are separated from tissue debris and spermatozoa by filtration through a Corning "medium" fritted disc. The filtrate so obtained is slightly opalescent and on darkfield examination[4] shows 2 or more organisms per field and an occasional red blood cell, but no appreciable tissue debris. As determined by calibration of the microscope, 2 treponemes per field is equivalent to 10^7 organisms per ml. When the number exceeds this value an appropriate amount of basal medium is added to dilute the suspension so that it contains 10^7 treponemes per ml. The percentage of motile organisms is also determined at this time by examining 50 successive treponemes in fields selected at random, and this is recorded as the 0-hour reading. Ordinarily, 90 to 98 per cent of the organisms exhibit active motility. While counts of 100 or more treponemes would yield more precise determinations of the percentage of motile organisms, the time required for such extensive counts would become so long that non-specific loss of motility might occur due to exposure of the organisms to atmospheric conditions other than those designed for optimal survival. This limitation is especially serious in reading a large series of test mixtures as are used in the immobilization tests described below, and has resulted in counting 50 organisms as routine in motility determinations.

Source of Complement.—Pools of about 30 to 40 ml. of serum from 10 to 15 guinea pigs are collected and distributed under aseptic conditions in cotton-plugged, rubber-capped tubes which are stored in solid carbon dioxide. As will be shown in the experimental protocols guinea pig serum in the low concentrations used exerts no effect on the motility of *T. pallidum.*

Collection of Sera.—Tests for immobilizing activity have been performed on five groups of sera: (1) from non-syphilitic, apparently healthy rabbits; (2) from untreated rabbits infected

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	Final concentration in test mixture
Choline·HCl	500 μg./liter
Inositol	500 μg./liter
Biotin	10 μg./liter
Folic acid	10 μg./liter
A stock solution is prepared in 0.85 per cent NaCl and contains 100 × the concentrations stated; sterilized by U. F. filtration; stored in brown bottles at −20°C. Use 1.0 ml. per 85 ml. of medium.	
Sodium bicarbonate	0.0072 M
1.26 per cent in distilled H_2O; sterilized by U. F. filtration; stored in pyrex glass-stoppered bottles at 5°C.; made fresh biweekly. Use 4.8 ml. per 85 ml. of medium. On admixture of serum and complement additional bicarbonate is contributed, resulting in a final concentration of approximately 0.0085 M, a value required for the maintenance of the system at pH 7.0 at 35°C. under 5 per cent CO_2. In runs at 30°C. the level of bicarbonate is raised accordingly.	

Add 0.85 per cent NaCl in distilled H_2O to bring medium to 85 ml.

[3] In several of the early experiments, extraction was carried out at 30°C., as noted in the experimental protocols.

[4] Five thousandths ml. of fluid (measured with a Kahn pipette) is placed under a 22 × 22 mm. coverslip and examined with a calibrated darkfield microscope. The number of treponemes in 25 high power fields in multiplied by a calibration factor of 200,000 to determine the number per milliliter. When accurate quantitation of the number of organisms per milliliter is not required, *e.g.* in reading the immobilization tests for the percentage of motile organisms, a drop of the fluid treponeme suspension approximating 0.005 ml. may be obtained by the use of a wire-looped inoculating needle.

with *T. pallidum* (Nichols strain) for 3 to 9 months; (3) from members of this department and the Medicine I Clinic of The Johns Hopkins Hospital, whose histories were reliable with regard to the absence of syphilitic infection; (4) from patients with darkfield positive primary or secondary syphilis examined either at the Medicine I Clinic of The Johns Hopkins Hospital or at the Rapid Treatment Center of the Baltimore City Hospitals; and (5) from presumably non-syphilitic patients either with acute febrile diseases or with chronic allergic diseases. Serum, obtained aseptically, is stored at −20°C. in cotton-plugged, rubber-capped tubes. Samples to be tested are heated at 56°C. for 30 minutes just prior to use.

Test Procedure.—One and seven-tenths ml. of the filtered treponeme suspension, 0.2ml. of the serum to be tested, and 0.1 ml. of guinea pig serum (complement) are pipetted into a 25 × 100 mm. pyrex culture tube. In each experimental series a control tube containing 1.7 ml. of treponeme suspension, 0.1 ml. of complement, and 0.2 ml. of ultrafiltrate of serum[5] is included. It is believed that the latter serves as an appropriate control substitute for whole serum, since the concentration of electrolytes is equivalent to that of whole serum, and since some of these electrolytes, *e.g.* Mg^{++} and Ca^{++}, may affect the outcome of the test (37). The tubes containing these mixtures are incubated at 35°C. in a Brewer anaerobic jar filled with a gas mixture of 5 per cent carbon dioxide and 95 per cent nitrogen. Percentages of motile organisms are determined as outlined above, at intervals of 4 or 8 hours, depending on the nature of the individual experiment, for a total period of 16 hours.

At the termination of each experiment all mixtures of treponemes, serum, and complement which fail to produce immobilization should be tested for the presence of active complement by addition of sensitized red blood cells, in order to guard against false negative results due to anticomplementary effects.

All manipulations are carried out with strict aseptic precautions. The glassware employed is cleaned with a dichromate-sulfuric acid mixture prior to sterilization. All steps in the procedure which involve exposure of the organisms to conditions other than those outlined for a given experiment, *e.g.* filtration of the treponeme suspension and reading the tests, are carried out as rapidly as possible in order to obtain optimal survival of the organisms.

Morphological Characteristics of Immobilization of Treponemes by Antibody and Complement.—Unlike treponemes observed in material from human or rabbit syphilitic lesions, those suspended in the fluid medium described above exhibit extremely active movements, which are often so rapid that the entire organism appears to vibrate. Moreover, translational or to-and-fro movements are infrequent, *i.e.*, most of the organisms contract and relax rhythmically in a relatively confined area. In addition, the treponemes bend or twist into circular, S-shaped, or V-shaped forms, but generally resume their characteristic motion along the longitudinal axis within a few seconds.

In the experiments previously reported (36) it was noted that sluggish forms are frequently seen when treponemes are exposed to unfavorable nutrient media. By contrast, when immobilization results from the action of antibody and complement, no intermediate phases of motility, *i.e.* sluggishly motile forms, have been noted. Since all the organisms seen are either fully active or completely non-motile, their differentiation presents no difficulty. Treponemes immobil-

[5] Simms ultrafiltrate of ox blood serum, prepared by Microbiological Laboratories, Flemington, New Jersey.

ized by antibody and complement suffer no appreciable distortion and may exhibit any of the several shapes described above for motile organisms. These facts, as well as the use of suspensions essentially free from tissue debris, render them easily identifiable. Counts of the total number of organisms have been made repeatedly, but no evidence of lysis has been noted, nor has agglutination been observed.

Since motile and non-motile organisms can readily be identified and differentiated, and since repeated counting of any one motile treponeme in adjoining fields is unlikely in view of the lack of translational movement, it is possible to make accurate determinations of the total number of organisms, as well as of the percentage of those motile.

TABLE I

Comparison of the Action of Normal and Syphilitic Rabbit Serum on T. pallidum in Vitro, with and without Complement. Initial (0-Hour) Motility of Treponemes: 98 Per Cent*

Serum pool tested	Final serum dilution	Motility of organisms after incubation at 30°C.			
		Without complement		With complement (1/20)	
		12 hrs.	24 hrs.	12 hrs.	24 hrs.
		per cent	*per cent*	*per cent*	*per cent*
Normal	1/10	92	94	96	84*
Normal	1/100	92	94	96	92
Normal	1/1000	90	94	96	92
Syphilitic*	1/10	96	84	2	2
Syphilitic*	1/100	92	96	50	10
Syphilitic*	1/1000	96	92	92	84
Serum ultrafiltrate control		94	96	92	92

* Syphilis serum pool 3: this pool gave a reagin titre of 24, expressed as the highest serum dilution producing flocculation with Eagle antigen. The Eagle test for reagin in the normal pool was negative.

EXPERIMENTAL PROTOCOLS AND RESULTS

The Comparative Action of Normal and Syphilis Sera on T. pallidum in Vitro.—

A pool of serum from 10 normal albino rabbits and a pool of serum from 10 rabbits infected with *T. pallidum* (Nichols strain) for approximately 6 months, were tested for immobilizing activity as described above, except that extraction of the treponemes and incubation of the test mixtures were carried out at 30°C.

As shown in Table I, neither serum pool exerted a significant deleterious effect on the motility of *T. pallidum* in the absence of guinea pig complement. How-

ever, in the presence of complement a marked reduction in motility of the organisms was produced by the syphilis serum pool, but not by the normal serum pool. Since this effect was manifested only in the presence of complement, it is highly probable that the component of syphilis serum responsible for the immobilization is an antibody against *T. pallidum*. Subsequent experiments appear to justify this assumption.

TABLE II

Influence of Serum Concentration and Time of Incubation at 30°C. on the Rate of Immobilization of T. pallidum by 3 Different Pools of Sera from Untreated Syphilitic Rabbits. 0-Hour Motility: 90 Per Cent

Serum pool No.	Final serum dilution	Complement added 1/20*	Motility of organisms	
			8 hrs.	16 hrs.
			per cent	*per cent*
1‡	1/10	−	90	78
	1/10	+	12	0
	1/30	+	20	0
	1/90	+	42	2
	1/270	+	60	6
2‡	1/10	−	90	80
	1/10	+	6	0
	1/30	+	22	0
	1/90	+	40	2
	1/270	+	60	8
3‡	1/10	−	90	80
	1/10	+	22	4
	1/30	+	40	10
	1/90	+	50	22
	1/270	+	72	40
Serum ultrafiltrate control........		−	90	80
Serum ultrafiltrate control........		+	88	80

* Addition of complement is indicated by +, while absence of complement is denoted by −.
‡ Reagin titres with Eagle antigen were 2, 4, and 24 for pools 1, 2, and 3 respectively.

The Rate of Immobilization as a Function of Time and Serum Concentration.—

Pools of sera were prepared from each of 3 groups of 10 syphilitic rabbits as follows: pool 1 from rabbits infected for 6 months, pool 2 from rabbits infected for 4 months, and pool 3 from rabbits infected for 3 months. Each pool was tested at final dilutions of 1/10, 1/30, 1/90, and 1/270. Motility observations were made after 8 and 16 hours' incubation at 30°C.

As shown in Table II, no immobilizing effect was manifested by the sera in the absence of complement. This confirmed the data in Table I. Moreover,

in the presence of complement the immobilizing effect increased with time and with serum concentration, and, in general, was manifested rather slowly.

Concentrations of serum above 10 per cent have not been used since in previous experimentation on survival of *T. pallidum in vitro* (36) inactivated normal serum at a final concentration of 40 per cent produced a significant inhibition of survival in the absence of complement. In the present experiments it was found that with occasional normal sera a small degree of immobilization occurred at a final concentration of 20 per cent, but none of the normal sera produced significant immobilization at a concentration of 10 per cent.

For these reasons, a final serum dilution of 1/10 and a minimal time of 16 hours' incubation have been used in the survey experiments recorded in Tables VII to XI. Under these conditions the sensitivity of the test is high, and yet control specimens containing normal serum plus complement, or complement alone, do not show an appreciable decrease in the percentage of motile organisms.

The Rate of Immobilization as a Function of Temperature.—

In this experiment treponemes were extracted from 2 rabbit testes at 30°C. Portions of the filtered treponeme suspension were pipetted into tubes containing serum and complement which had been placed at the designated temperature for the preceding 15 minutes. The rate of immobilization at 25°, 30°, 35°, and 40°C. was determined by reading the percentage of motile organisms at 4, 8, and 16 hours.

It is evident from the results given in Table III that the rate of antibody action increased with temperature, and as a result, the sensitivity of antibody detection at any given time interval also became greater. However, significant immobilization of the control suspension was manifest as early as 4 hours at 40°C., but not at 35°C., or lower. Therefore, an incubation temperature of 35°C. was chosen for subsequent experiments in the survey of human and rabbit sera (Tables VII to XI).

The Rôle of Complement and the Influence of Its Concentration on the Rate of Immobilization.—

Five ml. portions of a pool of fresh undiluted guinea pig sera were treated as follows: (*a*) heat-inactivated sample—heated at 56°C. for 50 minutes and then stored in solid carbon dioxide; and (*b*) decomplemented sample—mixed in the cold with a finely divided, washed specific precipitate consisting of 1.14 mg. of rabbit anti-egg albumin nitrogen and 0.24 mg. of egg albumin nitrogen. This mixture was left at 5°C. for 24 hours with occasional agitation, and then the precipitate was removed by centrifugation. The supernate was sterilized by passage through a Corning "ultrafine" bacterial filter. This procedure was designed to remove complement from the serum by fixation on an antigen-antibody complex (38).

The immobilizing activity of syphilitic rabbit serum (syphilis pool 4) was tested in the usual manner with untreated, with heat-inactivated, and with decomplemented guinea pig serum. In one series of controls, saline was substituted for guinea pig serum. Another set of controls contained ultrafiltrate of serum in place of the syphilitic rabbit serum.

The results shown in Table IV demonstrate that heat inactivation or removal of complement by absorption with a specific precipitate, renders guinea pig

serum incapable of immobilizing *T. pallida* in conjunction with syphilitic rabbit serum. In view of these findings, the immobilizing activity of fresh guinea pig serum has been attributed to its complement content.

TABLE III

Influence of Temperature on the Rate of Immobilization of T. pallidum by Varying Concentrations of Rabbit Syphilis Serum Pool 2 in the Presence of 1/20 Complement. 0-Hour Motility: 96 Per Cent

Temperature of incubation	Final serum dilution	Motility of organisms		
		4 hrs.	8 hrs.	16 hrs.
°C.		*per cent*	*per cent*	*per cent*
25	1/10	94	80	30
	1/30	96	90	60
	1/90	96	96	70
	Ultrafiltrate control	94	96	90
30	1/10	80	20	0
	1/30	94	30	0
	1/90	96	50	2
	Ultrafiltrate control	96	94	88
35	1/10	10	0	0
	1/30	40	10	0
	1/90	70	20	0
	Ultrafiltrate control	94	90	92
40	1/10	0		
	1/30	0		
	1/90	0		
	Ultrafiltrate control	50		

TABLE IV

The Identification of Complement as the Component of Guinea Pig Serum Which Immobilizes T. pallidum in Conjunction with Antibody in Syphilis Serum. 0-Hour Motility: 98 Per Cent

Guinea pig serum (1/20 final dilution)	Organisms motile after 8 hrs.' incubation at 35°C.		
	1/10 syphilis serum	1/50 syphilis serum	Ultrafiltrate control
	per cent	*per cent*	*per cent*
Untreated	6	24	86
Heat-inactivated	86	86	86
Decomplemented	84	86	90
None*	90	88	90

* Saline (0.85 per cent) substituted for guinea pig serum.

In order to test the effect of varying dilutions of complement on rate of immobilization, 1.6 ml. of treponeme suspension was mixed with 0.2 ml. of varying dilutions of syphilitic rabbit serum (syphilis pool 2) and 0.2 ml. of guinea pig serum (undiluted, 1/2, 1/4, and 1/8 dilutions

in saline). Motility readings were made on these mixtures after 4 and 8 hours' incubation at 35°C.

As may be seen in Table V, immobilizing activity was manifested only in the presence of complement and approached maximal intensity with a final guinea pig serum dilution of 1/10 to 1/20. While the difference between 1/20 and

TABLE V

Influence of Complement Concentration on the Rate of Immobilization of T. pallidum at 35°C. by Varying Concentrations of Rabbit Syphilis Serum Pool 2. 0-Hour Motility: 96 Per Cent

Final dilution of complement as guinea pig serum	Final serum dilution	Motility of organisms after incubation at 35°C.	
		4 hrs.	8 hrs.
		per cent	*per cent*
0	1/10	96	94
	1/50	90	94
	1/250	94	92
	Ultrafiltrate control	94	92
1/80	1/10	64	12
	1/50	94	54
	1/250	96	80
	Ultrafiltrate control	94	92
1/40	1/10	58	2
	1/50	84	18
	1/250	94	70
	Ultrafiltrate control	94	94
1/20	1/10	58	0
	1/50	84	4
	1/250	96	64
	Ultrafiltrate control	96	90
1/10	1/10	56	0
	1/50	78	0
	1/250	92	56
	Ultrafiltrate control	90	92

1/40 complement was hardly significant, it appeared advisable to use a final complement dilution of 1/20 in subsequent experiments in order to provide an adequate excess.

Immobilization as a Criterion for Treponemicidal Activity.—In previous experimentation carried out on the survival of *T. pallidum in vitro* (36), data were accumulated which demonstrated that motile organisms retain their virulence. In the present experiments it became necessary to establish the converse, *i.e.*

that non-motile organisms are dead, in order to prove that the effect of the antibody is treponemicidal.

Total counts and the percentages of motile organisms in 3 mixtures (*a*, *b*, *c*) of treponemes, serum, and complement were determined after incubation for 16 hours at 30°C. The total (motile plus non-motile) number of organisms was the same in each mixture, *i.e.*, 5×10^6 per ml. The motility readings were as follows: (*a*) complement plus serum ultrafiltrate—80 per cent; (*b*) complement plus normal serum—78 per cent; and (*c*) complement plus syphilis serum —4 per cent. One-tenth ml. samples of each mixture were injected intracutaneously into 4 sites on the backs of each of 2 normal rabbits. Thus, each rabbit received 12 inoculations, consisting of 4 test sites for each of the 3 mixtures. This procedure provided a comparative test of the 3 samples in a single animal, and therefore eliminated the variation in response of different individuals to inocula of the same size.

TABLE VI*a*

The Incubation Period and Size of Lesions Following Intracutaneous Inoculation of Rabbits with 5 × 10⁶ T. pallida per Site, as a Function of the Percentage of Motile Organisms. Inocula Obtained from Mixtures of Serum, Complement, and Treponemes after Incubation for 16 Hours at 30°C.

Rabbit No.	Treponemes incubated with	Organisms motile in inoculum	Proportion positive sites*	Incubation period		Average diameter of lesions
				Range	Mean	
		per cent		*days*	*days*	*mm.*
15-92	Complement only	80	4/4	6–7	6.5	13
	Normal serum + complement	76	4/4	6–7	6.5	13
	Syphilis serum + complement	4	3/4	13–14	13.5	3.5
15-93	Complement only	80	4/4	7–9	8	13
	Normal serum + complement	76	4/4	7–9	8	11
	Syphilis serum + complement	4	3/4	19–30	24.5	1.5

* Numerator denotes the number of sites showing darkfield-positive syphilitic lesions after 48 days' observation while denominator represents the total number of sites inoculated.

Significant differences in the incubation period for the development of lesions and in the size of the resultant lesions were observed between the sample containing 4 per cent motile organisms and the 2 samples containing 78 and 80 per cent motile organisms, respectively (Table VI *a*). Since it has been shown that the incubation period varies with the size of inoculum (39), and since the same total number of organisms (*i.e.* motile plus non-motile) was inoculated into each set of sites, it may be concluded that a significant proportion of the organisms in the mixture containing syphilis serum plus complement was rendered non-infectious and presumably dead, though the data do not suffice for quantitative estimation of the number of treponemes so affected. This result does not, however, furnish strict proof that the immobilized organisms are dead since it is possible that the immobilizing antibody renders the organisms highly suscep-

tible to the defense mechanism of the host. Nevertheless, either mechanism of action would implicate the immobilizing antibody as an important factor in the immune processes in syphilis.

In the second test, which was carried out with another batch of *T. pallidum*, one portion of organisms was exposed to complement plus serum ultrafiltrate (control) and another portion to complement plus syphilis serum. After 16 hours at 35°C., 90 per cent of the organisms in the control sample were motile, but all the organisms in the specimen containing the syphilis serum plus complement appeared to be non-motile. One-tenth ml. of each mixture was inoculated intracutaneously into 4 sites on the backs of each of 3 normal albino rabbits, *i.e.*, a total of 12 sites on 3 animals for the 90 per cent motile sample and 12 sites on 3 different animals for the 0 per cent motile sample. The incubation period was determined by daily observation for the development of lesions.

TABLE VI*b*

The Incubation Period of Lesions Following Intracutaneous Inoculation of Rabbits with 5×10^5 T. pallida per Site, as a Function of the Percentage of Motile Organisms. Inocula Obtained from Mixtures of Serum, Complement, and Treponemes after Incubation for 16 Hours at 35°C.

Rabbit No.	Treponemes incubated with	Organism motile in inoculum	Proportion of positive sites*	Incubation period	
				Range	Mean
		per cent		*days*	*days*
16-40	Complement only	90	4/4	6	6
16-41	Complement only	90	4/4	5-6	5.5
16-42	Complement only	90	4/4	5-6	5.5
16-43	Syphilis serum + complement	0	0/4	—	—
16-44	Syphilis serum + complement	0	0/4	—	—
16-45	Syphilis serum + complement	0	1/4	28	

* Numerator denotes the number of sites showing darkfield-positive syphilitic lesions after 48 days' observation while denominator represents the total number of sites inoculated.

As shown in Table VI *b*, all sites on the 3 rabbits inoculated with the control mixture (treponemes 90 per cent motile) developed typical darkfield-positive lesions within 6 days. On the other hand, only one of the sites inoculated with treponemes exposed to syphilis serum plus complement (0 per cent motile) developed a darkfield-positive lesion (on the 28th day) and all others were still negative at the time this report was compiled, *i.e.*, 89 days after inoculation. On the basis of dosage response curves published by Magnuson *et al.* (39) it may be estimated that in the 0 per cent motile specimen there were less than 500 viable treponemes in the total inoculum of 500,000 organisms. Thus, the percentage of viable organisms was less than 0.1, a value compatible with the 0 per cent motility observed in a count of 50 treponemes. With the qualifications noted in the preceding experiment, this result indicates that *T. pallida*

immobilized by antibody and complement, are non-infectious and presumably dead. Observations on these test animals continue.

Survey of Individual Rabbit Sera for Immobilizing Antibody.—

Individual serum specimens collected from a group of 20 non-syphilitic, apparently healthy rabbits selected at random from the laboratory stock and from a group of 20 rabbits with

TABLE VII

Survey of Sera from 20 Non-Syphilitic Rabbits for Immobilizing Antibody. All Sera Inactivated at 56°C. for 30 Minutes, and Tested at a Final Dilution of 1/10. 0-Hour Motility: 94 Per Cent

Serum No.	Reagin titre*	Motility of organisms after incubation at 35°C. for 16 hrs.	
		Without complement	With complement
		per cent	*per cent*
Ultrafiltrate control		—	90
101	0	90	90
107	0	88	88
108	0	90	86
112	0	90	90
113	0	92	70
114	0	90	92
115	0	92	92
116	0	92	90
117	±	90	88
118	0	92	90
119	0	92	92
139	0	92	90
140	±	90	76
141	0	92	92
142	0	84	80
143	0	90	82
144	0	82	84
145	0	86	80
146	0	88	80
147	0	82	70

* Reagin titre expressed as the highest serum dilution producing flocculation with Eagle antigen.

proved syphilis of 3 to 9 months' duration, were tested for immobilizing activity and for reagin titre. The tests for immobilizing antibody were carried out at 35°C. with freshly isolated, tissue-free *T. pallida* in the presence of 1/20 complement. All sera were used at a final dilution of 1/10, and readings were made after 16 hours' incubation.

The results of this survey, shown in Tables VII and VIII, were evaluated in terms of the ratio between the percentages of motile organisms in the tube containing serum alone and the tube containing serum plus complement. A per-

centage difference of less than 10 between these two tubes is not considered significant. If the difference is more than 10 but less than 25, a slight degree of specific immobilization is indicated, and if greater than 25, the test is interpreted as significantly positive. In addition, a control tube containing serum ultrafiltrate and complement was included in each experimental series in order to determine the degree of survival of the organisms in the absence of whole serum.

TABLE VIII

Survey of Sera from 20 Untreated Syphilitic Rabbits for Immobilizing Antibody. All Sera Inactivated at 56°C. for 30 Minutes, and Tested at a Final Dilution of 1/10.
0-Hour Motility: 94 Per Cent

Serum No.	Duration of infection	Reagin titre	Motility of organisms after incubation at 35°C. for 16 hrs.	
			Without complement	With complement
	mos.		*per cent*	*per cent*
Ultrafiltrate control	—	—	—	90
103	7	8	80	0
104	9	8	90	0
105	8	2	92	2
106	6	32	90	0
109	8	1	92	0
110	6	8	90	0
111	6	4	90	4
120	6	24	88	0
121	6	8	92	0
122	6	8	82	0
123	6	64	94	0
126	6	8	90	4
130	4	8	80	0
131	4	64	88	0
132	4	12	88	0
133	4	64	92	12
148	4	8	82	0
149	3	32	84	12
150	3	64	90	4
151	3	8	90	0

On the basis of this arbitrary classification, 17 of 20 sera from normal rabbits were negative and 3 showed a slight degree of immobilizing activity. On the other hand, all 20 sera from syphilitic rabbits showed a marked degree of immobilizing activity. With these 20 sera the failure of antibody to produce immobilization in the absence of complement was again verified.

Survey of Individual Human Sera for Immobilizing Antibody.—Since tests for immobilizing activity were negative in the majority of instances with sera from non-syphilitic rabbits, and consistently positive with sera from syphilitic rab-

bits, it became of obvious interest to carry out a preliminary survey of sera obtained from similarly classified human beings.

For this survey the cases for testing were carefully selected. The 20 sera classified as non-syphilitic were obtained from departmental personnel whose histories were reliable with regard to the absence of syphilitic infection. On the other hand, sera classified as syphilitic were obtained from proved cases of syphilis, *i.e.* darkfield-positive primary or secondary cases, with-

TABLE IX

Survey of Sera from 20 Non-Syphilitic Healthy Adult Human Beings. All Sera Inactivated at 56°C. for 30 Minutes, and Tested at a Final Dilution of 1/10 in the Presence of 1/20 Guinea Pig Complement. 0-Hour Motility: 94 Per Cent. All Sera Negative by Eagle Flocculation Test

Serum No.	Motility of organisms after incubation for 16 hrs. at 35°C.
	per cent
Ultrafiltrate control	92
7	90
9	88
10	90
11	92
12	90
13	92
15	90
16	92
17	94
18	92
31	88
61	80
62	90
63	88
64	90
65	92
66	90
67	88
68	80
69	90

out regard to the state of the standard serological test. Cases of latent syphilis have been avoided to date since frequently this diagnosis is necessarily made on the basis of standard serological tests, which may prove to be less definitive in this stage of the disease than the test here described. The technique for testing the human sera was identical with that used in testing the rabbit sera.

In general the results obtained with human sera (Tables IX to XI) correlate with those obtained with the rabbit sera (Tables VII and VIII). None of 20 normal sera showed significant immobilizing activity. Of 20 sera from cases of primary syphilis, 7 were negative, 10 were positive, and 3 showed a slight degree

of immobilizing activity. All 20 sera from cases of secondary syphilis, produced a marked immobilizing effect indicating the presence of significant antibody serum levels. In addition, 20 sera from patients with diseases other than syphilis were tested. Sixteen showed no immobilizing activity but the other 4 yielded inconclusive results since immobilization of the treponemes occurred both in

TABLE X *a*

Survey of Sera from 20 Human Cases of Darkfield-Positive Primary Syphilis for the Presence of Immobilizing Antibody. All Sera Inactivated at 56°C. for 30 Minutes, and Tested at a Final Dilution of 1/10. 0-Hour Motility: 94 Per Cent

Serum No.	Reagin titre	Motility of organisms after incubation for 16 hrs. at 35°C.	
		Without complement	With complement
		per cent	*per cent*
Ultrafiltrate control	—	90	88
28	32	94	72
33	32	88	0
35	8	88	88
41	16	92	64
70	1	90	72
71	1	86	80
72	1	88	70
86	16	82	4
89	24	92	10
92	0	86	88
93	0	88	90
94	16	82	0
95	32	84	18
102	0	86	88
115	8	88	62
116	0	90	86
122	4	80	80
123	0	86	50
134	96	88	22
135	32	84	44

the complement-free control tube and in the test sample containing complement. Since these 4 sera were obtained from patients receiving penicillin therapy it is possible that their sera contained sufficient amounts of this antibiotic to produce the immobilization observed in both tubes.

The Relationship of Immobilizing Antibody to Reagin.—In order to study the relationship of reagin to immobilizing activity of syphilis serum, the following absorption experiments were carried out.

A primary dilution of Eagle antigen was prepared by mixing 1 volume of an alcoholic solution of standard antigen with 2 volumes of 0.85 per cent saline. After this mixture had aged

at 5°C. for 24 hours, the lipoidal particles were sedimented by centrifugation at 20,000 R.P.M., and then brought to original volume with 0.85 per cent saline. The lipoidal particles were then sedimented again from 2 ml. and 5 ml. portions for the first and second absorptions, described below.

First Absorption.—Ten ml. of rabbit syphilis serum pool 4 was mixed with the washed sedimented lipoid from 2 ml. of Eagle antigen emulsion, and the mixture thoroughly agitated for

TABLE X *b*

Survey of Sera from 20 Human Cases of Darkfield-Positive Secondary Syphilis for Presence of Immobilizing Antibody. All Sera Inactivated at 56°C. for 30 Minutes, and Tested at a Final Dilution of 1/10. 0-Hour Motility: 92 Per Cent

Serum No.	Reagin titre	Motility of organisms after incubation at 35°C. for 16 hrs.	
		Without complement	With complement
		per cent	*per cent*
Ultrafiltrate control	—	92	88
19	64	90	62
22	64	88	0
32	64	92	4
34	128	88	16
36	32	88	4
37	48	92	8
38	64	88	20
42	16	80	0
43	24	92	0
54	24	90	4
77	64	94	0
78	24	84	20
79	64	92	6
97	6	86	6
99	128	84	0
117	128	86	0
119	64	80	0
136	12	82	0
138	96	78	0
142	64	78	0

10 minutes on a Kahn shaker. The resultant flocculate was separated from the serum by centrifugation at 20,000 R.P.M. for 30 minutes. The supernate (once absorbed serum) was pipetted off and filtered through a Corning "ultrafine" bacterial filter to remove any unsedimented floccules, as well as to sterilize the serum.

Second Absorption.—Five ml. of the filtrate obtained above (once absorbed serum) was mixed with the washed sedimented lipoid from 5 ml. of Eagle antigen emulsion, and treated exactly as in the first absorption.

As shown in Table XII, one absorption of the syphilis serum with Eagle antigen reduced the reagin titre from 16 to 2, while a second absorption removed all

titratable reagin activity. However, no detectable decrease in immobilizing activity resulted from this treatment when the sera were tested in the usual

TABLE XI

Survey of Sera from Patients with Diseases Other Than Syphilis for the Presence of Immobilizing Antibody. All Sera Inactivated at 56°C. for 30 Minutes, and Tested at a Final Dilution of 1/10. 0-Hour Motility: 90 Per Cent*

Serum No.	Type of disease	Duration of infection	Motility of organisms after 16 hrs. at 35°C.	
			Without complement	With complement
			per cent	*per cent*
Ultrafiltrate control	—	—	—	88
108	Acute streptococcal laryngotracheobronchitis	5 days	88	84
110	*H. influenzae* meningitis	9 days	90	86
114	Infectious mononucleosis	8 days	82	80
149	Eczematoid dermatitis	3 mos.	74	86
150	Atopic dermatitis	10 yrs.	76	84
151	Seborrheic dermatitis	3 mos.	70	82
306	Acute tonsillitis	2 days	80	84
307	Mumps	2 days	88	80
308	Measles	3 days	84	80
309	Cellulitis	7 days	84	78
313	Diphtheria	1 day	82	82
314	Poliomyelitis	7 days	96	90
316	Atypical pneumonia	14 days	88	80
318	Brucellosis	2 yrs.	84	86
319	Tuberculous meningitis	5 mos.	90	80
320	Pertussis	14 days	78	88
109	Lymphocytic meningitis (questionable etiology)‡	4 days	20	16
111	Pneumococcal meningitis‡	3 days	16	2
112	Diphtheria‡	16 days	14	12
113	Pneumonia‡	15 days	20	18

* All sera from this group of patients were negative for reagin with the Eagle and cardiolipin flocculation tests.

‡ These patients were receiving penicillin therapy at the time serum specimens were obtained. Inconclusive results were obtained since non-specific immobilization occurred in the absence of complement.

manner. Repetition of this experiment with another rabbit serum pool (syphilis serum pool 3) yielded similarly clear-cut results.

Therefore it may be concluded that the immobilizing and reagin activities are due to separate antibodies.

TABLE XII

Titration of Immobilizing Activity of Rabbit Syphilis Serum Pool 4 before and after Absorption of Reagin by Flocculation with Eagle Antigen. Complement Dilution—1/20. 0-Hour Motility: 92 Per Cent

No. of absorptions with Eagle antigen	Reagin titre	Final serum dilution	Motility of organisms after 8 hrs. at 35°C.
0	16	1/10	0
		1/50	0
		1/250	18
		1/1250	48
1	2	1/10	0
		1/50	2
		1/250	16
		1/1250	50
2	0	1/10	0
		1/50	0
		1/250	14
		1/1250	48

TABLE XIII

The Relative Heat Stability of Reagin and Immobilizing Antibody. Immobilization Test Carried Out with Rabbit Syphilis Serum Pool 2, with 1/20 Complement. 0-Hour Motility: 94 Per Cent

Serum heated	Reagin titre	Final serum dilution	Motility of organisms after incubation at 35°C.	
			8 hrs.	16 hrs.
			per cent	*per cent*
Ultrafiltrate control	—	—	88	90
Unheated control	—	1/10	14	2
		1/30	20	10
		1/90	50	14
56°C. 30 min.	4	1/10	10	2
		1/30	30	18
		1/90	54	18
60°C. 30 min.	3	1/10	12	6
		1/30	30	12
		1/90	54	18
64°C. 30 min.	1	1/10	10	2
		1/30	30	14
		1/90	54	14
68°C. 30 min.	0	1/10	14	2
		1/30	46	12
		1/90	60	16

The Relative Thermostability of Reagin and Immobilizing Antibody.—

Five samples of 3 ml. each from rabbit syphilis serum pool 2, were heated for 30 minutes at temperatures of 56°, 60°, 64°, and 68°C., respectively. A standard Eagle flocculation test was performed on each of the samples, and tests for immobilizing antibody were carried out with final serum dilutions of 1/10, 1/30, and 1/90. Motility readings were made at 8 and 12 hours.

The results shown in Table XIII demonstrate a progressive drop in reagin titre with increased temperature of heating, but no corresponding decrease in immobilization activity. In order to verify this striking difference in heat sensitivity this experiment was repeated with another sample of the same serum pool. Further to evaluate the stability of the immobilizing antibody at higher temperatures, 3 additional portions were heated at 70°, 72°, and 76°C. for 15 minutes. No decrease in immobilizing activity was noted with the samples heated at 70° and 72°C. The portion heated at 76°C. could not be tested because the serum proteins had coagulated.

Although the ability to withstand heating at 72°C. for 15 minutes appears unusual for an antibody, similar heat stability has been noted in other studies. For example, Jones (40) found that rabbit agglutinin against the flagella component of the hog cholera bacillus withstood heating at 70°C. for 20 minutes and was not completely destroyed in 20 minutes even at 90°C. Other examples are cited by Marrack (41).

Further studies on the physicochemical characterization of the immobilizing antibody are planned.

DISCUSSION

Although the number of sera tested in the present study is comparatively small, the results clearly show that sera from syphilitic rabbits or human beings with infection beyond the primary stage usually exert a marked immobilizing action on virulent *T. pallidum in vitro*, while this activity is virtually absent from normal sera. Like bactericidal and hemolytic phenomena, this immobilization effect occurs only in the presence of complement and therefore appears to be due to the action of specific antibody.

The immobilization of treponemes by antibody and complement is a relatively slow process, as contrasted to other specific *in vitro* antibody activities. As shown in Tables II and III, the speed of immobilization increases with the concentration of serum and with the temperature of incubation, but even with conditions designed for maximal velocity (1/10 serum dilution and 35°C. incubation) several hours are required for significant degrees of immobilization to occur (Table III). Concentrations of serum higher than 10 per cent or temperatures above 35°C. have not been employed since under these conditions non-specific immobilization of treponemes may occur in control suspensions.

The inability of previous investigators to obtain evidence of immobilization

of *T. pallidum* by immune serum may be attributed primarily to the use of poor experimental conditions resulting in the non-specific loss of motility of treponemes before the effect of antibody and complement could become manifest. This difficulty has been overcome in the present investigation by the use of specially designed procedures and media which permit the maintenance of *T. pallidum in vitro* in a highly active state for several days. Failures to obtain prolonged survival of control treponemes have been encountered on rare occasions in the present experiments, but it appears likely that these failures are due either to the deterioration of some labile constituent of the basal medium or to unknown factors inherent in testicular lesions which inadvertently have been allowed to progress beyond the stage of early syphilitic orchitis before removal.

Following the determination of conditions which appeared optimal for the detection of immobilizing antibody, a preliminary survey of animal and human sera was undertaken. While the antibody titre can be estimated with a fair degree of precision in terms of an end-point based on 50 per cent immobilization at a given time, limitations of time and material precluded such quantitation in these preliminary experiments. The surveys were therefore carried out under conditions of optimal sensitivity, *i.e.*, with a final serum dilution of 1/10 and incubation at 35°C. for 16 hours. The 16 hour incubation interval was selected so as to provide a sufficiently long period for low titre sera to exert a significant immobilizing effect, as well as to be convenient from the standpoint of carrying out the necessary laboratory procedures.

Of 20 cases of human primary syphilis tested (Table X *a*), 10 showed a marked immobilizing action, 3 exhibited weak activity, and 7 were without effect. In addition, a marked degree of immobilizing activity was exhibited by all the sera from 20 cases of secondary syphilis (Table X *b*). Since the infection had been present for several weeks longer in the secondary than in the primary cases, it is probable that the antibody level in a given individual increases with the duration of the disease. However, as yet no cases of late syphilis in human beings have been studied to verify this conclusion.

All the 20 sera from normal human beings examined (Table IX) were completely devoid of immobilizing activity, as were 16 of the 20 sera from patients with diseases other than syphilis (Table XI). The other 4 yielded inconclusive results since immobilization occurred both in the complement-free control tube and in the test mixture containing complement. These 4 patients were receiving penicillin therapy at the time the serum specimens were collected and the non-specific immobilization exerted by their sera was probably due to the penicillin present in the serum (36). Therefore, it appears advisable to collect sera prior to the administration of antibiotic or chemotherapeutic agents to the patient. Otherwise the risk of obtaining such inconclusive results is present, since at least 3 antibiotics, bacitracin (42), penicillin (36), and aureomycin (43), have been shown to immobilize *T. pallidum in vitro*, and would be present in the

sera of patients treated with these agents. However, such "non-specific" immobilization may readily be differentiated from the specific effect due to antibody, since the latter immobilizes treponemes *only* in the presence of complement.

Comparison of sera from 20 syphilitic rabbits infected for periods ranging from 3 to 9 months (Table VIII) with sera from 20 normal rabbits (Table VII) yielded similarly striking results. All the sera from syphilitic animals gave strongly positive reactions, while 17 of the 20 normal sera were completely negative. The other 3 normal sera exhibited low degrees of immobilizing activity. However, the presence of traces of antibody in the serum of an occasional rabbit might be expected in view of its possible infection at one time with *T. cuniculi* (rabbit venereal spirochetosis), an organism immunologically related to *T. pallidum* (44).

It appeared of prime importance to examine the relation of the immobilizing antibody to reagin. From the data presented in Tables II and VII to X, no direct correlation is apparent, except that both activities are more strongly manifest in secondary than in primary syphilis in human beings (Table X *a* and X *b*). The lack of relationship is shown clearly in the absorption experiment outlined in Table XII, which indicates that the immobilizing and reagin activities are due to separate antibodies. Moreover, since it is well known that the level of reagin is relatively low in late syphilis, when resistance to reinfection is high (26, 27), it apparently plays no rôle in immunity. On the other hand, the immobilizing antibody appears to kill *T. pallidum* (Tables VI *a* and VI *b*), and therefore its appearance may be associated with, and possibly responsible for, the development of the immune state. It may also follow that the immobilizing antibody is identical with the antibody demonstrated in human sera by means of passive protection tests in rabbits (6–10).

As an *in vitro* technique for the detection and measurement of specific antibody produced during syphilitic infection, the present immobilization test offers a convenient approach to the study of certain fundamental problems in the biology of the disease in animals and man. These include a study of: (*a*) the incidence and titre of immobilizing antibody in various stages of syphilitic infection in human beings; (*b*) the rate of appearance of immobilizing antibody during experimentally induced syphilis in rabbits, and its quantitative relationship to the development of immunity, as measured by resistance to reinfection; (*c*) the possible absence of immobilizing antibody in non-syphilitic individuals whose sera contain reagin, *i.e.*, the "biologic false positive" reactors; (*d*) the immunological relationships among experimentally induced *T. pertenue* (yaws), *T. cuniculi* (rabbit venereal spirochetosis), and *T. pallida* (syphilis) infections in rabbits; and (*e*) the possible immunological variations among different strains of *T. pallidum*.

In addition, it will be necessary to investigate thoroughly the various tech-

nical and theoretical aspects of the immobilization test before it can be applied to clinical diagnostic problems. For example, some diseases other than syphilis may produce antibody which cross-reacts with and immobilizes *T. pallidum*. If, however, the occurrence of such cross-reactions is rare the immobilization test may be of value in the study of sera from patients with so called "biologic false positive" reactions, *i.e.*, individuals whose sera contain reagin despite the absence of syphilitic infection. The perplexing problem encountered with this group of individuals involves the differential diagnosis of a "biologic false positive" reaction from the positive test for reagin found in patients with latent syphilis in whom no evidence of the disease is apparent on physical examination. Since reagin and immobilizing activities appear to be due to separate antibodies, it is possible that the stimuli which incite the production of reagin in a non-syphilitic individual may not give rise to immobilizing antibody. Moreover, in view of its probable association with active immunity, it may be anticipated that the titre of immobilizing antibody, unlike reagin, will remain relatively high in untreated syphilitics whose infection progresses to the latent phase. If this assumption proves correct in subsequent studies, the detection of immobilizing antibody in the sera of patients with latent syphilis should present no difficulty, particularly since the present preliminary studies indicate that the immobilization test as outlined is sufficiently sensitive to detect antibody in a fair number of sera from cases of primary syphilis in which comparatively low levels would be expected to be present. On this basis, the persistent absence of immobilizing antibody would be of aid in excluding the diagnosis of syphilis.

In addition, further studies are in progress in an attempt to define more clearly the factors which influence quantitation and reproducibility of the immobilization test, as well as to simplify the rather complex bacteriological procedures employed at present, so that the test may be applied more readily on a large scale in studies on the immunology of syphilis.

SUMMARY

Treponema pallida were extracted from rabbit testicular syphilomas and suspended in a special medium in which the organisms remain motile and infectious for several days. On incubation of such suspensions with syphilitic rabbit or human sera and guinea pig complement, the treponemes became non-motile and lost their capacity to infect rabbits. Various factors affecting this immobilization have been investigated.

In a preliminary survey of individual sera, immobilizing antibody could be detected in the majority of sera from syphilitic animals and human beings, but was absent in almost all the normal sera examined.

It could be demonstrated that the immobilizing and reagin activities of syphilis sera are due to separate antibodies.

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