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THE MICROBIOLOGY OF SYPHILIS

BY

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CHAPTER V

HISTORICAL ACCOUNT OF RESEARCHES ON THE MICROBIOLOGY OF SYPHILIS

THE study of the Microbiology of Syphilis is a subject which presents very considerable interest, not only from the point of view of the sciences of microbiology and syphilography, but also because it cannot fail to attract the attention of every one who is interested in the progress of knowledge in general, and the progress of scientific research and deductions in particular.

At one period of medicine, when it was thought that most maladies were caused by disturbances in the functions of the organism, although some, at any rate, of our affections were admitted to have their origin from the outside world, it was generally held that contagious diseases were caused by some living agent which was easily transmitted to the perfectly normal human frame.

This was from the very first the case in syphilis, for it was seen that without any predisposition of the organism it was quite enough for some trace of specific poison to penetrate into our tissues, for man with certainty to contract the malady. It was thus quite natural to suppose that some microscopical organism was the cause of syphilis, and that this was capable of growth and multiplication in the human body.

Indeed, we can say that the microbiology of syphilis dates back to a period even before that of microbiology itself ; for even at a time when we were still without a single precise idea with regard to the part played by microbes in fermentation, it was supposed that the real cause of syphilis was to be found in certain minute organisms which occurred in syphilitic lesions.

Indeed, no less than twenty years before the discovery by Pasteur of lactic acid fermentation, discussions were already

taking place with regard to the microbiology of syphilis. Pasteur's labours showed clearly that fermentation is due to the activity of a microbe, and this discovery really opened the way to scientific microbiology.

In 1837, the same year in which the first attempts of Caignard, Latour, and of Schwann appeared, in which they explained alcoholic fermentation by the development of the yeast cells, a French microscopist named *Donné* published his microscopical researches 'as to the nature of various genito-urinary discharges in both sexes'. This work was very remarkable indeed, considering the time when it was written. Donné described certain miscroscopical organisms which he discovered in chancres, and which he compared to vibrios (*Vibrio lineola* of Müller, Plate V, *a*). Having observed these vibrios in vulvar chancres, Donné asked whether these 'animalcules' had any important rôle in the causation of syphilis. Struck by the fact that these vibrios could not be found in extragenital chancres, Donné endeavoured to clear up the problem by experiments. He produced suppuration in syphilitic persons, and in the pus thus obtained he never succeeded in seeing vibrios; thus he concluded that these organisms had no intimate connexion with syphilis, and that though his results made him still sanguine that a search for vibrios might be of service in the diagnosis of syphilis. Donné proceeded with great prudence and with strict scientific deductions only; thus he finally had to abandon his hypothesis that a vibrio, and *Vibrio lineola* in particular, was the cause of syphilis. In his 'Course of Microscopy', published in Paris in 1844, he expressed his opinion that the presence of these 'infusoria' was only a pure chance and had no constant connexion with the malady.

In this, indeed, he was perfectly right, though we cannot doubt for an instant that the vibrios found by Donné in syphilis were certainly not the aetiological cause of the malady. They are, indeed, only some of the many vibrios which develop secondarily in chancres of the genital organs, but are wanting in a great number of true syphilitic lesions: they probably correspond to the *Spirochaete refringens* of Schaudinn, according to the name now given to it.

We must remember that in Donné's time microbiology as a science did not yet exist. It is true that a certain number of microbes were known in the various secretions of the body, as well as in the external world, but there was no sure knowledge as yet of the rôle played by micro-organisms both in fermentations and in general diseases, nor was there any certain method which could reveal the presence of organisms in those parts where they are difficult to recognize.

In the first place, very great progress had to be made in microscopical technique before it was possible to approach so delicate and difficult a subject as the search for the organism of syphilis : indeed a period of nearly half a century was necessary to render the work of the smallest value.

As soon as Pasteur had definitely settled that micro-organisms had an important part to play in fermentation, the attention of medical men was at once turned towards the microbiology of infectious disease : again the discovery of so many new methods of microscopical research, due more especially to the work of R. Koch and Pasteur himself, at last stimulated scientific study of the aetiological agent of syphilis.

When once micro-organisms had been discovered in a whole series of different infectious diseases, it was natural that search should be made for them in some of the most contagious, such as variola and syphilis. Thus a great number of bacteriologists have applied themselves for more than twenty years to discover the cause of the latter malady.

The late pathological anatomist, Weigert, who was particularly distinguished for his discoveries in the methods of staining tissues and organisms, and who gave the first lessons in this art to Robert Koch, inspired considerable research into the bacteriology of syphilis in his laboratory in Leipzig. His pupil, *Lustgarten* of Vienna, in 1884, executed under his direction a series of researches which resulted in the discovery of a particular bacillus in different syphilitic lesions. This bacillus was known under the name of ' Lustgarten's bacillus '.

The work of this distinguished scientist of Vienna appeared in 1885,[1] and produced a very great impression by its scientific

spirit and ingenious technique, as well as by the reserve with which he expressed his opinion.

By the aid of a method of staining closely resembling that used for the bacillus of Koch, Lustgarten succeeded in showing, both in indurated chancres and in syphilitic gummata, the presence of small bacilli, very fine and slender, closely resembling the tubercle bacillus. It was impossible to confuse these bacilli, however, with that of tubercle, as Lustgarten's organism could not be cultivated in any known medium.

This discovery was received in a very favourable way by the scientific world. It was confirmed by several observers, amongst whom Doutrepont and Schutz [2] should be particularly mentioned, and it appeared to be definitely established in science.

Although in the laboratory of the celebrated syphilologist, A. Neisser, no definite final pronouncement was made, the research of Marcuse,[3] proceeding from this scientific centre, confirmed Lustgarten's work and considered his bacillus as most probably the real cause of syphilis.

It is quite true that soon after the appearance of the work of the last-named Viennese syphilologist, Alvarez and Tavel [4] threw out the suggestion that the so-called bacillus of syphilis was nothing more than the microbe of preputial smegma, or of vulvar secretions which are to be found in healthy people ; for the general appearance, as well as the staining properties, were exactly the same in both kinds of bacilli.

After this statement the microbiology of syphilis was reduced to a study of the differential characters of Lustgarten's bacillus and that of smegma. Many workers, amongst whom we may quote G. Klemperer and Baumgarten, denied the identity of these two microbes, whilst others, notably Bitter, took an entirely opposite line.

Even if it were admitted that Lustgarten's bacillus, which was found in syphilitic products of the genital organs, was nothing but the ordinary microbe of smegma occurring in the same situation, there still remain those bacilli found by Lustgarten in syphilitic lesions of internal organs, where they could not be confounded with those of smegma as the latter could not have got into the depths of the diseased tissues.

It was now thought that the positive results of Lustgarten were due to a mixed infection in which the bacillus of tubercle was probably mistaken for the specific cause of syphilis.

The enthusiasm for Lustgarten's discovery did not last long; in a short time it was evident that his microbe was very far from being constantly present in syphilis, and in those cases even, where it was found, there was plenty of ground for believing that its presence was due to secondary infection by a bacillus which had really nothing to do with the original cause of syphilis.

This discouraging result showed very clearly the difficulty of the whole problem, and made many distinguished bacteriologists relinquish the search; but the discovery became all the more attractive to the most enthusiastic workers, men who never feared to face the greatest difficulties which microbiology could offer.

Thus we see, after the failure of Lustgarten's attempt, a series of works appearing in which many widely different varieties of microbes were presented as the causal agent of syphilis—some of them, it must be confessed, without any great reason.

Kassowitz and Hochsinger [5] described a streptococcus which stained by Gram's method as the cause of syphilis. Later, Disse and Taguchi [6] declared that the malady was due to a diplococcus surrounded by a capsule; these multiplied rapidly in the blood of the patient, and were pathogenic for different kinds of mammalia, such as rabbits, dogs, and sheep.

Dr. Max von Niessen [7] has insisted for a considerable number of years that the best way to elucidate the cause of syphilis consists in sowing either the products of syphilitic inflammation or the blood of syphilitics on the middle of a culture. The germs which grow under these conditions are, according to him, different stages in the organism of syphilis. He finds the most widely different organisms. Side by side with orange and yellow micrococci are many different forms of bacilli and even fungi. Despite the efforts Von Niessen has made to persuade scientific men of the accuracy of his results by making many communications at different scientific congresses, and particularly in distributing his cultures, the general opinion is that these represent a general hotch-potch of every kind of impurity introduced

into a nutritive medium. As far as the aetiology of syphilis is concerned, they are certainly not of the slightest importance.

In France, work of the same kind has been done by Dr. Quéry [8] who has cultivated many different kinds of micro-organisms from syphilitic products. These are just the same, and would appear to have nothing whatever to do with syphilis.

Amongst the bacteriological discoveries of recent years, those of Joseph and Piorkowsky [9] seem to agree best with the scientific requirements of microbiology. These inquirers also endeavoured to solve the problem by the help of cultures. But, considering very properly that ordinary methods could only bring them disappointment, they conceived an original and ingenious plan. Taking as a starting-point the fact of the infection of the foetus by a syphilitic father, they prepared cultures from a fresh human placenta sown with seminal liquid of persons affected with syphilis. By this means they obtained pure cultures of a bacillus, in which they believed they had found the specific cause of this disease. This organism was so distributed in the sperm of the patients that it was possible to isolate it in culture from no less than seventeen specimens of this secretion.

This same bacillus has, again, been found in cultures made from the blood of syphilitic patients, which fact has increased its value in the eyes of some scientists. At last, in the attempt to solve the problem, several doctors, themselves unaffected by syphilis, injected the bacillus of Joseph and Piorkowsky into their own arms. The negative result of this crucial experiment proved once and for all that there were no grounds for considering this microbe as the cause of syphilis. About this time De Lisle [10] commenced with great zeal to search in my own laboratory for the syphilitic micro-organism. After numerous experiments, carried out while taking into account the latest bacteriological work, De Lisle arrived at the conclusion that syphilis is a disease produced by large bacilli which were capable of cultivation on a certain nutritive media. Not being able personally to confirm this discovery, according to the etiquette of my laboratory I objected to its publication.

M. de Lisle then published it in collaboration with M. Jullien, without my sharing, in any way, their responsibility. It is very

probable that he was dealing with some common organism which got into his cultivations through some mistake in technique.

It must be said, moreover, that no one has ever been able to confirm M. de Lisle's discoveries.

The search for syphilitic bacteria having completely failed, scientists had come to believe that they might be more correct in supposing that the microbe of syphilis is a protozoon. In a series of publications by Professor Stassano [11] it was said to be due to a flagellated organism capable of reproduction by multiple division (Plate V, *b*). Schüller, in a series of publications,[12] demonstrated that this disease is due to a curious micro-organism which appears in the form of corpuscles surrounded by stiff hairs; these were capable of reproducing by being changed into sacs filled with spores. The author even describes the cultures of his organism, obtained either from rabbits, or from cultivations from the incubator. The detailed description and the numerous figures in Schüller's publications do not allow us to judge of the nature of these corpuscles. Here we find represented very different objects, some of which recall grains of pollen or spores of truffles, whilst others have no resemblance to anything that we know. One cannot see why the author has imagined these things to be protozoa at all, nor why he has attributed to them the title of the organism of syphilis.

One can understand that under these circumstances Schüller's discoveries were not accepted by any one, and that his work only increased the profound scepticism which already existed concerning the problem of the cause of syphilis.

It was in this state of mind that scientists heard of a new discovery of a protozoon of syphilis. This time it was Dr. Siegel [13] who published an article in the 'Mémoires de l'Académie des Sciences de Berlin', in which he declared that the blood and exudations of syphilitics contained a small protozoon which is stained by a mixture of azur and eosine, and which, according to him, ought without doubt to be considered as the real organism of syphilis (Plate V, *c*).

Siegel gives it the name of *Cytoryctes luis*. The description and photographs in this memoir showed nothing which could in the slightest degree carry conviction to the mind of the reader. The

photographs represented objects of uncertain shape, and did not resemble in the slightest degree any known form of protozoon. A great deal was said in the text about the ease of giving syphilis to rabbits and about the number of syphilitic organisms to be found in the kidneys of these animals. Although at the time of the publication of Mons. Siegel's book I myself was occupied in the study of syphilis, nothing seemed to me to point to the importance of this pretended 'Cytoryctes'. In my own mind I compared it to the hairy protozoa of Schüller. Without binding myself to go too deeply into research as to the aetiology of syphilis, I had been making some observations on my own monkeys, which had been infected by experimental syphilis. The resemblance of syphilis to dourine of horses, the parasite of which is well known, made us think that syphilis was caused by a trypanosome or some similar organism. When I met T. Bordet at the International Health Congress at Brussels, in 1903, he communicated to us the result of his researches in the microbiology of syphilis, made in co-operation with Gengou. These distinguished bacteriologists stained the syphilitic products with Kühne's carbol methylene, and afterwards with Nicolle's carbol gentian violet. By this method they found in an indurated chancre quite a large number of very fine spirilla, rolled like a corkscrew, and very faintly stained. These spirilla were found alone and not in association with any other microbe. Naturally, Bordet and Gengou were full of enthusiasm at this discovery, and continued the search for their spirilla in other cases of syphilis. But being unable to find this organism in five primary cases, either in the lymphatic glands of the groin, in the papules of the skin, or in the blood, they were discouraged, and did not pursue their investigations. It was only in a mucous plaque in the throat that they encountered the same spirilla, but as the throat, even in its normal state, contains very similar spirilla, they did not dare to attach any importance to their discovery. Despite this failure in the researches of Bordet and Gengou, and being fully alive to the difficulties I should probably find in my own way, I undertook the search for spirilla in syphilitic products obtained from my own monkeys.* In this

* The distinguished author omits to mention that in 1903, in conjunction with

work I was joined by *Levaditi*, who had a perfect knowledge of all the technical details of bacteriology.

Our first results were negative; we then thought that the best means of finding spirilla was to search for them in the small clear vesicles which sometimes form the primary lesion in the anthropoid apes. This form of lesion had been found by us more especially in the orang-outang. We obtained drops of clear exudation from these vesicles and examined them microscopically as suspended drops. As living spirilla are distinguished by their great motility, we believed that it would be easier to observe them by examining the exudate in a fresh state.

We were completely disappointed by our results; for the drops were always like a 'dead lake', and we were never able to observe the slightest movement in any of the minute particles which we found suspended in the fluid in great numbers. As a control we prepared hanging drops from the serum of fowls affected with spirillar disease; in these we could see without the slightest difficulty movements of the corpuscles caused by the spirilla, which were rushing about in all directions. The addition of a neutral red (Ehrlich's) stained the spirilla almost instantaneously, and they were then most clearly shown. The same stain added to syphilitic products never showed the presence of a spirillum. The methods of staining used by Levaditi gave the same result; in film preparations of syphilitic exudates we occasionally saw some few bacilli or micrococci which had got into the preparation by accident, but no spirilla.

After very many negative results, I was discouraged, and had not the energy to undertake a fresh research. Because of these results, Roux and myself, in our third 'Contribution on Syphilis'[14] ('Annales de l'Institut Pasteur,' 1904, pp. 661), expressed the opinion that the disease could not be attributed to any form of spirillum, but that it was probably caused by some non-motile organism. This view was soon to be proved to be erroneous, for it is indeed a spirillum that is the real aetiological cause of syphilis.

Roux, he first succeeded in inoculating a chimpanzee with syphilis; thus to their work we owe a large proportion of the recent advances in our knowledge of Syphilis.—TRANSLATOR.

CHAPTER VI

DISCOVERY OF THE SPIRILLUM OF SYPHILIS

ALTHOUGH Siegel's work produced little effect in most of the centres of research on syphilis, and though it was treated as yet another of the so-called discoveries of the organism of syphilis, in Berlin the work caused an actual sensation.

The members of the German academy of Science who had encouraged the author of *Cytoryctes luis* took great pains to show the truth of this work and to make it accepted by microbiologists. Franz Eilard Schulze, professor of Zoology at the University of Berlin, who is well known as the author of many important zoological works, was at the head of this movement. Thinking the whole matter to be of the greatest importance, he got together a commission of experts to study the question from all sides. *Schaudinn* was entrusted with the work, because he was considered one of the leaders in the study of Protozoa though he was still a young man. *Dr. E. Hoffmann*, a specialist in the study of syphilis, was associated with him in the work.

Their first care was to confirm Siegel's statement as to the presence in the blood and exudations of syphilitic patients of very minute organisms distinguished by their great motility. They very readily succeeded in proving that the so-called *Cytoryctes luis* was not really a micro-organism at all, but only some kind of organic débris like those found so often in all kinds of exudations. But at the very same time that they disproved this statement, Schaudinn found in the liquid taken from a syphilitic chancre some spirilla, exceedingly delicate in form, which swam about under the microscope with just the same movements which distinguish true spirochaetes.

An observer of such ability as Schaudinn, who had ignored the attempts of Bordet and Gengou which we spoke of in

chapter V, could not fail to at once ask himself if this discovery of spirochaetes in syphilitic products was merely accidental and of no importance or if he had actually discovered the true organism of syphilis. The work being carried on in conjunction with such an experienced syphilogist as E. Hoffmann, enabled many of the early difficulties to be cleared away.

We must regard it as a very fortunate chance that in their very first case they were able to find quite a considerable number of spirilla. The patient was a woman aged 25 who had suffered for ten weeks from syphilis of the genital organs. She was seen by them on March 3, 1905, and was found to have a primary chancre on the left labium majus and several slightly ulcerated papules on the vulva. One of these papules was examined, and in its fluid Schaudinn[15] discovered motile spirilla in sufficient numbers to enable him to find one in every four fields of the microscope. When he tried to stain them it was found that the organisms could be shown by Giemsa's method, which consists in a mixture of methylene blue and eosine.

In a second case, which was that of a widow with secondary syphilis in the form of large slightly ulcerated papules of the genital organs, the members of the Commission—which had now been joined by Drs. *Neufeld and Gonder*—could only find very few and exceedingly fine spirilla. To make up for this, in a third case of condylomata of the genital organs, tonsils, and mouth, the organisms were found in great numbers. Thus we see that, as far as the number of spirilla present is concerned, Schaudinn and Hoffmann had better fortune than Bordet and Gengou, as well as myself, in my own first attempts.

The presence of these very slender spirilla, readily stained by aniline dyes, in the whole of a large series of cases of primary and secondary syphilis at once aroused the feeling that we might well be here dealing with the specific cause of syphilis.

From the very first, however, there was a great difficulty, which had of necessity to be cleared away as a preliminary.

Many observers at different dates had already found spirilla in lesions of the genital organs in man. Apart from the work of Donné, which I have already mentioned in chapter V, p. 44,

large numbers of recent observations existed in the literature of the subject where the presence of wavy or spiral micro-organisms, found by different methods, had been noted. Thus Alvarez and Tavel [16] had found spirilla in the smegma, Berdal and Bataille [17] had found them in large numbers in the exudate from a case of ulcerating balano-posthitis. Later Rona,[18] of Buda-Pesth, had laid great stress on the spirilla which he had found in gangrenous lesions of the genital organs ; whilst, in spite of other observations, this author notes the presence of spirilla in nine cases of primary syphilitic lesions in man, although it did not occur to him to consider them as the actual cause of the syphilis, for he had already found the same spirillum in non-syphilitic lesions, and even in the normal genital smegma of both sexes.

Schaudinn and his fellow workers at once proceeded to attack the problem from this point of view. He examined for spirilla in different non-syphilitic affections, and he very soon found a large number in the exudation from a case of papillomata of the vulva in a woman aged 30 suffering from gonorrhoea, but having no sign whatever of syphilis.

We have already seen in the last chapter how hasty was the assurance with which some observers, but little qualified for a difficult research in microbiology, made their statements; on the other hand with what reserve those better qualified went to work —so much so that they were in the end too easily discouraged by the difficulties which came in their way.

We must consider it a particularly fortunate occurrence for Science that the discovery of the aetiology of syphilis fell into the hands of a man like *Schaudinn*, a zoologist by profession, who before this work had proved his ability by his research on the spirilla of birds and that of relapsing fever in man. From his familiarity with the characters of these particular micro-organisms, and as a result of patient inquiry, Schaudinn was able to announce that in the genital organs, both normal and diseased, two varieties of spirilla are to be found ; one of these is found in non-syphilitic affections as well as in some ulcerated syphilitic lesions, while the other is only found in syphilis. The first of these—which Schaudinn designated by the name of *Spirochaete refringens*—is much

larger than the second; its spiral turns are fewer in number, although better marked than those of the second; it is readily stained by different methods, more especially by that known as Giemsa's; it is much more intensely stained than is the spirillum of syphilis. On the other hand, the second variety, called the *Spirochaete pallida* by Schaudinn, is much smaller as well as more slender; its spiral turns are more numerous, although not so marked; it stains with great difficulty by Giemsa's method as well as by other methods of staining; owing to this peculiarity it has been called the 'pale spirochaete of syphilis'.

Owing to his differentiation of these two varieties of spirillar micro-organisms in the genital organs, Schaudinn was able to overcome the first difficulty which obstructed his work; and by so doing he may be said to have taken the position by an actual *coup de maître.*

Encouraged by their results, Schaudinn and Hoffmann continued their researches on the aetiology of syphilis. They were first able to prove that the *Spirochaete pallida* is found in all cases of this disease and is never found in any other affection. In a very short time they had collected twenty-six observations in which they had always been able to show the presence of these pale spirochaetes; in some of these cases the organisms were wonderfully few in number, but they were always able at the end of the examination to find some specimens. On the other hand, in not one single case, be it venereal or otherwise, which was not syphilitic could the presence of the *Spirochaete pallida* be proved.

Continuing their work, Schaudinn and Hoffmann came to this conclusion, that 'true spirochaetes are to be found in fresh preparations, not only on the surface of syphilitic papules and of primary chancres, but also in the depth of the tissues and in the enlarged inguinal glands of syphilitic cases, diagnosed clinically; they are also found in film preparations stained by Giemsa's method. The question as to whether it is possible to establish differences between the parasites found in marked cases of condylomata and those of early syphilis can only be settled by further investigation.' This first communication was dated April 10, and appeared early in May, 1905.

Some days before this was published I received a letter from Schaudinn, dated May 2, in which he told me of his discovery, and asked me to send him films taken both from the primary lesions and from the enlarged glands of syphilitic monkeys. He told me the difficulty he had sometimes experienced in finding these spirilla, as he had actually at times only found a single example after a whole day's search. At the end of this letter Schaudinn expressed his opinion in the following words as to the two different varieties of spirochaetes : ' I have at the present time no doubt at all that the *Spirochaete pallida* is distinct from the other varieties which are to be found in the genital tract, although up to the present I can adduce no definite proof of my opinion.'

It now became of the greatest importance to collect all new work which might throw light on the problem of the aetiology of syphilis. Schaudinn, before he asked for preparations from my syphilitic monkeys, had already received films from indurated chancres in two lower species of monkey; these had been inoculated with syphilis by Dr. Kraus. But he found it impossible to find in them the *Spirochaete pallida*, in spite of all his investigations, though he used the proper method of staining.

Kraus himself gave me these preparations, and at the same time informed me that Giemsa's method ought to be used to find the organisms. As I had at the time two chimpanzees infected with most typical primary chancres, I endeavoured to search for the pale spirochaete, using Giemsa's method of staining. But the results were absolutely negative just as they had been in my original attempts (p. 51), and the preparations were in every case devoid of spirochaetes although they were examined by younger and more competent eyes than my own.

Although the results of our original investigations had made me abandon the search after the micro-organism of syphilis, this second time—having in view the twenty-six positive cases of Schaudinn and Hoffmann—I continued the examination of specimens from syphilitic monkeys. After many unsuccessful attempts we at last came across one case where the pale spirilla were very clearly to be seen. It was in a macaque (*Macacus cynomolgus*) (see Plate XVII) with a typical primary lesion on the eyebrow ;

this was covered with a thick crust, and on removing this a reddish serum escaped; which was found to contain a considerable number of spirilla. Soon after this we found the same organisms in two lower species of monkeys, and again in one of the two chimpanzees which had given us a negative result on the former occasion.

Encouraged by these results, we were able to find the pale spirilla in the cutaneous papules of secondary syphilis in man; these were far separated from the genital organs—that is, from those places where the larger spirillum refringens is to be found.

Having settled these facts, I sent some of my best preparations to Schaudinn: he confirmed my discovery, and begged me to publish my results as soon as possible. This I did in conjunction with Dr. *Roux* at the meeting of the Paris Academy of Medicine on May 16. In our communication we gave our views on the then aspect of the question in the following words: 'The collection of facts which we have now been able to put together pleads strongly in favour of the theory that syphilis is a chronic form of spirillosis caused by the *Spirochaete pallida* of Schaudinn.'

The presence of *Spirochaete pallida* in the beginning of chancres on the face and penis of monkeys, in association with other micro-organisms, shows us the importance of Schaudinn's discovery; while the proof that the spirillum is to be found in papules from their very commencement, and in those entirely closed in from the exterior, confirms us in our opinion.

Saling,[19] in his critical account of the microbiology of syphilis, reproaches me with the readiness with which I changed my first opinion as to the rôle taken by spirilla in this affection, and would attempt to explain it by some psychological reason. The matter is, nevertheless, very simple. My own original work was done before the discovery of Schaudinn, and here I was, perhaps, too much influenced by failing to find any spirilla in my own syphilitic monkeys, perhaps also not a little by the failure of such able workers as Bordet and Gengou. But when I myself succeeded in observing these spirilla enclosed in syphilitic lesions, far distant from the genital organs, both in monkeys and man alike, and this in the absence of all other organisms of any kind —this, added to the original work of Schaudinn and Hoffmann,

proved at once to me that we had here a discovery of the very greatest importance.

The human syphilitic products which I had inoculated into my monkeys contained quite a microbic flora, in which spirilla, micrococci, and bacteria were to be distinguished. How, then, are we to understand that in the chancres produced by my inoculations I could only find Schaudinn's spirillum ? The other micro-organisms, not being pathogenic, had been destroyed soon after inoculation, while the *Spirochaete pallida* had resisted all the defensive arrangements of the organism ! It must be true, therefore, that it possesses a particular pathogenic power.

Thus I modified my own opinion with a full knowledge of my reasons for so doing. This, I confess, is a psychological process which can rarely be done without regret ; one tries to take so many precautions to avoid error that it becomes very difficult to have to recant what has already been stated.

While we were clearing the way for our communication to the Academy of Medicine, we heard that a communication by *Buschke and Fischer* [20] was shortly to appear, describing the discovery of a pale spirillum in the liver and spleen of a child ten weeks old, affected with hereditary syphilis ; they added that, though these spirilla were very abundant, it was impossible to find any other organisms in association with them.

Now, while Buschke and Fischer took great care not to interpret their discovery as being in favour of the spirilla of Schaudinn being the cause of the disease, it must be clear that this made the balance turn very considerably in favour of the view which held that syphilis was a chronic spirillosis. At the same meeting of the Academy of Medicine at which we made our communication, *Levaditi* brought specimens from an infant with hereditary syphilis, showing that the bullae of pemphigus were full of pale spirochaetes.

The facts that we have shortly detailed in this chapter may be said to have laid the foundation of the microbiology of syphilis. Schaudinn's discovery is by far the most fruitful of all made before him. In every bacteriological laboratory, and in a large number of clinics, men have set to work with wonderful enthusiasm to

find the *Spirochaete pallida*. Thanks to the perfection of their technique they have arrived at most accurate results, and in the short space of two years a quantity of facts has been accumulated about the microbe of syphilis which cannot be at all compared with the comparatively few we know about the bacillus of tuberculosis.

In this connexion it is worth noting that Lévy-Bing,[21] in detailing the literature of the *Spirochaete pallida* at the beginning of the present year 1907, mentioned 617 papers about this organism, whilst in the list of publications dealt with by E. E. Hoffmann,[22] completed up to December, 1906, the number was 750 articles.

Without entering too far into the subject of the microbiology of syphilis, I will content myself by giving the reader an account of the principal results we have obtained up to the present time.

CHAPTER VII

THE OCCURRENCE OF *SPIROCHAETE PALLIDA* IN SYPHILITIC LESIONS

As we have already seen, the spirilla of syphilis were first discovered in primary and secondary lesions of the genital organs. Soon after this their presence was proved in secondary lesions occurring in parts far removed from the genital organs; in syphilitic lesions in monkeys and in hereditary human syphilis.

At the present time, since our views about Schaudinn's spirochaete have grown in an extraordinary way, we can say, speaking generally, that this organism can be shown in every possible lesion which is definitely syphilitic. In the *primary indurated chancre* they have been found a great many times, and although we may meet occasionally with a typical primary lesion in which we search without success for these spirilla, such cases become more and more rare as our technique improves. Further, it is necessary sometimes to search for a very long time before we find a definite spirillum, and it is quite certain that many of those cases formerly described as being free from spirilla are to be explained by the entirely insufficient number of preparations examined in the investigation.

The same remarks apply to the *secondary lesions* in which it is sometimes impossible to find the spirillum. This has been more especially proved in the study of roseolar rashes. Very often we search in vain in these spots for the specific organism. But this does not interfere with our finding it in a certain number of cases.

Thus, Ploeger,[23] Bandi, and Simonelli[24] observed it in the blood of roseolar spots, while MM. Veillon and Girard[25] found it in sections taken from various roseolar spots, some of them of only a few days' duration. Spirilla have also been discovered in the *terminal capillaries* of the papillae of the skin, and in some

deeper capillary vessels below the papillae; and whilst the majority of these organisms are to be found in the blood, some of them have been shown to have already penetrated into the peri-vascular tissue.

The facts I have just mentioned are far from being the only ones which demonstrate the presence of the spirilla of syphilis in the *blood*. Amongst others which more particularly prove this point are the observations of Noegerrath and Stoehelin,[26] who found a few spirochaetes in the blood of three persons affected with secondary syphilis, who had not undergone any treatment. Indeed the presence of these organisms in the blood has now been shown a great number of times. Schaudinn himself demonstrated the presence of syphilitic spirochaetes in the blood of the spleen obtained by puncture.

Although they are to be found in the blood, these organisms are still more numerous in the *lymph* and *lymphatic organs* in general. In their very first searches Schaudinn and Hoffmann (loc. cit.) observed the spirilla in film preparations taken from the femoral glands of their patients. A large number of observations soon confirmed this result, and although it is by no means rare to search in vain in preparations of these organs for the organisms in question, still, on the other hand, their presence in lymphatic vessels may be said to be constant in syphilis, and it is at times possible to see a very large number in the peri-vascular spaces, although their number in the corresponding blood-vessels may be exceedingly limited.

Apart from these lymphatic glands belonging to the special regions attacked by the primary lesion, the presence of Schaudinn's spirillum has been shown many times in those more properly belonging to the secondary period, as in the case of Levoendowski, who found them in the epitrochlear gland, the enlargement of which is so characteristic of syphilis.

The attempts to find the spirochaete in the *cerebro-spinal fluid* have already been many in number without giving very positive results, except in the case of Dohi and Tanaka,[27] who found Schaudinn's spirillum in this fluid taken from a patient suffering from papular syphilis. It is by no means astonishing that many

other observers—amongst whom we may mention Widal, Ravout, and Siebert—have failed to do so, because the cerebro-spinal fluid has been shown, according to Hoffmann, to be free from any infective power except in very rare cases.

It is more astonishing that up to the present it has not been possible to demonstrate it in the *seminal fluid*, as this has been shown to be infectious by many experiments. Thus Finger and Landsteiner have obtained typical primary lesions in monkeys inoculated from the sperm of patients.[28]

It is not astonishing to learn that the *saliva* of persons suffering from syphilitic lesions of the mouth contains the syphilitic organism, although it is very remarkable to find that this fluid contains so many of Schaudinn's spirilla as Follet [29] would believe; this observer has stated that two or even three hundred specimens of the spirillum are present in each field of the saliva of persons who have not been treated by mercury. I must say, however, that in the preparations which Follet desired me to look at, I certainly saw a very large number of spirilla; but these belonged to many varieties, probably no less than four in number.

In the same way the presence of specific spirilla has been proved in the *urine* of persons infected with syphilis. Dreyer and Teuvel [30] have found them in the urine of a case of syphilitic nephritis; Maclennan [31] has described these organisms in the urine of a woman infected with syphilis, although it is true that he only found them in the neighbourhood of little corpuscles which he took for the *Cytoryctes luis* of Siegel, and on account of this the observations must be received with caution. It must not be forgotten that the female genital organs are the home of other forms of spirilla which may be at times mistaken for the *Spirochaete pallida*.

Now, although the presence of this organism has been proved without any great amount of difficulty by a great number of observers in both primary and secondary syphilis, it has not been the same in the case of *tertiary syphilis*. Here many observers have either reported a negative result or have given it as their opinion that the organism in tertiary syphilis is to be found in some form other than that of a spirillum. However, thanks to

untiring labour and improved technique, more accurate results have been arrived at. Spitzer in 1905[32] was able to show the presence of small numbers of the *Spirochaete pallida* in gummata, both closed and ulcerated. Since this work, several other observers have obtained the same results in a fair number of cases.

Schaudinn found a mass of the organisms in the peripheral part of a gumma of the liver, occurring in a patient infected at the age of twelve. Hoffmann and Feldmann,[33] after laborious work, found isolated individuals in the peripheral parts of tertiary syphilides ; in the same way Blascke found them in a lesion of the scrotum occurring sixteen years after infection. Again, we may quote S. Dudgeon,[34] who found a number of the spirilla in a suppurating gumma of the jaw ; also the work of Doutrelepont and Grouven,[35] who succeeded in showing the presence of Schaudinn's spirillum in sections taken from four cases of tertiary syphilis.

The *vascular system* in general and the arteries in particular are very seriously affected by syphilis in its different stages ; it has therefore been exceedingly important to prove the presence of the organism of syphilis in specific arterial lesions of the tertiary stage. Reuter of Hamburg[36] found the organisms in sections of the aorta of an old syphilitic, who had died suddenly in the street, after suffering from cardiac trouble. Schaudinn has confirmed this observation by examining his specimens.

Now, although the presence of the specific organism of syphilis in cases of primary, secondary, and tertiary infection is by this time a definitely established fact, in those complaints which are called by the general name of 'parasyphilitic affections' the many investigations which have been made up to the present time have been of no avail. All cases of general paralysis and tabes studied from this point of view have shown themselves to be free of pale spirilla, or indeed of any form of spirillum.

Although it is not remarkable that the aetiological cause of syphilis was only discovered when many very favourable circumstances were present, more especially owing to the discoverer having started on cases where the organisms of syphilis were particularly plentiful, yet we cannot but be surprised that the

pale spirillum had not been found in cases of *hereditary syphilis* before the work of Schaudinn. It is indeed amazing how large a quantity of these microbes are to be found in the foetus or newborn child infected with syphilis.

We have described in the preceding chapter the first cases of this affection in which spirochaetes were discovered. Since that time a large number of papers have been published on this subject, which, indeed, presents one of the very best worked out chapters in syphilography.

Although Schaudinn's spirochaete has not actually been discovered in seminal fluid, its presence in this secretion must be certain in many cases, owing to its infectious character; there is no reason to suppose that the spermatozoa are infected by this microbe, it is more probably to be found free in the surrounding fluid. On the other hand, there exists definite proof of the penetration of the *Spirochaete pallida* into the *ovum*. Thus Levaditi and Sauvage[37] studied the ovary of a child aged one month, whose mother was infected. They not only found *Spirochaete pallida* in the stroma of the ovary, but they also found some of these parasites in the protoplasm of the young ova themselves.

Independently of this discovery Hoffmann and Wolters[38] have observed the organisms surrounding the ova of a foetus of seven months, and some of them even in the interior of the ova. Thus there is nothing impossible in believing that this is one of the means of the propagation of syphilis, although there is still no actual definite proof of the fact.

From this point of view it is interesting to note the presence of the organisms of syphilis in a case apparently free from the disease. Buschke and Fischer[39] studied a typical papular eruption in a new-born child. The mother presented no suspicious symptoms whatever, except perhaps a certain amount of alopecia. On closer examination an indurated inguinal gland was found. This was punctured and found to contain most characteristic spirochaetes.

The *placenta* has been found by many observers to be a resting-place for these organisms. Ménétrier and R. Duval,[40]

Wallich and Levaditi,[41] Nattan-Larier and Brindeau of Paris,[42] Paschen of Hamburg,[43] Mohn of Leipzig,[44] all have proved their presence in the foetal placenta, though they are sometimes present in small numbers. Thus Wallich and Levaditi have only once been able to find them ; on the other hand Mohn discovered them nine times out of thirteen. The umbilical cord has very often been found to be invaded by spirilla.

The authors just named have recognized that the spirilla found in the infected placenta always belong to the foetal and not to the maternal part ; this agrees with the fact that these germs are only found rarely in the decidual membranes. The invasion of the foetal portion of the placenta is readily explained by calling to mind the extraordinary number of spirochaetes found in the tissues of a syphilitic foetus.

Thus, as we have just mentioned, the specific organism of syphilis is found in cases of *congenital syphilis*, very often in enormous quantities. While in *acquired syphilis* it would appear to be localized to the affected parts and its generalization seems to occur only in a very small degree, on the other hand in congenital syphilis this dissemination occurs to an extraordinary degree in both the organs and fluids of the body, which very often contain large quantities of the *Spirochaete pallida*. It has been found in the blood of the dead foetus, and also in that of the living child.

An observation of Buschke and Fischer[45] is of particular interest in this connexion. They observed a child six weeks old : it had not a single symptom of congenital syphilis, except perhaps some slight catarrh of the nasal passages, yet when examined clinically they found relatively large numbers of spirilla in the blood. Despite anti-syphilitic treatment, this child died, and at the autopsy very marked lesions of the liver and spleen, which were obviously syphilitic in nature, were found, and both organs were hypertrophied. Besides the blood, the liver, and the spleen, Schaudinn's spirilla have been found in the bile and in the deposit from centrifugalized urine.

We have drawn attention in Chapter VI (p. 58) to the discovery by Levaditi of numerous spirilla in the bullae of

Pemphigus neonatorum. Since that time a great number of observations has confirmed the fact, and they have been found not only in the liquid but also in the walls of the vesicles of pemphigus.

Without going into the question at length of other diseases of the skin and mucous membranes, we may say briefly that all the organs of a new-born child affected with syphilis have been shown to contain the spirillum. They have also been found in the superficial and deep lymphatic glands, in the liver (Plate VII), the spleen (Plates XIV and XVI), the lungs (Plate XV), the suprarenal capsules, and the pancreas. The thymus, the thyroid body, the testicles, and—as we have already mentioned—the ovaries.

In all these tissues a varying number of pale spirilla are to be found. Parts of the *skeleton*, such as the periosteum, cartilage, and marrow of the bones, have also been found to contain the organisms.

We should also note a peculiarly interesting fact—the presence of Schaudinn's spirochaete in the organs of circulation. In one case of a new-born syphilitic infant, who died of myocarditis, and was examined by Buschke and Fischer (loc. cit.), the quantity of these organisms was remarkably large ; the vessels and capillaries were surrounded by a thick mesh-work of spirilla, a certain number of them being found in the walls, as well as in the lumen of the vessels.

Thus it is not astonishing that, as far as aetiology is concerned, the study of the microbiology of syphilis has now come to occupy a very important position.

Side by side with this we must place *Experimental Syphilis*, a subject to which attention has already been directed. After the first proof of the presence of the *Spirochaete pallida* in syphilitic monkeys, a great number of new facts has been added to our knowledge.

It has been proved that the organism is to be found in all cases of primary infection, in anthropoid apes as well as in the lower orders of monkeys. Neisser, Baermann, and Halbenstädter in Batavia,[46] Finger and Landsteiner[47] as well as Kraus and Prantschoff[48] in Vienna, have proved this in a large number of cases. This observation, of course, is particularly important, in

view of the fact that apart from Schaudinn's spirilla the experimental lesions in monkeys (which are usually produced in the eyebrow) do not contain any other organism—at least during the earlier periods of the infection. The lymphatic glands which belong to this region contain in just the same way the specific spirillum, as has been shown by Levaditi and Manouélian.[49]

The same spirilla have been found in most of the examinations of the papules and mucous plaques of secondary syphilis in anthropoid apes.

The question as to the presence of spirilla in the internal organs of monkeys has been much discussed. Neisser with his fellow workers having shown that certain internal organs such as the spleen and the marrow of bones contain infectious virus, the question naturally followed as to whether spirilla were to be found in these parts. This has been answered by Zabolotny,[50] who found spirilla which stained readily in the spleen of a syphilitic monkey. He gave a demonstration of them at the Congress at Berne in 1906.

Lately several observers have published very interesting observations as to Syphilitic Keratitis, which was produced in rabbits and dogs by inoculating them with syphilitic products from men and monkeys. Bertarelli [51] was the first to show the presence of a large number of absolutely typical spirochaetes in these ocular lesions in the rabbit. This discovery has already been confirmed several times, amongst others by Hoffmann, who, working with Brüning [52] has added a new observation, that when a fragment scraped from an indurated chancre is inoculated into a dog's eye, very marked keratitis results, in which spirochaetes are found in fairly large numbers. This predilection of the organism of syphilis for the cornea merits particular attention, for inoculation into all other parts of the body of mammalia, except in monkeys, is usually without any effect.

All the preceding facts as to the presence of Schaudinn's spirillum in every kind of acquired syphilitic lesion, as well as in hereditary syphilis and in experimental syphilis, cannot but form, when taken together, proofs of the very greatest importance in favour of the aetiological part played by this organism.

CHAPTER VIII

TOPOGRAPHY OF THE *SPIROCHAETE PALLIDA*; ITS CONNEXION WITH THE CELLS OF ITS HOST

From the time when it was found possible to find the *Spirochaete pallida* in sections, we have been able to study the problems of its connexions with the cells of its host with considerable accuracy. Thus it has been shown most clearly that a certain number of the spirilla pass into the blood, shortly after the appearance of the primary sore, while others are to be found in the lymph; these facts were proved by Burnet and Vincent [53] and by Levaditi [54] in their first researches on the spirillum, in experimental chancres in monkeys.

The organism appears, however, to have a much greater preference for the blood-vessels and lymphatics; for in a very large number of cases the organisms have been found in the actual walls of the vessels themselves. In every sort of syphilitic lesion they have been found in the parts surrounding the vessels; here they often form a dense network.

Although they are rarely found in the superficial parts of a chancre, they are especially to be seen in the deeper parts of the epithelial layer of the skin, where they are to be found in the intercellular, as well as in the interfibrillary spaces. Very often they can be traced into the connective tissue, where they very curiously imitate the form and undulations of the wavy bundles of connective tissue fibrillae. All these facts, already well established, form an important link in the chain of the aetiology of syphilis.

A very general fact, although it is not without exceptions, is the simultaneous appearance of syphilitic spirilla together with the mononuclear leucocytes which are so characteristic of syphilis.

For a long time the lesions have been well known. Where, as a

result of this affection, there is found an abundant infiltration, in which we certainly do find polynuclear leucocytes, but in by no means such great numbers as that of the mononuclear elements which have often been described under the somewhat vague name of 'embryonic cells', those so-called 'plasmatic cells' would often appear to have an important relation to them.

As a general rule, whenever these changes are observed in a case of syphilis, we find at the same time spirilla. In those places only, where these organisms are particularly heaped up together, the mononuclear elements would seem to be much less in number than in the neighbouring parts, or else the wandering cells are much more numerous than the spirilla.

These facts may be interpreted in the following way. The spirilla produce some substance which promotes on the part of the leucocytes in general, and the mononuclears and other macrophages in particular, a chemio-taxis or positive sensibility; these cells are then directed towards the parts invaded by the micro-organisms; after this there is an actual contest between these two living elements. The organism puts into activity all possible means to maintain itself as well as to reproduce itself in the infected host, whilst the latter raises its army of defence to destroy the parasite as far as possible.

There were not sufficient facts to be able to judge of the intricate phenomena which take place in syphilis until after the revolution in syphilography which was brought about by the discovery of Schaudinn's spirilla in experimental syphilis. But a short time before this new discovery in science, Dr. Nagelschmidt[55] published a work on the immunity of syphilis, in which he affirmed that phagocytes certainly take no part at all in the defence of the organism against syphilis. After this, many observers have studied this problem, and their results show clearly that Nagelschmidt was by no means a true prophet.

Ehrmann,[56] a well-known syphilographer, has written the most complete work on this question. He worked on indurated chancres and primary lesions, inoculated living subjects, and studied under the best possible conditions. We add the conclusions which this observer came to in his last work: 'The

spirochaetes first penetrate into the interstices of the tissues, and after that reach the blood and lymphatic vessels ' (p. 240). ' The development of the capillaries, in just the same way as the migration of the leucocytes, we must regard as a phenomenon which depends on a chemio-tactic irritability. The leucocytes, which have passed right through the wall of the new-formed capillaries, now pass into the interstices of the tissues of the lymphatic ducts. Here they produce leucocytic infarcts, and these later on show all the stages of phagocytosis. This proves that the leucocytes form some means of protection against the propagation of the virus.' ' In the Tunica intima of the lymphatic vessels, the spirochaetes, which have come from without, cause the same changes as in the connective tissue; that is to say, increase in size and direct division of the fibroblasts, thus causing a thickening of the Tunica intima and the formation of new capillaries. These hypertrophied fibroblasts have exactly the same phagocytic action on the spirochaetes as that of the rest of the connective tissue, and by their proliferation they can bring about in a short time a more or less complete obliteration of the lymphatic vessels ' (p. 241).

In secondary lesions Ehrmann has been able to show spirilla enclosed in phagocytes. The phagocyte has been shown within papules in the stage of weakness of the general pathological process; and it has been found to be abundant in the skin, more especially in macular syphilides.

Later, Ehrmann expressed the opinion that in phagocytosis we have one of the opposing forces of the organism against spirochaetes. ' The infiltration is part of this phenomenon like the formation of the leucocytic infarcts in the smallest lymphatic channels, for these parasites are also found among phagocytic elements. Perhaps even the malignity of syphilis in cachetic persons depends on the feeble power of their white cells ' (p. 243).

The fact that phagocytosis has not been found in some cases of syphilis depends, according to Ehrmann, rather on the still imperfect state of our methods of research than upon their actual absence. Thus, ' In two fragments coming from one and the same chancre, which were stained at different times, although the

staining did not show spirochaetes, phagocytosis was only shown in one of these cases, although the spirochaetes were large in number and well marked in appearance in both' (note on p. 243).

This is perhaps the reason why many workers, amongst whom I can quote most able observers, such as Levaditi and Manouélian, failed in their attempts to find phagocytosis in primary and secondary syphilitic lesions. On the other hand, the former of these observers was quite certain that he had seen the engulfing of spirochaetes by phagocytes in hereditary syphilis. Levaditi [57] was struck when examining a case of pneumonic consolidation in a congenitally syphilitic child who died shortly after the mother's delivery, by the quantity of macrophages found in the pulmonary alveoli which contained spirilla. 'Here we have to do with the true phagocytic phenomenon followed by the digestion of the elements of the spirilla in the protoplasm of these macrophages,' —to quote again from the author,—'the proof that alterations are undergone by the spirilla when enclosed in this protoplasm is to be found in their varicose appearance and transformation into granules' (p. 66).

Amongst observers who have found *Spirochaete pallida* within phagocytes in cases of acquired syphilis I may quote Schaudinn himself,[58] who has seen them in the interior of a mononuclear corpuscle contained in a blood-vessel taken from a secondary papule in man.

The phagocytosis of syphilitic spirilla has been recently described by Gierke,[59] in a child who died four days after its birth. The organs were found healthy at the autopsy except the lung, which contained a yellowish-white focus of disease due to hereditary syphilitic pneumonia. In sections large quantities of spirochaetes were found. These were surrounded by leucocytes which had accumulated together in abundance, and were very often lodged in the actual interior of the cells. A more detailed examination showed that these cells were sometimes mononuclears though the polynuclears contained the organism much more frequently' (p. 351). Some of them only contained one or two spirilla, whilst others contained large masses of them.

In the preparations of Levaditi and Gierke, which I have

examined myself, I had no difficulty in being convinced of the accuracy of their statements (Plate VIII, *a*). The existence of phagocytes in syphilis therefore cannot be really called into question. This is quite in accordance with the way the principal pathological processes have been interpreted : these have been so well studied in the case of syphilis long before Schaudinn's discovery.

But although the presence of spirilla in the white cells is only a repetition of the phenomena of phagocytosis, so well known in other infectious diseases and now established in the case of syphilis, certain facts would appear to lead us in a new direction. While examining sections from the organs of hereditary syphilis in children, Levaditi seems satisfied that very often the spirilla are to be found contained in other varieties of cells, which have nothing to do with the white corpuscles nor with any other kind of phagocytes. ' We have to conclude ', says this author, ' that the spirilla of syphilis have the power of penetrating into the relatively intact protoplasm of certain epithelial cells, such as those of the liver and of the kidneys, the cells of the suprarenal capsules, and probably also those of the sweat glands ' (loc. cit., p. 63). These facts have been confirmed by several observers, and can no longer be doubted.

The only difficulty that remains is to interpret this fact. As Levaditi remarks, ' we still have to determine whether the intra-protoplasmic existence of the parasites is due to their active penetration into these cells, the cells retaining all their vitality, or whether there is in this, something of an agonic phenomenon, whose cause is, so to say, the state of inertia in which these cells are during the few hours which precede death ' (loc. cit., p. 63).

It is to be noted in this connexion, that in preparations taken from tissues removed during life, as in the researches of Ehrmann, which we have already quoted, the spirilla were only found in the interior of true phagocytes, and they have never been seen in the epithelial cells such as those of the skin or sweat glands.

On the other hand, demonstrations of spirilla in the interior of epithelial or other glandular cells have only been made from organs which have been removed after death. It is true that in

one case Levaditi was able to examine the tissues of a child very soon after death, at a time when the spirilla were still living, and in spite of this he found them in the interior of the epithelial cells, but of course it was quite possible that these cells had died before the death of the microbes.

The problem therefore cannot be very definitely settled. Gierke thinks there may be an actual phagocytosis of glandular cells, calling attention to the researches of Rössle on the phagocytosis of red cells by the epithelial cells of the liver, kidney, and pancreas. As the *Spirochaete pallida* is to be found in certain internal organs of monkeys, such as the testicle, it is in this direction we must look for the problem of its presence in those elements which usually have no phagocytic action. This subject is to be recommended for future research.

As syphilis is a malady which can undergo spontaneous cure, and which is nearly always very amenable to proper treatment, it ought to be very interesting to find out the ways by which a cure is effected. For this research monkeys should provide a good field, as in them syphilis is cured spontaneously in all but a few rare cases.

Levaditi has undertaken some work on this point; he has satisfied himself that the spirilla persist for a long time in primary sores, even when these appear to be completely healed. Many other observers have followed the life-history of the spirilla in human syphilis, when treated by mercury. Some have been struck by the long persistence of these organisms even when the cure seems to be quite perfect. Thus Galli, Valeris, and Lassueur[60] found a large number in an ulcerated papule which was nearly healed, and even after twenty centigrammes of sublimate had been injected. Levaditi and Sauvage[61] have found *Spirochaete pallida* in the blood and organs of a syphilitic infant whose mother had been treated with biniodide of mercury and iodide of potassium from the second month up to the end of her pregnancy.

On the other hand, some workers, among them Lévy-Bing,[62] have found that the spirilla disappear after mercurial treatment. The cause of the disappearance of these organisms has not been

settled, and it is quite possible that the action of mercury is towards strengthening the defences of the body rather than any direct lethal action on the spirochaetes themselves. With regard to the therapeutical action of atoxyl in syphilitic lesions, a fact that cannot be denied, the work of several observers has indicated that this substance does not kill the germs, but exerts its influence in strengthening the phagocytic defences of the host. This is also the opinion of Uhlenhut.[63]

CHAPTER IX

NATURAL HISTORY OF THE MICRO-ORGANISM OF SYPHILIS

The study of Schaudinn's spirillum in syphilis, together with the recognition of its constant presence in syphilitic lesions and its reappearance in an infected host are facts of the greatest importance in dealing with the great weight which should be attached to the presence of the organism. It is, however, desirable, in order to be able to form a conclusive judgement on this subject, first of all to know something about the natural history of the spirillum as well as its relations to the other micro-organisms which appear in a spirillar form.

We have already detailed in the second chapter (chapter VI) the very important part played in this question by the differentiation of the *Spirochaete pallida* from the other spirilla which are found in syphilitic lesions in man.

The following would appear to be the characteristics of the *Spirochaete pallida*, according to the work published by Hartmann and Prowazck, from writing found after Schaudinn's death.[64] In length it averages 6–15 μ, although some specimens are longer than this and measure between 16–26 μ. Its transverse measurement is so minute than it can hardly admit of measurement; it is about $\frac{3}{4}$ μ. Its undulations are extremely characteristic, especially as they remain the same during rest as well as in movement; this is what Schaudinn remarks himself: 'In comparative research we find at the very start that the *Spirochaete pallida*, which is only found in syphilitic lesions, varies extremely little in appearance; quite unlike the other known varieties of spirochaetes. As soon as one has had any experience, it is extremely easy to distinguish it from other forms when it is in the living state. The delicate shape and slight refractive power

of *Spirochaete pallida*, together with its typical spiral form with very numerous undulations (10–26), all perfectly regular, well marked and closely resembling each other, make it very difficult to mistake it for any other object whatever.

'The following fact is of particular importance, and is to be observed when examining the living organism—it is that the typical spiral form is to be seen not only when it is actively moving, but also when it is at rest; whilst all other analogous spirochaetes which present marked undulations show these only when they are moving vigorously, and when at rest have long flattened-out undulations, and indeed are very nearly straight. This particularly "stiff" appearance—so to speak—in *Spirochaete pallida* is due to the fact that in it the spiral arrangement seems to be definitely formed, and does not shift in any condition, whilst in other varieties the deep spiral is only produced during rapid movements and is straightened out at rest. There is only one spirillum, that of the mouth or *Spirochaete dentium*, which has a smaller fixed spiral arrangement, but this variety can be distinguished by other characteristics from the organism of syphilis.' 'The undulatory membrane has not yet been demonstrated in *Spirochaete pallida*.' 'At the present time I am inclined to think that the body of *Spirochaete pallida* may be round in section, that is to say, that the organism is cylindrical and not flattened as other spirochaetes are when observed from this point of view.'

The peripheral part (periplasium) is of equal thickness in the whole organism. It thins out at both poles and is continued in the form of a cilium whose length equals that of from four to six undulations. Schaudinn, at the end of his work, was able to recognize these cilia much better in the living organism than in stained preparations.

The largest individuals are distinguished by the presence of two cilia at one pole of the spirochaete. Schaudinn was able to confirm his speculation, that this was the beginning of a longitudinal division. These are his words concerning this in a report given in December, 1905: 'Owing to my somewhat long experience in this connexion, I am now able to recognize vibratile cilia in the living organisms at each pole of the spirillum, but, and

this is still more important, I have already succeeded in three cases in observing the longitudinal division of the organism. To follow out this process I have chosen individuals which already possessed two fine cilia at one of the poles, and I have seen longitudinal division progressing in a very few seconds beginning at this pole. At the moment of division, the organism abandons its marked spiral form and appears to be very irregularly contorted.'

All these details should be sufficient not only to distinguish the spirillum of syphilis from all related varieties, but also to give us some idea of its place among micro-organisms generally.

Here we touch on a problem which is very much debated in microbiology, as well as a subject where opinions are far from being unanimous. From the first observations on spirilla up to quite recently, these organisms were unanimously considered as belonging to the group of bacteria. In the attempts at classification made by Ehrenberg (1838), and Cohn (1875),[65] the spirilla were placed with the bacilli. The latter of these two great experts in microscopical organisms divided those bacteria which had a spiral arrangement into three classes :—

1. *Vibrio*, consisting of those microbes with a very simple spiral.
2. *Spirillum*, rigid and rolled in the shape of a corkscrew.
3. *Spirochaete*, a spiral organism with a flexible body.

The last class embraced the *Spirochaete plicatilis*, *Spirochaete denticola*, and *Spirochaete Obermeieri* of recurrent fever.

This distinction could not be maintained for any length of time, for amongst the bacteria there were some which, in a single species, contained the characteristics of the three varieties of Cohn.

Thus, for example, the organism of cholera is well known, owing to the great ease with which it can be cultivated. Now we find that it is usually seen in the form of a vibrio with a very simple curve (Plate VIII). On the other hand, under certain particular conditions it appears as a spiral filament, with either a simple or else a corkscrew curve. When these spiral filaments are short the body and organism show no signs of flexibility ;

but when they are very long they undergo the very flexible movements of a spirochaete (Plate VIII, *c*).

We have another example in the organism of recurrent fever; this, under ordinary conditions—that is to say, when it is found in the blood of patients during the attacks of fever—appears as a true spirochaete with a flexible body. But in patients who have malarial parasites in their system at the same time that they are suffering from recurrent fever, the spirillum of Obermeier, as has been shown by Karlinsky,[66] is divided into bodies which resemble much more closely the vibrios of cholera than any form of spirochaete.

More recently, Migula[67] has endeavoured to place the classification of spiral organisms on a more complete basis. For this he divides them into four varieties :—

1. *Spirosoma*, spiral organisms, immobile and without cilia.
2. *Vibrio*, comprising short vibrios curved on themselves and showing one, two, or even more than three cilia at their extremities.
3. *Spirillum*, micro-organisms of a spiral helicoid form provided at each end with several cilia.
4. *Spirochaetes*, very long *Spirilla* with more or less numerous and regular undulations and without cilia. Some of these spirochaetes have an undulatory membrane closely analogous to that of trypanosomes (*Sp. plicatilis*, Ehrenberg).

It is easy to see that this classification, which is really very little different from Cohn's, has indeed no foundation on fact. The organism of cholera would have to be classed among the vibrios when its numbers are small; at other times under *Spirillum* or *Spirochaete*. The true *Spirochaetes*, as, for example, those which cause septicaemia in fowls, or tick fever in men, seeing that they possess numerous cilia, should be excluded from this class altogether, as far as Migula's classification goes.

The latest modification proposed by Swellengrebel[68] has no better chance of success; he divides Migula's *Spirillaceae* into two sub-groups :—

1. *Spirillaceae*, whose bodies are not flexible, composed of

the genus *Spirillum* and *Vibrio*; and *Spirochaetaceae*, whose bodies are flexible, this class containing three sub-classes:

(1) *Spirochaete.*

(2) *Treponema* or *Spironema* (organism of syphilis).

(3) *Borrelia* (organism of septicaemia in fowls).

Unfortunately, in this classification again, no account has been taken of the vibrio of cholera, which is found at times in the form of long spiral flexible filaments.

The failure of all these attempts to classify spirilla is due to the very imperfect knowledge we really possess with regard to these organisms. I believe, therefore, under present conditions, that it is quite beyond our power to endeavour to imprison the few spirillar organisms which we now know within the very narrow limits of precise classification. Rather, at a later date, this should be the crowning-point of profound and extended study of the entire group.

It is on account of the imperfect state of our present knowledge that I have attached no great importance to the mere names proposed by different authors, and have purposely called the various spiral micro-organisms by the one name of *Spirilla.*

In the attempt to increase the sum of our precise knowledge of this microscopical world of its own, Schaudinn, whose loss we must ever deplore, was able to gather together a great number of new facts concerning it. In the course of his researches for parasites in the blood of certain birds,[69] he was struck by the fact that certain trypanosomes, or small flagellated protozoa, produced spiral enrolled filaments, each one resembling spirochaetes (Plate VIII, *d*). These were very minute organisms provided with an undulant membrane, which is very characteristic of true trypanosomes, and, like them, they were reproduced by longitudinal division. This discovery suggested to the distinguished author the idea that many spiral organisms, hitherto taken for bacteria, more especially the different spirochaetes of recurrent fever, septicaemia in birds, &c., were nothing less than very fine trypanosomes, and thus were flagellated infusoria, and not bacteria at all.

Working on this hypothesis, Schaudinn set himself to seek for

the true spirochaetes, and selected the study of *Spirochaete Obermeieri* of recurrent fever. It would seem that the facts which he collected on this subject were not very favourable to this theory. Nevertheless, his researches familiarized him very considerably with the world of spirilla. This was a fortunate circumstance, as he was thus able to recognize the spirilla in syphilitic products, when so many other observers had failed. If Bordet and Gengou had possessed the same skill and experience in observing spirilla, they would probably have found them in the preparations from chancres which appeared to them to be absolutely devoid of organisms.

When the discovery by Schaudinn of the organism of syphilis had attracted general attention, this able protozoologist set himself to follow out his idea as to the position of these spirals in the general system of micro-organisms.

We must remember that at the beginning of his task he was already very skilled in the identification of trypanosomes and spirochaetes. In an article [70] full of valuable suggestions, he expressed the opinion that very small trypanosomes, rolled in a spiral, had no very near genealogical connexions with the true spirochaetes (such as *Sp. plicatilis* and *Sp. Obermeieri*), and that, in consequence, any attempt to discover in them all the characteristics of trypanosomes was useless.

In the work published in Schaudinn's name after his death by Hartmann and Prowazck,[71] we find his opinion thus expressed on the question which is now occupying us: 'I must confess', says this lamented scientist, 'that the question of the place they occupy in the system of nature has but the smallest scientific interest. For a long time I have been convinced that bacteria and protozoa do not form two separate and distinct trunks, but are really two branches from the same tree, that of the unicellular organisms or "protista"; and that these two branches have many side connexions. However, scientific study in this domain may teach us to find one of these stages of transition among the spirochaetes, which may well lie between the flagellated organisms and the true spirilla which are reproduced by transverse division' (p. 16).

It appears to me that some observers in the pursuit of Schau-

dinn's original conception have gone very much further than the author himself. Schaudinn's successor in the Institute of Hamburg, Prowazek, attempts in many recent publications to prove that spirochaetes in general, are indeed trypanosomes or their nearest relations. In his argument a very important part is attributed to the longitudinal division which is typical in trypanosomes, but is believed to be absent in bacteria: so careful an observer of micro-organisms as Schaudinn has insisted that 'Longitudinal division is a process which is characteristic of flagellates, and up to the present has not been proved in any one bacterium. The latter always divide transversely' (Posthum. note, loc. cit., p. 16).

Nevertheless, it is well known that a great number of micrococci divide in two lines, perpendicular one to the other; some sarcinae even divide in three directions, but in the elongated forms there are undoubtedly some which undergo longitudinal division.

I myself described a bacterium in 1888[72] which I called *Pasteuria ramosa*, oval in shape, which divided longitudinally. It is true that Migula[73] has refused to believe that *Pasteuria* is a bacterium at all, although in this he is most assuredly wrong, because the production of spores shows clearly the bacterial nature of this organism. We cannot deny in some other cases that bacteria are really able to divide longitudinally.

Following the same suggestion of the connexion of spirochaetes with trypanosomes, Prowazek[74] affirms that the spirillum of septicaemia in fowls (*Spirochaete gallinarum*) divides longitudinally, contrary to the observations of other observers, amongst whom I may quote Levaditi.[75] The description of the division in Prowazek's papers, and also the pictures meant to illustrate it, are very far from being convincing.

On the other hand, the researches of Koch,[76] and Zettnow,[77] carried out with the greatest care, show clearly that the spirochaete of tick fever, which so closely resembles that of fowls, is reproduced by transverse division. The photographs in Zettnow's account, moreover, form a convincing argument. I may add that, in the course of my own research on the *Spirochaete Obermeieri*

and the organism of septicaemia in geese, I was able to find transverse division of these organisms without seeing the slightest indication of longitudinal division.

In a recent paper on spiral organisms found in the oyster, Borrel and Cernovodeanu [78] affirm categorically that this *Spirochaete Balbiani* (Plate IX, *b*) divides transversely, and yet here we have to do with an organism which approaches very closely to the flagellates, and which, for this reason, has often been taken for a trypanosome.

In a recent article, already quoted, Swellengrebel decided in favour of transverse division, not only of the parasite of the oyster but also of the *Spirochaete buccalis* (loc. cit., p. 580). Hartmann and Muhlens [79] have succeeded in cultivating *Spirochaete dentium*, and have given many details concerning its reproduction. But although they were then able, without difficulty, to observe transverse division, it was but rarely that they came across any stages which perhaps might be part of a process of longitudinal division. The authors themselves, however, make no final statement on this point.

Under these conditions it is quite clear that the whole question must be studied afresh, and that, despite the statements of Schaudinn, of Krzyztalowicz and Siedlecki,[80] and of Herxheimer,[81] the existence of longitudinal division in spirochaetes in general and in the *Spirochaete pallida* in particular, must be proved afresh by most certain and convincing arguments.

The study of the organs of locomotion of these organisms presents a second point which is a subject of much discussion. The bacteria move by means of cilia, whilst the organ of locomotion in trypanosomes is an undulant membrane. If the micro-organism of syphilis is a bacterium it ought to possess cilia ; but if, on the other hand, it belongs to the group of trypanosomes, we ought to find that it possesses an undulant membrane. In his first work Schaudinn, being rather inclined to associate spirochaetes with protozoa, believed that he had seen an undulant membrane in *Spirochaete pallida*, but at a later period, as I have already mentioned, he had to modify this opinion after having proved the existence in these organisms of two cilia at the opposite poles of

the body. In spite of his observations, partizans of the theory that spirochaetes are flagellated infusoria endeavour to show the presence in them of an undulant membrane. Thus Prowazck (loc. cit., p. 559) admits the presence of this membrane in the spirochaete of fowls, and this despite the discovery by Borrel[82] of vibratile cilia round the body of this organism (Plate IX, *a*, *Spirochaete gallinarum*, with vibratile cilia, after Borrel). Zettnow[83] was able to confirm this same fact by studying the connexion of this organism with the spirochaete of African tick fever.

It would thus seem to be proved that certain spirochaetes are indeed in possession of a true undulant membrane. During the last few years Schaudinn has described them in *Spirochaete refringens* and *Spirochaete plicatilis*; Hoffmann and Prowazck,[84] have seen the same in *Spirochaete buccalis*; Borrel and Mlle. Cernovodeanu (loc. cit.) have studied it in detail in *Spirochaete Balbiani* of an oyster (Plate IX, *b*, *Spirochaete Balbiani*, after Borrel). This curious organism, by its transverse division is closely connected with true spirilla, yet by its means of locomotion recalls the trypanosomes.

We cannot fail to see by all these facts that the discussion as to the place which the micro-organism of syphilis should take in the classification of organisms in general has brought about the discovery of a certain number of very important facts, and, although they are, up to the present, insufficient to deal definitely with the question, they have contributed most important results.

The more detailed knowledge of spirochaetes allows us to affirm with certainty that the spirillum of Schaudinn is a species quite distinct from the *Spirochaete refringens* and *Spirochaete balanitidis*, which have been found in certain syphilitic lesions and have been called secondary organisms.

This conclusion alone is quite important enough to console us for the impossibility up to the present time of obtaining a precise knowledge of the relations of the organism of syphilis to the other members of the legions of micro-organisms.

The *Spirochaete pallida* would appear to be an exceedingly delicate micro-organism which perishes very rapidly in the absence of moisture, and even in the presence of moisture it is only kept

alive with great difficulty. It is true that Hoffmann[85] has kept it in a motile condition for several weeks; on the other hand, it has been fully established by other researches that the virus of syphilis retains its pathogenic power outside the body for a few hours only, while Landsteiner and Mucha[86] have been able to keep the organism alive only for two days at the very most.

All attempts to establish the cycle of development in *Spirochaete pallida* have, up to the present, led to no satisfactory result. Krzyztalowicz and Siedlecki, in the memoir which we have already quoted (p. 82), have described a whole series of different forms which they believed to be stages in the development of the organism of syphilis. They observed very large spirilla which they recognized as macrogametes, or female elements, capable of sexual reproduction. They observed their union with very small spirilla which they took for the male elements. If this be correct the reproduction of *Spirochaete pallida* would closely resemble that of the protozoa.

Unfortunately the research of these Polish authors was effected by working with syphilitic lesions which were already of long duration, and therefore could not be considered to be free from contamination with organisms other than that of syphilis. Having in view the very great difficulty of this kind of observation, we must wait for results which will confirm these authors' work. This would seem to be all the more necessary, as in hereditary syphilis, where the organs are invaded by an enormous number of spirochaetes without being contaminated by any other organism, no one has yet found the particular stages described by the Polish observers.

Seeing the important part which this organism would appear to take in the aetiology of syphilis, it is clearly of great importance to be able to cultivate it in artificial media. Attempts to do this have been made in nearly every bacteriological laboratory in the world, yet up to the present none have been successful. Having regard to the great difficulty of obtaining anything like a proper quantity of Schaudinn's spirillum in a pure state with which to sow media, some observers have worked with other varieties of spirochaetes as closely as possible allied to

that of syphilis. Working on these lines, Muhlens[87] has obtained pure cultures of *Spirochaete dentium*, which appear to grow well, under anaerobic conditions, in serum agar. Levaditi,[88] on his part, has been able to make luxuriant cultivations of the spirillum of African tick fever, of the spirillum of fowls, and of the *Spirochaete refringens* by means of bags of collodion, placed in the peritoneal cavity of rabbits. In these cultures he has watched the development of several generations without observing the occurrence of any trypanosomic form. These results give reason to hope that the organism of syphilis may be cultivated at no distant date.

We may well conclude this chapter by adding a few words as to the actual name given to the organism of syphilis. Schaudinn, after having first given it the name of *Spirochaete pallida*, joined later on with Vuillemin in renaming it *Spironema pallidum*; however, after he had found that the name of Spironema had already been made use of to describe another kind of micro-organism, Schaudinn invented a new name *Treponema pallidum*. At the same time, however, Hoffmann—whose collaboration with Schaudinn had been already of such great value in the progress of our knowledge on this organism—abandoned this name, and used that of *Spirochaete pallida* or *Spirochaete pallidum*. I shall follow the example of this German syphilographer, as I consider that the time is still premature to make any classification of spirillar micro-organisms.

CHAPTER X

TECHNICAL METHODS IN CONNEXION WITH THE ORGANISM OF SYPHILIS

As we have already seen, the organism of syphilis was first discovered in a living state in a fresh preparation from a syphilitic lesion. This method, simple as it is, must be seen to have a much greater importance than all the complicated processes employed many hundreds of times before Schaudinn. It must, however, be said that examination in a fresh state is by no means easy, more especially in those cases (and these form the majority) where the organism exists in very small numbers. To be successful a very favourable light is necessary. I only succeeded in observing them when using a gas lamp with an Auer shade, although in the same preparation, when observed with the same lenses, but using daylight or an ordinary electric light, I was unable to see a single pale spirillum. In the presence of these difficulties we must regard the discovery of a simple and precise method of looking for the organism in fresh preparations as a great advance in our knowledge. Landsteiner and Mucha (loc. cit.) have done this. These observers have applied the principle of the ultra-microscope of Siedentopf and Zsigmondy for the examination of small particles in a dark field. To do this they used a condenser made by Reichert, of Vienna, which would appear to be the best of these instruments. The illumination is given by an arc lamp of 20 ampères. The most satisfactory combination of lenses has been found to be that of a dry objective, No. 5, with Reichert's compensatory ocular, No. 18. An examination is made by taking a small amount of syphilitic secretion, which is then rubbed between cover slips into a film; the cover glasses protect it against desiccation. In those cases where the material contains too many solid particles it is treated with

normal saline solution; in these conditions the syphilitic spirilla are seen on a dark background between all sorts of corpuscles, in the form of motile organisms, strongly illuminated and easily recognizable (Plate X, *b* and *c*). The extraordinary mobility, combined with the spiral appearance, enables us very readily to distinguish the spirochaete of syphilis from other spirillar organisms. Usually an examination of a few minutes is enough to confirm our diagnosis. This very interesting and convenient method, which has already been confirmed by Hoffmann and other specialists, may now be considered as a necessary part of daily practice.

Thanks to the ease with which we are now able to recognize the organism of syphilis with a dark background, methods of staining syphilitic products in film preparations have been recently relegated to a second place. Nevertheless, their importance remains, not only in observing the minute anatomy of the spirilla, but also in all those cases where we desire to keep preparations for some time. Before staining the films, in each case we have, of course, to obtain the necessary material for their preparation.

As has already been shown many times, the *Spirochaete pallida* is much more abundant in the deeper parts of syphilitic lesions, whilst on the surface its number is very small. Sometimes they are not found at all in the peripheral parts of the lesion. This peripheral part is, on the other hand, very often the seat of secondary organisms, amongst which we may meet the *Spirochaete refringens* together with bacilli and micrococci. For this reason we should as far as possible take material from the deeper parts of syphilitic lesions in order to make film preparations.

Sometimes it is useful, in connexion with syphilitic lesions of the skin, and even with portions of healthy skin in the immediate neighbourhood of these lesions, to use *vesicants* after the methods of Levaditi and Petresco.[89] After the surface of the skin is washed, a vesicant is applied either on the lesion or on the neighbouring parts, over a surface of about two square centimetres; this is left in position for from six to eight hours. The liquid which accumulates can now be removed by a sterile pipette introduced under the skin. Again, the vesicle may be

excised and the bare surface thoroughly rubbed: the serum which exudes will serve for other film preparations.

In the study of *tertiary lesions*, material must be removed from the peripheral parts and not from the mass of the caseous gumma itself. With regard to the vesicles of pemphigus in new-born children and the pustular eruptions in adults, the contents should be removed for making film preparations.

The best method of finding the spirilla of syphilis in the *blood* is that of Noeggerath and Stoehelin.[90] The blood of the ear is taken, or, better still, one of the veins of the arm is punctured, and diluted with ten times its volume of a solution of acetic acid of one in a thousand. At least 1 c.c. of blood must be removed. The blood diluted in this way should be thoroughly centrifugalized, after which it can be used to make film preparations.

It is often very important to make a microscopic examination of the juice of glands; this should be removed from the living subject. For this purpose, as Hoffmann has shown, the inguinal region must first be shaved, then disinfected and washed with saline solution. After this the sharp needle of a syringe, holding 5 c.c., is introduced into the inguinal gland, which is fixed in position by the left hand. We should endeavour to pierce one of the largest of the glands and remove its contents by slow aspiration. If the gland follows the movement of the needle it proves that the needle is actually in the interior of the gland. After a sufficient quantity of the juice of the gland has been taken away, the syringe is emptied on to a clean plate and the film preparations are made with fine layers of the extracted fluid.

In taking material from an autopsy, the technique is so simple that there is no need for any further explanation.

The films obtained by these methods should be stained by one of the methods which have been found best in studying the organism of syphilis. Giemsa's method is the one which, undoubtedly, takes the first place amongst these methods of staining. The films are fixed in strong alcohol and then treated for one or several hours with a mixture composed of a solution of azure blue and eosine. Giemsa's mixture, ready prepared,

can be easily bought.* Fifteen drops of this solution are taken and mixed with 10 c.c. of distilled water. The spirilla of syphilis are feebly stained by this method, and usually show a clear rose colour, whilst the other spirilla, especially *Spirochaete refringens*, are coloured violet. We should, however, mention that this staining is not sufficiently constant to allow us to use it to differentiate the microbe of syphilis from others.

Instead of fixing the films by alcohol, Hoffmann and Halle advise another method. They put in a flat plate 5 c.c. of a one per cent. solution of osmic acid, and add to it ten drops of glacial acetic acid. The films are then exposed for at least two minutes to the mixed osmic and acetic vapours. For this method the syphilitic products are rapidly spread out on to slides which have been exposed to the osmic acid vapour, and before the films are dried they are again exposed to the same vapour for one or two minutes. The films, being thus fixed, are now treated for about a minute with a very weak solution of permanganate of potash, washed in water, dried, and finally stained.

The osmic acid may be without inconvenience replaced by formalin.[91]

Instead of staining by Giemsa's method as explained above, one of the following methods may be used:—

Giemsa's New Method.[92] As soon as the material is obtained, it is treated with a freshly made mixture, obtained by adding 5 to 10 drops of a solution of carbonate of potash of 1 in 1,000 to 10 c.c. of distilled water. While the liquid is being constantly shaken, 10 drops of Giemsa's commercial stain are added to it. With this method it is found sufficient to leave the slides in the staining bath for from three quarters of an hour to an hour. Herxheimer[93] has proposed to stain the films when fixed with alcohol with a hot solution of gentian violet. A quarter of an hour is enough to obtain a good staining of spirilla. By heating the preparations

* Giemsa's latest formula is the following:

Azur ii, Eosin	3 grms.
Azur ii	8 „
Glycerine (Merck, chemically pure) .	250 „
Methyl alcohol (Kahlbaum i) . . .	250 „

these organisms can even be sufficiently stained in half a minute. Preis [94] has further modified Giemsa's method for films of syphilitic products; these are thrown on to a mixture of 20 to 25 drops of Giemsa's stain diluted by 10 c.c. of distilled water. The plates are then kept about 5 cm. above a Bunsen's flame and heated until vapour comes from them, taking care that the liquid does not boil. The same process is repeated twice, and in five minutes the preparations are finished; in them the organisms of syphilis are stained quite a *deep red.*

Marino's method [95] is also distinguished by its rapidity. The films are first dried without being fixed in any way, and then are treated by a mixture of Marino's blue (0·1), and of methyl alcohol (20·0). After three minutes some drops of a watery solution of eosin (0·05 in 1,000) are added. Two minutes after this the films are thoroughly washed and are now ready to be examined. I have myself found this method of the greatest use.

In order to stain the terminal cilia of *Spirochaete pallida, Loeffler's method* should be used. A freshly made mixture is used, consisting of—

10 c.c. of a 20 per cent. solution of tannin;

5 c.c. of a cold saturated solution of sulphate of iron;

1 c.c. of a saturated alcoholic solution of fuchsin.

This mixture is put on the film, and the whole is heated three times until vapour comes off. It is then washed in distilled water and stained with Ziehl's solution of carbol-fuchsin, gently heating it at the same time. By this means the organism of syphilis is stained a dark red, whilst its cilia are shown in a light rose colour. Care must be taken in all these methods to dilute the syphilitic products with some drops of distilled water.

Many other methods have been proposed for the staining of films; but those which we have shown are quite enough to obtain good preparations of Schaudinn's spirilla.*

* Plate XIII shows a film preparation stained by Leishman's 'serum method', which may be briefly described: The film is fixed in methyl alcohol for half a minute, this is blotted off with cigarette paper and a thin layer of fresh blood serum is run over the surface of the film. Only a single drop of serum is required, and this is drawn over the surface of the film with a needle. The excess is then removed by allowing the slide to stand on one end, and the film is

Formerly the greatest difficulty was found in obtaining a proper staining of various spirochaetes in sections, and only a very partial effect was obtained in the case of the organisms both of recurrent fever and of septicaemia in geese and fowls. But since the study of the organism of syphilis first attracted the attention of a great number of workers, an extraordinary amount of progress has been made in this branch of micro-biological technique.

Bertarelli and Volpino,[96] were the first to apply Van Ermenghem's method of staining cilia to syphilitic tissues. Their sections are treated from 24 to 48 hours with a solution of nitrate of silver (0·2 to 0·5 per cent.). After washing it rapidly with distilled water, it is then treated for a quarter of an hour with Van Ermenghem's solution, which is composed of—

5·0 acid gallic.
3·0 tannin.
10·0 acetate of soda.
350·0 of distilled water.

The sections, as soon as they are stained yellow in this solution, are again placed in the nitrate of silver solution until they are stained brown; after being again washed in distilled water they are passed in the usual way through alcohol and xylol, and finally mounted in Canada balsam.

After some time Bertarelli and Volpino[97] modified their original method and perfected it in the following way: Very thin sections (not more than 7 μ in thickness) are first fixed in alcohol, and then put into a bath composed of—

3 gr. nitrate of silver.
50 c.c. of alcohol (96 per cent.).
50 c.c. of distilled water.
4 or 5 drops of pure acetic acid.

The whole is put in a deep glass and gently heated between 35° and 37° C. (95°–98° F.) for three or four days. As soon as a precipitate is formed the fluid to be renewed. After repeated

then allowed to dry in the air. The film is now stained with a mixture of equal parts of Leishman's stain and distilled water. After twenty-five minutes the stain is washed off and the film dried and examined. The serum intensifies the action of the stain and prevents the occurrence of any deposit.—TRANSLATOR.

washing in distilled water the sections should remain in Van Ermenghem's reducing solution for 24 hours ; this is the same as that used in the last method. The sections are again washed with distilled water, and then dehydrated with alcohol in the usual way.

Whilst Bertarelli and Volpino's two methods are modifications of Van Ermenghem's method of staining cilia, Levaditi's method[98] is a modification, adapted for syphilis, of that of Ramon-y-Cajal for staining the terminations of nerve-cells.

Fragments of the tissue about 2 to 3 millimetres in thickness are fixed in 10 per cent. formalin solution for 24 hours ; they are then kept in 96 per cent. alcohol for the same time, then washed in distilled water until they fall to the bottom of the vessel containing them. They are then put into the following solution :—

1·5 to 3 grs. of nitrate of silver.
100 c.c. of distilled water.

The fragments are put with this solution into a wide-mouthed bottle with a tightly-ground glass stopper ; in this they should remain for three days in an incubator at a temperature of 38° C. (100° F.) *in the dark* : after this they are washed with distilled water and then put into the reducing liquid. This contains—

4 grams pyrogallic acid.
5 c.c. formalin.
100 c.c. distilled water.

The reduction is allowed to continue for 24 hours ; the fragments are now dehydrated, passed through xylol, and cut in paraffin. The sections (which should not be thinner than 5 μ) may be stained by Giemsa's method or by toluidine blue and neutral red, combined with methyl blue. This method should be that used by preference for staining spirilla in the organs of inherited syphilis. Many observers have already made use of it for all sorts of syphilitic tissues with the greatest success. (See Plates XIII to XVI.)

Levaditi, in conjunction with Manouélian,[99] has suggested another method, which they especially recommend for those tissues which are taken from the living body. Small pieces of

tissues are fixed in formalin, 10 per cent. solution, from 24 to 48 hours, and then kept in 96 per cent. alcohol for 24 hours : they are then washed in distilled water and placed in the following solution :—

10 c.c. pyridine (added to silver solution at the moment of use).
90 c.c. of a 1 per cent. solution of nitrate of silver in distilled water.

They are kept in this solution, first at the temperature of the laboratory for two to three hours and afterwards from three to five hours at a temperature of from 45° to 50° C. (113–122° F.).

After being washed with 10 per cent. solution of pyridine they are passed into the reducing solution, which must be freshly made and is as follows :—

17 c.c. pyridine.
10 c.c. acetone.
99 c.c. of a 4 per cent. solution of pyrogallic acid in distilled water.

The reduction should be allowed to continue for several hours, after which the fragments are dehydrated, treated by xylol, and cut in paraffin ; the sections should not be thinner than 5 μ. Staining is done by the same method as the original one of Levaditi. Some other writers prefer that of staining the sections with iodine green ; while others again advise freeing the preparations from reduced silver before using the ordinary methods of staining.

As many observers have taken objection to those methods by which spirilla are shown, not by staining only, but by reduced silver, some have endeavoured to discover a method of staining spirilla in sections other than those of Bertarelli and Volpino and that of Levaditi and Manouélian.

Schmorl,[100] whose talent in technique is appreciated by all the world, would appear to have recently solved this problem. He fixes his sections in a solution of 4 per cent. formol ; from this they are removed by a sterilized glass rod and put into a solution of sterilized water contained in a perfectly clean glass dish. They are then put into Giemsa's staining solution, con-

tained in a drop of water on a slide; there they are allowed to remain for an hour. They are then put into a second staining mixture, and there allowed to remain for at least five hours. They are then washed rapidly in either distilled water or in a concentrated solution of potash alum; in which latter case they must be again washed in distilled water. They are now ready to be stuck to slides, and mounted in either cedar oil or Canada balsam.

The author has succeeded by this method in staining the spirilla of syphilis in two cases of congenital syphilis.

Just as this method of Schmorl has allowed us to stain Schaudinn's organism by those aniline dyes which we use for staining film preparations, it has been lately shown to be possible to demonstrate the presence of spirilla on films by the silver nitrate reduction method. Although this has never yet succeeded in films when used according to the methods we have just described, Stern [101] has described a method which can be well used with films of all sorts of syphilitic products. In this method the films containing the syphilitic material are kept for several hours at a temperature of 37° C. (98° F.), after which they are plunged for some hours into a 10 per cent. solution of nitrate of silver; when the preparation is slightly brown in colour it is taken out of the bath and washed in distilled water.

Thanks to the various methods we have just described for the reader, science now possesses very complete methods for studying the *Spirochaete pallida* in the various conditions in which it occurs in the body.

CHAPTER XI

CRITICISMS AS TO THE PART TAKEN BY THE SPIRILLUM OF SCHAUDINN IN THE CAUSATION OF SYPHILIS

THE last methods, of which we have just given an account in the preceding chapter—those of Schmorl and Stern—have a special importance when considering the objections which have been made to the part played by Schaudinn's organism in the aetiology of syphilis.

One would have thought that so many precise facts all in agreement as to the presence of this spirillum in syphilis, and its differentiation from other spirillar forms, would have sufficed to satisfy us that it is really the specific cause of syphilis, despite the impossibility, up to the present, of obtaining pure cultures of the organism, by which means syphilis could be reproduced. Nearly forty years have passed since the discovery of the spirillum of recurrent fever, during all which time, although no cultures were obtained, it was without hesitation considered to be the cause of the disease; and yet no contradiction of the accumulated facts was made. Nay, further, it is the same in the case of trypanosomes, whose importance as the cause of so many infectious diseases is admitted by everybody.

Yet, despite all this, ever since the publication of Schaudinn's discovery there has been no cessation to the objections which have been made as to its value in connexion with the etiology of syphilis. These objections more especially came from authors who had already pleaded the importance of *Cytoryctes luis* as a cause. First among these was *Thesing*,[102] who declared that Schaudinn's spirillum did not really come from syphilitic products at all, but was simply introduced in the various solutions made use of in the work. After having found some organisms in

Giemsa's solution, Thesing declared that the *Spirochaete pallida* came from this mixture in the same way. It was, however, very easy to refute this objection, as Schaudinn had actually made the discovery when using fresh material, in which medium the spirilla were living and moving under the eye of the observer.

However, this refutation did not discourage his adversaries: far from it; they proceeded to frame a regular indictment gainst the *Spirochaete pallida* as the cause of syphilis, and although at the present time only a very few observers of any note remain on their side, it is, all the same, proper that we should take some notice of the principal objections.

This task becomes more easy, thanks to the appearance of two recent papers by *Saling*,[103] in which he has collected together all that can possibly be said on the subject. According to this author, the *Spirochaete pallida* is a saprophyte which develops with great ease in the cutaneous lesions of syphilis, and is only found in internal organs in hereditary syphilis of the foetus and the new-born child, where it is generalized in the same way as the common bacillus of the digestive tract.

The principal argument by which Saling tries to prove his contention is the following:—The spirillum of Schaudinn has never been obtained in the internal organs of syphilitic monkeys, which organs are nevertheless very virulent. Saling applies himself to many experiments to prove this fact, which he considers to be absolutely established. Here is an extract from his work:—'Although every year hundreds of syphilitic monkeys are examined, no spirilla are to be found in the "extremely virulent organs", except in one case of Zabolotny [which we have already quoted in the third chapter (chapter VII)]. This case is merely an exception to the rule.'

I myself can testify, from a good deal of knowledge in the matter, that of the large number of syphilitic monkeys which are under observation in different laboratories by far the greater number have never been examined from the point of view as to whether their internal organs contained spirilla at all. Indeed it would have been a useless waste of time to constantly make

this examination, for these organs are very far from being 'extremely virulent', as Saling so often affirms. Indeed, it has already been shown by Neisser and his fellow workers that there are only some special internal organs of monkeys which contain the virus at all. These are, more especially, the spleen, the marrow of the bones, the lymphatic glands, and the testicle. Moreover, in order to produce a primary lesion, it is necessary to inoculate a very considerable quantity of the pulp of these organs, and in order to get a positive result, material must be taken from many cut surfaces of an organ, or else the pulp of an organ must be inoculated after the whole organ has been thoroughly shaken up, yet even in these cases inoculation does not always produce a primary lesion.[104] Now as the syphilitic virus is so active that the very smallest quantity will produce infection, we can well believe that spirochaetes must be very rare indeed in the internal organs of infected monkeys. In order to justify his research it should be the case that the virus of these organs is exceptionally strong, and this is far from being the case, despite Saling's assertions.

The specific spirillum has been searched for in tertiary lesions in man far more than in the organs of syphilitic monkeys. For a long time the attempts to prove its existence in tertiary lesions failed, and this led us to believe that the organism is to be found here in some other stage of existence. But, as we have before mentioned, in the end it has been found in its spiral form, only the organisms are very few in number and very unequally distributed in tertiary syphilis.

These facts will be seen to be in perfect accordance with the knowledge that tertiary products are only virulent in exceptional cases, and that in order to produce a lesion by inoculation the material used must be taken not only from the softened parts but from the very same peripheral layers in which the syphilitic spirilla have been found to exist. If these were nothing else but simple saprophytes, why is it that they do not increase in gummata, and are only to be found in syphilitic lesions of all kinds, and never in other cutaneous affections? The *Spirochaete refringens*, on the other hand, which *is* a saprophyte, is found in

lesions other than syphilis, and in the different products of the genital organs. There is, therefore, no justification to the theory of the saprophytism of *Spirochaete pallida.*

A great number of observers have endeavoured to deny the microbic nature of the spiral filaments which are stained by aniline dyes. Thus *Omeltchenko*[105] affirmed that they are nothing else but fragments of connective tissue which have taken a spiral form. There is no difficulty in disproving this objection ; every observer who has any skill in work knows well the pseudo-spirilla which are sometimes found in preparations. But it is only necessary to exercise a very little practice and attention in order not to confound them with micro-organisms in general and with the spirilla of syphilis in particular.

Many have been the discussions on the subject of the interpretation of spirilla in sections stained by means of nitrate of silver. *Walter Schulze*[106] called them by the name of *Silver spirochaetes* (Silberspirochaete), and denied that they have any importance in the study of syphilis. He regards them as being the débris of nervous filaments and of elastic connective fibrils which resemble spirilla but have no true connexion with them at all. Saling[107] and some other workers agreed with this opinion, which they held with great tenacity.

Now it cannot be denied that certain filaments of the tissues can be stained black by silver nitrate, and may then somewhat resemble spirilla. But it is not difficult to distinguish them, and those observers who have some experience in the study of spirilla have been able to do this without hesitation.

Schulze's first paper immediately provoked Levaditi's reply,[108] in which he brings a number of arguments to bear which ought to suffice to refute all objections. The black spirilla in sections of syphilitic lesions are found exactly in the zone which is affected by the syphilitic lesion, whilst the secondary organisms and, amongst others, the *Spirochaete refringens*, are to be found in the superficial layers. The *Spirochaete pallida* of syphilis is to be found not only in the tissue, but also in the lumen of the blood and lymphatic vessels, where it closely corresponds with the spirilla of septicaemia in fowls, and of recurrent

fever, both of which are stained black in just the same way by nitrate of silver.

It is still harder to understand the zeal with which the microbic nature of '*Silver spirochaetes*' has been denied, as the very same tissue, when examined in a living state or stained in film preparations by Giemsa's method, shows a large number of spirochaetes absolutely characteristic in appearance.

In his later work Saling insists most of all on the importance of *Karlinsky's* preparations, which show '*Silver spirochaetes*' in the macerated foetus of a pig. To judge from his photographs [109] these filaments closely resembled true spirochaetes, but Saling is quite wrong in thinking that there is anything in this fact which can impeach the value of Schaudinn's spirillum.

The sow in question was killed in the end, and the autopsy and microscopical examination showed no lesion at all, but the entire litter of six were dead and macerated. In sections from one of these treated by Levaditi's method, spiral filaments were found in great abundance. Hoffmann, to whom these preparations were submitted, did not hesitate to declare that these closely resembled true spirochaetes. Instead of drawing the conclusion from these facts that the pig should be joined to other mammalia, such as man, the ape, the horse, the ox, the sheep, mice, &c., all of which are subject to spirillar diseases, Saling endeavours to use it to refute the microbic nature of the spirillum of syphilis. He rests his opinion on the fact that the mother sow had no lesion at all, and that her body did not contain spirilla. But, while we admit the accuracy of this fact, there is ground for thinking that the mother was able to overcome the same spirilla which brought about the death of the offspring. It is true that Saling affirms that a farm hand had beaten the sow, and that 'the death of the foetus could only have been brought about by the injury' (p. 343), but it must be evident that this theory cannot possibly be considered as a well established fact.

It is quite useless to enter here more fully into the details of the question of the '*Silver spirochaetes*', as a great number of scientific men of very great note, amongst whom I may quote Benda,[110] Blaschks,[111] Gierke,[112] Hoffmann,[113] Levaditi (loc. cit.),

Orth,[114] and others, have to a great extent refuted all the arguments of Schulze and Saling.

Amongst other objections taken to the aetiological part played by Schaudinn's spirilla, great capital has been made of the strong resemblance of this organism to other spirochaetes. Thus, *Kiolemenoglou and Cube*[115] affirmed that the spirilla observed by them in fragments of tissues which had nothing to do with syphilis—such, for example, as in ulcerated cancers—could not be distinguished from *Spirochaete pallida*. Schaudinn and Hoffmann, who were able to observe the preparations of these authors, declared, on the other hand, that there was an absolutely distinct difference between the two varieties of organisms.

It is, however, certain that among the large number of spirochaetes now known there are some which resemble very closely that of syphilis. Among these are some of the spirilla of the mouth.

But it is more especially the organism described by Castellani[116] in Yaws, or the framboesia of tropical regions, which presents a very striking analogy to Schaudinn's spirillum. Castellani has called it by the name of *Spirochaete pallidula*, and considers it as the aetiological cause of this disease. This discovery has been confirmed by several observers, amongst whom is Prowazck,[117] who recognized, nevertheless, some morphological differences between the two species. According to him, in the spirillum of Yaws the curves are less steep and more irregular than those of the organism of syphilis; the former spirillum has flattened curves which distinguish it from the latter. I myself have had the opportunity of observing the spirilla of Yaws in Levaditi's films; and I also was able to distinguish the differences between it and that of syphilis.

But in spite of all this, the relationship between the two species is very great. Nevertheless, very far from serving as an argument against the aetiological rôle which is attributed to them, the resemblance would seem to me only to confirm it the more. Here, indeed, we have two different chronic affections whose very close analogy must be clear to every eye, and in both of them we constantly find in the depth of the lesions two different

forms of a spirillum which present an astonishing resemblance to each other. Surely then it is clear that we have here to do with two infective agencies whose relationship is similar to that which we know so well exists between the bacilli of tuberculosis and leprosy.

It should be quite enough to look round at the whole of the bacteriological facts which have been collected on the subject of syphilis, in order to be able to assure oneself that, in spite of the impossibility of cultivating Schaudinn's microbe and of obtaining experimental syphilis by means other than that of inoculation with syphilitic products, it is indeed the *Spirochaete pallida* which is the microbe of syphilis. We should recognize that the description of *Cytoryctes luis* was very useful in stimulating the research which brought about the discovery of the cause of syphilis, and acknowledge the value of the large number of objections raised against the aetiological part of the spirillum. For the refuting of these objections has, in turn, very much increased the importance of this organism.

CHAPTER XII

PRACTICAL APPLICATION OF THE RECOGNITION OF THE ORGANISM OF SYPHILIS

ALTHOUGH the microbiology of syphilis cannot possibly be considered as a closed chapter, it is nevertheless certain that it has now gained possession of certain facts of very great importance, not only from a theoretical point of view, but also because of their practical application. It is perhaps the diagnosis of syphilis which profits most of all from the progress which has been made in its bacteriology.

It is by no means rare to meet cases of venereal disease where it is difficult to be sure of the nature of the infection. Sometimes we have to deal with mixed infection, and at other times with lesions whose characteristics are often very indefinite or are wholly absent. Much valuable time is often lost in doubtful cases by waiting for the secondary manifestations of syphilis, and for this reason anything that can make the diagnosis more clear in these cases should be most favourably accepted.

Soon after Schaudinn's discovery, Hoffmann [118] submitted to bacteriological examination many primary lesions taken as soon as possible after their first appearance. He has more especially studied some whose clinical diagnosis could not have been made very definitely; and he found in six of these cases that where the microscope revealed the presence of *Spirochaete pallida* the subsequent course of the disease justified the diagnosis of syphilis.

After these facts had been confirmed in a very large number of cases, Hoffmann [119] formulated the following opinion: 'The diagnostic value of *Spirochaete pallida* in primary lesions, and, above all, in recent chancres, is of great practical importance, for a clinical diagnosis is very often imperative in these cases.

The presence of these organisms justifies a decisive conclusion, although the negative result of the bacteriological examination has no great importance. In those cases where we have to do with the differentiation of exuding papules, with more or less ulcerated surfaces, or with herpetic erosions which resemble them very closely clinically, the presence of *Spirochaete pallida* is of very definite value. In those cases which are not suspected to be the cause of the glandular enlargement, the result of their puncture according to my method should settle the question.'

In a very detailed and careful work, Roscher[120] gives us many facts which confirm the diagnostic importance of *Spirochaete pallida*. Heller and Lydia Rabinowitsch[121] are rather less encouraging, for they think the search after this organism, from the point of view of practical diagnosis, is not to be recommended. But we must observe that their results were obtained with quite an insufficient number of preparations, and they used exclusively films stained by Giemsa's mixture. Much more positive are the facts given by Danziger,[122] who was able by bacteriological examination to make a diagnosis in five cases of syphilis, where clinical examination was useless: usually he examined insignificant erosions of a very indeterminate nature, sometimes even without a trace of induration. Where the presence of *Spirochaete pallida* showed the syphilitic nature of the lesions, the ultimate course of the disease in every case confirmed the diagnosis.

Besides the case of lesions at the very outset, in which bacteriological examination can be of the very greatest use, the certainty of the presence of Schaudinn's spirilla may again be very useful in those cases where syphilitic lesions are well developed, and which may even be in process of cure, although they do not present sufficient clinical characteristics for diagnosis. It is exceedingly interesting in this connexion to note the cases studied by Danziger; he had to do with a case which entered the hospital for the cure of leucorrhoea. On examining the patient, a flat ulceration, very little indurated, was found on one labium minus; the patient was ignorant of its presence. Some short time afterwards typical spirochaetes were found in this ulcera-

tion, which was by that time completely healed. Later on the patient had a papular eruption of the trunk and extremities, which confirmed the diagnosis.

It is thus clearly shown that in all cases, easy though they may be to distinguish by ordinary clinical examination, a microbiological diagnosis is indispensable. Thanks to the use of the ultra-microscope, this work is now rendered rapid and easy. Indeed every clinic should introduce this apparatus, which should be used by some one who has experience in searching for spirilla and distinguishing their different species. Very often a glance through the microscope or an examination of a very few minutes' duration will suffice to give a most definite opinion.

With regard to prognosis, although according to some observers the disappearance of spirilla should indicate a cure, a great number of facts in our possession contradict this assertion; for we may often find that the cure is of a most temporary character, and almost at the very moment that they disappear the spirilla may reappear afresh.

Hoffmann (loc. cit.) insists on the utility of bacteriological diagnosis in the treatment and prophylaxis of syphilis; for once the lesion has been diagnosed at its very beginning, during that phase which, so far as clinical diagnosis shows, is indefinite, we can at once proceed to excise the lesion, and this may, perhaps, facilitate the cure. This accurate diagnosis must of course be of enormous value in prophylaxis, by eliminating from the beginning an important source of infection.

Thus we see, by all the facts which have been quoted in this article, that in the light of recent events, up to the present time, the microbiology of syphilis *has already rendered enormous service to humanity.*

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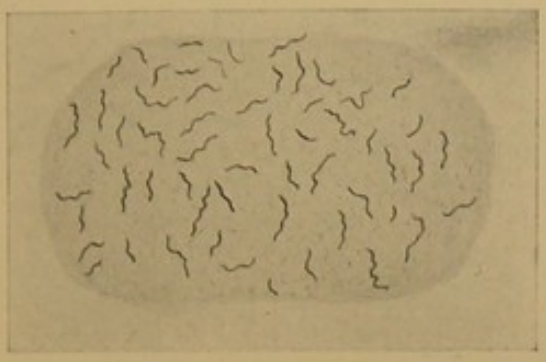
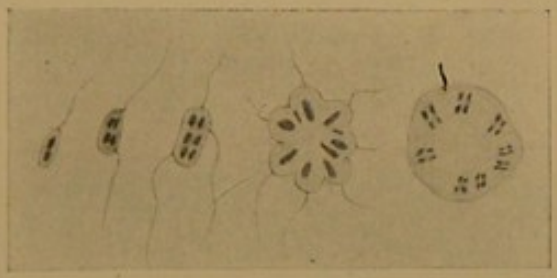
115. Ibid., 1905, p. 1275.
116. British Medical Journal, November 11, 1905, p. 1280; November 18, 1905, p. 1330; November 25, p. 1430; Deutsche medecin. Wochenschrift, 1906, p. 132.
117. Arbeiten a. d. k. Gesundheitsamte, 1907, vol. xxvi, p. 28.
118. Deutsche medicin. Wochenschrift, 1905, No. 43, p. 1712.
119. Dermatologische Zeitschrift, vol. xii, p. 224.
120. Berliner klinische Wochenschrift, 1906, No. 28.
121. Medicinische Klinik, 1906, No. 28.
122. Berliner klin. Wochenschrift, 1906, p. 1365.

Plate V

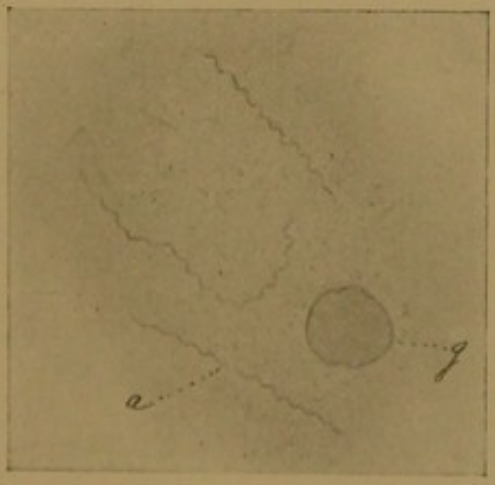


a. Donne's '*Vibrio lineola.*' 1834.

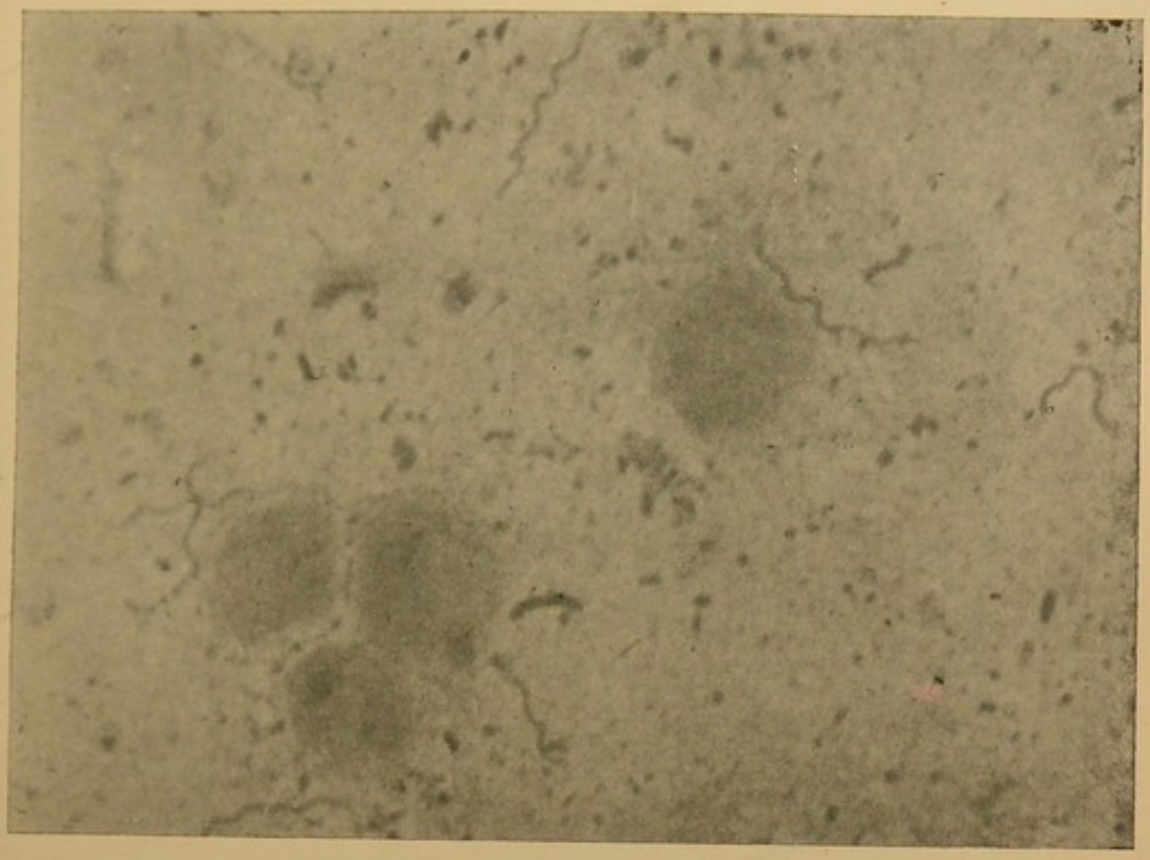
b. Stassano's parasite of syphilis. 1901.



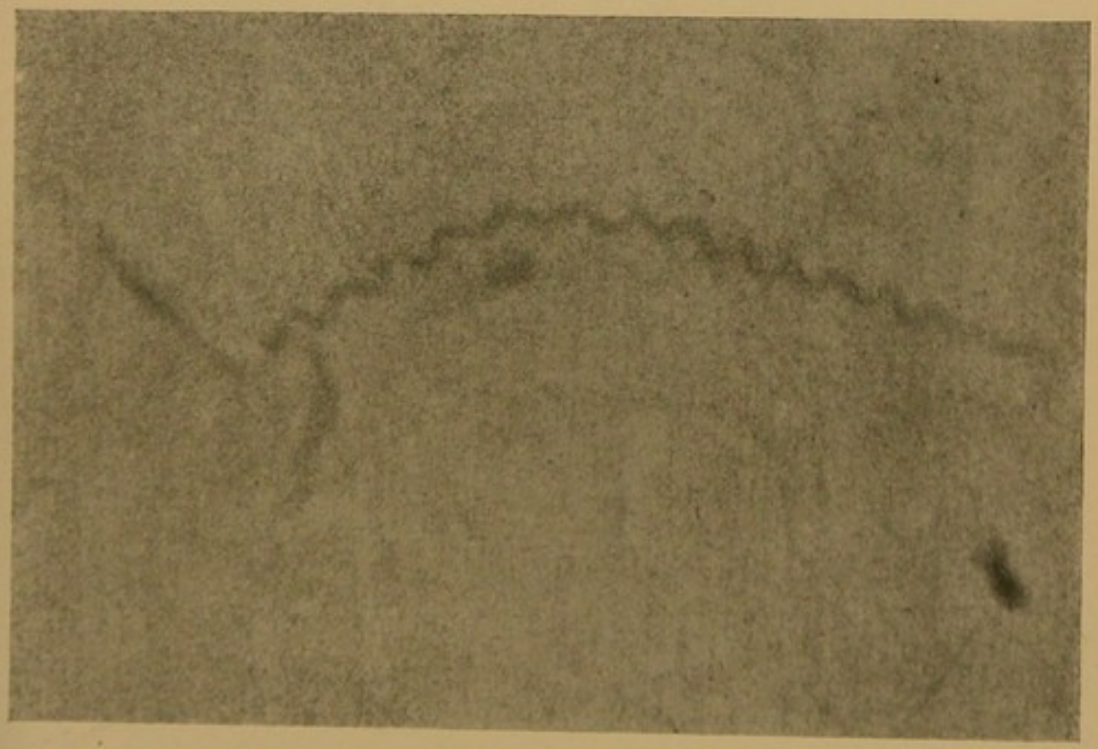
c. Cytoryctes luis, after Siegel. 1902.



d. Spirochaete pallida from a primary lesion in a chimpanzee.
a Spirillum undergoing division; *g* red corpuscles. (Giemsa's method.)



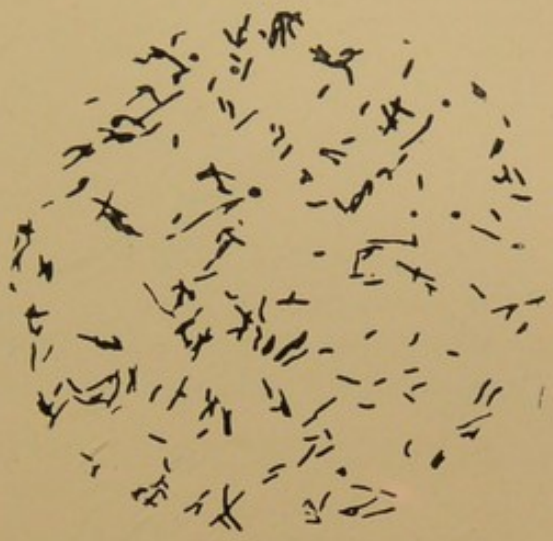
Spirochaete refringens. Photomicrograph of a film preparation. Magn. 7000 diameters.



Spirochaete pallida. Photomicrograph from the fluid of a vesicle. Magn. 7000 diameters.
From photomicrographs by Staff-Surgeon Richards, R.N.

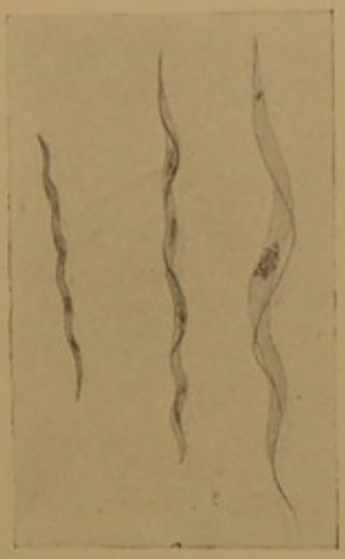
Spirochaete pallida in the liver of a new-born child.
(Stained by Levaditi's method.)

a. One mononuclear and two polynuclear leucocytes, all containing the spirillum. After one of Dr. Gierke's preparations, stained by Levaditi's method. 1907.

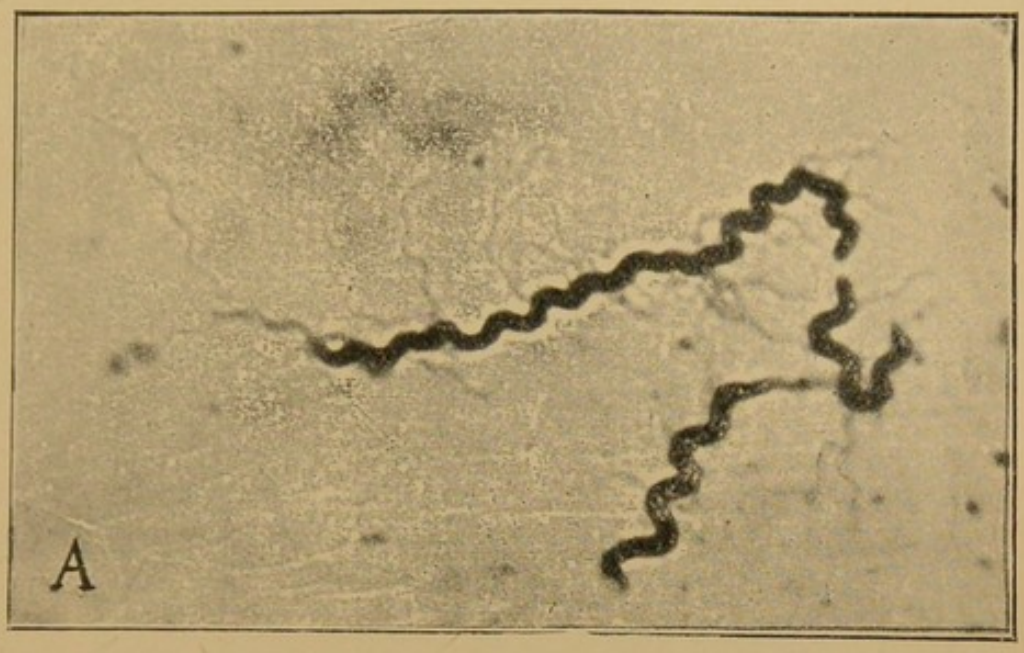


b. *Cholera vibrios* in culture.

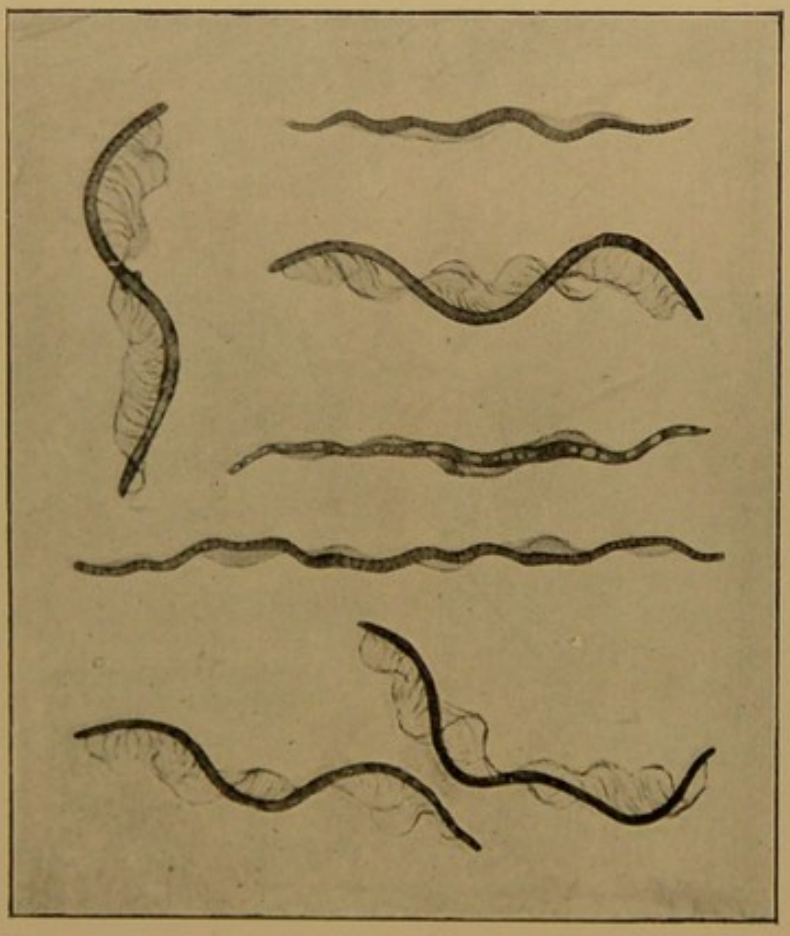
c. Vibrio of cholera in the form of a flexible spirochaete.



d. Trypanosomes passing into the shape of a spirochaete. (After Schaudinn, 1905.)



a. Spirochaete Gallinarum, with *Vibratile Cilia.* (After Borrel.)

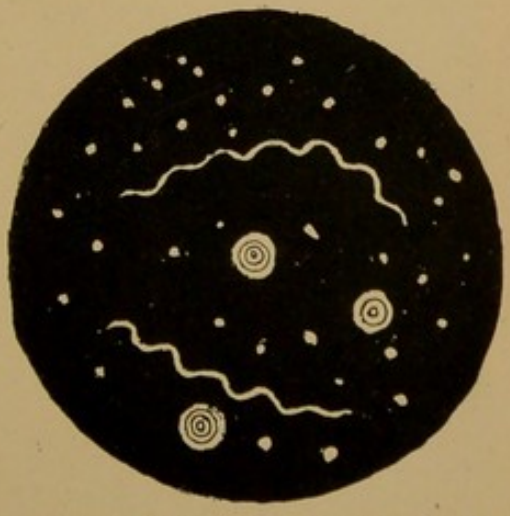


b. Spirochaete Balbiani. (After Borrel.)

Plate X

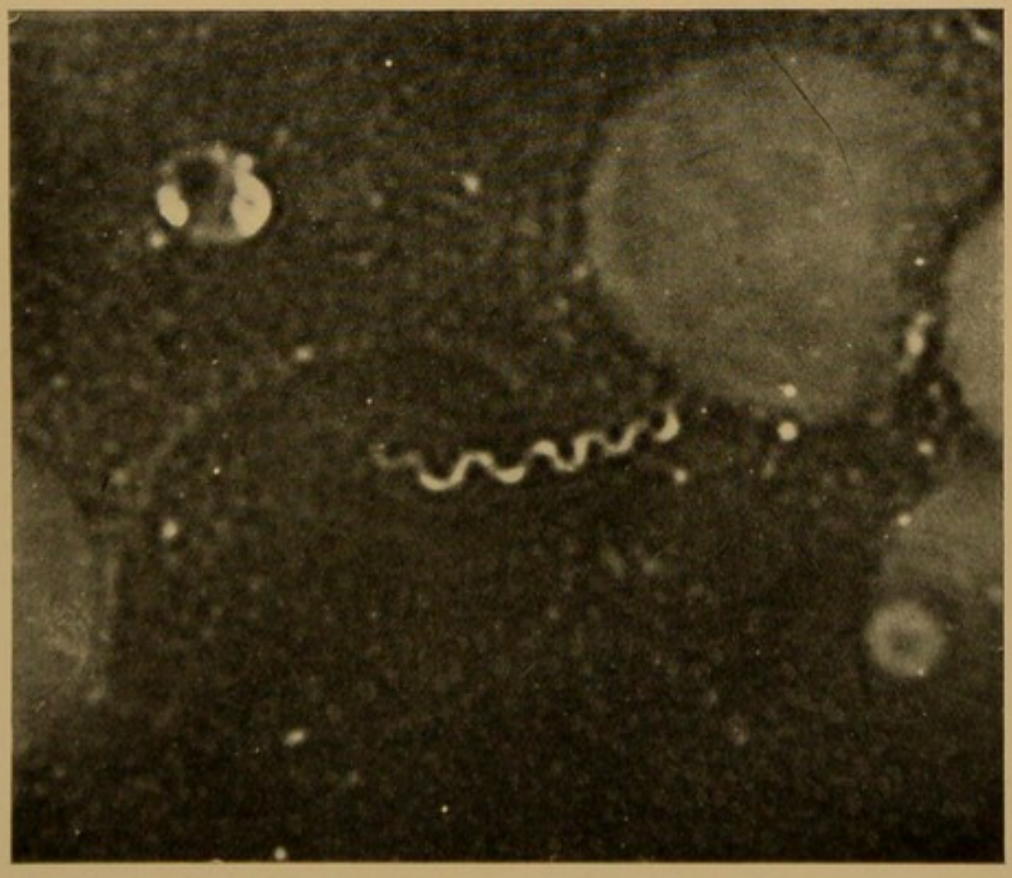
a. Trypanosoma Gambiense; from rat's blood film preparation. Note undulant membrane.

b. Spirochaete pallida, on dark field in Reichert's ultra-microscope.



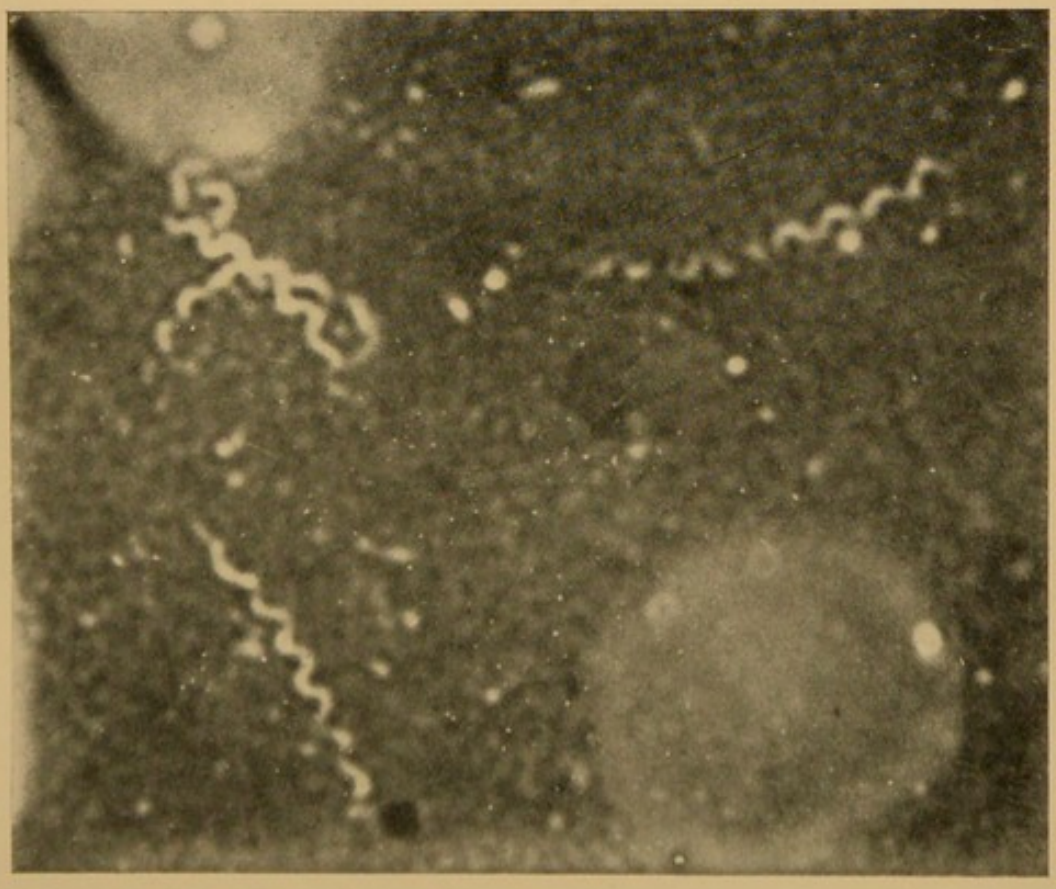
c. Spirochaete refringens, on dark field in Reichert's ultra-microscope.

a. *Spirochaete pallida*. Photomicrograph under high magnification. Photographed by reflected light.



b. A specimen of *Spirochaete pallida*. Photomicrograph. Magn. 4500 diameters. Photographed by raflected light. The circular body above the *Spirochaete* is a red blood corpuscle.

a. Spirochaete pallida.



b. Spirochaete pallida.

Plate XIII.

Spirochaete pallida from a condyloma where the organism was unusually plentiful. Film preparation stained by Leishman's method. × 1000.

(*From a film kindly lent by Lieut.-Col. W. B. Leishman, R.A.M.C.*)

(*A. M. Kelley, del.*)

Plate XIII.

Spirochaete pallida from a condyloma where the organism was unusually plentiful. Film preparation stained by Leishman's method. × 1000.

(*From a film kindly lent by Lieut.-Col. W. B. Leishman, R.A.M.C.*)

(*A. M. Kelley, del.*)

PLATE XIII.

Spirochaete pallida from a condyloma where the organism was unusually plentiful. Film preparation stained by Leishman's method. × 1000.

(From a film kindly lent by Lieut.-Col. W. B. Leishman, R.A.M.C.)

(A. M. Kelley, del.)

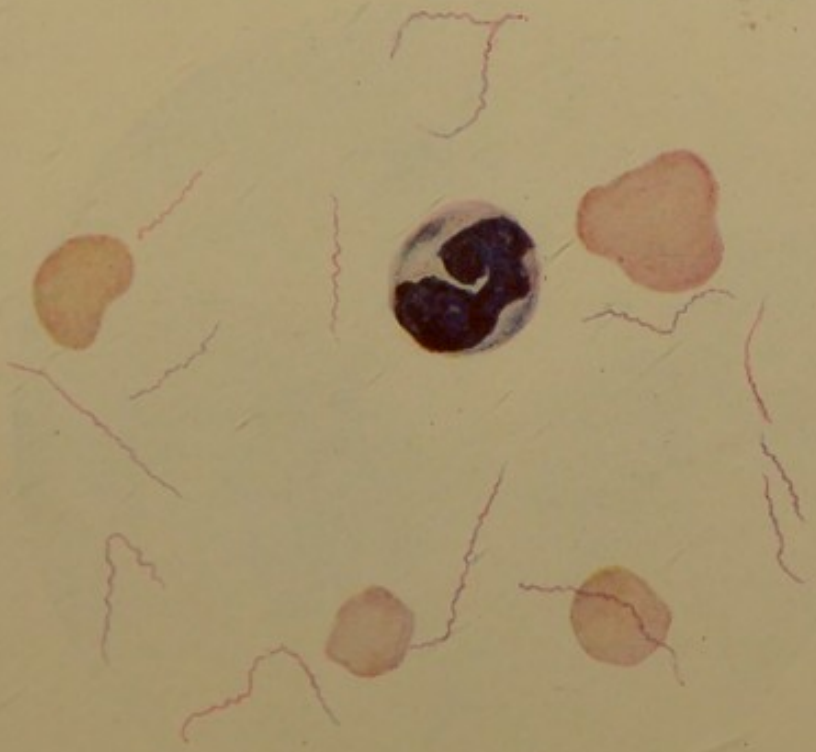


Plate XIV.

Spirochaete pallida in the spleen of a macerated foetus, stained by Levaditi's original method. × 1000.

(*From a specimen kindly lent by Lieut.-Col. W. B. Leishman, R.A.M.C.*)

(*A. M. Kelley, del.*)

PLATE XIV.

Spirochaete pallida in the spleen of a macerated foetus, stained by Levaditi's original method. × 1000.

(From a specimen kindly lent by Lieut.-Col. W. B. Leishman, R.A.M.C.)

(A. M. Kelley, del.)

Plate XV.

Spirochaete pallida from the lung of the same case shown in the preceding plate. Stained by Levaditi's original method. × 1000.

(*From a specimen kindly lent by Lieut.-Col. W. B. Leishman, R.A.M.C.*)

(*A. M. Kelley, del.*)

Plate XV.

Spirochaete pallida from the lung of the same case shown in the preceding plate. Stained by Levaditi's original method. × 1000.

(From a specimen kindly lent by Lieut.-Col. W. B. Leishman, R.A.M.C.)

(A. M. Kelley, del.)

Plate XVI.

Spirochaete pallida from the macerated spleen of a syphilitic infant, stained by Levaditi's method and counterstained with polychrome methylene blue. × 1000.

(*From a specimen of Lieut.-Col. W. B. Leishman, R.A.M.C.*)

(*A. M. Kelley, del.*)

PLATE XVI.

Spirochaete pallida from the macerated spleen of a syphilitic infant, stained by Levaditi's method and counterstained with polychrome methylene blue. × 1000.

(From a specimen of Lieut.-Col. W. B. Leishman, R.A.M.C.)

(A. M. Kelley, del.)

Plate XVII.

Drawing of *Macacus cynomolgus*, showing typical primary lesions over eyebrows, six weeks after inoculation with syphilitic products.

PLATE XVII.

Drawing of *Macacus cynomolgus*, showing typical primary lesions over eyebrows, six weeks after inoculation with syphilitic products.