CHARACTERISTICS OF PATHOGENIC SPIROCHETES AND SPIROCHETOSES WITH SPECIAL REFERENCE TO THE MECHANISMS OF HOST RESISTANCE

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That the spirochetes are among the neglected and forgotten children of the family of pathogenic microörganisms is reflected in the meagre information available on the biology and chemistry of these organisms. It is not surprising, therefore, that the mechanisms of host resistance in spirochetal infections are poorly understood. This situation is attributable partly to the difficulty in cultivating pathogenic spirochetes, the chronicity of some of the spirochetoses, and the apparent rarity and consequent lack of interest in certain spirochetal diseases in some parts of the world. Recently the ability of physicians successfully to treat leptospirosis, relapsing fever, and syphilis with penicillin and other chemotherapeutic agents has, unfortunately, forced fundamental studies of these organisms and the mechanisms of resistance into the background. It is desirable to have a fundamental basis for the treatment or at least understanding of cases of these diseases refractory to chemotherapy. Also, a study of this group of relatively rare diseases and disease-producing agents may shed some light on the

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mechanisms of more common or related diseases and organisms. Henrici has wisely said (74): "It is a truism of science that a study of rare and curious events in nature often brings to light general phenomena or principles which may be exaggerated in the rare but overlooked in the commonplace."

A valuable aid for the further study of antispirochetal resistance is the analysis and synthesis of the available data on the subject. The author, aware of no critical, up-to-date survey of the material on spirochetal resistance, has attempted to prepare such a review. Rigorous selection of material was necessary, as there is a great deal of published information on the individual spirochetoses, much of it in monograph form (25, 34, 78, 101, 142, 143, 150, 151, 204, 219, 225). In general the data are confined to leptospirosis[2], relapsing fevers, and syphilis as the major spirochetoses, but pertinent facts on the other spirochetal infections occasionally are introduced[3]. *Treponema pallidum* and related organisms causing chronic granulomatous diseases are considered together with the Borrelias and Leptospiras which produce acute diseases. The inclusion of the syphilis spirochete is justified by its many similarities to the other groups of spirochetes, aside from morphology. These similarities are described in later sections.

Although the histological changes in the spirochetoses, particularly syphilis, may enable the pathologist to distinguish one disease from the other, description of the pathological picture in these diseases has been kept down to a minimum in the review. The author considers it dangerous to use this picture alone as evidence upon which to base a decision as to whether a life cycle of spirochetes exists or as to the mechanisms of infection or host resistance. In so far as pathological studies contribute to the elucidation of the fundamental mechanisms of pathogenesis and host resistance in the spirochetoses, an effort is made to incorporate them in this review.

It is hoped that this review will provide a stimulus to research in antispirochetal resistance by furnishing a survey of past research and discussion and by pointing out possible pathways of future investigation. An ulterior motive, perhaps, is to stir up interest in the spirochetes themselves by stressing their qualities apparently unique among microörganisms.

I. MICROBIC FACTORS THAT MAY INFLUENCE HOST RESISTANCE

It is very difficult to divorce host from microbic factors in resistance to disease. Some of the host reactions may eventually be shown to be reflections of certain chemical and physical characteristics of the organisms. This interdependence

[2] Unless otherwise indicated, data for leptospiras or leptospirosis refer to the species *Leptospira icterohaemorrhagiae* and diseases of man and animals caused by this species.

[3] The reader unacquainted with spirochetes and spirochetoses is referred to any standard textbook of bacteriology for a brief description of these organisms and diseases. Following are the genera and major species of spirochetes and the diseases they cause: *Treponema pallidum*—syphilis; *Treponema cuniculi*—rabbit venereal spirochetosis; *Treponema pertenue*—yaws; *Leptospira icterohaemorrhagiae*—infectious jaundice of Weil's disease in man and animals; *Leptospira canicola*—leptospirosis in dogs and man; *Borrelia duttoni, novyi,* and *recurrentis*, African, American and European relapsing fevers, respectively, in man; *Borrelia anserinum*—avian spirochetosis.

has been well expressed by the eminent French immunologist, Bordet: "Immunity is the organism's virulence to the microbe, just as virulence is the microbe's immunity to the organism."

A. Morphology and life cycle

The manner in which morphology of spirochetes may affect phagocytosis of the organisms is discussed in section II D.

Striking morphologic changes in spirochetes, presumably due to the action of agglutinating or lytic antibodies, have been observed in the course of natural and experimental infections or *in vitro* upon contact of organisms and specific antibodies (18, 62, 78, 85, 101, 106, 219). The organisms tend to become agglomerated, lose their motility, and appear granular. These alterations in morphology have been associated with a reduction or loss in virulence of the organisms (18). Agglutination and granulation of spirochetes may occur also in degenerating cultures in the absence of antibodies (185, 208, 219). The granules appearing spontaneously *in vitro* have sometimes been considered not to be the result of degeneration but homologous to bacterial spores, providing a means of maintaining life under adverse environmental conditions (78, 131, 208, 219). Recently, investigators using the electron microscope have observed spheroidal bodies at the sides of intact *T. pallidum* and have called them asexual reproductive bodies (146). Similar bodies have not been seen in electron microscope studies of Leptospira (141) or Borrelia (122). Other workers have described *in vivo* experiments which may be explained by the postulation of a granular or infra-visible stage in a spirochetal life cycle (78, 131, 219, 230). Still others believe that Borrelias may have a developmental life cycle in their vectors, lice and ticks (190), including an infra-visible granular form. However, despite the impressive array of scientists supporting a granular form as part of a life cycle (78, 219), the evidence for such a stage or life cycle is not altogether persuasive. This is regrettable as this interpretation, if correct, might aid in the understanding of enigmatic aspects of latency, periodicity, and antibody and drug-fastness in spirochetal infections.

B. Electrokinetic potential[4]

Most bacteria possess a negative electrokinetic potential at or near pH 7 (1, 48). However, there is no general agreement on the sign of the surface potential of pathogenic spirochetes (56). Part of the confusion undoubtedly has arisen from the lack of standardization of technique, age and dissociative state of organisms, the growth and suspending mediums in determination of the potential. Kligler and Aschner (98) stated that as a rule spirochetes and protozoa have a positive charge at the pH of blood in contradistinction to bacteria which are generally negatively charged at this pH (1, 48). If substantiated, this is a very basic distinction, as it would imply a fundamental difference in the chemical composition of at least the surface of the spirochetal or protozoan cell as compared with that of the bacterial cell.

[4] Also termed zeta or surface potential, or, loosely, electric charge.

Kligler and Aschner (98) reported, on the basis of cataphoretic studies, that *L. icterohaemorrhagiae* possessed a positive charge at the pH of blood. Timmerman (206) found these organisms to be positively charged in respect to dilute rabbit serum in which they were grown. Other authors have claimed that Leptospira is negatively charged (56). Brown and Broom (14) studied the effect of negatively charged colloids on Leptospira in the presence of immune and normal serums. They observed that the negatively charged spirochetes adhered to bacteria when treated with specific immune serum containing complement. Adhesion did not occur in the presence of normal serum. Negatively charged colloids could replace the bacteria; and when the colloid was, for instance, a copper sol, the organisms were killed.

Similar divergent observations as regards surface potential are recorded for Borrelia and Treponema (56). Culwick and Fairbairn (42) claim that *Borrelia recurrentis* may have "electrical variants" so that some strains are positively, others negatively charged. However, their technique for determining the surface potential is open to criticism. They examined stained films of infected blood and recorded the percentages of spirochetes adhering to red blood cells and the percentage not sticking. Presumably the spirochetes which adhered to the negatively charged erythrocytes were positively charged; those which did not were negatively charged. However, in careful cataphoretic experiments with various strains of trypanosomes, Brown and Broom (15) found that some of them carried positive and others negative charges. With the same strain a change in charge was observed after relapse and after treatment with arsenicals (16).

In section II B the possible relationship of zeta potential to ability of organisms to pass through the walls of the cerebral blood vessels and the significance of this observation are discussed. The possible influence of electrokinetic potential on the phagocytability of spirochetes is considered in section II D.

C. Motility and sedimentability

Motility and sedimentability of spirochetes may influence the process of phagocytosis of the organisms by host cells. The point is taken up in section II D.

D. Cultivation and preservation

Of the three genera of pathogenic spirochetes, the Leptospira are the most readily cultivated *in vitro* (208). Borrelia is very difficult to cultivate in initial culture and cannot consistently be maintained in serial passage. Although there are many so-called culture strains of *T. pallidum*, it is doubtful whether virulent strains of the organism have ever been cultured successfully *in vitro* (63, 93). *L. icterohaemorrhagiae* (140) and Borrelia (152) have been grown without loss of virulence in the chick embryo. Wile and Snow (231) have reported experiments suggestive of proliferation of *T. pallidum* in the embryo, but their evidence is not conclusive. *T. pallidum* has not been grown in tissue culture (94), a technic often successful for the growth of organisms which are not cultivable on lifeless media.

All of the mediums which have proved satisfactory for the cultivation of patho-

genic spirochetes have contained native animal protein in the form of blood, serum or ascitic fluid (99, 101, 147, 150, 208, 219). Whether the protein acts chemically as a nutrient or physically as a protective colloid against noxious substances is unknown. It is known that soaps on glassware are rapidly lethal to Leptospira (194). The protein may protect the organisms against fatty acids as albumin protects tubercle bacilli and other mycobacteria from harmful fatty acids in culture (49). Leptospira and Borrelia are obligatory aerobes (150, 208).

Chang (30) has recently found that the virulence of *L. icterohaemorrhagiae* may be preserved *in vitro* if a small amount of emulsion of fresh guinea pig liver is added to the medium. The mechanism of this effect is unknown.

The pathogenicity and parasitic ability of spirochetes may be correlated partially with their cultivability and growth requirements *in vitro*. *T. pallidum* which multiplies with great difficulty or not at all *in vitro* or in the chick embryo, is a typical tissue parasite which produces chronic manifestations similar to those of tuberculosis. Borrelia is principally a blood parasite, whereas Leptospira invades both blood and the tissues. Recent studies indicate that the hemoglobin or hematin of red blood cells is necessary or helpful for the cultivation of Leptospira (31, 163, 177, 185) and certain species of *Borrelia* (99). This requirement might explain in part the affinity of these organisms for the blood stream of the host. The greater propensity for multiplication might enable the Leptospira and Borrelia to proliferate more rapidly *in vivo* and to produce a more acute infection. Such an infection might evoke a more acute host reaction and hence a more prompt termination, fatal or otherwise. The more fastidious growth requirements of *T. pallidum* probably restrict its growth *in vivo*, resulting in a more lesisurely development of the infection and consequently of host reaction to it.

Levaditi and Vaisman (116) found that intratesticular injection into rabbits of *T. pallidum* suspended in carbon enhanced growth of the organisms as compared to injection of organisms alone. Presumably the granuloma produced by the carbon provided a favorable medium for growth of the spirochetes. Chesney and Kemp (35) found that a non-specific inflammatory reaction induced by trauma or coal tar and involving the skin and subcutaneous tissue fostered the initiation and development of a primary syphilitic lesion in the rabbit. The most favorable time for inoculation of a wound was when it was granulating nicely and was well on its way to healing. The authors also described experiments by Levaditi and Yamanouchi (118) on growth of the spirochete in inflamed rabbit eyes and postulated that the inflammatory exudate furnished an increased food supply for the organisms. Chesney and Kemp, therefore assumed that the substances ("trephones" (29)) present which stimulate the growth of fixed connective tissue cells and the abundance of nutrients for new cell growth might also favor survival and growth of the Treponema. Such an hypothesis is in close agreement with the suggestion of Theobald Smith (183a) that the syphilis spirochete exerts a metabolic activity so close to that of the host that the latter reacts slowly and slightly to infection. Whether this means that the organism is intracellular at some time during its residence in the host or whether it may de-

rive its sustenance extracellularly remains to be determined. In either event, the close association of spirochetes and actively proliferating cells finds some confirmation in that the sites where the organisms are extremely numerous, such as the testicle of the rabbit and the organs of the human syphilitic fetus, are also centers of active host cell growth (35).

The rôle of the inflammatory reaction in enhancing infection by furnishing a site wherein the spirochetes settle preferentially from blood and lymph must also be considered (137).

It is interesting that *T. pallidum* despite its fastidious growth requirements will survive for months or possibly years in the animal host (66, 193). Perhaps this organism possesses the same faculty as the human tubercle bacillus (121) under adverse nutritional conditions of reducing its metabolism to a low level with the retention of respiratory and proliferative capacities.

T. pallidum rapidly succumbs to drying outside the body (66). Even drying from the frozen state thus far has been unsuccessful for preservation of these organisms (217), of Leptospira (189), and of *T. pertenue* (217).

E. Metabolism

Knowledge of the biochemical and metabolic characteristics of pathogenic spirochetes is very meager, particularly as providing a basis for identifying various species, distinguishing virulent from avirulent strains, developing synthetic mediums, or explaining factors in resistance. This may be attributed to the difficulty in cultivating and counting spirochetes and in obtaining sufficient numbers of them for metabolic studies. A few observations on spirochetal metabolism are summarized here. Further data, including a description of a partially successful synthetic medium for Leptispira (177), may be found in references (30, 31, 70, 163, 172, 176, 177, 195).

Noguchi (149) reported that addition of carbohydrates to the medium had no effect on the growth or morphology of *L. icterohaemorrhagiae*. Recently Chang (31) found that this organism did not utilize the simple sugars provided but probably subsisted on proteins or amino acids. He was unable to detect any consumption of oxygen by large numbers of leptospiras during a period of several hours in the Warburg apparatus. Supniewski and Hano (195) observed that Leptospira utilizes L-arabinose but no other pentoses; it hydrolyzes galactose and glucose and to a smaller extent fructose and mannose. It breaks down urea but not uric or lactic acid. Scheff (196) made observations on *Borrelia recurrentis* and *Borrelia anserinum*. He found that when these organisms were grown in a medium containing glucose, they broke down the sugar to lactic acid without using up any of the oxygen. Fenyvessy and Scheff (54) made comparative studies of the metabolism of Borrelia and species of trypanosomes. They observed that Borrelia apparently did not use molecular oxygen and derived their chief energy from sugar fermentation, whereas trypanosomes used oxygen and obtained energy from oxidation of sugar.

There are some data in the literature on substances, usually vitamins in animal and microbial nutrition, which foster the growth of *L. icterohaemorrhagiae* and

L. canicola. Space is not available here for presentation of these data. However, it is of interest to note that many of the same vitamins which are growth-promoting factors for other microörganisms (ascorbic, nicotinic and pimelic acids, biotin, thiamine, calcium pantothenate, para-aminobenzoic acid, pyridoxine, nicotinic acid amide, riboflavin) also enhance the growth of Leptospira (31, 172, 176). These results indicate that enzyme systems similar to those in other microorganisms probably operate in Leptospira. Unfortunately, there are no comparable data on the spirochetes which are more difficult to cultivate *in vitro.*

F. Antigenicity and chemical composition

Early work indicated that serological cross-reactions could be obtained between spirochetes and trypanosomes but not between spirochetes and bacteria (46). In view of the significance of these findings in suggesting an antigenic relationship between spirochetes and trypanosomes, they should be confirmed by means of more accurate and refined modern immunological methods.

Spirochetes are generally considered to be gram negative in tinctorial properties (150, 208, 219). It has been suggested (208) that they stain poorly with methylene blue because of a lack of nucleoprotein in their protoplasm, but ability to stain with methylene blue is not definite evidence that an organism contains nucleic acid. Mudd (144) has found evidence that cultured spirochetes of the genus *Treponema* have surfaces whose wetting properties are suggestive of a high lipid content. Sensitization of these organisms with homologous immune serum results in an altered surface whose wetting properties are like those of protein.

Leptospira. Among spirochetes, Leptospira perhaps resembles the bacteria most closely in antigenic and possibly chemical properties. Following inoculation of killed or live organisms or during natural infection agglutinating, lytic, complement-fixing and protective antibodies are produced in high concentration (110, 219). These antibodies may persist in detectable titer for long periods of time, up to many years, in the blood of man and animals (110, 219). The immunity they engender is of correspondingly high quality and long duration (219). The antibodies are often highly specific, so that failure to obtain a demonstrable serological reaction against one antigen is not conclusive evidence of absence of infection with an organism containing another antigen (219).

There are different antigenic strains of *L. icterohaemorrhagiae* (66, 219). The specific antigens have not, however, been isolated. The only substance isolated in some reasonable degree of purity from spirochetes is a specific soluble carbohydrate from the non-pathogenic saprophytic *L. biflexa* (79). This carbohydrate, like the type-specific pneumococcal carbohydrate haptens, did not give rise to antibodies upon injection but was precipitated by rabbit antiserums to the homologous strain, though not by antiserums to pathogenic strains of *L. icterohaemorrhagiae* (79). Whether similar carbohydrate haptens exist in pathogenic Leptospira is unknown. The author (185) has immunized guinea pigs against fatal leptospirosis by repeated injections of cell-free supernatant from leptospiral cultures; this suggests that there is a soluble protective antigen in cultures of *L. icterohaemorrhagiae.* Hollande (83) has shown by the effect on tinctorial

properties after lipid extraction that there is lipid in cells of this organism. Carlinfanti (27) has presented serological evidence for the presence in Leptospira and in Wassermann beef-heart antigen of a common alcohol-soluble partial antigen. This is not surprising as lipid antigens of the Wassermann type have been found in a number of bacterial, plant and animal tissues (108, 226).

Borrelia. The pathogenic Borrelia induce the formation of lytic, agglutinating, complement-fixing and protective antibodies upon introduction into a suitable host (147). These antibodies are produced in high titer and apparently are closely connected with the mechanism of effective host resistance to infection.

There are no data available on the chemical composition of the relapsing fever spirochetes.

Treponema. The greatest chemical and antigenic non-conformist among the spirochetes is *T. pallidum.* This organism may unobtrusively enter the body causing little inflammatory reaction (55, 133, 137) and, in fact, may evoke no histological response by the host during its residence in the body during a period of months or years (34). Peculiar antibodies termed "reagins" are eventually produced by the host in response to infection (43, 208, 226). These "reagins" yield flocculation, precipitation, and complement-fixation reactions with spirochetal or tissue antigens. Avirulent culture strains of *T.* pallidum induce antibodies which give agglutination and spirocheticidal reactions *in vitro* with avirulent but not with virulent strains of the organism (239). There is growing evidence that protective antibodies are produced very slowly and in low concentration in the course of experimental or natural syphilis (18, 39, 212, 216).

T. pallidum and possibly other pathogenic treponemas (218) possess a Wassermann-type lipid hapten which is "exceptional among serologically active materials on account of its widespread distribution, surpassing in this respect even the Forssman antigens." (108). This ubiquitous hapten is found in proteus bacilli, Leptospira, trypanosomes, tubercle bacilli, plants, milk, egg yolk, and in normal tissues of man and animals[5]. Bergel (9) has shown that *T. pallidum* contains a great deal of lipid in the cell membrane. He believes (9) that chemotactically this lipid produces a lymphocytic reaction which in turn, via lipolysis, leads to disintegration of the spirochetes. He considers the "reagins" or antilipoids as products of lipolytic lymphocytes which appear as reaction against the lipid antigens.

That there are heterologous antigenic strains of *T. pallidum* is suggested by the relatively specific character of the resistance of rabbits to reinfection (38). The resistance against the homologous strain of organism originally employed for inoculation usually is greater than against heterologous strains (38).

Turner and his co-workers (218), by cross immunity studies of experimental syphilis, yaws, and venereal spirochetosis of rabbits, have found evidence to indicate that the treponemas causing these infections are biologically and antigenically related. Rabbits infected by intratesticular injection of *T. pallidum*, or

[5] For further information on the Wassermann antigen and its relationship to biologic false positive serologic reactions in syphilis, the reader is referred to two recent reviews (43, 226).

T. pertenue, or *T. cuniculi* developed within 6 months a substantial amount of cross immunity to challenge intracutaneous injection of each of the same species of spirochetes.

G. Toxins and aggressive substances

Sanarelli and Pergher (175) believe that spirochetes do not themselves produce pathological changes but only render the host more susceptible to damage by secondary invaders. The observations of Zavagli (234) that dead cultures of *L. icterohaemorrhagiae* increase the severity of *Trypanosoma brucei* infection in mice while dead bacteria had no effect support the supposition of Sanarelli and Pergher. However, Taylor and Goyle (204) and Stavitsky (185) cultured organs of guinea pigs experimentally infected with leptospiras and isolated only the spirochetes, except when the organs were cultured many hours after death and were consequently contaminated with intestinal bacteria. When paratyphoid bacilli or streptococci were introduced into guinea pigs approximately at the height of leptospiral infection, these organisms either did not affect the course of the spirochetosis, or the new infection supplanted the old, only the bacilli or cocci being present in the organs at necropsy (185).

Leptospira. Pettit (157) gave guinea pigs up to 10 ml. of filtrates of cultures of *L. icterohaemorrhagiae* with no ill effects. Fukushima and Hosoya (61) found substances toxic for guinea pigs in cultures of these spirochetes which, having been maintained under anaerobic conditions, contained dead and dissolved organisms. Higuchi (76) also demonstrated a toxin in pure cultures of leptospiras which had been kept at 37 C *in vacuo* for 2 to 3 days. Injection of this toxic material into guinea pigs produced an increase in the bilirubin of the blood, hyperemia of the bulbar conjunctiva, and fever (76). However, attempts to confirm these studies were negative (186). Further efforts to demonstrate toxin in various cultures or tissue extracts from infected animals were unequivocally negative. Stavitsky's experiments (186), although not excluding the existence of a spirochetal toxin, make it unlikely that it is a potent exotoxin such as is produced by *Corynebacterium diphtheriae*, or a stable endotoxin such as can be extracted from cultures of *Vibrio comma*. The toxin or toxic antigen of *L. icterohaemorrhagiae* may be extremely labile and rapidly destroyed in the presence of the chemical and physical agents used in preparing the test spirochetal extracts.

Attempts to demonstrate hyaluronidase (50), fibrinolysin, leukocidin or coagulase in leptospiral extracts were negative (185). As far as is known no attempts have been made to demonstrate these enzymes in other spirochetes.

Borrelia. No clear-cut demonstration of a toxin from borrelias has been found in the literature.

Treponema pallidum. Brown and Pearce (19) consider the possibility of a toxin as a factor which is distinct from the spirochetes in exciting infection. They believe certain features of the syphilitic reaction may find their explanation in the action of a toxin. However, Kolmer (104) could not demonstrate filtrable or soluble exogenous toxins in cultures of spirochetes alleged to be *T. pallidum* but avirulent for rabbits, or in filtrates of the tissues of acute testicular

syphilis in rabbits. Feebly toxic substances, possibly endotoxins, were obtained from cultures of spirochetes subjected to autolysis or desiccation, grinding and extraction (104).

H. Tropisms

1. Chemotropism (chemotaxis). The apparent lack of chemotropic properties in pathogenic spirochetes is discussed in section II D.

2. Histo- and organo-tropisms. The concept of selective affinity of infectious agents for specific organs, tissues or even cells where they find conditions favorable for survival and proliferation has been strengthened considerably by recent studies on these tropisms of viruses (224).

Leptospira. *L. icterohaemorrhagiae* appears to have an affinity for the liver in experimental infection of the guinea pig. Within 7 hours after injection into this animal the organisms may be recovered from the liver (25). The extensive damage done to the liver in leptospirosis (219) is further evidence of some special suitability of this organ for multiplication of the spirochetes. In this connection, the finding that virulence of Leptospira may be maintained *in vitro* if a small amount of emulsion of fresh guinea pig liver is added to the medium (30) is interesting. Experimental and clinical observations have suggested that strains of this organism may localize in the meninges in the course of infection (158, 210). However, attempts to develop a "meningotropic" strain of *L. icterohaemorrhagiae* by repeated subdural passage in guinea pigs were unsuccessful (186). Other affinities of these organisms for the eye (186, 187, 219), adrenals (186, 219), bone marrow (47, 186), and kidney (219) require further study.

Borrelia. There is considerable material in the literature on latent infection of the brain of experimental animals with these spirochetes (26, 143). Whether this represents a true tropism is not known. It is known that certain species of these organisms may pass through the blood-brain barrier (26, 60).

Treponema. There has been much research on the question of neurotropic strains of *T. pallidum* (114, 193). This work has given rise to the postulate (193) that prolonged residence in a particular tissue induces permanent changes in this organism. Thus, sojourn in the mouse brain may prepare the spirochete for the development of a neurotropic reaction in the rabbit (193). Presumably the pronounced neurotropic tendencies often manifested by *T. pallidum* in man (114, 193) are attributable to infection with or development of a neurotropic strain of the organism. *T. pertenue*, causative agent of yaws, is often considered a variant of *T. pallidum* which, by residence in the negro, has developed dermotropic affinities (155). Bessemans (11) found that low temperature in tissues favors the multiplication of *T. pallidum* in experimental syphilis. The organisms apparently settle in those tissues which have lower temperatures, such as the testis.

I. Spirochetal variation

This is certainly one of the most important phases of spirochetal biology as it influences all aspects of spirochetal behavior and, therefore, the pathogenesis and resistance in these diseases. Braun (13) and Luria (126) have discussed various

phases of bacterial dissociation or variation. These discussions have proved useful for providing at least a tentative understanding of the possible mechanisms of spirochetal variation. In the absence of significant information on spirochetes, therefore, we shall have to rely upon reasoning by analogy, always a hazardous procedure, in the attempt to understand spirochetal antigenic variation.

1. Antigenic variation. The data on antigenic variation among spirochetes are most complete for the borrelias (143, 147, 180). The generally accepted facts of the phenomenon of antigenic variation of borrelias and the apparently related clinical relapses are presented in Section II G. Whether these antigenic variations are spontaneous, that is occurring continuously at a certain rate regardless of whether the organisms are in contact with specific antibodies or substrate, or are induced by contact of organisms with specific antibodies or substrate, is not known. In the first case the antibody acts merely to select the new variant, permitting it to outgrow the original normal form. In the second case the antibody itself induces the variation resulting in adaptation. In analogous cases in bacteria the new variant has appeared independently of the substrate to which it was adapted. Resistance to penicillin of *Staphylococcus aureus* (44), resistance to phage of *Escherichia coli* (125), and the requirement for uracil by clostridia (174) have been found to occur spontaneously, independently of the environmental agent.

2. Variations in virulence. There are strains of spirochetes of different degrees of virulence (63, 143, 219). As with other microörganisms, virulence is gradually lost upon cultivation *in vitro* and often regained upon animal passage. An exception is the maintenance of virulence by *L. icterohaemorrhagiae in vitro* if a small amount of emulsion of fresh guinea pig liver is added to the medium (30). The basis for the variation in virulence is obscure. Many factors, such as antigenic variation, variation in chemical composition, and possibly variations in the magnitude or sign of the electrokinetic potential may be involved. The virulence of the organisms may influence the development of the carrier state or relapses by the host. If the organisms are virulent, they may stimulate the defensive responses of the host and conduce to a more solid immunity or else overwhelm the host's defenses and kill the animal (97). Thus mildly virulent strains of *Leptospira* lead to relapses (5), whereas virulent strains rarely do.

An arresting observation is that syphilitic organisms from rapidly growing chancres in rabbits tend to give rise to more rapidly growing chancres with a shorter incubation period in normal rabbits than do spirochetes from slowly growing lesions (18). It is not known whether this effect depends on the alteration of the organisms' potential for proliferation *in vivo* or on the carry-over of nutrients or protective colloids with the chancre material.

Turner and his associates (218) believe that stability rather than variability is generally the rule with regard to pathogenic properties of treponemas. If variants do occur, either the rate at which they appear is small, or conditions for their survival are unfavorable.

3. Variations in susceptibility to drugs and antibodies. These variations are

known to occur in spirochetes and to persist through succeeding generations of the organisms (97, 193, 219). Basically they probably are related to variations in antigenicity or chemical structure (including enzyme systems), so that drugs or antibodies cannot be adsorbed at certain of the "active centers" (162) of the organisms and, therefore, cannot accomplish their effects.

II. HOST FACTORS THAT MAY INFLUENCE HOST RESISTANCE

A. Natural resistance

The separation between natural and acquired resistance is artificial and arbitrary, as these properties are obviously interdependent. This point is particularly well clarified in Rich's recent monograph on the pathogenesis of tuberculosis (168). For example, the apparent greater resistance to an infection of older compared to younger individuals may be simply the consequence of prompter and greater antibody production by the elder animals (6). On the other hand, the ability of the rabbit by raising its body temperature to resist infection with pneumococcus III (170) seems to be an unquestionable example of natural resistance. It is, therefore, imperative that the basic mechanisms involved be understood before native and acquired resistance can be separated rationally.

Leptospirosis. Apparently there is no complete natural resistance to leptospirosis in man and animals (219, 225). Sex differences in resistance have not been demonstrated. An increase in resistance with increased age has been shown in the guinea pig (185, 192), white mouse (109), dog (219, 232), and rabbit (219). Species resistance to the disease is generally recognized in the rat, mouse, rabbit and dog (219, 225). Symptomless infection is particularly common in these species. There are also differences in resistance of various genera of mice of which the deer mouse (*Peromyscus*) is most susceptible (154). Within various species, moreover, there appear to be strain differences in resistance which have been most clearly defined among strains of white laboratory mice which vary from extreme susceptibility (109) to great resistance (71, 188). The guinea pig and hamster are the species of laboratory animals most susceptible to leptospiral infection (113, 186). There are individual differences in susceptibility to this infection among animals of the same species (185, 219). However, it is difficult to exclude inapparent or past infection and the consequent production of some active immunity in these individuals. Moreover, few strains of laboratory animals are genetically homozygous, and it is probable that these individual differences in response to infection are merely expressions of genetic strain differences in the animals.

There have been a few studies of the mechanisms of age and species resistance in leptospirosis. Some of the data are included in the following sections B, C, and D. Here only the most recent data are given.

Higuchi (75) attributed the great resistance of rats to fatal leptospirosis to strong antibody formation and destruction of organisms by lytic antibodies. He found that rats were easily infected, but their symptoms were light. Stavitsky (187) studied peripheral barriers, non-specific inflammation, normal and immune whole blood and serum, cell-free inflammatory fluid and phagocytic cells

in old and young, normal and immune animals of resistant and susceptible species which were injected with Leptospira (187). In all groups only lytic antibodies exerted a significant effect on the organisms *in vitro* or *in vivo*. The organisms did not appear to damage the tissues of rats and mice as much as those of guinea pigs and hamsters (185). Although these strains of rats and mice rarely succumbed to the infection, the ease of invasion and spread of the spirochetes in these hosts, the findings of hemorrhagic pulmonary lesions, and the development of a carrier state (185, 186, 187, 219) indicated that resistance of these species to infection is relative rather than absolute. In view of the importance of antibodies in resistance to this disease, it was suggested (187) that larger numbers of animals be studied to determine the comparative ability of young and old animals and susceptible and resistant species to form antibodies against the spirochete.

Relapsing fever. Relapsing fever apparently is primarily a disease of lower animals; its occurrence in man is accidental (143, 147). The disease does not seem to make distinctions as to age, sex, color or social status of man (143, 208). All animals except apes, rabbits, guinea pigs, mice, and rats are considered naturally resistant (143, 147, 208).

There are some data on the relationship between age and resistance to relapsing fever (147, 156). In 95% of old mice inoculated with a strain of *Borrelia*, organisms were in the blood in 3 to 10 days, while of young animals 64% showed no infection, 24% light infection and 12% medium infection (147a). In the younger animals the infections were never serious or fatal and the period of incubation was 1.8 days shorter. On the other hand, an increased resistance to Borrelia of guinea pigs with increasing age has been shown (69). Interesting studies have been made of the comparative susceptibility of the chick embryo, the chick, and the adult bird to infection with *Borrelia duttoni* (152). The embryo was susceptible but the newly hatched chick and adult bird were not. Some inoculated embryos harbored organisms after hatching but these were disposed of by the rapidly developing defensive mechanism of the hatched bird. The circulating blood was eliminated as a basis for the natural immunity of the chick when phagocytosis and spirocheticidal effects of serum and by cells of whole blood of embryo or chick could not be shown.

There are species differences in response to relapsing fever spirochetes although relapses are common in many species (78, 143, 209). However little is known of the basis for these differences.

Syphilis. Syphilis is naturally acquired only by man (34). The hypothesis of early South American explorers that syphilis existed in llamas there and that infection was originally acquired by natives from these animals has not been substantiated (34). South American workers (87) reported the successful reproduction in llamas of all of the essential features of human syphilis, but Zinsser (236) states that this work has never been supported by subsequent investigation. Monkeys and rabbits can be infected and the organism transmitted indefinitely in them (34). Other species are more or less resistant to frank infection (34). Rats and mice, for instance, invariably develop an asymptomatic infection in which the spirochetes invade the tissues without the production of lesions or

clinical signs of illness (66, 208). Most other species, though occasionally reported to have been infected, usually manifest complete resistance to syphilis (34). Experimental infection in monkeys and man corresponds very closely to natural human infection (208). In rabbits many of the lesions seen in human infection are often duplicated (208). The organisms may remain latent in the lymph nodes (22) or in one or more of the internal organs (184). A positive Wassermann reaction is usually produced (34). Late lesions are not produced in the monkey and rabbit (34).

Rosahn (171) has presented evidence for breed, race or family resistance to syphilis in rabbits. In studies of the reaction of standard breeds of rabbits to experimental syphilis, he found that Havana and Dutch animals were relatively resistant whereas English, Himalayan and Rex rabbits were relatively susceptible. Frazier and Mu (57) found albino rabbits less resistant than brown rabbits. Brown and Pearce (20) obtained poor results with scrotal inoculation of Belgian and Flemish giants while small albinos, grays, browns and Dutch belts gave satisfactory results. In general the "higher" apes such as the chimpanzee react more like man to infection (34, 208) whereas in "lower" apes like macaques the disease is less easily produced and on the whole milder (34).

Age appears to be a factor in experimental rabbit syphilis (33). Inoculation apparently produces lesions more often in younger rabbits (33, 34, 184). Sexual differences in susceptibility to syphilis are particularly outstanding in man and animals (156). The female exhibits a markedly greater resistance than the male (156). Unquestionably, this exalted resistance is somehow related to the effects of the female sex hormones, the estrogens, as will be elaborated in a later section.

There appear to be no naturally immune individuals among man or susceptible species of animals, although there are individual human, monkey, and rabbit reactions to infection.

Although the mechanisms of natural resistance to *T. pallidum* have not been studied extensively, there are suggestive data on the influence of physical constitution of rabbits on their resistance (23). In these experiments rabbits were subjected to varying forms of light. It was found that the light increased resistance to infection in proportion as it affected the physical constitution of normal rabbits, and the organs most affected were those concerned in the animal's reaction to syphilitic infection. Zinsser and Hopkins (237), studying the mechanism of natural resistance of mice against *T. pallidum*, could not detect phagocytosis of the organism in the peritoneal cavity of this species. They concluded, therefore, that phagocytosis is not primarily responsible for the marked resistance of this host.

B. Peripheral barriers of portals to infection

The first reaction of the host is usually an attempt to localize the infective agent at the site of entrance into the body (166). It is therefore of interest to determine how effectively spirochetes are localized at the various portals of entry to the body.

All of the pathogenic spirochetes appear to invade the body rapidly and un-

obtrusively by most portals of entry (85, 147, 150, 208, 219). This may in part be due to their rapid motility which is most readily observed *in vitro*. The apparent boring of the avian spirochete into macrophages in tissue culture (77) supports this suggestion. *T. pallidum* has been known to bore through a solid medium, and *Borrelia recurrentis* through a semi-solid one (150). The boring of Leptospira through agar has been compared aptly to 'the gyring and gimbling of Lewis Carroll's slithy toves.'

Leptospira. In the guinea pig and hamster Leptospira invades the body quickly after injection by peripheral routes including cutaneous, meningeal, intraperitoneal, intraanal, intravaginal, conjunctival and intraocular (85, 186, 187, 221). They may enter the human body through intact skin (85, 150). Schüffner (178) has taken advantage of the great invasiveness of these organisms to devise a method for separating them from contaminants. The contaminated spirochetal suspension is inoculated intraperitoneally into a guinea pig and 10 minutes later the animal is bled from the heart. Invariably the spirochetes may be isolated from this blood. It also has been shown by extirpation of the injection site (186) that 5 minutes after intradermal injection of these organisms into guinea pigs, enough have left the area of injection to cause death of the animal. Only intact skin and nasal mucous membrane seemed to afford an effective barrier to invasion of the body of the guinea pig (187). The oral route was not as effective as the other peripheral routes (187), probably due to destruction of the organisms by gastric juice (208).

Since "every intradermal injection is truly intralymphatic" (136) the spirochetes probably escaped from the site of intradermal injection through the superficial lymphatics. Attempts to demonstrate fibrinolysins and "spreading factor" (50) in filtrates and autolysates of leptospiral cultures were unsuccessful (185). It was, therefore, suggested (186, 187) that the great speed with which the organisms revolve in cork-screw fashion enables them to bore through connective tissue as well as fibrinous networks and other localizing barriers erected by the host (185, 186, 187). The organisms were not localized at the site of intradermal inoculation (186, 187). Nor could they be isolated from regional lymph nodes after peripheral injection (186, 187), suggesting that they pass rapidly through these structures into the blood, few of them being trapped in the nodes. The eye is a particularly favorable portal of entry to the body as the organisms appear to multiply abundantly there (186).

When injected into the dermis, leptospiras produced a very mild inflammatory reaction there during the first 24 hours after injection as indicated by histological studies and by the free diffusion to the tributary lymphatics of trypan blue (137) inoculated into the area (187). When injected intraperitoneally, intraocularly and intrameningeally, they produced progressively more intense inflammatory reaction and correspondingly more localization of spirochetes in that order (187). However, it is not known which is primary, rapid escape of the organisms from the injection site before causing much local cellular injury or their non-irritant quality *per se*. It is possibly significant that the organisms escaped from a peritoneal cavity which was artificially inflamed so that intraperitoneally

injected streptococci, horse serum and trypan blue were unable to spread through the regional lymphatics (185). *In vitro*, the spirochetes wriggled through rather dense networks of fibrin which trapped many other species of motile microörganisms (188). Perhaps a very closely-knit fibrin coagulum and complete lymphatic blockade are required to restrain their spread (137).

After inoculation into the brain or meninges, *L. icterohaemorrhagiae* often can be isolated from the blood; and occasionally they have produced generalized leptospirosis in guinea pigs and red foxes (185). However, despite positive blood cultures, these spirochetes do not readily, if ever, reach the brain or spinal fluid through the blood vessels, as judged from negative brain and meningeal cultures (185). This paradoxical situation is, nevertheless, only apparent. The walls of the meningeal blood vessels provide an impenetrable barrier for foreign particles which gain entrance into the general circulation (132). The passage of substances from blood to spinal fluid is mediated by the choroid plexus. This structure, unless damaged, is impermeable to colloidal substances (59). On the other hand, it can be shown with the aid of aniline dyes that in small animals intracranially injected material always reaches the lateral ventricles (59). The passage of substances from the ventricles to blood takes place through the venous sinuses which are permeable even to corpuscular elements (59). It is quite understandable, therefore, that spirochetes go from spinal fluid to the blood but not in the opposite direction.

The negative brain cultures in guinea pigs after peripheral injection of leptospiras are in keeping with a concept of Friedemann (60). This worker has explained the impermeability of the capillaries of the central nervous system (blood-brain barrier) to aniline dyes, toxins, viruses, antibodies and drugs on the basis of the electrochemical properties of these substances. This barrier appears to be permeable to substances carrying a positive or no surface potential while it is impermeable to those bearing a negative charge at the pH of blood. He feels that this permeability possibly may be correlated more correctly with the magnitude rather than the sign of the zeta potential. However, as previously summarized (Section I B) there is no agreement on the surface potential of Leptospira. Therefore, careful cataphoretic studies are required to establish the zeta potential of leptospiras under various conditions. In that way it may be decided whether the impermeability of the cerebral blood vessels to the spirochetes is correlated with the electrokinetic potential of the organisms. It may be that different species of animals vary in the permeability of their blood-brain barrier to leptospiras. Also, the spirochetes may be able to change the sign of their zeta potential as some trypanosomes do (16).

In view of these facts, investigations of the correlations between electrokinetic potential and ability to invade the central nervous system would be of great interest. The oft-discussed problem of neurotropic strains of the syphilis and other spirochetes may thus be related to the surface potential of the organisms.

Borrelia. These also are rapidly invasive, apparently through the unbroken skin (147). Twenty-four hours after intraperitoneal inoculation into mice, the organisms may be demonstrated in the blood. There are no data on their local-

ization in the skin or in regional lymph nodes. Schuhardt (179) has seen no evidence of a prompt and severe inflammatory response to artificially injected Borrelia.

Treponema pallidum. This organism invades the body rapidly after peripheral inoculation. It can pass through apparently intact skin and mucous membrane (35), though it is impossible to exclude the presence of minute breaks in these tissues (150). The organisms also can pass through granulating wounds (35). Trauma and abrasion favor invasion (35, 36) but apparently are not always necessary. There is evidence of invasion of the body through the lymphatics and blood since there is an inflammatory reaction in the perivascular lymphatics (193, 208). The organisms settle out in inflammatory areas and produce lesions there when inoculated intraperitoneally or intratesticularly (36).

Kolle and Evers (103) infected rabbits by cutaneous or subcutaneous inoculation into the scrotum, removed the inguinal lymph nodes after varying periods, and injected these into fresh animals. By this means they found that the nodes were infective within 30 minutes of the injection. They were able to show that in guinea pigs the spirochetes reached the focal lymph nodes 5 minutes after cutaneous inoculation of the scrotum. In apes the time between inoculation and invasion is probably longer. For example, a chimpanzee anointed locally with calomel ointment 1 to 2 hours after cutaneous inoculation never developed syphilis (138). During the primary stages organisms are found in the local chancre and sometimes can be demonstrated in the blood (193). Mahoney (130) deposited a suspension of *T. pallidum* on intact genital mucosa of male rabbits, and at successive intervals killed animals for histologic study. One hour after exposure began the organisms occupied a more or less protected position in the crypts of the mucous membranes. In 2 hours there was evidence of penetration of the deeper tissues, and in 3 hours the organisms had penetrated to a depth that would preclude direct influence of any chemotherapeutic agent applied to the surface. *T. pallidum* apparently can traverse the blood-brain barrier and pass from blood into the brain substance (60).

C. Humoral mechanisms[6]

1. Normal blood. Taylor and Goyle (204) reported that freshly drawn human blood had spirocheticidal properties for *L. icterohaemorrhagiae in vitro*. There was no study of the effect of blood on the organisms *in vivo*. Stavitsky (187) was unable to detect any spirochetostatic, lytic, or agglutinating effect on the spirochete *in vitro* of normal defibrinated whole blood from adult man, rhesus monkeys, dogs, foxes, guinea pigs, rabbits, hamsters, white mice, and white rats. Oag (152) observed that mouse, chick embryo and chick bloods were spirocheticidal *in vitro* for *Borrelia duttoni*, possibly by a direct lytic effect. However, such a property apparently was absent from the blood of mouse and chick embryo *in vivo*. Turner and Diseker (215) demonstrated that *T. pallidum* loses its virulence and, probably, infectivity after 72 hours' storage in citrated human or rabbit blood at 2 to 4 C; and Bloch (12) found that *T. pallidum*, stored in citrated

[6] Antibodies are discussed under Acquired resistance, Section II E.

blood at 5 C, retained its virulence for 3 but not 4 days. He stated that the presence of tissue may favor the maintenance *in vitro* of virulence of the organisms.

2. Normal serum or plasma. Marked differences exist in the ability of serums derived from different species to support the growth of Leptospira *in vitro;* rabbit and horse serums favor growth while most other serums support poorer or no growth (186, 219). However, no correlation may be made between the ability of their serums to support growth and species susceptibility to Leptospira (186, 219). Normal dog serum has no protective power against this organism (219). Normal serum from man, horse, goat, rabbit, guinea pig, white rat, hen, and sheep contain no agglutinins for Leptospira (221). According to Corrales (40), the serum of horses, lower apes, rabbits, guinea pigs, or mice does not exert a spirochetolytic effect *in vitro*. Hen and eel serums were found feebly spirochetolytic (40). Normal serums from man, monkeys (*Macacus rhesus*), dogs, foxes, guinea pigs, rabbits, hamsters, white mice, and white rats were without demonstrable effect on the morphology or growth of the spirochetes *in vitro* (187). Turner, Bauer, and Kluth (217) reported that *T. pallidum* and *T. pertenue* suspended in blood serum were killed during freezing in carbon dioxide and alcohol and desiccation. Selbie (182) found that *T. pallidum* kept in rabbit plasma at 5 C maintained its virulence for 6 but not for 10 or 21 days. Ravitch and Chambers (162a) showed that *T. pallidum* and relapsing fever spirochetes lost their virulence gradually over a period of days or weeks during storage in frozen plasma at −12 and −20 C. Probey (161) observed that *T. pallidum* suspended in saline-normal horse serum is apparently killed during the deep freezing and drying process since material restored immediately after processing was not infective for rabbits.

3. Bile and bile salts destroy *L. icterohaemorrhagiae* rapidly *in vitro* (150). Taylor and Goyle (204) believe that the bile which is formed as a result of destruction of blood cells or through some other mechanism in leptospirosis is at least partly responsible for the disappearance of the organisms from the blood. According to Noguchi (150), *T. pallidum* is disintegrated by 10% bile salts. There are no data on the effect of bile on Borrelia.

4. Human gastric juice destroys Leptospira in 30 minutes (208). No data were found on the effect of gastric juice on Borrelia and *T. pallidum*, but it might be expected that they too would be destroyed in this highly acid medium.

5. Inflammatory fluid. Corrales (40) reported that rat, rabbit, and mouse inflammatory fluid devoid of cells was spirocheticidal for leptospiras *in vitro*. The organisms became deformed and granular, their mobility decreased and finally they disappeared. However, Stavitsky (187), using cell-free inflammatory fluid from guinea pigs, mice, and rats could not confirm these observations. Nor could he discern any effect of a peritoneal exudate on Leptospira *in vivo* (187). There are no data on the effect of inflammatory fluid on the other pathogenic spirochetes.

6. Hormones. There has been a great deal of research on the influence of various hormones on syphilis (156). Women react to syphilitic infection as

though a different species from man; they are so much more refractory to this infection (193). There is little doubt that pregnancy is an important factor in altering the course of syphilis (95, 156, 193); it may be responsible for the comparative freedom of women from neurosyphilis (193). Infections in a group of male and non-pregnant female rabbits were more severe than in a group of females in which impregnation and infection occurred at about the same time (17). Lowered resistance to syphilitic infection was observed in ovariectomized rabbits (84).

A hormonal basis for these phenomena has been made very plausible by studies in experimental syphilis and tuberculosis. Estrogens given to male and female rabbits appear to inhibit the syphilitic infectious process (58, 95). The most striking modification of response was the resistance to the disease developed in the testis (58). Lurie (128) has found that estrogens also exert a profound inhibitory effect on experimental tuberculosis in the rabbit.

Experimental syphilis of the rabbit has been reported to be more severe in animals after complete thyroidectomy than in controls (159). Partial thyroidectomy resulted in a milder disease than in control animals. Complete thymectomy produced a less pronounced effect than either partial or complete thyroidectomy but, in general, the syphilis resembled that in partially thyroidectomized animals (159).

It seems, therefore, that in syphilis the integrity of the endocrine glands and balance of endocrine functions are valuable adjuncts in defense of the host.

D. Cellular mechanisms

No feature of anti-spirochetal resistance is so controversial as the cellular mechanisms. In part this is due to incomplete knowledge of the diverse ways in which cells may take part in host defensive reactions. The all-important question of cells in defense through their rôle in antibody formation will not be considered as it is an extremely broad and disputatious subject in itself. The reader is referred instead to a recent paper (53) which discusses cells in connection with antibody production. Here attention is focussed mainly on the thorny question of phagocytosis as a defensive weapon in spirochetal infections.

It is very difficult to determine whether or not phagocytosis of spirochetes occurs either *in vitro* or *in vivo*. Many spirochetes are lysed extracellularly and possibly intracellularly upon contact with specific antibodies, and may leave within phagocytic cells no clearly recognized vestige which may be distinguished from normal intracellular granules. It is very difficult to recognize a spirochete within a cell by means of the dark-field technic. Moreover, one has to distinguish between adherence of the organisms to the leukocytes and actual ingestion, also between the active boring of the organisms into cells (77) and their passive ingestion. It is often not possible to determine whether a spirochete is within a cell or above or below it in wet or fixed preparations. However, one might expect in the course of numerous dark-field examinations to witness the actual engulfing of the organisms, if the process occurs at all frequently.

The great length of some strains of pathogenic spirochetes (4 to 30 μ) 98, 208,

219) might preclude their ready phagocytosis even by large macrophages without: *a.* lysis or curling of the organisms; *b.* agglomeration and fusion of phagocytic cells (foreign-body giant cells) about the spirochetes. Phagocytosis of spirochetes accompanying their lysis has been reported (97, 101, 106, 107, 151, 219). The presence of coiled intracellular organisms likewise has been noted (117). Phagocytosis of spirochetes by foreign body giant cells has not been described. Partial ingestion of spirochetes may be seen in illustrations to an article by Kritschewski and Sinjuschima (106).

The active motility of spirochetes may render phagocytosis difficult in the absence of some immobilizing agent such as a specific agglutinating or lysing serum (233). However, Himmelweit (77) has described the apparent active boring of the avian spirochete into macrophages giving the appearance of phagocytosis.

Teale (205) has emphasized the importance to the phagocytic process of sedimentation of fibrin meshworks with subsequent trapping of the organisms in them. It may be significant, therefore, that leptospiras, probably due to their motility, do not sediment readily. Approximately 5 hours' centrifugation at 3000 rpm was required to clump them completely from a suspension (185).

There has been no definite demonstration to date that the pathogenic spirochetes are positively chemotactic. *L. icterohaemorrhagiae* did not attract phagocytic cells *in vitro* or *in vivo* despite the coexistence in one tube or in a preformed inflammatory exudate of large numbers of spirochetes and phagocytes (185, 187). There was no evidence that the spirochetes produced leukocidin, (208 because the phagocytes engulfed cocci introduced into the *in vitro* mixture (187). Novy and Knapp (151) considered as chemotactic the distorted Borrelia in rats which have recovered from relapsing fever. However, it is questionable whether this term may be applied to organisms sensitized and degenerated by contact with specific antibodies. McCutcheon (134), in a review of chemotropism, states that specific antibodies appear to play no part in chemotaxis which is a nonspecific phenomenon often displayed by antigens. Bergel (9) believes that the lipid cell membrane of *T. pallidum* chemotactically induces a lymphocytic reaction, but McCutcheon (134) states that lymphocytes do not exhibit chemotaxis to any substance yet studied. He knows of no evidence that Leptospira or Treponema causes a chemotropic response and considers it unlikely that they do (133).

There are at least three possible explanations for the apparent lack of chemotropic properties in pathogenic spirochetes. First, the organisms may not produce substances in the course of their metabolism that attract phagocytes (134). Second, they may not injure tissues, which may then release chemotactic substances (134). Third, the organisms may escape from the focus of infection without having released chemotropic substances. Under such conditions the tissues may also fail to produce these agents.

Leptospirosis. Only the humoral aspects of host resistance in leptospirosis have been given much attention (187, 219). The rôle of the fixed and mobile phagocytes has been almost entirely neglected except insofar as investigators

have studied these cells in stained smears or sections of tissues from naturally or experimentally infected men and animals (219).

Inada *et al.* (85) found spirochetes in epithelial and phagocytic cells in man. Degenerated organisms were also observed in lymph nodes and spleen. The occurrence of intracellular spirochetes was attributed to the spirochetes' invading the cells in order to escape the action of the antibodies; while the presence of spirochetes in epithelial cells of glands perhaps indicated their way of escape from the body. Vanni (223a) observed that leukocytes between liver cells and in blood vessels were filled with nests of spirochetes in which individual organisms were still recognizable. Corrales (40) has described phagocytosis of leptospiras by phagocytic cells in the peritoneal cavities of mice, rats, guinea pigs, and rabbits following intraperitoneal injection of the organisms. He concluded that natural resistance of such species as mouse, rat, and rabbit is due to phagocytosis, destruction of spirochetes by substances elaborated by the leukocytes, and the inability of the organisms to injure the tissue cells of these species. Hindle (78) states that phagocytosis of the spirochetes is extremely active in monkeys, rabbits, rats and mice, both *in vitro* and *in vivo*, but presents no experimental basis for the statement.

In view of the significant nature of Corrales' work (40), Stavitsky (187) attempted to confirm and extend his studies. However, not a single instance of phagocytosis of Leptospira was observed in the course of numerous examinations, *a*, of exudates from infected eyes, peritoneal cavities, or blood containing spirochetes, *b*, of *in vitro* mixtures of serum (normal and immune), polymorphonuclear and mononuclear leukocytes (from normal and immune animals), and spirochetes, and *c*, of stained smears and sections of brain, meninges, liver, spleen, adrenals, bone marrow and kidneys of infected animals. The organisms were agglutinated or lysed only in the presence of specific antibodies. Mononuclear cells in cell cultures were without effect on the organisms. When Leptospira were introduced into a skin area containing a preformed predominantly polymorphonuclear inflammatory exudate, no localization or phagocytosis of the spirochetes in the injection site was observed (185).

Tscherikower and Rubinstein (211) found no significant difference in the response to leptospirosis of splenectomized and normal guinea pigs, and concluded that there is only a slight protective function of the reticulo-endothelial system in this disease. In another study (233) in which the reticulo-endothelial system of guinea pigs was blocked by intracardial injection of ferric sucrate 2 days after splenectomy, the surviving animals behaved much as did the normal ones as regards active and passive immunization and response to bismuth therapy. Splenectomy in mice did not alter the course of subsequently introduced infection (185). However, these experiments are open to the criticism that blockade and splenectomy may not have lowered the functional activity of the reticulo-endothelial system sufficiently to produce a noticeable effect.

In contradistinction to most microörganisms (205, 208), *L. icterohemorrhagiae* apparently was not cleared by cells of the reticulo-endothelial system from the blood of normal guinea pigs, mice, and rats during the first 48 hours after intra-

cardial injection; there was no appreciable fluctuation in the number of organisms in the blood during this time (187). In other experiments (185), organisms were cultured from the blood up to 120 hours after injection. Chang (30) has reported that avirulent leptospiras may be found in the blood of guinea pigs for a week or more after inoculation.

Benzene administered to produce a profound artificial leukopenia in mice was without detectable effect on leptospiral infection in naturally resistant strains of white mice (188).

Relapsing fever. In no field of immunology has the question of cellular versus the humoral basis of immunity been asked so persistently as in relapsing fever studies (143, 147). Some have considered resistance as due to phagocytosis, and others to lytic action of specific antibodies with phagocytosis a secondary phenomenon (66, 143, 147). It is well, therefore, to review the evidence on the question.

It is generally agreed that Borrelia are usually seen extracellularly in tissue sections (97, 143, 147). Many intracellular fragments or granular corpuscles may be observed occasionally, but there is no conclusive evidence that these are related to the spirochetes (97, 147). Dark-field examination may show spirochetes entangled in the pseudopodia of poymorphonuclear leukocytes, but there is little to suggest that ingestion ever occurs (97). Fixed preparations do not reveal phagocytosis by wandering phagocytes (97). Spirochetes are invariably extracellular in blood films (97). Histological sections of infected tissue likewise give no indication of phagocytic action by mobile leukocytes (97). Oag (152) did not observe phagocytosis of *Borrelia duttoni* by any circulating cell in the blood of chick embryo, chick, or mouse. Other investigators have reported phagocytosis of Borrelia by wandering cells in blood and tissue (66, 143, 147). However, there seems to be more general agreement on phagocytosis of the spirochetes immobilized and sensitized by specific antibodies (143, 179). In the classical experiments of Novy and Knapp (151), phagocytosis and intracellular digestion by macrophages of organisms altered by immune serum were very rapid. Kritschewski and co-workers (106) found that phagocytosis is usually an accidental phenomenon in experimental relapsing fever. When organisms were injected intravenously in immune animals only lysis was observed; following injection into skin and peritoneal cavity, some phagocytosis was seen. In tissues, only lysis of organisms and no phagocytosis were the rule. The monocytes seemed less important than the microphages in phagocytosis.

The rôle played by fixed phagocytic cells in relapsing fever is difficult to establish despite the many papers attributing a protective function to the reticuloendothelial system in this disease (66, 147, 156). Spirochetes have been described within cells by many workers (66, 143, 147, 156), but their work is open to the objection that histological studies are difficult to interpret precisely. Spirochetes are occasionally seen within the Kupffer cells of the liver but none in the macrophages of the spleen (97). Only granular forms are seen in the reticuloendothelial macrophages of the spleen (97, 147). It is probable that fixed

phagocytes are not very active against living forms of the spirochetes although these organisms are disseminated widely throughout the body (97). Certain macrophages, particularly the splenic cells, may be packed with argentophilic granules; but if phagocytosis of living organisms does occur, spirochetolysis must take place very rapidly because forms recognizable as spirochetes are never abundantly present in these cells (97). The significance of fixed phagocytes in relapsing fever apparently is still an open problem.

One group of workers (97, 147) has presented circumstantial evidence that the spleen is involved in the destruction of the spirochetes. The spleens of severely infected animals did not show enormous numbers of morphologically normal spirochetes while the other organs were loaded with them. In the spleen many intracellular fragments and extracellular granular, degenerated forms were noted. On the other hand, in animals in which not many organisms were seen, the normal organisms were most numerous in the spleen and often could not be seen in other organs. However, these suggestive studies should be confirmed, preferably by other than histological methods, a technic open to considerable danger of misinterpretation.

Kritschewski and his co-workers (107) have made exhaustive studies of the rôle of phagocytic cells and reticulo-endothelial system in resistance to borrelias. It was found that after splenectomy great multiplication of the spirochetes occurs. However, splenectomy is without effect in experimental infections with some species of *Borrelia* (156). There are many observations on the effect of splenectomy or blockade of the reticulo-endothelial system on infection with relapsing fever spirochetes (66, 156), but they do not contribute much of significance to an understanding of the problem. The decreased resistance upon splenectomy or blockade may be a manifestation of decreased antibody formation incident to the removal of large numbers of antibody-forming reticulo-endothelial cells or lymphocytes (53).

Avian spirochetosis. Levaditi and Stoel (115) have observed phagocytosis by macrophages of *Borrelia anserinum* in tissue culture. However, Kritschewski and Rubinstein (107) have found phagocytosis to be a secondary phenomenon, occurring after the death or immobilization of the spirochetes. Himmelweit (77) believes spirocheticidins play an active part in the destruction of the organisms in hens. In tissue culture experiments (77) the spirochetes were seen to bore into macrophages from normal spleen cultures but not into fibroblasts and small round cells. In spleen cultures from previously immunized hens the organisms began to bore into macrophages one hour after infection. No phagocytosis was shown by fibroblasts and round cells in these cultures. Twelve hours after infection the spirochetes lay in a braid around macrophages and showed degenerative forms. After a while no live organisms were seen. Upon addition of immune serum to infected macrophages from normal cultures the spirochetes immediately bored into the macrophages. Himmelweit questions whether phagocytosis is the all-important factor, since lytic and immobilizing antibodies are important factors in overcoming the crisis in the infection. Knowles *et al.*

(101), in a recent review of the literature on avian spirochetosis including original experiments on the mechanism of immunity, conclude that immunity in the disease is basically humoral in nature.

Syphilis and yaws. A number of investigators have reported finding typical syphilis spirochetes within various cells in tissue sections of natural or congenital human syphilitics (236) or of experimentally infected rabbits (cf. 34). The tissues included liver, lung, and kidney from congenital syphilitics, and testis, liver and kidney epithelial cells, adrenal capsular cells, sweat glands, and cytoplasm of nerve cells from syphilitic rabbits. Bergel (10) reported that phagocytosis of treponemas by lymphocytes and large mononuclears could be demonstrated in the peritoneal cavity of rabbits, guinea pigs and mice inoculated intraperitoneally. However, Chesney (32) states that he has never seen a single instance of phagocytosis of *T. pallidum* either in human syphilis or in experimental syphilis of the rabbit, and he does not attribute an important rôle to phagocytosis in anti-syphilitic resistance. Ferris and Turner (55) have noted no evidence of ingestion of spirochetes by mononuclear or any other cell in cutaneous lesions of syphilis or yaws in man or rabbits. Zinsser and Hopkins (237), in studying the mechanism of natural resistance of mice against *T. pallidum*, observed actively motile unphagocyted organisms surrounded by masses of leukocytes in the peritoneal cavity as long as 3 days after injection. They could not convince themselves that any significant amount of phagocytosis had occurred. Hoff and Silberstein (81) found some indication that the sera of malaria-treated general syphilitic paralytics contained opsonizing substances for treponema. Beck (7), however, has reinvestigated this matter and concluded that such sera contain no opsonic elements. Occasionally he noted what appeared to be partial ingestion of an organism by a leukocyte but attributed this to an active boring movement of the spirochete into the cell. Most workers, in fact, found the syphilis spirochete in a predominantly extracellular position in the tissues (184). The ability of these organisms to penetrate granulating wounds (35) also speaks against the occurrence of phagocytosis on a very large scale.

Although it is commonly assumed that the fixed tissue elements play an important rôle in defense of the host against syphilis, there is little factual material to indicate that this is true or what this rôle might be. Phagocytosis has not been shown to occur to any great extent in these tissues (184). Although the spleen and lymph nodes usually contain only a few organisms, this may not be due to phagocytosis by macrophages. It may rather be that the extracellular fluids in these organs afford a poor medium for growth of the spirochetes (41, 48, 164). Jungeblut (90) observed that blockade combined with splenectomy in mice in no way altered the course of subsequently induced syphilitic infections. The disease was latent, as in the control animals, with localization of the organisms in the lymph glands.

Phagocytosis may be of great importance in cleaning up the lesions in syphilis and thus assisting in the removal of toxic products from the body. The importance of an increased number of large macrophages in the resolution of syphilitic lesions in the rabbit has been emphasized by a number of investigators (124).

E. Acquired resistance

Specific antibodies appear to be primary elements in acquired resistance to leptospirosis and infections produced by species of *Borrelia*. However, until quite recently there was no evidence for the participation of antibodies in the resistance which is acquired in the course of syphilitic infection in man and animals.

Leptospirosis. In natural and experimental leptospiral infection the host apparently becomes resistant to infection coincident with the development of specific agglutinating and lytic antibodies during the second week of the disease. The resultant resistance may persist for months or even years paralleling the persistence of the antibodies in the blood. According to Kaneko and Okuda (92) the antibodies are capable of destroying the spirochetes found within the organs of man, with the exception of those in the kidney. Similar results have been obtained in experimental infections in guinea pigs (187) and hamsters (185) whether the antibodies were formed actively or acquired passively. Specific antibodies have a definite therapeutic effect on leptospirosis in susceptible strains of mice if given as late as the fourth day after infection (111).

It is not known definitely whether complement must be present for lysis of Leptospira by specific antibodies (185, 219).

Active and passive immunization are quite successful in leptospirosis (219). Immunity may be transferred to the fetus apparently by way of the placenta (219).

It has been claimed that antisera to leptospirosis exert their beneficial effect by virtue of their antitoxic content (219). However, final judgment on this view must await conclusive demonstration of a spirochetal toxin.

Relapsing fever. Human cases of relapsing fever characteristically result in long lasting acquired resistance to reinfection (150). Moreover, human immunity seems to be more lasting, relatively, than that observed in the lower forms where after some months it is sometimes possible to demonstrate a certain amount of susceptibility (143). However, second infections may occur in human cases where specific therapy has interrupted the natural processes of immunization (143).

The important rôle of spirocheticidal antibodies in infections with Borrelia was first recognized by Gabritschewsky (62) and later put on a firm experimental foundation by the classical experiments of Novy and Knapp (151). It was shown that the spirochetes will remain alive for forty days in blood drawn before the onset of an attack, but in blood drawn during the decline of an attack or after recovery they die out in less than an hour (151). When the organisms were examined *in vivo* during the decline phase of infection, they were observed to lose their motility and become agglutinated, degenerated and granular (151). This also could be observed when blood from an immune animal was inoculated into an infected rat. In rats that had recovered from the infection, Pfeiffer's phenomenon could be produced *in vivo*. Injection of infected blood into such animals was followed by agglutination and granular degeneration of the spirochetes. The course of antibody production and action in human beings seems to resemble

that in experimental animals. It was suggested by Novy and Knapp (151) that the relapse is the consequence of the survival of a few individuals which are resistant to the specific spirocheticidin and which multiply to give rise to a new serum-fast strain. This train of events is repeated in each relapse. After a number of relapses the active immunity developed by the host is sufficient to prevent further invasion of the blood by the spirochetes and an apparent cure results. Antibodies may be transferred passively to the young of man and animals (147).

Some workers believe that immunity in relapsing fever depends on the virulence and numbers of spirochetes introduced rather than on the highly complex antigenic structure of the organisms (97). Many virulent organisms may elicit an active immune reaction from the host while smaller numbers or mildly virulent strains may not arouse the host so markedly (97). However, it seems that the relapse phenomenon is of greater significance for the outcome of this infection, as a sudden shift in antigenicity of the organisms may negate the acquired immunity of the host.

The participation of complement in antibody reactions with borrelias has not been studied. The benefits of active immunization in relapsing fever are less definite than in leptospirosis. The beneficial effects of specific antiserum may be nullified by the failure of the antibodies to correspond to the newly formed antigenic variant.

The rôle of residual infection in immunity to relapsing fever is discussed in Section II G.

Avian spirochetosis. The phenomena and mechanisms of host resistance in avian spirochetosis are essentially the same as in relapsing fever.

Syphilis. Acquired resistance in syphilis is quite different from that in the other spirochetoses as well as in most other infectious diseases. It perhaps resembles most closely resistance in tuberculosis (168). It is, therefore, pertinent to note the salient features of acquired resistance in natural and experimental syphilis (34, 222).

Compared with most infections, resistance in syphilis is acquired very slowly. Nevertheless, in man reinfection has not commonly been proved to occur (8, 34). In the rabbit it requires about 6 weeks following inoculation for a state of partial resistance to be achieved (51). This resistance at its best is not always complete as the organisms often persist in a latent state in the tissues of rabbits and conceivably of man (34). At some time between the 45th and 90th day following infection the rabbit acquires more effective resistance against syphilitic infection. It appears to be strain-specific in rabbits (38) and possibly in man (5). Resistance is shared by most of the tissues of the body (39) with the eye a prominent exception (39). However, some tissues appear more resistant to the organisms than others (34, 193). Brown and Pearce (24) believe that the testis, bones, skin, and eye successively take up defense of the body and provide by local reaction much of the general physiologic defense. Resistance persists at a high level throughout the secondary or most active period of the disease and into the tertiary stage. During the latter it appear somewhat to wane as reinoculations

are often more successful at this period (5). At its height, the resistance is considered not sufficient to eradicate infection (5, 34, 66, 193), but sufficient to restrain multiplication of small numbers of organisms and prevent the development of an inflammatory lesion. The number of spirochetes in the challenge inoculum is important in determining whether asymptomatic infection or solid resistance to reinfection is manifested in rabbits (129).

Rabbits treated with arsphenamine before the 45th day of the disease can be reinfected successfully as the organisms have been destroyed before producing sufficient active immunity (34). Between the 45th and 90th days, treatment has a variable effect on reinfectability (34). If treatment is postponed until after the 90th day the animal often is refractory to a second infection with the homologous strain of organism providing there is no trauma at the site of injection (34). In monkeys, the earlier treatment is begun the more apt the animal is to yield a positive result upon reinoculation.

The question as to the persistence of active immunity in syphilis following drug treatment is of more than theoretical concern. Chesney (34) believes that animals treated in the late stages of syphilis are biologically cured and at the same time actively immunized. Neisser and Kolle (34, 102, 222), on the other hand, deny that infection has been eradicated from these animals and claim that they harbor latent organisms as indicated by positive lymph node or organ transfer tests. The interested reader is referred to material on both sides of the controversy (34, 102, 193, 208, 222, 236).

What physiological changes in the host are responsible for the increase in resistance in the course of syphilitic infection? Several investigators have noted an enhanced physiological activity of large mononuclear cells during early acute syphilis (124). An inverse relationship between the intensity of the local lesion and the intensity of secondary lesions has been reported and may be related to this enhanced cellular activity (18). However, in the absence of evidence that these cells are engaged in extensive phagocytosis of treponemata, some other function must be assigned them. These cells have been considered to be active in resolving or clearing up the debris in syphilitic lesions rather than in disposal of the spirochetes themselves (124). Other possible activities of the cells have been taken up in previous sections. There remains only the subjectof acquired humoral resistance. This subject will be discussed quite thoroughly as it commonly has been discarded as a factor in syphilitic immunity[7]. Although this phase of resistance seems feeble when compared to the powerful influence of acquired antibodies in other infections (89, 143, 168, 208, 236), it is in fact the only concrete factor in resistance to syphilis which has been established up to the present time.

In the past, experiments have been described which suggested that protective antibodies are developed in the course of syphilitic infection. Eberson (52)

[7] For example, in an editorial in the Journal of the American Medical Association for August 23, 1947, it was stated: "Details of the mechanism of the defensive reaction against syphilis are still unknown. *It is definitely not humoral* but is probably a tissue or cellular reaction." (Italics by author).

mixed virulent *T. pallidum* and serum from persons with late syphilis and from rabbits with syphilis of 6 months' duration or longer and incubated them for 2 hours at 36 C. When inoculated intratesticularly into rabbits, these mixtures produced no lesions, whereas control mixtures containing serum from normal rabbits and rabbits infected for less than 6 months produced lesions. Tani and Oginti (202) reported similar results in experiments in which mixtures were injected intracutaneously. Of greater interest, however, are parabiotic experiments performed by Tani and Aikawa (203). They parabiosed rabbits with active syphilis of 9 to 94 days' duration either with rabbits infected with syphilis for 99 to 459 days or with normal rabbits. In general it was found that rabbits joined to immune rabbits showed definite healing of their active lesions while most of those joined to normal rabbits did not show any healing.

Turner (214) has criticized the techniques and lack of suitable controls in previous experiments which led him to his studies on the question. He realized that any test to demonstrate protective antibody in syphilis must be delicately designed so as to bring out the small differences in antibody content that probably exist between the serums of normal and syphilitic animals or human beings. He worked out a technique to satisfy this requirement (214). An emulsion of virulent *T. pallidum* was added to serums from normal rabbits and from untreated immune syphilitic rabbits, infected with an homologous strain of *T. pallidum*, and the mixtures, after incubation at 37 C for 6 hours, were injected intracutaneously into normal rabbits. Typical syphilitic lesions developed at the site of inoculation of the normal serum-spirochete mixtures, while at the sites of inoculation of the immune serum-spirochete mixtures there was either no manifest response or a reaction marked by a longer incubation period and smaller lesions than in rabbits injected with normal serum-spirochete mixtures. Ten out of eleven syphilitic persons with negative reactions to serologic tests for syphilis were positive for protective antibodies by Turner's test (216). Complement seemed necessary to demonstrate the action of these protective antibodies. The fact that positive reactions for protective antibodies were found along with negative reactions to serologic tests for syphilis suggests a probable lack of identity between antibodies for positive serology and those necessary for protection. In any event, these studies indicate that the protective antibodies are associated with a high degree of acquired immunity to the disease.

In recent studies on a larger scale with serums from human beings with syphilis, Turner (212) essentially has substantiated his earlier results: "The serum from syphilitic patients showed a substantial degree of protection as compared with serum both from non-syphilitic hospital patients and from the normal pool. Serum from patients with latent and late syphilis as a group exhibited a higher degree of protection than serum from patients with early syphilis. Serum from patients with secondary syphilis as a group was more protective than serum from patients with primary syphilis, but here the figures are smaller...." The results of the protection test did not correlate closely with the complement fixing titer using ordinary beef heart antigen, but no tests were made in which the so-called

Wassermann antibody was removed by adsorption. Turner (212) has no direct information as to whether the antibody would be protective regardless of the route of inoculation of the spirochete-antibody mixture

In their extensive studies on experimental syphilis in the rabbit, Brown and Pearce (18, 20, 21) observed that the lesions exhibited a relapsing character paralleled by agglutination and degeneration of the spirochetes. Though less regular and marked these observations recall the situation in relapsing fever. Moreover, the agglomeration and lysis of *T. pallidum* are highly suggestive of the operation of specific agglutinating and lytic antibodies such as have been produced in relapsing fever and leptospirosis, and with culture strains of *T. pallidum* (239).

Chesney and co-workers (39) have presented indirect evidence for the humoral nature of host resistance in syphilis. It has been known for some time that the cornea of the syphilitic rabbit does not share to the same extent as other tissues in the general resistant state which develops in that animal during the course of syphilitic infection. It was assumed that this was connected with the absence of a blood supply to the cornea so that the corneal cells either did not receive an antigenic stimulus sufficient to induce immunity or did not receive through the circulation enough of syphilis antibody to endow them with resistance. However, when the cornea was vascularized by previous inoculation with an irritant (39) (dead tubercle bacilli), it manifested greater resistance against a second inoculation with homologous *T. pallidum* than did the normal eye. This result might be due either to exposure of corneal cells in the vascularized eye to a greater amount of antigen with consequent more active cellular response, or to the presence of increased concentration of circulating antibodies. As the rabbits were treated with arsphenamine prior to vascularization, it seems unreasonable to suppose that the eyes had been exposed to an undue amount of circulating antigen. Therefore, the authors were inclined to the view that the greater resistance on the part of the vascularized cornea was more likely due to an exposure to increased circulating syphilitic antibody.

Reynolds (165) cites experiments from the literature which suggested that *T. pallidum* inoculated into immune animals were not only restricted in spread but destroyed locally. He criticized these experiments as dependent on histologic observations for their validity and hence unreliable. In his own studies, Reynolds followed the fate of homologous strains of the syphilis spirochete inoculated subcutaneously into immune rabbits. These organisms did not migrate to the regional lymph nodes but were immobilized and destroyed *in situ* by the immune mechanism of the host, probably by a local antigen-antibody reaction.

Despite the extensive literature recording negative attempts to demonstrate protective antibodies in syphilis (34), the positive and suggestive experiments summarized above force the conclusion that immunity in syphilis rests on at least a partly humoral basis. Possible difficulties in demonstrating protective antibodies are indicated in Turner's work in which these antibodies were shown to be of low titer and probably distinct from the antibodies detected by the usual

serologic tests for syphilis. Moreover, the apparent strain specificity of resistance in experimental syphilis (38) points to the importance of employing homologous organisms for the demonstration of strain-specific antibodies.

Whether the protective antibodies in syphilis exert their effect at least partly by their opsonizing properties is open to further study.

The results of vaccination in syphilis using various kinds of living, attenuated and dead vaccines have been essentially negative (34). There is no evidence for the transfer of immunity from syphilitic man or rabbits to their off-spring (96, 184). However, the positive parabiosis experiments of Tani and Aikawa (203) bring up the possibility of passive transplacental transfer of protective substances in syphilis.

The rôle of infection-immunity or latent infection in acquired resistance to syphilis is discussed in Section II G.

F. Hypersensitivity

The importance of hypersensitivity in the pathogenesis and immunology of infectious diseases is a question of tremendous theoretical and practical consequence. There are reliable students of disease on all sides of the question, some holding that hypersensitivity is definitely advantageous and even necessary, others that it is decidedly deleterious or at least unnecessary, and still others that it is without appreciable effect for the effective operation of acquired resistance (166, 167, 168, 169, 222). It would be unrewarding to enter into an extended discussion here of this acute controversy. At least some of the difficulty arises from lack of general agreement on the definition of hypersensitivity (167, 222). While it is convenient to explain the pathogenesis and acquired resistance in syphilis in terms of hypersensitiveness, it would be preferable for this concept to rest on a firm factual foundation.

Although skin tests employing antigenic material derived from bacteria, fungi, viruses and helminths have proven useful in the diagnosis of many diseases, this method has not been employed with any great success in any of the spirochetoses. There is no report of hypersensitivity in the literature on relapsing fever. The only report of a skin test in leptospirosis is that of Jacobsthal in 1917 (86). However, his observations were only preliminary in nature and never confirmed. Stavitsky (185) noticed that four or five days after intradermal inoculation of *L. icterohaemorrhagiae* into guinea pigs concentric hemorrhagic lesions often appeared at the site of injection. It is not known whether this represents an allergic response (45) or is due to hemolytic or toxic substances of spirochetal origin concentrated in the injected area by virtue of increased capillary permeability there (137). There are no further data on hypersensitivity in leptospirosis. The luetin test (148) employing killed suspensions of *T. pallidum* as a skin test agent for the diagnosis of syphilis was found to be extremely non-specific. The organic luetin reaction (105) using extracts of mature syphilomas from rabbit testes is apparently more specific under certain conditions.

The exalted or at least altered reactivity of a host upon second contact with a foreign protein of bacterial or non-bacterial origin has been noted repeatedly

(156, 167, 168, 208, 236). Consequently, it is not surprising that the syphilitic rabbit may react differently at a second encounter with *T. pallidum* than at their first meeting. Lisi (119) found that when injections of virulent spirochetes were given to vaccinated rabbits, the lesions were larger and the period of incubation shorter than when injections were given to control animals. Aronson and Meranze (3) studied the lesions produced by injection of tubercle bacilli intracutaneously into syphilitic and non-syphilitic rabbits. The authors concluded that the cellular reaction in the syphilitic rabbits was an "anamnestic reaction", i.e., the cells of the syphilitic rabbits were so modified that injection of an unrelated organism provoked a prompt inflammatory response characteristic of the initial syphilitic reaction. The perivascular focal character of the lesions, the presence of large mononuclear cells and fibroblasts, and the formation of new vessels suggested syphilis; on the other hand, the subsequent appearance of epithelioid cells and of caseation and softening were more reminiscent of tuberculosis.

Rich *et al.* (169) have conducted extensive experiments to clarify the rôle of hypersensitivity in syphilis. Noguchi (148) had shown previously that rabbits injected repeatedly with virulent *T. pallidum* gave allergic skin reactions to a spirochetal suspension ("luetin reaction"). However, Rich *et al.* (169) found immunized rabbits refractory to large test doses of spirochetes without displaying any microscopically discernible inflammatory reaction. In no instance was there the slightest gross or histologic indication of a more prompt or exaggerated hypersensitive inflammation in the immunized rabbits as compared with the controls. On the contrary, after the initial minimal inflammatory response which followed injection of the spirochetes, and which never was more prominent in the immunized than in the control animals, the sites of inoculation in the immunized animals promptly became and remained normal, whereas the lesions in the controls progressed steadily to chancre formation. Rich therefore was led to conclude that although hypersensitivity may appear under certain conditions during certain stages of infection in some individuals, experimentally it had been demonstrated that acquired resistance in syphilis is not dependent upon hypersensitive inflammation; nor does hypersensitivity necessarily develop concurrently with acquired resistance. Acquired resistance and hypersensitivity are distinct and unrelated phenomena.

Gastinel *et al.* (65) recently have presented evidence for the thesis that immunity and hypersensitivity are different manifestations of the same process. He produced both immunity and hypersensitiveness in the same animal by the following procedure: he first reinoculated (without eliciting a local reaction) the testis on the side previously injected; then he reinoculated the other side, promptly producing a chancre with pronounced necrosis, suggesting localized hypersensitiveness of the testicular tissues. Low (123) has considered that "the relative immunity in syphilis is really a hypersensitiveness. . . . Persons with syphilis are protected from reinfection by the local hypersensitive reaction which occurs at the seat of reinoculation." According to this view the organisms are localized at the site of reinfection by the allergic inflammatory response. Rey-

nolds (165) has, in fact, observed such a localization of the organisms of reinfection. However, he attributed this localization to the immobilizing action of specific antibodies on the organisms by a local antigen-antibody reaction, probably agglutination, as has been observed in other infections (166).

The results of the studies of Gastinel *et al.* (65) recall the experiments of Lisi (119) and suggest that hypersensitivity may at times influence the development of pathological alterations in the syphilitic host. The evidence (156, 167, 168) that hypersensitivity may condition the pathological response of the host in tuberculosis and other chronic granulomatous diseases lends weight to the postulated rôle of allergy in the pathogenesis of syphilis. In all of these diseases the rôle of hypersensitiveness is to intensify the response of the host to the pathogen and to act to the apparent detriment of the host. The destructive tertiary lesions in syphilis especially have been considered the result of an altered response (hypersensitivity) toward the spirochetes (193). It is obvious that considerable precise study and agreement on definitions of basic terminology are called for to settle the question of the rôle of hypersensitivity in syphilis.

G. Carrier and relapse states and infection-immunity

These phases of resistance to spirochetal infection are important but as yet inadequately understood.

Leptospirosis. Leptospira usually does not give rise to relapses (219). However, there are mildly virulent variants of the organism which do (5). In these so-called "kurzfristigen" leptospiral infections in man the fever curve in general is of the same type as that in relapsing fever.

The symptomless carrier state often is observed in natural and experimental leptospirosis, usually in relatively resistant species of animals such as mice and rats (219). However, individuals of susceptible species may under some circumstances become carriers. According to Tjong (207) the carrier state is established as follows. During the acute period of the infection the spirochetes pass from the interstitial renal tissues through the wall of the tubules into their lumen. The organisms are then swept into the lumen of the distal convoluted tubules and lodge there because of the weak flow of urine and the tortuosities in these tubules. The organisms then maintain themselves by multiplication there and are shed continuously in the urine.

It is well established that the carrier of leptospira in the kidneys, whether man, dog, or rat, often has a considerable concentration of anti-leptospiral immune bodies (agglutinins and lysins) in the blood and urine (80, 112, 183). It is difficult to understand how the organisms survive in the urine in the presence of antibodies unless the organisms have undergone antigenic variation and are no longer homologous for the antibodies. However, it has not been shown that Leptospira undergoes antigenic variation *in vitro* or *in vivo* to any great extent.

The factors responsible for the latent state in leptospirosis are obscure at present. The relapses due to mildly virulent strains are probably reflections of the mild virulence with its consequent mild stimulus to active resistance on the part of the host, giving rise to inadequate immunity to prevent relapses. The

apparent ineffectiveness of phagocytosis as a defensive weapon in leptospirosis may contribute further to the inability of the body to eradicate the infection.

Relapsing fever. Before considering the possible mechanisms of the relapses in this disease, the generally accepted facts of this phenomenon (66, 97, 143, 180) will be stated. For convenience and clarity they are listed:

1. In the course of natural human or experimental infections Borrelia produces one or more, usually several, relapses.

2. A typical relapse episode is characterized by the appearance of organisms in the blood, persistence there for many days, disappearance from the blood for a variable period of days and then reappearance in the blood.

3. The number of times this episode is repeated is variable in different species and dependent on many factors.

4. During each episode the antigenic composition of the organism is different from that of strains participating in past or future relapses in that host. Antigenic variants do not recur (181). However, all possible antigenic varieties may not appear in the course of an infection.

5. Upon introduction into a suitable host, a single spirochete may produce all the antigenic variations which characterize that particular strain during infection in that host (181).

6. The succeeding antigenic variants may tend to become weaker in virulence (143).

7. The relapse strains of spirochetes reappear against a definitely rising titer of antibody which is able to confer a considerable amount of immunity against any relapse strain (181).

8. If the antibody-forming mechanism of the host suffers any substantial impairment, as by daily injections of foreign red cells, the relapse phenomenon may be reduced in number of relapses, or entirely absent; and the host may undergo a violent infection (97). If the animal does not succumb to this nearly fatal attack, a single prolonged period of reaction with organisms in the blood ensues, after which the spirochetes cannot be found in the peripheral circulation. Occasionally two severe reactions occur lasting as long in total duration as the single reaction but separated by 1 or 2 negative days. After recovery the resulting immunity is broad in its specificity and enduring in quality.

9. Artificial crises can be produced by injection of specific antiserum, provided it is active against a sufficient majority of the antigenic varieties of the spirochetes involved in the attack (179).

10. The great majority of the surviving organisms are destroyed at the crisis of each attack (97).

11. The antigenic variants may be detected by agglutination or adhesion tests or spirocheticidal action of immune serum (78). Cross-immunity tests may have limited usefulness for this purpose (78).

Some workers have presented evidence which refutes the concept that antibodies are involved in the relapse phenomenon (180). However, taken as a whole, the above data, especially points 8, 9, and 10, support the generally accepted concept that relapses in this disease are somehow bound up with antigen-

antibody reactions. More specifically, relapses appear to be dependent on the inherent ability of borrelias to undergo one or more antigenic phase variations during the course of the disease. The new variant is resistant to the action of the highly strain-specific antibodies elaborated in response to the previous antigenic varieties of *Borrelia*. However, it appears that only a very small percentage of the many organisms participating in an attack are capable of accomplishing this variation as the great majority are destroyed at the crisis of the attack. Presumably the spirochetes which finally succeed in producing antigenic variants are some of those which may leave the blood stream during an attack to seek a haven in the brain, spleen or other tissues from lytic antibodies. Additional antigenic variations account for succeeding relapses until the capacity for variability of the spirochete is spent. Complete recovery then ensues.

It should be stressed that, beyond the stated facts of antigenic variation, the above picture of the pathogenesis of the relapse phenomenon in relapsing fever is at present far too impressionistic. It is difficult to account for all of the facts of the relapse phenomenon solely on the basis of successively distinct antigenic complexes of spirochetes. If the relapses are closely dependent upon antigen-antibody combinations, how does it happen that these reactions become effective at such regularly predictable intervals? Moreover, how can these antisubstances be effective enough to clear the blood stream of enormous numbers of organisms and yet fail to be effective against a certain few? Where or how do the few organisms escape destruction? Also to be explained is the demonstration that succeeding antigenic variants tend to become weaker in virulence and that they appear against a definitely rising titer of antibody capable of transferring immunity against any of the relapse strains. Satisfactory answers to these and other questions require considerable further study. It is a fact, however, that, regardless of the variations in antigenicity or virulence, relapses occur at regular intervals under natural conditions.

Various species of *Borrelia* may become latent in the brain and possibly other tissues of experimental animals during the interval between pyrexial attacks or after the blood is no longer infective (66). Some workers (143) go so far as to state that it is doubtful whether a true cure occurs in relapsing fever, in the sense that infection has been eradicated completely from the body. This is difficult to determine as it seems certain that even though organisms give no indication of their presence, they may remain alive in the tissues for weeks or months (143). The mechanisms responsible for this latency are poorly delineated. Among the possible mechanisms are the antigenic variation of organisms, intracellular habitat of the organisms (coccoid phase?) and consequent protection from noxious influences, change in electrokinetic potential of the organisms which permits them to pass through the blood-brain barrier while antibodies do so only very slowly.

This latent or residual infection is the subject of a heated controversy as to the nature of immunity in relapsing fever (66). As in syphilis, some students connect the immune mechanism with residual infection; in other words, the immune state is maintained only as long as there is residual infection. This argu-

ment will be discussed more fully in the section on infection-immunity in syphilis. However, it may be stated here that supporters of this idea claim that the refractory period of immune animals bears a much closer relationship to the demonstrable presence of viable organisms in the tissues than to the concentration of antibodies in the blood (143).

Syphilis. Relapses which occur in human syphilis are often probably related to inadequate chemotherapy with resultant production of drug-resistant variants or to premature treatment before adequate active immunity has been established (34, 193). In extensive experiments on rabbit syphilis, Brown and Pearce (18) found that the lesions exhibited an essentially relapsing character paralleled by cyclic degenerative and agglutinative changes in the spirochetes. In view of these studies, it is possible that the relapsing nature of experimental syphilitic lesions has a basis similar to the relapses in borrelia infections—antigenic lability of the spirochetes and the development of lytic antibodies by the host. However, further study is required to establish this hypothesis.

Symptomless and lesionless infection with *T. pallidum* for many months, particularly in lymph glands (22, 34), but also in one or more internal organs including spleen and brain (34, 184, 209), occurs in experimental infections of the monkey, mouse, and rabbit and probably in human infection. In the mouse, (208) inoculation with syphilitic material is usually without result; no lesions or symptoms develop (208), although the organisms apparently persist and multiply in the tissues (208). This may be tied up with failure of the mouse to develop much hypersensitivity in experimental infections. A similar failure of the rat to develop the characteristic epithelioid cell lesions in tuberculosis has been correlated with failure of this species to develop a high degree of hypersensitivity to the tubercle bacillus (228).

There is a great argument as to whether rabbits or man are ever cured spontaneously or after treatment in the late stages of syphilis, or whether the organisms are always latent in the tissues (34). Some workers (34) have presented evidence for biological cures in rabbits under these circumstances. Cures with arsphenamine and penicillin apparently are possible in the early stages of infection (222). However, it is known that infection may become latent during any of the three stages of human syphilis (193).

The concept of infection-immunity has been developed in connection with a number of diseases: protozoan, bacterial, and spirochetal. It maintains that immunity in these diseases corresponds to a situation within the host in which, together with humoral immunity, there is a latent tissue infection. It is a state of equilibrium between host and invader which, like other equilibria, may be disturbed. The parasite does not make its presence very obtrusive to the host. The result is practically complete absence of tissue reaction. This concept is supported by Neisser and is based on two sets of evidence (34). The first determined that untreated syphilitic apes which were refractory to reinfection could be shown to harbor spirochetes in their internal organs. The second type of evidence supported the view that infected apes treated with various antisyphilitic drugs and thought to have been cured, were at once susceptible to reinfect-

tion, whereas other apes treated but thought not to haven bee cured remained refractory. Although the first point is well corroborated, Chesney (34) casts doubt on the validity of the second point. The crux of the argument seems to lie in justification of the assumption that animals later shown to be susceptible to reinoculation had been cured of their first infection by treatment, and that those which later proved refractory to re-injection were not cured. Recent studies in rabbit syphilis would seem to controvert Neisser's views and indicate that an immunity not dependent upon persistence of foci of infection may be developed in syphilis. Treatment late in the disease, when rabbits had had opportunity to develop active resistance, abolished disease as shown by negative lymph node transfer experiments, and yet left resistance to reinoculation intact (34). Clinical evidence (34) in man also supports Chesney's ideas. Moreover, Chesney (34) considers Neisser's concept as strange in the light of general immunological principles. One would hardly expect that resistance acquired as a result of syphilitic infection and great enough to protect an animal against large doses or reinfection would vanish at once after the first infection had been eliminated.

There are few data to support further speculations as to the mechanisms of infection-immunity. It is conceivable, for instance, that persistence of organisms in the body might stimulate the maintenance in the peripheral circulation of a titer of antibodies adequate to cope successfully with any spirochetal invader. These organisms might be more or less tolerated without active reaction by the host or might stimulate defensive mechanisms more than is readily apparent. However, in the absence of a solid foundation for the concept it would be wisest to defer final judgment on infection-immunity until supportive data are obtained. For, convenient as it might seem for the depiction of the latent state in syphilis, the concept of infection-immunity suffers from the same serious lack of concrete supportive evidence as it does in relapsing fever.

It is important to know whether patients with latent syphilis are infective, but unfortunately information on the point is meager. Animal experiments (209) indicate that female rabbits with latent syphilis may transmit the disease to healthy bucks.

It is of great practical significance to know whether biological cure of natural syphilis with drugs, that is eradication of infection, results in persistence of active immunity. Opinion on this question is divided (222). It is to be hoped that experiments with penicillin, an extremely effective chemotherapeutic agent in syphilis, will provide an unequivocal answer to this question (222). At the same time, the question as to the necessity for persistence of foci of spirochetes for acquired resistance ("infection-immunity") may be answered.

H. Local and tissue immunity

It often has been claimed that true tissue immunity apart from circulating antibodies may be developed upon infection with spirochetes, especially the syphilis organism (78, 143, 193, 208, 219, 153). However, there is little sub-

stantial evidence on which to base this claim. The evidence for the existence of local in the absence of general immunity is also weak.

Leptospirosis. There are no data to indicate that local immunity is developed in leptospirosis. However, Ono (153) reported experiments which suggested that a true "cellular or tissue immunity" apart from demonstrable circulating antibodies might be operative in this disease. He claimed to have produced a high degree of resistance in guinea pigs by subcutaneous, intramuscular, or intraperitoneal injection or peroral administration of a weakly virulent culture. This immunity was apparent 24 hours after inoculation and lasted for at least 16 weeks. No symptoms or histological changes accompanied the immunization. However, Stavitsky (185) repeated Ono's experiments but was not able to confirm the establishment of the resistant state so soon. Hindle (78) attributes the persistence of spirochetes in the kidneys of rats to "tissue immunity" but presents no further evidence for this concept.

Relapsing fever. Data on cellular factors in latency and infection-immunity, in resistance to Borrelia, have been presented in Sections II D, and G. However no data have been found to suggest the occurrence of true tissue or local immunity in the course of natural or experimental infections with Borrelia.

Syphilis. Zinsser (238, 239) has conducted experiments which indicate that local tissue immunity and susceptibility may occur in syphilis. Twenty rabbits were reinoculated into the testes after primary unilateral chancres in these organs had healed. It appeared that the opposite testis could be successfully infected before, during and after the existence of a testicular lesion on one side, but reinfection of the same testis which had apparently returned to the normal state, at periods from 6 weeks to 1 year, was unsuccessful. Kolle (102) confirmed and extended these observations and termed this local resistance "chancre immunity." This type of immunity renders animals resistant only in so far as the formation of cutaneous lesions is concerned; it fails to prevent invasion of *T. pallidum* into the internal organs (34). Therefore, Chesney (34) regards "chancre immunity" as only partial and but a step in the direction of complete immunity. This partial immunity is not sufficient to eradicate the infection but sufficient to suppress the multiplication of small numbers of organisms and thus prevent development of inflammatory lesions (34, 51, 222). Chesney (37) has presented evidence for the wide distribution of the resistant state among different tissues after intracutaneous injection in rabbits. However, local resistance might be associated with local formation of antibodies (64) or their concentration in the local inflamed area (137). On the other hand, more vigorous mobilization of physiologically hyperactive cells in a tissue upon second exposure to an organism (127), may underlie the enhanced local resistance to infection. Manifestly, the entire problem of local as distinct from general resistance requires further careful study.

Until recently, in the absence of evidence for the rôle of humoral antibody in syphilis immunity, it usually has been stated that immunity rests in the cells or tissues. However, with the acquisition of evidence of several types for the

development of protective antibody in syphilis (39, 52, 212, 214, 215), it is only reasonable to demand that comparable evidence be presented for a concept of immunity of cells as distinct from humoral antibodies.

I. Diet, fatigue, trauma, temperature, and light in resistance

The manner in which these factors may influence host resistance is difficult to ascertain as they may be acting upon the microbes, the host, or both.

Leptospirosis. There was no discernible effect on leptospirosis in guinea pigs of raising their rectal temperature to 40–42 C by incubation in a hot chamber at 42 C (160).

Relapsing fever. No data were noted on these factors in relapsing fever.

Syphilis. The data on dietetic factors in syphilis are meager. It has been claimed that green fodder protects rabbits to some extent (193), but the factor in the fodder is unknown.

It has been claimed that physical strain may predispose man to cardiovascular lesions, arthritis, aortitis, and other complications of syphilis (193).

Trauma predisposes to syphilitic infection in rabbits in an unknown way (35, 36). It is known that the organisms settle out and multiply profusely in inflammatory areas (36). Whether this is a consequence of increased capillary permeability in the local region (137) or some other mechanism is unknown.

Temperature may influence strikingly the course of syphilitic infection. It has been noted that the period of incubation of experimental rabbit syphilis is increased during the summer (193). Artificial short wave fever may destroy the organisms and cause healing of the lesions in experimental or natural syphilis (28). The mechanism of the beneficial effect of short wave fever in rabbit syphilis (28) is not completely known. It may activate the body defenses through the effect of heat on the adrenal cortical-pituitary system in reaction to stress (229). Bessemans (11) claims that *T. pallidum* in experimental infection displays a predilection for localization and multiplication in those tissues having the lower temperatures in the body.

In general, the reaction to syphilitic infection is increased in proportion to the amount of light and the constancy of exposure of rabbits to that light (23). The mechanism is unknown, but it was observed that the light modifies the physical constitution of normal rabbits, and the organs most affected were those concerned with the response to syphilis. Brown and Pearce (23) believe that the effects are probably manifestation of functional activity closely related to changes in physical constitution.

Ultra-violet radiation lowered the weights of normal and syphilitic rabbits (72), but the significance of these observations for an understanding of syphilitic resistance is questionable.

III. DISCUSSION

The increasing number of factors which must be considered in interpreting the data presented caution us that we are first beginning to acquire enough information to start to put together the picture of the pathogenesis of and host re-

actions to infections. Nevertheless, some generalizations from these data would seem to be justified if only for their heuristic value.

It is clear from this survey of the main features of the spirochetoses that in general they conform very closely to the well-established principles of pathogenesis and host resistance in infectious diseases (6, 66, 156, 166, 168, 197, 208, 236). What, then, is so unique about spirochetes and the diseases they produce? Is it true, as Henrici (74) has noted, that "infectious diseases present specific earmarks which are determined by the species which cause them; nosology reflects taxonomy"?

There seem to be three groups of pathogenic spirochetes from the standpoint of nosology as well as taxonomy (66). The treponemas, including the organisms of syphilis, yaws, rabbit venereal spirochetosis (135, 218), and possibly pinta (82), are well-adapted tissue parasites showing highly individual species adaptation to man and rabbit (in case of venereal spirochetosis) and giving rise to incompletely developed acquired resistance to reinfection. The borrelias are blood parasites which produce the relapsing phenomenon most strikingly. They are mainly parasites of lower animals which probably infect man accidentally. Antibodies against them resemble bacterial antibodies in their great specificity and concentration. The leptospiras infect the tissues and blood and are widely promiscuous in species adaptation (139), infecting a variety of lower animals and man. They produce acute diseases which usually terminate quickly in death or solid and enduring resistance to reinfection. Of the spirochetes, the leptospiras most closely resemble bacteria in general biological properties.

There seems to be an essential similarity in the pathogenesis and host resistance in spirochetal infections. This similarity involves the cyclical character of symptomatology and lesions in these diseases and the parallel cyclic agglutination and degeneration of the spirochetes, a phenomenon most regularly and readily observed in relapsing fever. In acute leptospirosis there is only one attack recovery from which is preceded by agglutination and lysis of the organisms. However, in a type of human leptospirosis caused by mildly virulent variants, clinical relapses commonly are observed (5). In experimental syphilis of the rabbit, the concurrence of retrogression of the lesions with degenerative changes in the organisms have been described by Brown and Pearce (18–21), and compared to similar phenomena in relapsing fever. Human syphilis is particularly characterized by a series of progressive and retrogressive phases.

Kligler *et al.* (100) have suggested that the characteristic antigenic and pathogenic properties of trypanosomes were conditioned by the predominantly lipoidal type of cell low in glucoside, as compared to the usual bacterial cell which is mainly glucosidic and low in lipid. They cited the tubercle bacillus with its high lipoidal content (2) as another example of a microbic cell against which immunizing antibodies are produced slowly and with difficulty. The similarity in the pathogenesis of trypanosomiases and spirochetoses might be due at least partly to a common lipoidal Wassermann antigen which has been demonstrated in trypanosomes and spirochetes (226). It may be surmised further that the "antigenic inertia" of *T. pallidum* and possibly other spirochetes is a reflection

of their high lipid content. This assumed low antigenicity of spirochetal lipids might be due to their low solubility in body or intracellular fluids and similarity to antigens present in normal tissues. This would be in accord with the great difficulty of obtaining auto-antibodies (120). The failure of lipids to elicit an active inflammatory response also might contribute to their poor antigenicity. The protective antigens may be subsurface and dependent on breakdown of the spirochetal cell to make them available to antibody-forming cells. In line with this idea is the observation that spirochetes have lipid surfaces (144). Slow multiplication of the organisms with low production and slow release of protective antigen may also underlie the meager and slow protective antibody formation in syphilis. The possible relationship of lipoidal antigens of the Wassermann type of microbic origin to the antigenic and pathogenic properties of the organisms seems worthy of careful study.

Many authorities (32, 39, 97, 101, 107, 212, 214) agree that on the basis of present knowledge immunity in spirochetal diseases seems basically humoral in nature. Chesney (32) states: "I doubt that phagocytosis is the primary element in host resistance to syphilitic infections. I am much more inclined to think that such resistance is dependent upon humoral factors although for a time the evidence of humoral antibodies was nil." Admittedly, the resistance attributable to circulating antibodies in syphilis seems weak by comparison with that in other diseases. Nevertheless, at this time it is the only mechanism of acquired resistance which has any foundation in experimental studies of syphilis. Kritschewski *et al.* (106, 107), on the basis of some of the most recent experimental evidence, conclude that immunity in relapsing fever, avian spirochetosis, and syphilis is primarily due to destruction of spirochetes by specific lytic antibodies with phagocytosis of the organisms being only an accidental or secondary phenomenon. The importance of specific antibodies in acquired resistance to leptospirosis is clear.

There does not seem to be the obvious joining or cooperating of cells in immune activities in spirochetal infections that there is in many bacterial infections (89, 208, 236). However, in view of the new trends in the study of the contribution of the host cell to resistance to infection[8], a full understanding of the rôle of the cell in spirochetal infections must await further study.

There is no major conceptual difficulty in transposing the picture of spirochetal resistance into the framework of antimicrobic resistance. The basic factors, antibodies and cells, are the same although employed to different degree and in different ways than in other infections. There is nothing particularly unique about the importance of antibodies in spirochetal resistance. Rich (168) has remarked: "In the attainment of her ends Nature may be prodigal of materials, but she is rigorously economical of methods, and in one infection after another, whether caused by bacteria, fungi, filtrable viruses or rickettsiae acquired resistance has been found to depend upon antibodies." The uniqueness would appear to lie rather in the ability of lytic antibodies to destroy the spirochetes with little or no apparent aid from phagocytic cells. The resemblance

[8] See references 41, 68, 88, 91, 127, 134, 164, 166, 173, 191, 200, 227, 229.

between extracellular lysis by antibodies of spirochetes, generally considered gram-negative, and similar lysis of gram negative typhoid and cholera organisms is particularly arresting. One is tempted to suspect a common chemical factor for this similarity. This idea may be related to the recent demonstration of a difference in the surfaces of gram negative and gram positive bacterial cells; the former are devoid of a protein-ribonucleate complex which the latter cells possess on their surfaces (48).

Goodpasture (68) in a discussion of the cell-parasite relationship in bacterial and virus infections has noted that "those infectious agents that are able to

TABLE 1

Outline of some problems in the study of the mechanisms of host resistance in spirochetal infections

I. Parasite factors
A. Morphology and life cycle. Significance of coccoid bodies.
B. Electrokinetic potential. Does it change in sign in spirochetes? If so, how? Importance of potential in relation to blood-brain barrier and phagocytosis.
C. Methods of accurately counting spirochetes.
D. Cultivation of Borrelia and T. pallidum. Growth factors and metabolism, especially as related to non-specific inflammation in support of growth of *T. pallidum.*
E. Antigenicity and chemical composition. Relation to pathogenesis of infection.
F. Toxic and agressive substances. Presence of hyaluronidase, fibrinolysin, leukocidin, coagulase.
G. Tissue tropisms. Do Borrelia and Treponema exhibit a tropism for brain tissue?
H. Variation. Is it induced or spontaneous? Does it occur in Leptospira and Treponema, and is it related to relapses and carrier state in these infections?
II. Host factors
A. Natural resistance. What are the mechanisms of age and species resistance? Is antibody formation a crucial factor in natural resistance to leptospirosis?
B. Fixed cells. How often does phagocytosis occur spontaneously without opsonins?
C. Antibodies. Is complement necessary for lysis of spirochetes?
D. Hypersensitivity. Is it necessary or helpful for operation of acquired resistance in syphilis?
E. Mechanism of carrier and relapse states.
F. Infection-immunity. Is it necessary for acquired resistance and, if so, how does it operate?

survive and persist even to grow, within living cells are most apt to escape their antagonistic effect (*circulating antibodies*) (italics by author); and taking advantage later of a lowering of the antibody concentration and other favorable factors, then may renew the attack . . . and indeed infections with these agents . . . may be of relatively long durations, and in some cases recurrent." Thus far spirochetes, at least in their characteristic spiral morphology, have seldom been noted intracellularly. Indeed, the organisms are seen mainly extracellularly *in vivo* and would seem at most to be only facultative intracellular parasites.

The importance of the antigenic stability of the microbe to the effective operation of acquired resistance should be stressed. Antigenic variability is a property of the parasite whereby, by a change in antigenicity, it may frustrate completely

the solidly acquired resistance of the host. This antigenic lability may render it difficult or impossible to actively immunize a host effectively. It may account also for the failure of chemotherapy. After a non-sterilizing dose of drug, the acquired resistance of the host cannot complete the task of destruction. It is suggested by studies of Borrelia as well as trypanosomes that variability of organisms may include the ability to change the sign of its electrokinetic potential.

As has been noted by others (89), many characteristics of trypanosomiases (4, 198, 199) are very much reminiscent of those of spirochetoses. The relapsing character of trypanosomiases, particularly its connection with the development of antigenic variants and specific lytic antibodies (197, 201) comes to mind first, as it has been considered here as a typical feature of spirochetoses. The morphology, serological cross-reactivity of spirochetes and trypanosomes (46), presence of a common Wassermann antigen (108, 226), positive zeta potential of some strains of spirochetes and trypanosomes (15, 16, 42), are further indications of a possible biological relationship between these two groups of microörganisms. Whether this relationship has evolutionary significance remains to be determined by further investigation.

In the field of spirochetes and spirochetoses many problems await study. Some of them are summarized in table 1. Interestingly enough, many of them, including infection-immunity, carrier and relapse state, relation of hypersensitivity to problems of infection and immunity, are being debated also in relation to other microbic agents.

In view of the singular properties already recognized in the biology and chemistry of the pathogenic spirochetes, the author commends these organisms as an intriguing virgin and fertile field of study for general microbiologists as well as for immunologists.

IV. CONCLUSIONS

1. The pathogenic spirochetes and spirochetoses conform very closely to the established principles of pathogenesis and host resistance in infectious diseases.

2. The spirochetes are heterogeneous and from the standpoint of pathogenesis of the infections they cause, as well as taxonomy, may be divided into treponemas, borrelias and leptospiras.

3. There seems to be an essential similarity in the pathogenesis and host resistance in spirochetal infections which involves the cyclical character of symptomatology and lesions in these diseases and the parallel cyclic morphologic changes in the spirochetes.

4. On the basis of present knowledge antibody formation with lytic properties seems to be the most important factor in acquired resistance in spirochetoses. Phagocytosis apparently is a secondary or accidental phenomenon.

5. Some of the characteristics of spirochetes such as antigenic variability, and capacity to produce relapses may be related to their possession of the ubiquitous Wassermann type of lipid antigen.

6. The resemblance of trypanosomes and trypanosomiases to spirochetes and

spirochetoses on the basis of points 3, 4, and 5 suggests that these two groups of microörganisms may be biologically related.

7. The spirochetes must be commended as a virgin field of study for general microbiologists as well as immunologists.

Acknowledgement. The author is very grateful to Ruth Stavitsky for careful reading and aid in revising the manuscript.

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