

Part III

RECAPITULATION AND DISCUSSION

Chapter 10

RECAPITULATION AND DISCUSSION

Most of the studies included in this monograph represent the investigations conducted in this laboratory during the past decade, but a few are of an earlier date. One wishes that much more had been accomplished, yet on the whole it can fairly be stated that our knowledge of the treponematoses is now greater than it was, and that this knowledge inevitably will help in the very practical problem of the control of these diseases, leading it is hoped to fewer cases of infection and less suffering among the afflicted.

In this chapter we shall summarize the principal points brought out during the course of these studies, and in so far as it is possible we shall attempt to fit them into some reasonable pattern in terms of the fundamental biology of the treponematoses.

I. BIOLOGY OF TREPONEMAL INFECTIONS

Sources of Strains Studied

As was seen in Chapter 1 altogether 76 strains of treponemes have been isolated, 70 of these from human beings and 6 strains of cuniculi treponemes from rabbits. Among the strains isolated from human beings were 39 from patients with a clinical diagnosis of syphilis; 20 from patients with yaws; 3 from bejel patients; and 8 from patients with one of the syndromes classified as endemic treponematosis. While there were some failures in attempts at isolation, these failures occurred either early in our experience when less information about the factors that affect the results of isolation was available than at present, or under circumstances where sources of strains were plentiful, and one could therefore be less meticulous and use fewer animals than in situations where sources of treponemes were less abundant.

Viewing the experience as a whole we believe that the hamster is the most satisfactory laboratory animal in which to isolate treponemal strains. All the human strains studied, with the exception of pinta treponemes, either induced lesions in the hamster at the site of inoculation, or else involved the regional lymph nodes, from which treponemes could easily be

recovered. Transfers of hamster material to rabbits then usually resulted in the establishment of the strain in the latter species.

Direct transfer of material from man to the rabbit was likewise successful in a high proportion of instances. On the whole inoculation of the material into the body of the testis yielded the best results, but when secondary contaminating organisms were present, purulent lesions often developed in the testis, thus reducing the chances of successful isolation. Injection of material into the skin of the back or by scarification into the skin of the scrotum usually circumvented difficulties due to secondary infection, but the proportion of positive results tended to be lower than when intratesticular inoculation was used. In attempting to isolate strains in rabbits it is recommended that all three routes be employed.

In most of the inoculations of hamsters in this laboratory, material has been injected intracutaneously into the groin region. There is no proof that this area is especially favorable, and yet our impression is that it is somewhat more satisfactory than the skin of the back, for example. In the first place, the fur of the animals is less abundant in the former area, so that a developing lesion can be better visualized. Moreover, the lymph drainage from this area is into the inguinal lymph nodes, which are readily accessible at operation. Finally, the animal can conveniently be grasped by one hand and inoculated with the other.

At times inoculations have been made into the lips of the hamster, but we do not recommend this because the resulting lesions often interfere with the animal's eating, and it increases the danger of laboratory infection in the event of laboratory personnel's being inadvertently bitten by the animal.

Our experience in the attempted isolation of pinta treponemes has been disappointing but perhaps not altogether hopeless for the future. Transfers have been made from 3 patients into a total of 30 hamsters and 2 rabbits. As pointed out in Chapter 1, darkfield examination revealed the presence of treponemes in the regional lymph nodes of 3 hamsters, but subsequent passages into hamsters were negative. Perhaps other attempts should be made using not only hamsters and rabbits but other laboratory animals as well.

Pertinent information on patients from whom the newly isolated strains of treponemes were obtained is given in Appendix 1.

The Experimental Disease in Laboratory Animals

Much, of course, was already known about the characteristics of experimental treponemal infections in laboratory animals, and our own efforts, described in Chapter 2, have been directed largely to the exploration of poorly understood aspects.

In studying the experimental disease in rabbits attention has been directed, first, to investigation of certain fundamental biological processes relative to treponemal infections, and, second, to the development of techniques

which would permit better quantitation of experimental results. It has often been possible to pursue these two objectives at the same time.

Following testicular or intracutaneous inoculation syphilis treponemes induce an indurated type of initial lesion. Other species of treponemes induce a less indurated type of lesion; these differences are described more fully in Chapter 7.

The clinical evolution of experimental syphilis in rabbits has been correlated with the concomitant histopathological changes. Prominent among these is the initial production of a mucoid material identified as hyaluronic acid. A second stage in the evolution of the syphilitic lesion is characterized by the influx of mononuclear cells probably as a manifestation of the immune response on the part of the host. In a third stage polymorphonuclear leukocytic infiltration becomes prominent, presumably as a reaction to injury, to necrosis, and sometimes to secondary infection in the lesion. Comparative features of the histopathology induced by different species of treponemes are discussed in Chapter 7.

Among the developments in respect of better quantitative methods were, first, the utilization of a "pattern" method of multiple intracutaneous inoculation, especially in immunity experiments. This method, by simplifying the reading of results, has the virtue of being much more precise from the standpoint of uniformity and reproducibility than the method of inoculation into the testis, eye, or bloodstream. Secondly, studies were made on the rate of multiplication of treponemes in the hours and days following inoculation of rabbits. It became clear from these studies that after a time lag of 24 to 48 hours, treponemes multiply in a logarithmic pattern, until interrupted by the immune processes of the host, or unless some other extraneous factor, such as the administration of antibiotics or an unfavorable environmental temperature, intervenes. It is in the nature of these methods that the more that is known about the optimum conditions for the multiplication of treponemes the more precise can be the quantitation when any single factor is examined.

From these studies it has become clear that the incubation period can be regarded as an index of the number of viable treponemes inoculated, and this constitutes an indirect method of some value in estimating the number of viable treponemes in a given inoculum.

On the basis of extensive observations in this laboratory, it has been shown that there is a direct straight-line relationship between the number of treponemes (Nichols strain of *T. pallidum*) inoculated into rabbits and the incubation period of the resulting lesions. With an intradermal inoculum of 500 treponemes per site the incubation period is approximately 17 days; for each tenfold increase or decrease in the number of treponemes the incubation period is shortened or lengthened, respectively, by about 4.5 days. A generation time of 30-33 hours for *T. pallidum* can be computed from these data.

Direct treponeme counts made on rabbits' testes following inoculation with a known number of organisms reveals that after the first 24 hours there is a regular logarithmic increase in treponemes; computations based on these data likewise indicate a division time of 30-33 hours for the Nichols strain of *T. pallidum*.

Assuming that each treponeme divides into two, a single treponeme multiplying logarithmically will yield about 100 000 000 organisms, the number believed necessary to produce a macroscopically recognizable lesion in approximately 32 days. These methods were applied in detail mainly to syphilis treponemes, but in broad outline the findings are probably valid for other strains of pathogenic treponemes.

The time of development of generalized lesions appears to depend on two factors: one of these is the time at which the treponemes migrate from the local focus, a process which probably begins within the first few hours or days of infection and continues until retarded by the development of the immune mechanisms; the other factor is the time required for the multiplication of the few treponemes at the remote focus.

From a technical standpoint our exploitation of the hamster as an experimental animal has given rise to many practical advantages in the isolation and maintenance of strains of treponemes. In addition, the use of the hamster has revealed strain differences among treponemes which, while as yet not fully understood, must be regarded as a consequence of underlying biological differences. Three types of reaction in the hamster are recognized: an Sh type characterized by few local lesions, but involvement of the regional lymph nodes; an Mh type in which both local skin lesions and involvement of lymph nodes are observed; and a Yh type in which local lesions are common but involvement of the regional lymph nodes rarely occurs.

Limited studies on the monkey as an experimental animal for the treponematoses have been made. In the monkey syphilitic lesions at the site of inoculation tend to remain small; generalized lesions seldom occur and are not prominent. *T. cuniculi* was shown to be pathogenic for the monkey, but the lesions produced, and indeed the whole disease process, were insignificant. The results suggest, however, that *T. cuniculi* may likewise be pathogenic for human beings; the potentiality of this organism as an immunizing agent against syphilis, yaws and related syndromes has not been fully explored.

For the study of most problems the monkey offers no advantages and indeed serious disadvantages in comparison with the smaller and less expensive rabbit and hamster. However, there are undoubtedly problems which could be studied more definitively in monkeys than in these other laboratory animals; such studies should ideally be conducted where monkeys are plentiful and where they can be maintained under conditions of good general health and nutrition.

Limited studies on the course of experimental treponemal infections in guinea-pigs and mice are also presented.

From a more fundamental standpoint, our studies have emphasized the critical importance of hyaluronic-acid-like substances in the pathogenesis of treponemal infections. While this mucoid material has repeatedly been described in the pathological process of syphilitic infections, we were perhaps the first to suggest, on the basis of some experimental and circumstantial evidence, that hyaluronic acid is probably produced by the treponeme itself and is in the nature of capsular material; that there may be a direct relationship between the abundance in which this material is produced and the virulence of a particular strain of treponeme; and that this attribute may be one of the principal differences among treponemal strains of various origins. It is further suggested that the conversion of hyaluronic acid to a sulfated form, which partakes of the characteristics of chondroitin, may be related to the damage and scarring of tissues following infections, and this supposition leads in turn to a hypothesis that the strains of treponemes which produce the most hyaluronic acid can ultimately cause the most damage to the animal or human body. Finally, from these studies has come a conviction, as yet not substantiated by adequate experimental evidence, that the conversion of mucopolysaccharide to a sulfated form, as postulated for treponemal infections, will provide a model for certain other infectious processes, notably streptococcal infections, in which the same sequence of events may occur.

Factors Affecting the Evolution of Experimental Treponematosis

The study of factors (other than the parasite or the host *per se*) which influence the course of treponemal infections in laboratory animals was chiefly concerned with environmental temperature, certain hormonal effects, antibiotics and related substances, and the early operation of the immune mechanism. These investigations are discussed in Chapter 3.

Environmental temperature

Our studies on the effects of temperature in experimental treponemal infections were initiated largely with the limited objective of determining at what temperature the treponeme seemed to thrive the best *in vivo*. As usually happens, however, the results of these studies had implications beyond the original objective.

To summarize a whole series of experiments, it appears that the environmental temperature favorable for treponemes is approximately 35°C. Even only slightly higher temperatures are unfavorable and when the

temperature rises to near 40°C progressive destruction of the treponemes occurs. Likewise as the temperature falls inhibition is noted, although the lower limits of multiplication of treponemes have not been so well defined as the upper. The evidence indicates that pathogenic treponemes probably multiply slowly if at all at temperatures below 30°C.

Most of these studies were made with the Nichols strain of *T. pallidum*, but the evidence suggests that other species and strains of treponemes are influenced in much the same manner as the Nichols strain; and while most of the studies on the effect of temperature were made on the experimental disease in rabbits, it appears that the same factors are also operative in hamsters.

From these investigations has come the realization that the localization of treponemal lesions is influenced perhaps in a major way by the local temperature of the animal or human host. The internal body temperature of the rabbit, for example, is normally about 39°C—a temperature which is higher, of course, than that of man—and lesions of internal organs of the rabbit are rarely if ever encountered. By contrast, however, certain areas of the rabbit's body, notably the skin, ears, testes and extremities, are significantly cooler than the internal body temperature and it is in these areas that treponemal lesions occur readily, either as a result of direct inoculation or by metastasis from focal lesions elsewhere.

One can only speculate concerning the effects of long-continued environmental temperature on the treponematoses in human beings. It can be deduced from these studies on the experimental disease that consistently high environmental temperatures such as are met with in the tropics, which in turn account for slight but definitely higher skin temperatures, may have a slightly adverse effect on treponemes infecting individuals inhabiting the areas concerned; and this thesis can reasonably be extended to visualize a substantial modification of those strains of treponemes, after years and years of exposure, towards a less virulent variety. Carrying this idea a step further it may be postulated that treponemes of lowered virulence likewise provide a lesser antigenic stimulus to the host. This in turn may account for poor development of the immune response, with a tendency to chronicity and relapse over a period of many years.

Hormonal effects

Earlier studies in other laboratories have indicated that male rabbits tend to exhibit a more extensive disease picture of experimental syphilis than do female rabbits. Moreover, the administration of female sex hormones (estrogens) to male rabbits induces a milder type of disease than is seen in normal male controls. These studies were made before some of the modern techniques of quantitating treponemal infection were developed, so that the differences are often not too sharp or too definitive. Our own

experience in this field, presented in detail in Chapter 3, has been limited largely to a study of the effect of certain of the steroid hormones, notably cortisone and related products.

The general action of cortisone in animals and human disease conditions is now well known. Important among its effects are that it suppresses inflammatory tissue reactions of all kinds, including those due to hypersensitivity; in large doses it tends to suppress antibody production. Through one or both of the foregoing mechanisms, it tends to favor the excessive growth of many bacteria in the animal or human body.

These effects were early noted in experimental syphilis. The administration of cortisone to animals infected with syphilis leads to a tremendous over-growth of treponemes in both initial and secondary foci. Concomitantly there is a great increase in the amount of hyaluronic acid in these lesions, which lose their typical firm, often stony-hard characteristics, becoming soft, spongy and filled with mucoid material.

As mentioned in the preceding section, there is good indirect, but unfortunately not direct, evidence that this mucoid material is produced by the treponemes themselves. A hypothesis put forward from this laboratory, which has not yet been substantiated, postulates that under ordinary conditions this hyaluronic acid is converted rather rapidly to a sulfated compound similar in its properties to chondroitin sulfate; under the influence of cortisone, according to the hypothesis, the sulfating of hyaluronic acid is inhibited, thus giving rise to the characteristic changes observed in these lesions.

It has been suggested, too, that an increase in the amount of hyaluronic acid may favor the growth of treponemes. At the same time there is perhaps a less favorable situation from the standpoint of the defenses of the body, in that phagocytosis may be inhibited by the mucoid material, and even the penetration of antibody may be inhibited. It is well known that in large doses cortisone suppresses antibody production, but the changes noted in treponemal lesions occur so rapidly after the initiation of steroid therapy that this phenomenon does not appear to play a major role in the appearance of such changes.

Withdrawal of cortisone in experimentally infected animals often leads to the so-called rebound phenomenon in which there is a rapid and tremendous increase in the size of lesions, often with development of extensive generalized lesions. This phenomenon can probably be explained by the presence in the lesions of enormous numbers of treponemes, which are then freed from the suppressing effect of cortisone.

The demonstrated action of cortisone in experimental treponemal infections has been put to practical use in all procedures in which large numbers of treponemes are desired, such as the preparation of treponemal antigens. Cortisone has also been used in attempts to enhance the virulence of newly isolated strains of treponemes, but with less successful results.

Antibiotics

The effect of antibiotics on treponemal infections is considered principally in Chapters 6 and 9; in Chapter 3 we are concerned more with the incidental effects that arise from contacts with antibiotics other than those intentionally employed for therapeutic or prophylactic purposes.

The very complexity of our civilization makes it extremely difficult to keep traces of unwanted elements from reaching the experimental animal, or indeed man. This has been true of the antibiotics, for time and again one or another of these drugs has inadvertently been introduced, either directly or indirectly, into the food of our experimental animals. For example, many prepared animal foods contain antibiotics, either as a result of deliberate addition, or because they contain meat and milk products obtained from animals to which antibiotics have been fed. Repeatedly, our experiments or routine TPI tests have been ruined by the inadvertent and, at the time, undiscovered introduction of antibiotics into our animals' food.

It is interesting to speculate on the extent to which the treponematoses are now being affected by the widespread use of antibiotics primarily for non-treponemal conditions. Nor is it altogether fantastic to postulate that even before antibiotics were discovered some of the geographical and epidemiological peculiarities of treponemal disease may have resulted from antagonistic bacterial or fungal flora in certain areas of the world.

Sensitization of treponemes

While the general picture of immunity in treponemal infections is presented in Chapter 5, one effect of immunity on the genesis of the infection is considered in Chapter 3. This occurs principally when small amounts of specific antibody, perhaps produced in or in proximity to the local treponemal lesion, become intimately associated with treponemes. Such sensitized treponemes, which constitute the earliest detectable evidence of an immune response, have a decreased vitality.

When such treponemes are tested *in vitro* the mere addition of complement will induce immobilization of the treponemes. To what extent a similar phenomenon takes place *in vivo* is difficult to determine, although it may be the basis for the earlier observations that when treponemes from long-standing infections are transferred to a new host the incubation period is commonly longer than that produced by approximately the same number of treponemes obtained from very early lesions.

This same phenomenon may account in part for the difficulty encountered at times in successfully transferring treponemes from one animal species to another; or in transferring the organisms to laboratory animals from human beings with long-standing infections. *In vivo* sensitization of treponemes can likewise be a problem in the preparation of antigens for the treponemal agglutination test or for the immobilization test.

The influence of prior cuniculi infection

As pointed out in Chapters 7 and 8, there is a substantial degree of cross-immunity between the treponematoses of man and the natural rabbit disease caused by *T. cuniculi*. Pre-existing cuniculi infection of laboratory rabbits therefore significantly modifies the response of these animals to inoculation with other species of treponemes. Indeed, at times it appears to account for the complete suppression of lesions in supposedly normal rabbits.

Pre-existing cuniculi infection has been particularly troublesome in the production of treponemal antigens for the treponemal agglutination test or the treponemal immobilization test, since naturally occurring cuniculi infection appears to be widely prevalent among domestic rabbits in the United States of America. The trouble seems to arise from the accelerated production of specific antibody following the initiation of the experimental infection; the treponemal inoculum serves as a booster dose in rabbits which have had what amounts to a basic immunizing infection, through the previously naturally occurring cuniculi infection. While such animals customarily show negative standard serological tests, they can now be identified by prior testing of their serum with the treponemal agglutination test.

Other factors influencing the course of experimental treponemal infection

Considered also in Chapter 3 are other factors which are known to influence the course of treponemal infection in the rabbit, including the breed, age and sex of the animal; intercurrent infection in the host animal; and the site of inoculation. As for the influence of breed, age and sex of the rabbit, little additional work has been done in this laboratory. The influence of intercurrent infections appears to rest largely upon the extent to which the body temperature of the animal is raised, thus bringing into play the adverse effect of elevated temperature on the treponemes, as mentioned above.

The influence of site of inoculation likewise seems to depend largely if not entirely on the suitability of local conditions at the site, the skin and testes being favorable sites, because their temperature—several degrees below the internal body temperature—is particularly suitable.

Inoculation of treponemes directly into the blood-stream of rabbits leads to a highly selective localization of lesions. Although with large inocula treponemes are presumably carried to all parts of the vascular bed, lesions occur principally in the distal portions of the extremities, and on skin surfaces of the trunk from which the fur has been removed. The temperature in all of these areas is known to be lower than the internal body temperature of the rabbit, and it is concluded that the localization of the treponemal lesions is determined largely by this factor.

Characteristics of Treponemes "In Vitro"

Since pathogenic treponemes cannot be cultivated on artificial media, all *in vitro* studies of this organism must be made with material containing relatively large amounts of host tissue, thus complicating antigenic studies and chemical analyses. The methods and problems of *in vitro* studies are examined in Chapter 4.

Large numbers of treponemes for experimental purposes can best be obtained from testicular lesions of rabbits following the use of cortisone. Methods of separating treponemes from host tissue are described.

Recent studies with the electron microscope (carried out in laboratories other than our own) confirm earlier observations concerning the presence of an axial filament, and give validity to Noguchi's classical description: "The essential structure of a treponema is a spring-like axial filament and a layer of contractile protoplasm enclosed in a delicate periplast."

It is clear that treponemes may exhibit capsular material, and this is probably made up largely of hyaluronic acid. Characteristic spiral motility is snake-like in viscid media, and rotatory in more fluid media. Mention is made of miscellaneous observations on the staining of treponemes, on centrifugation, and on the refractive index of pathogenic treponemes.

A medium which permitted the survival of pathogenic treponemes for periods up to two weeks has been developed in this laboratory and modified here and elsewhere. This development has paved the way for the demonstration of treponemal immobilizing antibody. Subsequent study of this "survival medium" has shown that, while the media must be anaerobic in nature, the maintenance of anaerobiosis is not sufficient; it appears that compounds containing sulfhydryl groups are essential, possibly because these compounds participate directly in the metabolism of the treponemes.

Studies on the survival of treponemes *in vitro* were undertaken in the hope that they would provide a rational approach to the cultivation of the organism on artificial media. It may be, however, that optimum conditions for survival are altogether different from those required for multiplication. For example, it seems probable that factors, such as decrease in temperature, which slow the metabolic rate of treponemes, tend to prolong survival, and yet these same factors may well be unfavorable for multiplication.

To the earlier studies on long-term survival of treponemes in the frozen state have been added new observations of greatly enhanced survival. When frozen at approximately -70°C both syphilis and yaws treponemes have been found to be virulent for rabbits after storage for over 9 years. However, when frozen in aqueous suspension the treponemes undergo a considerable loss in viability due probably to damage at the time of either freezing or thawing rather than during the storage period. The addition

of glycerol in a concentration of approximately 15% to the suspending medium appears to prevent this damage, and survival results are considerably superior to those obtained with aqueous suspension. This same phenomenon is observed with many other species of micro-organisms.

Damage to treponemes during the storage period is however largely a function of temperature. When frozen in 15% glycerol, pathogenic treponemes were non-infectious after one month's storage at -15°C ; at -40°C there was good survival for one month but not for two months; while at -70°C an aliquot of this same material was not perceptibly diminished in virulence after 9 months.

The conclusion is reached on the basis of a review of the literature and the experiences in our own laboratory that no reliable method now exists for the cultivation *in vitro* of pathogenic treponemes. Studies on cultivation of the non-pathogenic Reiter spirochete are described and the various forms of the organism observed during successive stages of cultivation are illustrated.

Immunity Phenomena in the Treponematoses

Because of the peculiar chronicity of syphilis, the long-continued precarious balance between host and parasite, the inability readily to demonstrate serum antibodies in infected humans or animals, and perhaps because of the enormous scientific authority of Neisser's observations, the treponematoses, particularly syphilis, were regarded for many years as being immunologically unique among infectious diseases. The work of Chesney and his associates and, stemming in a straight line from Chesney's findings, the studies in this laboratory, have had the effect of bringing these diseases once again into an immunological pattern common to other infectious processes.

Our own particular contribution to this broad problem has been largely in the field of the humoral expression of immunity in treponemal infections. A by-product of these fundamental studies has been the development of several serological tests which have come to play an important role in the clinical management of patients with one or another of the treponematoses, particularly syphilis.

Evolution of the immune state

In Chapter 5, after outlining briefly some of the earlier work on the evolution of the resistant state in the treponematoses we have illustrated these phenomena by experiments reported from other laboratories as well as our own. From these experiments, which deal almost entirely with experimental syphilis, certain basic facts emerge, the most noteworthy of which are the following.

Immunity in syphilis develops comparatively slowly over a period which is best measured in weeks rather than in days, as in most of the acute infections. This period is determined in part by the extent of the infectious process since this determines the degree of antigenic stimulus. Experiments were reported from this laboratory in which a minimal infection was maintained in the rabbit for periods up to 20 weeks, without the induction of significant degrees of resistance. In other words, the mere presence of infection is not sufficient in itself to stimulate the development of immunity.

The persistence of the immune state is dependent upon a number of factors. Once immunity has developed to its highest point—ordinarily within 3 months after initial infection—a high level of resistance is usually maintained as long as the infection, even though latent, is present.

The persistence of immunity, however, after elimination of infection by therapy seems to be a function of time plus the degree of immunity attained at the time of treatment. For example, it was shown that a group of rabbits treated 2 months after infection and challenged shortly after the termination of treatment had a significantly higher degree of immunity than another group of animals similarly inoculated and treated but challenged one year after treatment. Animals in which immunity is fully developed at the time of therapy, however, commonly show no evidence of diminished resistance after one year.

The whole question of latency in treponemal infections remains rather an enigma. Rabbits with untreated syphilis may show a high degree of immunity to challenge inoculation, as indicated by the absence of lesions, yet at the same time they may be harboring virulent treponemes in their lymph nodes immediately prior to challenge inoculation. The same phenomenon can be observed in treated immune syphilitic rabbits, in which, despite the failure of lesions to develop on challenge inoculation, latent infection may be established. It is difficult to envisage the biological mechanism that is the basis of this phenomenon, whereby the defenses of the host are capable of restraining the invading parasite to the point where it can do no harm, but at the same time are incapable of destroying the parasite altogether. The study of treponemes from animals with latent syphilitic infection has failed to reveal biological differences in these organisms that might account for their seeming resistance to the immune defenses of the host.

A new observation stemming from the experiments referred to above is that reinfection in rabbits with a high degree of immunity seems to occur largely without reference to the size of the challenging inoculum, within the limits of the experimental techniques employed. Again, it is difficult to explain the biological basis of this observation.

While most of the investigations on the evolution of the immune process in treponemal infections have been carried out with the Nichols strain of *T. pallidum*, what evidence there is indicates that other strains of syphilis treponemes behave in essentially the same manner. The same statement

can be made for strains of treponemes belonging to the yaws, bejel, and endemic treponematoses groups.

Humoral expressions of immunity

From this laboratory has come the demonstration that human beings and animals infected with treponemes develop antibodies that are specific for this group of organisms. It is interesting to recall that Wassermann, in his original investigations on syphilis serology, was looking for a rich source of treponemes to serve as a specific antigen, and extracted fetal liver from congenital syphilitics for that purpose. The presence of the same or a cross-reacting alcohol-soluble substance in normal tissue led to the substitution of this more convenient but perhaps less specific material as a diagnostic antigen. Concentrated attention on the tissue antigen over a period of years has progressed to the point of chemical identification of a serologically active phospholipid fraction in normal tissue. In the past few years attention has been re-directed to the study of the treponeme itself as a potential antigen, thus in a sense completing the circle initiated by Bordet and Wassermann and their associates.

The earliest work in this laboratory on the general subject of humoral immunity resulted in the demonstration of the suppressive effect of serum from syphilitic animals and human beings on the development of lesions, when serum and treponemes were mixed *in vitro* and inoculated into test animals. The development of methods for maintaining motile treponemes *in vitro* over a period of days made it possible to observe the effects of normal and immune sera on these organisms without resorting to the more cumbersome method of animal inoculation. From this evolved the treponemal immobilization test which has been widely used both in clinical medicine and in the study of certain fundamental aspects of the treponematoses.

Considered from a basic biological standpoint, the TPI test clearly measures an antibody to pathogenic treponemes which differs from the so-called Wassermann antibody. In the vast majority of patients infected with one of the treponematoses and in many experimentally infected animals the two antibodies exist simultaneously, although often in differing quantities.

Existing evidence indicates that immobilizing antibody is a highly specific index of past or present treponemal infection; it has also been invoked by the injection of killed treponemes. There is evidence, gained mostly from study of the experimental disease, that treponemal immobilizing antibody plays a significant role in immunity to treponemal infections. However, enough discrepancies are noted between the presence of immunity and the presence of immobilizing antibody to indicate that this antibody is perhaps not the sole factor in the immune process.

At the clinical level the TPI test has proved to be a noteworthy aid in the management of treponemal infections, and there are some indications that

it may have even wider application in the study of other chronic disease processes.

Because Wassermann antibody occurs in some human beings in whom all the clinical and epidemiological evidence indicates the absence of past or present treponemal infection, the TPI test has been a valuable means for identifying these so-called biologic false positive Wassermann reactors. There are suggestions that the presence of detectable amounts of Wassermann antibody in persons who have never been infected with one of the treponemal organisms may be indicative of some other underlying disease which in time will become clinically overt. At the time of writing, however, we are only on the threshold of knowledge in this field.

Because of the complicated technical features of the TPI test, intensive search has been made in this laboratory for other methods of detecting specific antibody to pathogenic treponemes. Among the approaches made have been (a) attempts to simplify the TPI test, without however signal results; (b) study of the phenomenon of treponemal agglutination as a specific antigen-antibody reaction; and (c) study of adhesion phenomena. In addition, attempts in another laboratory to obtain a serologically active chemical fraction from pathogenic treponemes appears at the time of writing to have met with some success.

With the use of cortisone to enhance the *in vivo* growth of treponemes, and with improved methods of extraction and separation of treponemes from tissue elements, it has been possible to prepare highly concentrated suspensions of treponemes suitable for agglutination studies.

These treponemal suspensions, when killed by heat, agglutinate both with Wassermann antibody and with another and seemingly specific antibody probably identical with treponemal immobilizing antibody. In the agglutination test developed in this laboratory Wassermann antibody is first removed from the serum by absorption with cardiolipin antigen or crude powdered beef-heart, thus leaving the specific antibody to react in the agglutination test.

Difficulties have been experienced in the preparation of agglutinating antigens, since some batches are quite satisfactory while others are not. The factors influencing these results have not been fully identified. While, therefore, much valuable information has been acquired through the use of the treponemal agglutination test, unfortunately at the present time it is not sufficiently standardized as a test procedure to render it useful as a routine diagnostic method. However, the simplicity of the test procedure commends it for practical use, provided that the more fundamental difficulties are overcome.

The immune adherence phenomenon described from this laboratory, and confirmed by other investigators, consists in the addition of treponemes to human red blood cells, complement and serum. If the serum contains specific antibodies, the treponemes presumably adhere to the red cells,

and upon centrifugation are cleared from the supernatant fluid; but they remain in suspension in mixtures containing normal serum.

Current studies in this laboratory indicate that the red blood cell adherence is essentially a manifestation of the type of adhesion reaction, described by Rieckenberg, between trypanosomes and specific antibody in the presence of complement. Adhesion of this character has been demonstrated between treponemes and erythrocyte ghosts and blood platelets from man, the rabbit and the guinea-pig; collodion particles; and the bacteria *Escherichia coli*, *Alkaligenes faecalis*, *Streptococcus pyogenes*, *S. lactis*, and *Spirillum rubrum* in the presence of complement and syphilitic immune sera. The difficulties inherent in such complicated systems, however, have not been fully examined, and the uses and limitation of this phenomenon as a practical test remain to be explored.

Published reports concerning the utilization of serologically active chemical fractions of pathogenic treponemes are not yet detailed enough to determine the potential value of this approach.

Finally, it should be noted that newer methods of extraction of treponemes have permitted the development of better antigens for use in skin-test procedures. While studies of this nature are in preliminary stages they already confirm and strengthen older observations to the effect that among persons with syphilis a large proportion of those who have late syphilis show a positive skin reaction of the tuberculin type, while most of those with early syphilis, as well as normal persons, do not.

Response of Treponemes to Drugs

In studying the comparative effectiveness of various drugs, particularly the antibiotics, on treponemal organisms, and the comparative response of various strains of treponemes to the same antibiotic (Chapter 9), it was necessary first to develop satisfactory methods of assay, since the methods previously used were expensive and time-consuming.

Both *in vivo* and *in vitro* methods have been developed in this laboratory; while these procedures, which are described in Chapter 6, are perhaps accurate only within a two- to threefold range they seem to be as good as any other heretofore used, and have the virtue of being comparatively simple and inexpensive.

In the *in vivo* method developed in this laboratory treponemal lesions are induced by intracutaneous inoculation of the strain of organisms to be tested. When these lesions have reached their maximum development, treponemal counts by darkfield examination of material taken directly from the lesions are made before and after the administration of graded doses of the antibiotic to be tested. Critical readings are customarily made 24, 48 and 72 hours after administration of the first dose of the drug.

The *in vitro* method developed was patterned after that used for certain other bacteria, although adaptation of the method to the study of treponemes required the utilization of specialized and on the whole rather complicated procedures. The method consists essentially of extractions of pathogenic treponemes from rabbits' testes, separation of the organisms from tissue debris in so far as is practicable, and maintenance of the treponemes in a medium that will permit retention of motility for at least 18-24 hours. The particular antibiotic to be assayed is then introduced into this system. A useful end-point is that concentration of drug which immobilizes (and presumably kills) 50% of the treponemes within a given period of time, commonly 18 hours. This method has been used principally in determining the comparative susceptibility of various strains of treponemes to graded doses of penicillin; the results are presented in Chapter 9.

With the use of the *in vivo* method referred to above various antibiotics and certain other therapeutic agents were tested against the Nichols strain of *T. pallidum*. Of the penicillin fractions assayed, penicillin G was found to be significantly more active than fractions F and X, and much more effective than fraction K. It is of interest to note that the results obtained by this short *in vivo* method of assay were similar from a comparative standpoint to those obtained by much more time-consuming and expensive methods of *in vivo* assay.

Assays of some of the newer antibiotics by this short *in vivo* method showed that while none was as effective as penicillin, a number did have significant therapeutic potentialities against the Nichols strain of *T. pallidum*, and, in view of the results of *in vitro* studies presented in Chapter 9, presumably against other species and strains of treponemes as well.

Among the newer antibiotics tested Magnamycin was the most active, while Aureomycin, Terramycin and erythromycin were therapeutically active in doses approximately 100 times greater than that of penicillin on a mg/kg body-weight basis. Chloromycetin and streptomycin on the other hand were only slightly active in much larger amounts. Magnamycin and penicillin when used together appeared to exhibit an additive effect.

In view of the wide range of antibiotics that have some bactericidal or at least some inhibitory effect on treponemal infections, it is probable that these diseases in man are continuously being subjected to minor therapeutic effects. What role this may play in their control or modification is difficult to assess. In areas where penicillin is widely available, as is now the case in many countries, its widespread use through lay as well as medical channels may well be highly influential in modifying the epidemiological and even the clinical picture of these diseases.

In addition to the use of antibiotics in the treatment of human disease it must not be overlooked that antibiotics are now widely used in the dairy livestock and poultry industries, so that milk and meat products, in certain geographical areas at least, often contain traces of these substances. Again,

to what extent exposure to these minute traces of antibiotics over long periods of time may modify the picture of treponemal disease can be only a subject for conjecture. It is possible even that fundamental changes might be induced in a strain of treponemes by this means; but factual data on these points will be extremely difficult if not impossible to acquire.

Attempts to induce increased resistance to penicillin in one or another species of treponemes under experimental conditions have given no indication that such a phenomenon does or can occur. It is known that some micro-organisms, particularly the staphylococci, readily develop resistant forms, probably as selective mutational phenomena, while other organisms, as for example the hemolytic streptococcus, seem to develop little or no increased resistance to penicillin under either natural or experimental conditions. It will be a fortunate circumstance if in this respect treponemes behave like the latter groups of organisms.

Studies on the mode of action of penicillin on pathogenic treponemes have failed to reveal the basic nature of this phenomenon, although some useful knowledge has been acquired. From *in vitro* studies it has been found that regardless of the dose of penicillin there is a time-lag of a minimum of about 4 hours before any treponemicidal effect can be noted. After this, however, the rate of action of penicillin is a function of the concentration of the antibiotic and of the temperature.

In the case of staphylococci it is known that penicillin acts principally on organisms that are actively metabolizing, rather than on those which are more or less in a resting stage. Good data on this point in respect of treponemes are not available. Since all suspensions of pathogenic treponemal organisms are obtained from animal tissues it is assumed that any one suspension will contain treponemes in various stages of growth and reproduction. All treponemes, however, appear to be susceptible to penicillin when exposed to sufficiently large doses; with borderline amounts of penicillin, there is a substantial differential in time between the first and last treponemes to be immobilized, but the factor or factors determining this differential susceptibility are not understood.

From both *in vitro* and *in vivo* experiments it appears that penicillin exerts its lethal effect on treponemes without bringing about lysis. True enough, in treponemal lesions of man or of experimental animals a marked reduction in the number of treponemes is commonly observed after appropriate penicillin therapy. However, in infected animals treated with cortisone in which there are excessive amounts of mucoid material in the lesions, penicillin renders the treponemes immobile and presumably dead, without effecting a substantial decrease in their number. This suggests that the essential action of penicillin does not invoke the phenomenon of lysis; it is postulated that dead treponemes are normally cleared from lesions through phagocytosis, which is inhibited in the cortisone-treated animal by the presence of large amounts of mucoid material and decreased numbers of phagocytes.

II. COMPARATIVE STUDY OF TREPONEMAL STRAINS

Comparative Characteristics of the Experimental Disease Invoked by Various Strains of Treponemes

It has been observed in this and other laboratories over many years and by a succession of investigators that there are qualitative differences in the disease picture invoked in rabbits by different strains of treponemes. These differences are examined in Chapter 7.

At one end of the scale there are strains which when inoculated either by the intratesticular or by the intracutaneous route quite uniformly invoke extensive lesions which are characterized by the presence of firm indurated tissue, having at times the consistency of cartilage. This type of reaction in rabbits has been referred to as the syphilis or Sr type, since it was first observed with strains derived from typical cases of venereally acquired syphilis. At the other end of the scale are strains of treponemes which commonly invoke only minimal lesions when inoculated intratesticularly or intracutaneously into rabbits; these lesions rarely contain the indurated tissue referred to above. Moreover, on testicular inoculation a peculiar granular involvement of the surface of the testis is often observed. The strains that belong at this end of the scale are more difficult to maintain in rabbits, and on the whole can be clearly distinguished by all observers from the syphilis or Sr type strains. This type of reaction has been designated as the yaws or Yr type, since it is most often observed in rabbits inoculated with strains of treponemes derived from typical cases of yaws. Strains of treponemes which appear to be intermediate between the Sr and Yr types have been designated Mr types.

Clear differences have also been noted in the reaction produced by various strains in hamsters. The Nichols syphilis strain and many other strains isolated from typical cases of venereally acquired syphilis commonly induce no lesion at the site of intracutaneous inoculation, although the regional lymph nodes show large numbers of treponemes. This disease picture in hamsters has been designated the Sh type. By contrast, strains of treponemes isolated from patients in Western Samoa with typical signs of yaws invoke in hamsters large, spreading lesions at the site of intracutaneous inoculations, with few or no treponemes demonstrable in the regional lymph nodes. This disease picture in hamsters has been designated, perhaps rather arbitrarily, as the Yh type. A third type of reaction, which partakes of some of the features of each of the other two types, is characterized both by large, spreading lesions at the site of intracutaneous inoculation and by the presence of large numbers of treponemes in the lymph nodes. This disease pattern has been designated the Mh type, and has been observed with a number of strains isolated from patients with yaws, and with all the strains isolated from such syndromes as endemic syphilis, bejel and dichuch-

wa. Strains of cuniculi treponemes isolated from the naturally occurring diseases in rabbits seem to produce a disease picture in both hamsters and rabbits which differs somewhat from the foregoing ones, although studies on this group of strains are not definitive. These strains have been tentatively designated as the C type.

In an effort to apply these particular indices in a quantitative manner, each of the 17 newly isolated strains of treponemes, together with several older laboratory strains, has been analysed according to the type of reaction which they invoke in rabbits and hamsters. A summary of these findings will be found in Table XLVII, page 199.

It would doubtless be gratifying if each of these strains fitted neatly into the classification which we have devised. Such is not the case, however, for although by and large the strains do follow a pattern roughly corresponding to the clinical and epidemiological syndromes from which they were isolated, the classification of strains by rabbit reactions is not identical with the classification obtained by hamster reactions. Thus, only 3 of 4 strains isolated from typical cases of venereally acquired syphilis conform to the S type in both rabbits and hamsters, and only 3 of 7 strains from typical yaws cases have shown the extreme yaws type of reaction in both rabbits and hamsters. It can be stated that many other syphilis strains isolated from patients living in North America or Jamaica (see Tables IA and IB, Chapter 1, pages 21, 22), but not included in this analysis, likewise conform to the Sr type as observed in rabbits, but most of these latter strains were never observed in hamsters.

We have, therefore, ill-defined but none the less real differences in certain biological characteristics of these treponemal strains. Furthermore, such characteristics seem to be reasonably stable, although rapid passage of a strain of the Yr type using large inocula often leads to a progressive increase in the proportion of animals showing the Mr or Sr type of lesion. The fundamental basis for these observed differences is not clear, nor do we know what circumstances have led to their development. There is some evidence suggesting that the key to the differences in behavior in rabbits is the ability of the strain of treponeme to produce hyaluronic acid; and in general more hyaluronic acid is present in lesions of the Sr type. We have no corresponding hypothesis to offer as a possible explanation for the difference in behavior of these strains in the hamster.

The study of the histopathology of lesions induced by each strain of treponeme adds little to the information derived from observation of the gross lesions in rabbits and hamsters. There are no qualitative differences in the tissue response to the different strains, which are indistinguishable on the basis of histology alone. Nevertheless, real quantitative differences do occur, and these also seem to be largely a reflection of the amounts of hyaluronic acid produced by the treponemes. In consequence the relative amounts of metachromatic staining material, and the relative amounts of

cellular infiltration present in lesions may also be a rough guide to the classification of the strains.

Observations have been made which are relative to the question of variation and mutation of strains upon continued passage in laboratory animals. It is noted that at least one strain—the Nichols syphilis strain—has been shown through accidental laboratory infections to be still pathogenic for man 42 years after its original isolation in rabbits.

With rapid animal-passage most treponeme strains appear to assume enhanced virulence for the rabbit, to the extent that the incubation period of the initial lesion becomes shorter and the lesion becomes larger and more indurated. Strains of the yaws type tend to produce a higher proportion of lesions resembling the intermediate and syphilis types. Commonly there is no indication that these changes reflect a permanent alteration in the basic characteristics of the strain. Likewise, the maintenance of two typical syphilis strains in rabbits subjected to a high environmental temperature for 10 passages over a period of one year failed to induce recognizable changes in the strains when tested at the end of that period in animals subjected to a low environmental temperature.

However, in two instances changes believed to be in the nature of mutations have been observed, each involving the conversion of a yaws-type strain to one of a syphilis type. The first observation was made by Chesney with strain Y9, which had been isolated in Haiti. This strain had been propagated in rabbits for three years through 9 passages, when transfers were made from the lymph nodes of rabbits with a long-standing infection; subsequently the lesions induced by two sub-strains approached in character those invoked by a syphilis strain which had also been isolated in Haiti.

The second observation of this nature involved our YD strain. Beginning with the 16th passage in rabbits this strain began to induce in some animals lesions resembling those of syphilis; the reaction in hamsters at this time was of the Mh type, which is often produced by yaws strains. A sub-line YD-post-1949 derived from the lymph nodes of an animal of the 26th passage, 27 months after its initial syphilis-like infection, invoked syphilis-type reactions in all subsequent transfers both in rabbits and in hamsters.

The suggestion is offered that long-standing infection of rabbits tends to select from populations of yaws treponemes those organisms having characteristics of syphilis treponemes.

Antigenic Relationship between Strains of Treponemes

Comparison of newly isolated strains of treponemes is continued in the material presented in Chapter 8, the approach here being directed to a study of the antigenic relationship existing between strains or groups of strains. Methods available for such investigations are crude when compared with

those used in the study of many other organisms— crude, primarily because pathogenic treponemes can be obtained only from mammalian tissue, and even the most highly “purified” concentrates of treponemes still contain proportionately larger amounts of host cellular material.

Despite these limitations, useful information has been obtained, first, through cross-immunity studies and, second, through the utilization of *in vitro* antigen-antibody reactions.

Relationship as revealed by cross-immunity studies. Cross-immunity studies have been made principally by testing newly isolated strains of treponemes against a single “standard” reference strain—the Nichols strain of syphilis treponemes—on the basic assumption that strains which show some degree of cross-immunity with the Nichols strain will likewise show some degree of cross-immunity with each other. Even with such a simplified system, cross-immunity tests carried out in rabbits are time-consuming and expensive, thus imposing limitations on the extent to which conclusive answers can be obtained.

Perhaps the most impressive result of these studies has been the clear demonstration that there is some degree of reciprocal immunity between all the pathogenic treponemes studied, even between strains isolated from the natural rabbit disease (non-venereal spirochetosis) and strains isolated from typical cases of syphilis in man. It can be stated unequivocally therefore that all the treponemal strains studied have some antigenic components in common.

The degree of cross-protection varies from strain to strain; despite the limitations of the test methods some notion of qualitative relationships can be obtained. For example, it is clear that the cuniculi strains have a lesser degree of reciprocal immunity with the Nichols than do the other strains of treponemes tested. It is not always certain, however, to what extent quantitative factors enter the picture, since in general the syphilis strains gave better immunity against the cuniculi strains than the cuniculi strains gave against the syphilis strains.

Altogether 23 strains isolated from patients with typical venereally acquired syphilis, most of them living in North America, have been tested against the Nichols strain in this laboratory. Of these, 18 showed a high order of cross-protection, in 2 data were equivocal or inadequate, while 3 strains, all from the United States, showed significantly lower degrees of cross-protection. The experience of other laboratories, while less extensive in this respect, indicates also that most syphilis strains show good cross-protection with each other. It would seem to be a fair summary statement that the vast majority of syphilis strains are sufficiently closely related antigenically to give good cross-immunity by the usual infection-challenge test in rabbits; strains do exist, however, which seem to be less closely related antigenically than the majority.

Data on cross-protection among yaws strains are much less extensive, but what data there are suggest that there is good reciprocal immunity among yaws strains.

Most of our recently isolated strains have been tested against the Nichols strain. Data for any one strain are perhaps inadequate to permit definitive characterization of the immunological relationship of that particular strain. But when strains from a given clinical syndrome or a given area are considered as a group (Table LVII, page 223) the results of these tests become more significant.

For example, the Nichols strain without exception showed a high degree of cross-protection against all the syphilis strains. Rabbits infected with the Nichols strain, however, exhibited a much lower degree of immunity when challenged with a group of 6 newly isolated yaws strains, although against 2 of these strains there was good protection in the limited number of animals tested. It is interesting too that protection was less than complete against each of the 3 bejel strains employed for challenge, while against each of 2 endemic syphilis strains, and each of 2 dichuchwa strains there was a high order of cross-immunity.

With respect to cuniculi strains, while significant degrees of cross-immunity existed between 2 of these strains tested and the Nichols strain and other syphilis and yaws strains, the cross-protection was not of a high order.

In order to study the antigenic relationship between culture strains of treponemes and pathogenic varieties, rabbits were given a long series of injections of the former organisms and challenged with small doses of the Nichols strain of pathogenic treponemes. No significant degrees of cross-immunity were observed.

While, therefore, no precise patterns emerge from all the cross-immunity tests described in Chapter 8, some outlines are clearly detectable. Just as strains of treponemes differ in their capacity to evoke a particular disease picture in rabbits or hamsters, so they may differ in their capacity to evoke cross-protection in a rabbit host; moreover, as a generalization these variations in antigenic capabilities go hand in hand with those biological characteristics which determine tissue reaction.

Before leaving this subject special comment should be made on the arresting fact that a naturally occurring treponematosis of rabbits—if such a term is permissible in connexion with an animal disease—not only is closely related in many biological characteristics to all the treponematoses known among humans, but this cuniculi infection is also capable of evoking significant degrees of cross-immunity to infection with syphilis and other pathogenic treponemes.

As noted in Chapters 2 and 8 we have been much interested in the potentialities of this relationship, and have sought to explore certain practical problems incident thereto. The studies in question have by no means been

definitive, partly because we believed that some of the more important of these problems can be examined best in the monkey and the higher apes, and there are serious limitations on such studies in the circumstances under which we work. Should other investigations have better opportunities for experimental work in monkeys consideration should be given to pursuing further some of these problems.

Relationships as revealed by serological tests

As pointed out in Chapters 5 and 8, no differences were noted between strains of treponemes in respect of the titer of Wassermann antibody developed.

Likewise, no clear differences between strains were detected by studies made with the treponemal immobilization and the treponemal agglutination tests. The technique of the quantitative TPI test is not sufficiently accurate to obtain results which are entirely satisfactory from the standpoint of reproducibility. The TPA test on the other hand utilizes the same antigen in successive tests with satisfactory reproducibility of results. Even under these circumstances no constant differences were noted. Type antigens for the agglutination test were prepared from syphilis, yaws, bejel and cuniculi strains; sera from rabbits infected with most of the recently isolated strains were tested against these antigens. While the agglutinating titer varied from serum to serum there was no pattern of variation in titer according to the type of antigen employed, with the possible exception of the cuniculi antigen. Determination of both immobilization and agglutination titers on sera from cuniculi-infected animals revealed in general higher titers with cuniculi antigens and lower titers with syphilis, yaws and bejel antigens. The trend was toward a reverse relationship in respect of the sera from animals infected with strains of syphilis, yaws and bejel and endemic syphilis treponemes.

Comparative Susceptibility of Strains of Treponemes to Penicillin

As explained in Chapter 9, both *in vivo* and *in vitro* methods were used to determine the susceptibility of newly isolated treponemal strains to penicillin. While these methods are subject to fairly wide variation, the studies in question may be briefly summarized by stating that the experimental data failed to reveal significant differences in susceptibility to penicillin among the strains tested.

The experimental methods were regarded as accurate within a threefold limit of variation. Since most treatment schemes with penicillin make use of an excess of the drug the tentative conclusion is drawn from these experimental results that a treatment scheme which has been demonstrated to be therapeutically effective in man in one disease syndrome will be equally effective in other treponemal syndromes.

It should be pointed out that the data on the upper limits of the effective dose of penicillin are perhaps more accurate than similar data bearing on the least amount of the antibiotic that is effective. In other words, while all strains responded well to that dosage level which was found to be effective for most strains, it is possible that some strains might respond well to substantially smaller amounts of penicillin.

III. CONCLUSIONS

In this monograph we have been concerned with studies on the fundamental biology of the treponematoses, particularly those which have been carried out at the International Treponematoses Laboratory Center, Johns Hopkins University. While these studies cover a wide range, obviously unequal attention has been given to some facets of the over-all problem. Even within this context, however, there emerges a picture, vague in many details, but nevertheless whole as regards the basic biology of this great group of diseases.

This picture differs in certain important respects from that which might have been drawn a decade or two ago. The main effect of this re-orientation is to bring the treponemal diseases more closely in line with other infectious processes. Thus research on the treponematoses has not diverged from the main stream of medical and biological research, but has formed an integral part of it, taking from other fields of investigation and making contributions to them. We may anticipate that this fruitful exchange of new knowledge will continue.

It is clear that, except in the case of pinta, a treponemal disease which we have not been able to study for lack of successful reproduction in laboratory animals, treponemes from treponemal syndromes when established in experimental hosts behave according to a common pattern, which varies in detail but is essentially similar in its broad outline. A relatively few organisms, perhaps even a single treponeme, can produce infection. Multiplication ordinarily occurs at a regular although a comparatively slow rate, and grossly discernible lesions are produced by the mass of growing treponemes. The immune reaction of the host, which in its essential features seems to be similar to that invoked during the course of other infectious diseases, begins to develop early during the course of infection, increases in a leisurely fashion, and reaches a degree in which it is serviceable to the host, but inadequate to rid the host completely of the invading treponemes. The balance struck between host and parasite may be exquisitely fine, although wide fluctuations in this balance are probably the rule rather than the exception.

Many more or less extraneous factors affect this balance; some of them—temperature, for example—occur as a result of the natural environment; others, such as the antibiotics, may be artificially interposed between parasite

and host. Less well understood but probably none the less real are the inherent capabilities for adaptation on the part of the treponeme, so that caution must be exercised in assuming that a balance established in favor of the host by artificial means will remain thus for long periods of time; constant vigilance concerning the mutational capabilities of the treponeme should be the order of the day.

That adaptive mechanisms reside in both parasite and host is suggested by a study of the clinical and epidemiological features of the various recognized treponemal syndromes, and support for this basic biological observation is forthcoming from the results of laboratory studies. There can be no question that strains of treponemes isolated from patients with different syndromes do differ in certain fairly stable biological characteristics.

The outcome of this interplay between parasite and host over centuries, in situations which differ in their physical and ecological characteristics, has brought about not only modifications in the human host—however slight and difficult to measure—but also changes in the treponeme. Perhaps the most astonishing aspect of this situation is not that there are differences in treponemes, but that during the countless generations of straight-line descendancy strains have survived in which these differences are slight.

The pathogenic treponemes which we have been able to study in the laboratory both *in vivo* and *in vitro* are very closely related in their essential biological characteristics, in the disease picture they invoke in man and in experimental animals, in their immunological features, and in their reaction to antibiotics. As mentioned above, however, certain relatively stable differences have been observed. These differences relate particularly to the kind of lesions invoked in rabbits, the disease picture in hamsters, and certain immunological patterns as determined by challenge inoculation of rabbits.

On the basis of those criteria, strains of treponemes from various parts of the world have been placed with some unexplained overlapping into one of the three following categories: (a) the S-type, comprising most but not all of the strains isolated from patients with the classical disease syndrome of venereally acquired syphilis; (b) the Y-type, comprising most but not all of the strains isolated from patients with the classical disease syndrome of yaws; and (c) the M-type, which in the foregoing characteristics occupies an intermediate position between the S and Y types. The M-type comprises most but not all of the strains isolated from patients with disease syndromes of bejel, endemic syphilis, and locally designated syndromes of endemic treponematosis, such as dichuchwa. Perhaps strains of treponemes from naturally occurring cuniculi infection in rabbits should be placed in a fourth category, which we might designate the C-type. Serological tests, including the treponemal agglutination and the treponemal immobilization tests, have failed to reveal serological differences between the S, Y and M types, and have demonstrated only qualitative differences between these three

types and the C-type. It is apparent that the tests detect antigens which are common to the several types.

On the basis of limited evidence we believe that a fundamental biological difference between these types does exist, residing in the character and the amount of capsular mucopolysaccharide that each strain produces. Under laboratory conditions of animal passages which favor the treponeme over the host, we have noted an increase in production of mucoid material and a shift toward the S-type reaction. To what extent such shifts either toward the S-type on the one hand or toward the Y or C types on the other may be taking place in nature can be only a question for speculation.

What of the future? What main lines of basic inquiry does it seem profitable to pursue? The field of prophesy is traditionally unrewarding for the scientist, and yet perhaps one may say with some degree of confidence that without a minimum of laboratory research in this broad field we can expect little accretion to our knowledge of the biology of the treponematoses. As a corollary, it may be predicted on the basis of experience that continuing investigation at the fundamental level by alert and imaginative investigators carries always the germ of unanticipated and possibly highly significant advances in knowledge, advances which may contribute to biology and medicine as a whole, as well as to the conquest of the treponematoses.

Within this context, we suggest that four main lines of investigation may be worthy of continued exploration:

1. Study of the adaptive and mutational patterns of the pathogenic treponemes. Particular attention might be paid to the potential impacts of antibiotics and radioactivity.
2. Immunochemical studies of pathogenic treponemes, with particular reference to identification of serologically active antigenic components both in the body of the treponeme and in the capsular material, with a view to improved methods for demonstrating group- or species-specific antibody.
3. Study of the nature of Wassermann antibody and the mechanism of its production, with a view to elucidating the significance of its occurrence in treponemal and other chronic disease processes.
4. Continued efforts to cultivate pathogenic treponemes *in vitro*.