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T H E        S P E C I F I C        N A T U R E

of

T R E P O N E M A        P A L L I D U M .

by

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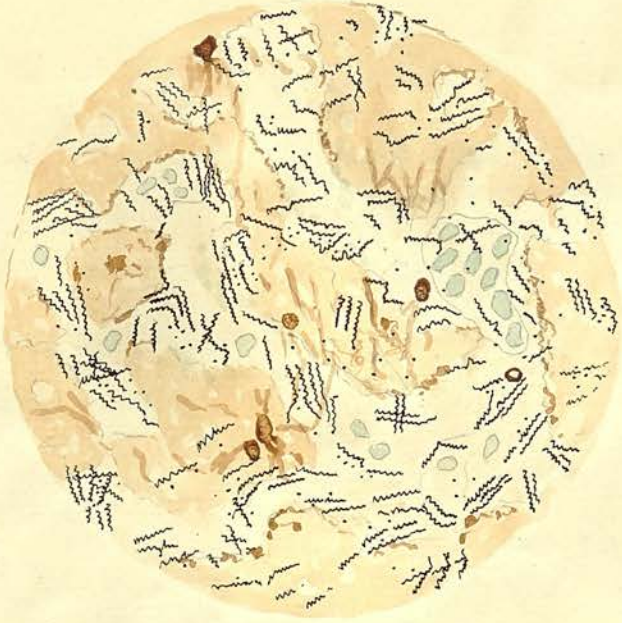


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C H A P T E R S.

1. Introduction.
  2. Historical.
  3. Inoculation of animals.
  4. Discovery of *Treponema pallidum*.
  5. Morphology.
  6. Cultures.
  7. Bacteriological Technique.
    - (a) Smear preparations.
    - (b) Sections.
  8. Verity of Silver Spirochaetes.
  9. Differential diagnosis.
  10. Presence in syphilitic lesions.
  11. Control experiments.
  12. Distribution in syphilitic lesions.
  13. Influence of mercurial treatment.
  14. Natural History.
  15. Conclusions.
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X 1000.

TREPONEMA PALLIDUM

IN

LIVER OF SYPHILITIC FOETUS.

from own preparation

stained by Levaditi's method.

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## THE MICRO-ORGANISM OF SYPHILIS.

*roduction* - On the 23rd April 1905, appeared the memoir in which Schaudinn and Hoffmann announced that they had discovered in syphilitic lesions, a micro-organism which they then called the Spirochaeta pallida<sup>(1)</sup>, but to which they subsequently gave the name of Spirochona pallidum, and finally of Treponema pallidum.

Since Schaudinn's discovery an astonishingly large number of articles and papers have appeared on the subject, which bear eloquent testimony to the great amount of interest which the history of this organism has elicited.

We propose in this thesis to give a critical survey and digest of our present knowledge of the Treponema pallidum.

After a brief historical review of the whole subject, we propose to deal with the morphological and other characters of the Treponema, laying special stress on the factors which have enabled us to consider this micro-organism as being the true causal agent of syphilis.

As soon as the important part played by micro-organisms in the pathology and causation of diseases had become an established fact, syphilis was one of the many diseases to which bacteriologists immediately directed their attention. Everything in the study of the disease, its contagious nature, its clinical and anatomic-pathological analogies to other bacterial diseases, its general evolution, all tended to prove that it was probably a germ disease. In

spite

(1). Spirochaeta being a Latin feminine, the adjective is pallida, while it is pallidum in the case of Spirochona and Treponema, which are Greek neuters. Spirochaeta pallida is the name by which the organism is usually known, but as Lévy-Bing remarks, "Treponema pallidum", being Schaudinn's latest nomenclature, should be used instead. It is the term which we shall employ in this thesis, in speaking of the organism of syphilis.

spite however, of the most rigid bacteriological technique, all attempts at discovering the causal organism failed. It is true that a large number of organisms, cocci, bacilli, spirilla amoebae, protozoa, trypanosomata, in fact nearly every known form of micro-organism, were found and described, to which their discoverers attributed ~~the causation of the disease~~. But none of these have ever withstood the test of further and more thorough researches. Most of the organisms described, are now buried in the limbo of a forgotten past, from which we do not intend to resuscitate them. We shall confine ourselves to mentioning a few of those which at the time excited a certain amount of interest, and may <sup>have</sup> achieved some ephemeral success in being considered the specific germ of ~~the disease~~.

Historical  
The first mention made of a syphilitic microbe was that described by Donne in 1837, under the name of Vibrio lineola. This organism was of the nature of a spirochaete, and was probably no other than the Spirochaeta refringens, of which we shall speak later. In 1884, Lustgarten described an organism, which for a long time obtained a large measure of success. He had found it on 16 different occasions, in chancres and gummata. In 1885, Babes confirmed these results, and considered Lustgarten's bacillus as diagnostic of syphilis. It was an acid-fast bacillus resembling Koch's bacillus. It is now believed that this organism was probably the harmless smegma bacillus.

During the last 12 years Von Neissen with an uncommon tenacity of purpose, has maintained the specificity of an organism which he discovered in 1896; it belongs to the group of pseudo-diphtheritic bacilli, and is essentially polymorphous. By means of cultures supplied by Neissen himself, Neisser in Java, failed to inoculate the disease <sup>into</sup> monkeys.

Another diphtheria-like bacillus was described by Paulsen in 1902. Max-Joseph and Piorkowsky have also described a similar bacterium. They obtained it in cultures by placing fresh human placenta, collected aseptically, in a medium to which they added syphilitic sperm obtained during the secondary period. <sup>In</sup> 1901 Lisle and Jullien obtained an organism from the blood, which they easily succeeded in cultivating on the ordinary media, and which they considered as being

specific

II  
Historical

specific, since it was agglutinated by the serum of syphilitics.

The least objections that can be brought forward against the specific nature of these organisms, is the fact that they are not found in all syphilitic lesions; they are moreover obtained by methods which do not entirely exclude the possibility of secondary infection.

These continual failures had naturally led to a spirit of discouragement among bacteriologists; experiments and researches on the subject were for a time almost abandoned; it was thought by some that if there really existed a germ, it was probably ultra-microscopic; while others seemed to think that the disease was probably not of microbic origin, but due to something in the nature of an enzyme. Conjectures and theories took the place of experimental observation. After years of barren and fruitless research, Bacteriology, on which such ~~past~~ hopes had been based of solving the problem of the pathology of a disease which had for so long baffled all the efforts of science, had also to acknowledge itself vanquished.

inoculation of animals.

One great factor which seriously hindered experimental observation and research, was the fact that it had been found impossible to inoculate the disease into animals. Many attempts were made at various times to inoculate syphilis ~~to animals~~, birds, cold-blooded animals, rabbit, guinea-pig, mouse, dog, cat, goat, sheep, hare, cow, pig, and the inferior monkeys, but none of these proved successful.

III  
Inoculation  
of  
Animals

Hunter in 1789, and Ricord in 1833, had already made attempts in this direction but failed. We shall confine ourselves to giving a short description of a few of the rare cases where a certain measure of success seemed to have been achieved.

In 1878, Klebs announced that he had succeeded in inoculating syphilis ~~to~~ a monkey by injecting a fragment of a hard chancre beneath the animal's skin. In spite of his great reputation, no credence was attached to Kleb's statement, the general opinion being that he had merely succeeded in inoculating tuberculosis.

Four years later, in 1882, ~~Martin~~<sup>neau</sup> and Hamie presented to the Société Médicale des Hôpitaux of Paris, a monkey named Fracastor, after the author of

the

the poem on syphilis, which they had successfully inoculated with the disease, and in which later they described the presence of secondary lesions.

In 1881, Paul Hansell made some experiments on the rabbit, which he inoculated with products from secondary syphilitic lesions. After a certain incubation period, the animal presented lesions which histologically seemed of syphilitic nature.

Sperck in 1883, after a large number of failures among several species of monkeys, stated that he had been successful in inoculating the disease to a macacus.

Maurice Nicolle in 1893, after a large number of unsuccessful attempts, succeeded in producing in a macacus, syphilitic papules at the points of inoculation, but without causing a general infection. His brother, Charles Nicolle, in 1899, obtained similar results among 3 macaques (M. sinicus), after incubation periods varying from 15-19 days; in one case only was there any lymphatic enlargement.

None of these experiments had however destroyed the dogma of the non-inoculability of *animals* to syphilis ~~to animals~~. Journier in his well known treatise (Traitement de la Syphilis, 1899). wrote "Syphilis is a disease exclusively proper to the human species". This indeed was almost the universal opinion held on the subject when in July 1903, Metchnikoff and Roux made their classical communication to the Academy of Medicine in Paris, and definitely proved that syphilis could be transmitted to the anthropoid apes. We had the occasion whilst working at the Pasteur Institute, to examine some of the inoculated apes, and can testify as to the specific character of the sores. They subsequently showed that the disease could also be communicated, though in a less degree, to the inferior catarrhini. Their results have since been amply confirmed by Neisser, Lassar, and others, and have now passed into the domain of established facts.

The lesions are, as one might expect, much more typical of the human disease in the case of the anthropoid apes, than they are in the case of the

inferior



inferior catarrhini. The former are not only very similar to man in their general structure and anatomy, but there is also a very close blood relationship. Their blood serum is very similar if not identical to the human blood serum, to such an extent, that of all sera, it is only the sera of the anthropoid apes, which do not exercise any destructive influence on the human red cells. Their haemolytic properties are absolutely identical. Grunbaum (Lancet, January 18th, 1902), found that the serum of animals injected with human blood, gives a precipitate, not only with this blood, but also with that of the anthropoid apes, the chimpanzee, gorilla and ourang-outang. "It was impossible to distinguish this precipitate either by the quality or the quantity with that obtained from human blood".

I have entered with some detail into the subject of the experimental inoculation of <sup>animals with</sup> the disease ~~to animals~~, because Metchnikoff and Roux's discovery opened up a new era in the study of syphilis. Fresh impetus was given to the study of the disease, great interest was naturally once more aroused in the search for the causal agent, as a result of which, numerous investigations were again made in this direction.

In 1905, a paper was read in Berlin by Siegel, in which he stated that he had found in the blood and exudate of syphilitics, a minute ciliated protozoa, Cytorrhycles luis, which he considered to be the specific germ of the disease. This communication created at the time a great deal of interest, especially in German medical circles, where it was held by many that the true microbe of syphilis had at last been found. The inoculation of syphilitic products <sup>to</sup> animals gave rise to a proliferation in their blood and tissues of the cytorrhycles luis. The inoculation of the pulp of these infected organs <sup>to</sup> healthy animals produced the same symptoms in the latter.

It is somewhat difficult to state the part played, if any, by this organism in syphilis. (It may possibly be some involution form in the life history of Treponema pallidum.) The general opinion however, is that it bears no causal relation to the disease, as it is stated that it has also been found outside of syphilis.

The great interest in the discovery of this organism lies in the fact that it led to the discovery

IV  
Discovery of Treponema  
Pallidum

covery of  
truma pallidum

of

of the Treponema pallidum. It was in trying to verify Siegel's results that Schaudinn was struck with the large number of a small spiral corkscrew shaped organism which he met with in all the syphilitic products he examined. In his first memoir in connection with Hoffman, he described two organisms, a larger easily stained spirillum to which he gave the name of Spirochaeta refringens, and a smaller feebly stained spirillum to which he gave the name Spirochaeta pallida, and which Hoffman and himself thought might possibly be the causal agent of syphilis.

Schaudinn's and Hoffman's observations have since been confirmed by observers in all parts of the world. During the last two years the links in the chain of evidence have gradually been forged, and little doubt is now left that science has at last succeeded in laying her hands on the true specific germ of syphilis, which had for so long eluded her grasp.

It is true that the organism has not as yet fulfilled all of Koch's essential conditions, the tripod of ideal certainty as regards the specificity of any organism.

1. It should be found in all undoubted cases of the disease, either in the adult form, or in some involution form.
2. It should be obtained in pure culture;
3. The inoculation of these pure cultures should determine in living beings a disease similar to that from which the microbe is obtained.

To these we may add a fourth, viz.; That it should not be found outside that particular disease.

It is easy to understand that these proofs at times are of unequal value, and that we may under certain conditions ascribe to an organism a pathogenic relation to a disease, without it, absolutely fulfilling all these requirements. <sup>e.g. proteus, sp. malariae, Hansen's bacillus of leprosy.</sup> It is nevertheless towards the realisation of this end that all bacteriological enquiries should tend. We shall see that in the case of syphilis, this desideratum has not yet been attained.

Before proceeding any further, we may pause and enquire why the organism of syphilis was not discovered long ago, why was so much time required to arrive at what apparently seems such a simple solution? Bacteriological technique was sufficiently advanced to have enabled us to do so. The Treponema pallidum has it is true little affinity for most stains. One of the

best stains for demonstrating it, is Giemsa's stain; it can however be equally stained and demonstrated by such substances as fuchsine, methyl violet, gentian, stains commonly employed in laboratories, and which must have been used in the search for it. It had indeed been seen by Bordet and Gengou in 1903 in a scraping from a chancre; they had not however attributed to it any etiological relation to the disease, as they had never succeeded again in seeing it.

Many a discovery is but the resurrection of facts already known or something previously seen. It however often requires a fortuitous combination of circumstances before a discovery can make any headway. Schaudinn's discovery arrived at a time when great interest had just been revived in the study of syphilis, and there were many who were only too eager to prove or disprove the specific nature of the organism he described. When once success had been attained, astonishment was expressed that it had been so long in coming. "Rien n'est plus claire que ce qu'on a trouvé hier", says Biot, "et rien n'est plus difficile à voir que ce qu'on trouvera demain".

*Morphology*

The organism described by Schaudinn is a very fine corkscrew-shaped spirillum, the thickness of which is never more than  $\frac{1}{2}\mu$ , and sometimes cannot even be measured. Its length varies between  $4\mu$  and  $14\mu$  according to the number of spirals present. The spirals are very variable in number, the average being 8-12; as many as 30 have however been counted (Betarelli and Volpino). These spirals are very fine, regular, set closely together almost at an acute angle, and are always present even though the organism be at rest or dead. The extremities terminate in very fine endings, so fine indeed that it is almost impossible to state exactly where they end (Milian). It is motile, and moves by rotation on its longitudinal axis, as well as by flexion of its body. Very fine flagellae one at each extremity have been described (Hexheimer and Schaudinn). It is apparently a very frail organism. "The syphilitic virus" says Metchnikoff, "remains virulent only for a few hours outside the organism; drying destroys its virulence. When kept at a low temperature it soon ceases to be

active

*Morphology*

active. Heated to 43°C the virus no longer transmits syphilis."

*Several observers state that the serum of syphilis agglutinates the Treponema, but this requires confirmation (1)*

Cultures  
 All attempts at cultivating the organism have hitherto failed. Levadite and Mc Intosh however state (Annales de l'Institut Pasteur, Oct., 1907) that they have succeeded in cultivating the Treponema by means of the collodion sac method. The material which was obtained from the primary lesion of a rhesus monkey was enclosed in a collodion sac and placed in the peritomal cavity of a macacus cynomolgus. Subsequent passages were effected by successive transferences of the material into collodion sacs, which were inserted into the peritomal cavity of rabbits. Up to the time of writing, twelve passages had been effected, and fifty-nine sacs successfully inoculated during a period of 74 days. In spite of the progressive dilution of the virulent material, the late cultures were found to be richer in treponemata than the earlier. Various other bacteria were also found in the sacs, among which was the streptococcus pyogenes. When tested on monkeys and on a chimpanzee, the parasites recovered from the sacs, were found to have completely lost their virulence, but their morphological, staining, and biological characters were very similar to those of the Treponema. The authors therefore conclude that they have succeeded in cultivating a genuine though non-virulent form of the Treponema pallidum.

Levadite had previously succeeded in cultivating some allied spirilla, viz., the Sp. gallinarum, spirillum of tick fever, and the Spirochaeta refringens.

Bertarelli also states that he has succeeded in cultivating the Treponema in the rabbit's eye through five generations; inoculation of these cultures into the monkey produced syphilis.

These results however require confirmation before they can be finally accepted.

Microscopical Technique  
 The organism may be examined in the living state by means of fresh preparations in saline solution, and their movements watched. Hoffman and Hale state that experienced observers will find this method the best and most useful. To differentiate such living spirochaetes however, is a matter of extreme difficulty and according to Schaudinn

impossible

(1) Niesser in gave has shown from a large number of experiments on monkeys that the serum reaction is useful in diagnosis and offers a test of complete cure. (Jan. 1908)

impossible.

It is by means of smear preparations stained by Giemsa or sections impregnated with silver nitrate, that the best results have hitherto been obtained.

In making smear preparations, the following precautions should be taken in order to obtain satisfactory results.

Smear Preparations  
The sore, cutaneous lesion or mucous patch from which the preparation is to be made, should be thoroughly cleansed; this can easily be done by means of a piece of sterilized gauze or lint soaked in ~~sterilized~~ distilled water. We should also bear in mind that in the chancre, the syphilitic lesion is localised in the dermis, where sections have shown that the Treponema is most abundant. The nearer we get to the surface of the epidermis, the scarcer are the organisms; they are not usually found beyond the stratum granulosum. (Ehrman) Should the epidermis be injured, the surface of the papule or chancre becomes the seat of a secondary infection; the consequent presence of cellular detritus, of leucocytes, albuminous precipitate, the large number of organisms of suppuration, and lastly the presence of large spirochaetes, which are not uncommon in ulcerative lesions, all tend to render the search for the treponema in these cases extremely difficult. To avoid this, we should neglect the superficial layers of the lesion and try to obtain our material from the deeper layers, and in the case of the chancre especially from the papillary layer of the dermis (Lévy-Bing). We can usually do this after a preliminary washing, by scraping the lesion with a needle or fine knife or *histology*, until a serous exudate appears, care being taken not to cause any bleeding. In the case of the roseolar rash, a small blister should be applied to a macule; the serous exudate as well as a scraping from the denuded surface should then be examined. It seems however that it is with the greatest difficulty that the treponema can be demonstrated in this lesion. We have only once succeeded in seeing it.

In the case of a crustaceous papule, the scab should first be removed, and a scraping made from the denuded surface.

In all cases, the films should be made as

thin

thin as possible.

The stain first used by Schaudinn to demonstrate the Treponema was Giemsa's original stain. It is essentially an aqueous solution of azure, methylene blue, and eosine. After fixation in absolute alcohol for about 10 minutes, the preparation was placed in this solution for 16-24 hours.

It was however soon found that equally good results could be obtained with much more rapidity by using a solution of Giemsa in methyl alcohol instead of water. This stain is now found all prepared on the market.<sup>(1)</sup> The solution before use is diluted with 3-4 times its volume of distilled water, <sup>to which a few drops of a 1 per 1000 solution of Potassium carbonate has been added</sup>. The treponemata are already stained after 15 minutes, but the best time is about an hour. The preparations <sup>preferably</sup> should be placed face downwards in the solution on account of the large amount of deposit which is formed; after removal ~~it~~ <sup>they</sup> should be thoroughly washed in water.

Hoffmann and Hale recommend fixation by osmic acid vapour instead of alcohol. Five cubic centimetres of a 1% osmic acid solution is placed in a small watch glass, and to this ten drops of glacial acetic acid is added, and some clean cover-slips are then exposed to the osmic acid vapour over the watch glass for 2 minutes. The secretion to be examined is then applied to the cover-slips on the exposed surface, and again exposed to the osmic acid vapour for 1-2 minutes <sup>and washed in a very dilute solution of Potassium Permanganate for 10 minutes</sup> before being stained. By this method thicker smears can be examined and the spirals and extremities of the Treponema are shown with more detail.

It was thought for a comparatively long time that Giemsa's stain was the only stain for which the treponema had any affinity. It has now been shown that satisfactory results can be obtained with a large number of other stains, e.g., all the various derivatives of the Romanowsky method, Marino, Leishman, Jenner, Laveran, etc., various solutions of gentian violet, and carbol fuchsine (hot), <sup>and even methylene blue (heated)</sup> (2)

Giemsa's method is the only one of which we have had any practical experience. By this method the treponemata are stained a light red, while the large spirochaetes are stained blue.

We shall now proceed to the study of sections. The silver impregnation method is the only one by which

the

(1) Giemsa'sche Lösung für die Romanowsky Färbung, Dr. Grüber et Cie, Leipzig.

(2) See also Addendum

Sections

the organism can be demonstrated in the tissues. This method has greatly advanced our knowledge of the distribution of the treponema, and its etiological relation to the disease.

In smear preparations it is uncommon to see more than one <sup>two</sup> treponemata under ~~one~~ field of the microscope, while we may often examine dozens of fields without coming across a single specimen. On the other hand, in silver preparation, it is by hundreds that they are seen ~~under~~ every field; at times so numerous are they that it is impossible to count them.

There are several silver methods in use, the best known being that of Levaditi. It is based on the same principle as ~~Ramon~~ <sup>Ramon</sup> ~~of~~ <sup>of</sup> Cajal's stain for nerve fibrils. The following is a short description of the method:-

1. Fixation of fragments not more than 1 mm thick in 10% formol for 24 hours.
2. Wash and harden in alcohol at 96° for 24 hours.
3. Wash in distilled water for a few minutes until the fragments have fallen to the bottom of the recipient.
4. Impregnate with silver nitrate (1½-3%) at 38° C for 3-5 days.
5. Wash in distilled water and reduce with the following-
  - Acid pyrogallic. . . . . 2%-4%
  - Formol. . . . . 5cc.
  - Distilled water . . . . . 100 cc
6. Wash in distilled water, *dehydrate* in alcohol, xylol, paraffin, and then <sup>cut</sup> sections <sup>of</sup> 5 μ maximum thickness.

The preparation may be counterstained by Giemsa, but this is unnecessary.

~~Mandriélian~~ in connection with Levaditi has modified the method by using pyridin, which allows the silver salts to penetrate more rapidly into the tissues and so reduces the time required for impregnation. It appears that a larger number of treponemata can be brought into view by this method.

The treponemata are stained black on a yellow background. *When counterstained, the nuclei of cells appear blue, while the connective tissue is stained green.*

ity of silver  
spirochaetes

The specific character of these silver spirochaetes has however been denied by several authorities. Schulze (Berlin Klin. Woch. Sept. '06)

VIII . Verity of Silver Spirochaetes.

states

states that until Levaditi introduced his silver impregnation method, the find of the spirochaetes were too small to justify drawing conclusions. He considers that the employment of strong alcohol without preliminary dilution in the silver method, is liable to contract the tissues and to tear the nerve fibres. The staining follows irregularly. He carried out some experiments with the cornea of a healthy rabbit, and when he injected some street mud, which produced an inflammatory reaction, he was able to find a large number of silver spirals, which were extremely like treponemata. He claims that the treponemata demonstrated in tissues are not treponemata at all, but are only broken off nerve fibres, fibres of connective tissue, etc., which have been impregnated with silver.

Friedenthal in the same journal employs the same arguments. He claims that the so-called spirochaetes seen in silver preparations are not necessarily nerve, elastic or other fibres; they can also simply be metallic deposits. Under all conditions, he states they are not parasites.

We have had the occasion to examine a good many preparations stained by Levaditi's method, and find it difficult to believe that such can possibly be the case. Mc Lennan (Brit. Med. Journ., Nov. 23, 1907,) advances the following points as substantiating the verity of silver spirochaetes.

1. The similarity of silver spirochaetes to nerve fibrils, etc., is merely apparent. The spirochaetes are found where no nerve fibrils exist, for example, granulation tissue, Wharton's jelly, and above all in the blood.
2. The increased size of the silver spirochaetes compared with those stained, is due to the fact that the silver spirochaetes are not stained with silver, but are coated with particles of that metal, and further all other organisms so treated are <sup>similarly</sup> ~~so~~ enlarged (Orth).
3. Nerve fibrils are <sup>not</sup> distributed in the tissues as are the silver spirochaetes.
4. Acetic acid, soda and salt solutions have no obliterative action upon silvered nerves or elastic fibres, but they cause the immediate disappearance of silver spirochaetes (Bab).



5. Silver spirochaetes are not found in non-syphilitic tissues.
6. Silver spirochaetes have been shown in sections of tissue from which previously stainable spirochaetes had been expressed.
7. Silver spirochaetes have been affirmed to be maceration products, yet syphilis gives rise to sclerosis—anything but maceration.
8. Successful inoculation experiments have been made with spirochaete-infiltrated tissue, and the resulting lesion has revealed the spirochaetes by the smear as well as by the silver method. (Bertarelli, Benda, Thomasczewski, and many others).

The attempts to obtain these smears leads to the mechanical destruction of cells and nuclei, and in this way all sorts of threads, granules, and other detritus are found which may give rise to difficulty in searching for the treponemata.

Filaments of fibrin, of mucus, or chromatin, or fibrils of connective tissue may in certain cases simulate very closely the undulating and fine characteristics of the treponema. We have occasionally found great difficulty in distinguishing between them. The filaments derived from most of these histological debris usually end in a ~~thickened~~ <sup>blunt</sup> extremity, or else terminate in the nucleus from which they have arisen; their extremities are often sharply broken off, and do not present the fine ending of the treponema.

In sections, as previously stated, fibrin, elastic and especially nerve fibrils may lead to error. Buschke and Fisher state that the most expert may sometimes be deceived.

The second cause of error <sup>is</sup> ~~are~~ due to the presence of spirochaetes which bear a more or less close resemblance to the *Treponema pallidum*. The most common of these organisms is the *Spirochaeta refringens*. This is a larger organism which takes the stain much more readily than the *Treponema pallidum*. The spirals are ~~less~~ <sup>fewer</sup> in number and more undulating, and its extremities are usually blunt. It is usually stained blue by the different stains derived from the Romanowsky method, while the treponema is usually of a rosy tint.

If the two are seen side by side there

can

Differential  
Diagnosis

IX

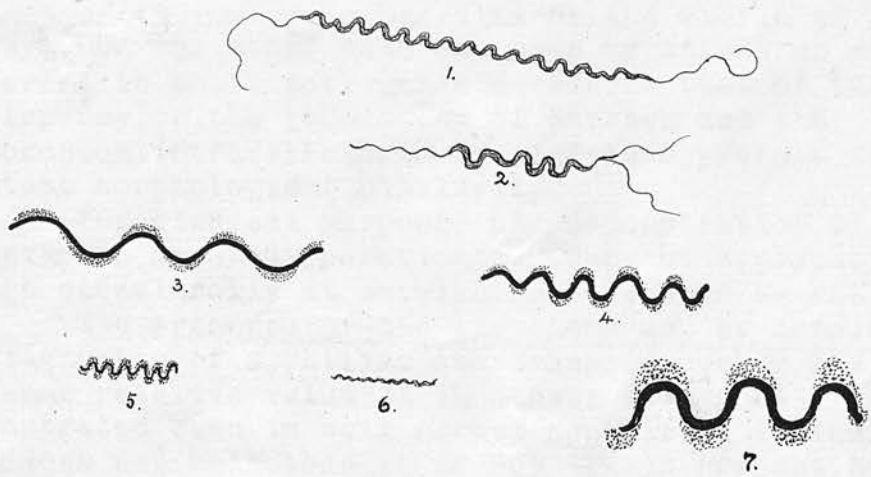
— 41m

fewer  
41m

can be no difficulty in distinguishing them, but aberrant forms of either organism may render the diagnosis doubtful.

The following organisms which occur in the mouth are to be distinguished from the Treponema, the Spir. buccalis, Spir. dentium, and Spirillum Vincenti. They all take the stain much more readily and are stained blue. Their extremities are blunt, and the spirals are not so closely set, being more <sup>an</sup> undulating. Sp. dentium is much smaller (it is the smallest spirochaete known), while the other two are much larger.

Certain spirochaetes have been found in ulcerated carcinomata which in some cases bear a very close morphological analogy to the Treponema, on account of their tenuity and very large number of spirals. Hoffmann states that an experienced observer can find points of distinction especially in the arrangements of <sup>the</sup> spirals. Neisser, Baerman and Halberstadter, however report cases of malignant papilloma of the penis, and Kriemtz a case of cancer of the stomach, in which spirochaetes were found impossible to differentiate from the Treponema.



MORPHOLOGY OF PRINCIPAL SPIROCHAETES.  
(after Schaudinn)

- 1 & 2. Treponema pallidum.
- 3 & 4. Spirochaete refringens.
- 5. Spirochaete from carcinomatous ulcer.
- 6. Spirochaete dentium.
- 7. Spirochaeta plicatilis.

There is one other form of spirochaete to be described, viz., the Spirochaeta pertenuis seu pallidula discovered by Castellani in the lesions of yaws. This organism is identical <sup>with</sup> to the Treponema. Schaudinn, and Castellani himself, state that they have been unable to detect any differences. According however to Blanchard, Mesnil, and others, slight morphological distinctions can be made out. Though morphologically so similar, the two organisms must be biologically different, as Castellani and Neisser have definitely proved by inoculation ~~to~~ monkeys, that yaws and syphilis are two different diseases.

The fact that spirochaetes have been found outside of syphilis bearing the closest analogy to the Treponema pallidum, has been one of the arguments brought forward against the specific nature of the organism. The morphological similarity of two organisms does not in the least imply identical biological characters. The study of the morphology of organisms has made great progress in recent years and we now know the great polymorphism there exists among many forms of life, whose etiologi- cal specificity is as well established as that of the tubercle bacilli or the vibrio of cholera. On the other hand one sees organisms as widely different in their pathogenic action as that of tubercle and leprosy, or the gonococcus of Neisser and the diplococcus intracellularis meningitidis, present the greatest morphological similarity.

For clinical purposes the demonstration of the organism in smear preparations is easy of application, though occasionally it entails considerable search.

The presence of the treponema may be considered as diagnostic of syphilis. Its absence however has not the same relative value, as it cannot always be demonstrated even in well marked syphilitic lesions. This does not mean that it is not always present, but merely that our imperfect methods do not permit us to bring it to light.

The Treponema has been found in nearly every known syphilitic product. We can give in this thesis but a brief summary of the results that have been obtained in this direction.

absence in syphilitic lesions

presence in syphilitic lesions

Primary period.

Primary Period. It has been constantly found in genital as well as extra-genital sores. Certain statistics show that it has been found by some observers in 100% of cases examined. It has been found by Queyrat and Levaditi in chancres of 45 days duration. It has been seen only on a few occasions in primary syphilitic lymphangitis (Shuman), but it has been demonstrated extremely frequently in primary enlarged syphilitic glands.

Secondary period. In the secondary period the treponema has been found with almost equal frequency. Its presence has been detected in all the various manifestations of cutaneous syphilis, in the ~~microbe~~<sup>acute</sup> of the roseola rash, the different papular, vesicular, or pustular syphilides, in condylomata, in mucous patches whether genital or extra-genital, as well<sup>as</sup> in the ulcerative lesions of the tonsils.

Though capable of demonstration in the blood, it does not seem that the micro-organism of syphilis finds there suitable conditions for its existence, and the media probably serves more as a means of transport, than as a habitat to the organism.

It has not been found in the spleen or the spinal fluid during this stage. It has however been detected in the urine of patients suffering from secondary manifestations. It has also been found in the syphilitic lesions of the hair and nails.

Tertiary Period. It is a well known fact that the lesions of tertiary syphilis are but very slightly contagious; indeed it was stated almost as a dogma at one time that they were non-contagious. Neisser, Finger and others, have however observed that ~~syphilis~~<sup>treponema</sup> could be successfully inoculated ~~to man~~<sup>with syphilis</sup> with products taken from the periphery of gummata. Gummata are only contagious when the tertiary neoplasm is not in a state of degeneration or of suppuration. "We may state that all ulcerated and soft gummata no longer contain any inoculable virus" (Neisser, Congress of Lisbon, 1906).

What we know of the presence of the treponema in these lesions, seems quite in accordance with facts gleaned from clinical and pathological experience. It has been extremely difficult, in spite of many attempts to do so, to demonstrate the organism in the gumma. Schaudinn was however able to find 5-6 treponemata at the

periphery

periphery of a gumma of the liver. Since then several more successful cases have been recorded of its presence in gummata and ulcerative tertiary lesions. All attempts to discover it in parasymphilis (General paralysis and tabes) have hitherto failed.

Hereditary syphilis. It is principally in cases of hereditary syphilis that success has been most marked in the search for the treponema. The parasite has not only been found with extreme frequency, but in astonishingly large numbers. It is in hereditary syphilis that the treponema seems to attain its greatest generalisation; the whole organism becomes literally infected. There is barely an organ in which it is not found in large numbers in some cases the whole microscopic preparation of the organ seems nothing but one mass of treponemata.

It occurs in the blood, spleen, liver, pancreas, thyroid, thymus, kidney, supra-renals, testicle, and even in the bony skeleton and bone-marrow in cases of syphilitic affections of the bones.

It has equally been found in all the cutaneous and other manifestations of this type of the disease.

It has been detected in the placenta and umbilical cord, as well as in the meconium of syphilitic fetuses.

Experimental syphilis. Experimental inoculation of monkeys has amply confirmed clinical observation as regards the presence of <sup>the</sup> treponema in syphilitic lesions. Metchnikoff and Roux, as well as other observers have succeeded in demonstrating its presence in the primary sore, in the enlarged lymphatic glands, as well as in the secondary cutaneous and mucous lesions of the anthropoid apes. This <sup>fact</sup>, along with its ~~constant~~ presence in such large numbers in the organs of syphilitic fetuses, are perhaps the strongest proofs that have ~~not~~ yet been brought forward in support of the specific etiology of the treponema.

The constant presence of the treponema in syphilitic lesions would not of itself be conclusive of the diagnostic value of the organism. To be so, it is equally necessary to prove that it is not found outside of the disease. With this end in view a large number of experiments have been performed. We have already mentioned the Spirochaeta pertenuis as well as some other spirochaetes found in cancerous lesions which <sup>have</sup> a strong morphological resemblance to the treponema. The result of all

these

control  
Experiments

XI  
Control  
Experiments

these experiments however with a few rare exceptions which we shall mention, has been to establish the fact that the treponema is not found in any normal or pathological conditions.

Thesing in the staining solution itself, Kiolmenogloo and Von Cube in balanitis, in scrofulous abscess and in an ulcerated carcinoma, Scholtz in a non-specific condyloma, and Heidingsfield and Markley in soft chancres, described spirochaetes which they stated were similar to the treponema pallidum.

It was shown that Thesing had used an old prepared solution of Giemsa containing dextrine, which can serve as a medium of culture to a large number of micro-organisms, including spirochaetes. Besides, the spirochaete he described was quite different from the Treponema pallidum.

The organisms described by Kiolmenogloo and Von Cube were shown to Schaudinn who proved they were quite distinct from the treponema. The same may be said of that described by Heidingsfield and Markley who at first described as treponemata any organisms which bore a similarity to that germ.

In Schultz's case, the organism seemed to have been almost morphologically identical to the treponema, but there was a doubt as to the specific nature of the lesion.

In the healthy human subject, a large number of experiments were carried out which all proved negative. These experiments included the skin, mouth, dental tartar, the expectoration, vagina, uteri, and preputial smegma.

The following cutaneous affections gave negative results, acne, eczema, epithelioma, carcinoma, ulcer, sarcoma, folliculitis, herpes zona, scabies, impetigo, sycosis, lichen, psoriasis, lupus, seborrhoea, molluscum, contagiosum, hyperidrosis, erythema, non-specific pemphigus, ulcers of the tongue, leg, etc.

Negative results were equally obtained in cases of stomatitis, angina of Vincent, various kinds of expectoration, ordinary pus, leucoma, in the following genital affections, chancroid, venereal warts, gonorrhoea, balanoposthitis, non-syphilitic erosions, or ulcerations of the male or female generative organs, vaginitis and the various vaginal discharges.

Finally a large number of examinations were

carried

carried out post-mortem on normal organs and various pathological lesions such as broncho-pneumonia, carcinoma, melano-sarcoma, septicaemia, tuberculosis caries, inflammations of the knee, spleen, liver, etc., which all proved uniformly unsuccessful.

We may state that most of these experiments were made by various observers on different occasions, giving a vast number of normal or pathological conditions examined, all with negative results.

We may therefore conclude, that, up to the present at least, the treponema pallidum has never been definitely demonstrated outside of syphilis.

The impregnation method has enabled us to study the distribution of the treponemata in the various syphilitic lesions.

In the syphilitic chancre, the hard base is due to a thickening of the dermis, in which there is a dense infiltration of embryonic cells and hyperplasia of connective tissue. In the centre of the lesion, the infiltration is more diffuse and chiefly centred round the blood vessels, and becomes denser the nearer we get to the periphery.

The lesion of the dermis is soon accompanied by that of the epidermis, which becomes thickened by the presence and migration of cellular elements, the proliferation of which penetrate between the papillae of the dermis. At the free surface, the corneal and granular layers gradually disappear giving rise to an erosion.

The distribution of the treponemata is found to correspond very closely with the pathological processes. At the periphery of the lesion they are found chiefly around and in the walls of the blood vessels. In the centre of the erosion, they are found deeply in the connective tissues of the dermis, where the thickening is most marked; they become less numerous as we approach the surface, where they are entirely replaced by organisms of secondary infection.

In the roseolar rash the organism is found with difficulty; it is present in the terminal capillaries of the papillae, which shows that the lesion is probably due to congestion brought about by an embolism of parasites, rather than toxic in character.

In the secondary lesions, the pathological process and the distribution of the treponemata are very

similar

XII  
Distribution  
in Syphilitic  
lesions

Distribution  
in  
Syphilitic lesions

similar to what is found in the primary lesion. In the papules they are however chiefly found in the epidermis below the level of the stratum granulosum. There evidently seems to be a migration of the parasites from the dermis when they are brought by the blood to the epidermis, unlike the chancre when migration takes place in the opposite direction, that is from the deeper layers of the epidermis, where proliferation first takes place, to the dermis.

In all the lesions examined the treponemata are usually found at the spot where the pathological process is most marked and active. "There is no infiltration of leucocytes or hyperplasia of connective tissue due to syphilis which has not a direct relation to the presence of the treponema." (Levy-Ding).

The treponema has never yet been found in the nervous centres, though it has been discovered in the blood vessels of the brain in cases of the tertiary disease of that organ.

The organism evidently multiplies in the chancre where, to judge from the number of division forms, it shows a period of hyperactivity. It is in the deeper layers of the epidermis that inoculation takes place, and in which the parasite first begins to proliferate. From there it passes to the dermis, and sections show that it spreads from these both by the lymphatics and blood, though chiefly by the former. It then goes to the nearest lymphatic glands in which atypical forms have been seen, which are possibly the results of the contest which probably takes place between the leucocytes and the organism, in which the latter is finally victorious. It again shows active multiplication in the glands, from which it enters the general circulation and infects the whole system.

It is however a well known clinical fact that syphilis has become generalised long before the sore has appeared.

The exact period at which the generalisation takes place is badly defined to man. Neisser has however shown that the blood of two monkeys inoculated since 16 and 18 days respectively, was virulent for other monkeys. Th experiments of Metchnikoff and Roux tend to show that the virus has probably already infected the organism even before that period.

On the other hand, the treponema has never

been



been found in the blood, before the appearance of the secondary lesions. During the first incubation period before the appearance of the chancre, it is probably multiplying at the point of inoculation. Why however do the tissues take such a long time as three to four weeks to react to their influence? At what psychological moment do the treponemata pass from the dermis into the general circulation? After the appearance of the sore, we have to await a further period of 40-45 days before the treponemata manifest themselves in the blood and cutaneous system. We know that a certain number of treponemata pass directly from the dermis into the blood *stream*, without going through the lymphatics. What becomes of these organisms? Do they go through some process of involution in the blood or in some organ of the body before showing again signs of their activity?

All these are questions, the replies to which are still in the realms of conjecture, and to which we can give no definite answer.

According to the researches of Hexheimer and Opificius, the organism, judging by the presence of forms of division, seems to show a greater activity during the night than the day, which would accord with nightly predominance of all pain of syphilitic origin.

Levy-Bing has made some interesting observations on the influence of mercurial treatment on the treponemata. In a series of six cases which he examined before, during and after treatment, he found that under the influence of mercury, the organisms gradually decreased in number until they finally disappeared, though the lesions were not yet healed. Untreated cases after the same lapse of time, still showed no diminution in the number of treponemata present. Most other observations on the subject, tend to show that mercury has a distinct influence on the treponema, causing its disappearance from syphilitic lesions.

Whether the drug acts directly on the treponemata, or whether it does so indirectly by increasing the defensive powers of the tissues is still a question sub judice. The disappearance of the organism under treatment with mercury may only be apparent; it may take on some involution form which escapes our method of investigation. We know full well that even after years of apparently complete cure, the disease may again manifest itself. In what form or in what part of the body

do

*Influence of  
mercurial treatment*

XIII  
*Influence of  
mercurial treatment*

do the organisms remain during that long period, and what excites them to renewed activity, are still solutions which lie in the womb of the future.

To the action of mercury on the treponema, provided we admit its etiological relation, is allied directly the question of the prophylaxis of syphilis by means of mercurial ointments or lotions. Metchnikoff and Roux after having prevented the onset of syphilis in the monkey in thirteen cases out of thirteen, by using a 53% calomel ointment on the points of inoculation a few hours after that inoculation, successfully performed the same operation on M. Maisonneuve a medical student who had voluntarily offered himself for the purpose. (1)

The good results obtained in the treatment of sleeping sickness with atoxyl, and the analogies in structure between the organism (*Typanosoma gambiense*) of that disease, and that found in syphilis, led many pathologists to try this drug in the treatment of syphilis, in which it has apparently given excellent results. Experiments on monkeys by Metchnikoff and Salmon appear to indicate that atoxyl administered after an exposure to infection is capable of aborting the disease. M. Metchnikoff thus describes the experiment that shows this. "Seven monkeys (macacus) were inoculated with the same syphilitic virus; during the incubation period of syphilis two of these animals were given by injection a certain dose of atoxyl. While the chancre appeared in the five monkeys not subjected to the arsenical treatment, the two monkeys treated did not show any visible signs of syphilis. It is, then, possible, by the use of arsenic to prevent the development of the syphilis inoculated and to cause it to abort. Besides its curative properties on established lesions of syphilis, arsenic shows preventive properties".

nal History

The study of infectious diseases had long shown that spirochaetes were the causal agents of many diseases especially those of tropical countries. They had been discovered in epidemics affecting fowls (*Sp. gallinarum*) geese (*Sp. anserina*), cattle (*Spirochaeta* of tick fever), and sheep; Obermeyer had since 1873 proved that recurrent fever of man was due to a spirochaete inoculable to monkeys.

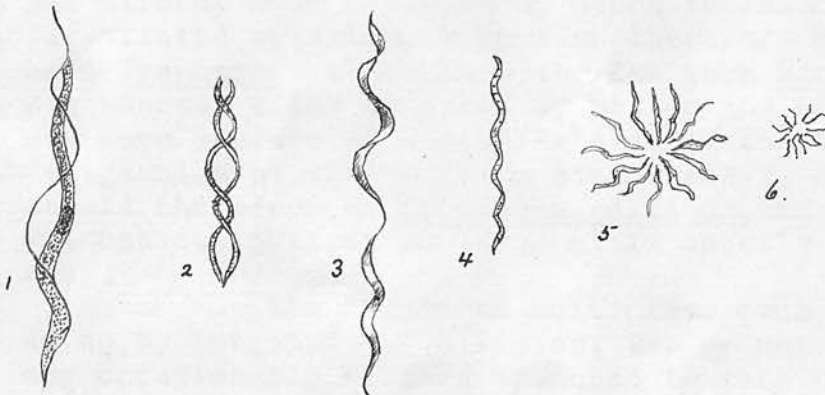
XIV.  
Natural  
History

When

(1) The full details of these experiments with subsequent criticisms will be found in the authorised English translation by ourselves, of Dr. Maisonneuve's thesis, Experimental Prophylaxis of Syphilis, J. Wright & Co. Bristol, January 1908.

When Schaudinn first discovered the Treponema, he noticed that it was a motile spiral, which might be classified as belonging to the Genus Spirillum or Spirochaeta. Spirilla possess cilia at their extremities, and though they move along their axis, this is rigid and shows no *movements* of flexion. On the other hand, spirochaetes do not possess any cilia; progression takes place around their axis from behind forward, and movements of flexion occur; they also possess an undulating membrane. On further study Schaudinn found that his organism possessed these latter qualities (though the possession of an undulating membrane seems doubtful), and he therefore called it, on account of its very pale appearance, the Spirochaeta pallida.

Until 1904 the genus Spirochaeta was considered as belonging to the bacteria. Schaudinn at that time published some very interesting observations he had made while studying the evolution of the haematozoa of an owl Haemamoeba *Liemanni*. He found that the fertilised gamete gave rise to one or more trypanosomes, which in their turn by repeated longitudinal division gave rise to very fine spirals, very similar to spirochaetes. He therefore classified the genus Spirochaeta as belonging to the Protozoa. Schaudinn's observations however have never been confirmed, and he himself in a subsequent report throws some doubt on the spirochaeta stage of Haem. *Liemanni*.



DEVELOPMENT OF TRYPANOSOME INTO SPIROCHETE. (AFTER SCHAUDINN.)

In connection with this it may be interesting to note the curious fact that the disease called Dhourine is due to a trypanosoma (*Tryp. equiperdum*). This disease occurs among horses in Algeria and India,

1. Trypanosome
2. " " dividing.
3. " " joined at extremities.
4. Spirochaete.
5. Agglomerated spirochaetes.
6. " " minute forms.

and

and may be described as syphilis of the horse. It has the following close analogies to that disease. It is conveyed under natural conditions by coiters only. After an incubation period of 11-20 days, oedematous swellings of the genitalia appear. In 40-50 days this is followed by the appearance of characteristic "plaques" on the skin; the animals become anaemic, complete paraplegia sets in, and death in two to ten months. The trypanosomes are most easily found in the "plaques" but with difficulty in the blood.



*Trypanosoma of Stovaine*

(*Tryp. Equiperdum*).

Stained by Giemsa.

Subsequent researches by Schaudinn and *Hensheimer* showed that the Spirochaeta pallida possessed two flagellae one at each extremity. As the organism could no longer be classified among the spirochaetes or the spirilla, a new genus Spirochona was created. It was however soon found that this term had already been employed by Klebs in 1893 for a flagellated organism. Schaudinn therefore created the word Treponema to replace it. The term Microspironema was shortly after proposed by Stiles and Pfender, who were unaware of Schaudinn's note. The only correct designation of the organism discovered by Schaudinn should therefore be Treponema pallidum, and not Spirochaeta pallida as it is still usually called (Levy-Bing).

The Treponema multiplies, or appears to do so, by longitudinal division; two or more treponemata may occasionally be seen attached to each other by one of their extremities; sometimes two or several may appear partly fused, or merely interlaced, or lying very closely parallel to each other.

This is the only method of reproduction we know of, but Krzystalowicz and Siedlecki have from

the

the study of some special forms, described a sexual<sup>or</sup> agam-  
etous cycle in which the organism would at a certain phase  
phase of its existence, pass through the stage of a  
trypanosoma. This however requires confirmation.

The whole subject of the classification  
of the different forms of spirochaetes, is still in a  
condition of chaos into which order is being gradually  
instilled, as their morphological characters are being  
studied and becoming better known.

Opinion seems still fairly divided as to  
whether the Treponema should be classified among the  
protozoa or bacteria.

Sambon classifies them among the blood-  
inhabiting protozoa in the Spiroschaudinnidae. The  
following is Sambon's classification of the Haemprotozoa.

Haemogregarinidae	One Genus	Haemogregarina.
Plasmodidae	Two genera	{ Plasmodium.
		{ Laverania.
Spiroschaudinniidae	Three genera	{ Leucocytozoon.
		{ Spiroschaudinnia.
		{ Treponema.
Haemoproteidae	Two sub- families	{ Haemoproteinae One genus. Haemoproteus.
		{ Trypanosominae Four genera { Babesia.
		{ Leishmania. Trypanosoma. Trypanoplasma.

The new generic name Spiroschaudinnia is  
here proposed for the blood spirochaetes, which differ  
greatly, both morphologically and biologically, from the  
free-living form Spirochaeta plicatilis, on which  
Ehrenberg established the genus Spirochaeta.

The chief arguments in favour of the pro-  
tozoal nature of the treponema are the following -  
the evolution of trypanosomes into treponemata (this  
still remains however very doubtful), longitudinal div-  
ision, the presence of an undulating membrane (also very  
doubtful) great similarity of syphilis to dhourine, a  
protozoal disease, and difficulty of cultivation.

Metchnikoff on the other hand thinks that  
it should be classified among the bacteria. "The great

analogy

26. 26  
analogy of the microbe to the spirillum of recurrent fever and the spirillosis of foub, leads one to think that the first is as much as the other two, a true bacterium. The discovery of numerous ciliae in the spirillum of foub by Borrel, and in Spirochaete Obermeyer's by Zettnow also tends to prove this".

um Levaditi, who was able to demonstrate a terminal flagellum in Sp. refringens, would classify all the blood spirochaetes in one group that of ciliated spirochaetes, which would include Sp. Obermeyer's, Sp. of tick fever, Sp. gallinarum, Sp. refringens, and finally Treponema pallidum.

The exact classification of Treponema pallidum, remains as we see, still unsettled.

conclusions The large number of researches and experiments which have been carried out on the Treponema pallidum, enables us to formulate the following conclusions.-

XV  
Conclusions

1. The Treponema has been found in all the various lesions of syphilis, in the acquired, hereditary, as well as the experimental disease
2. It has never been demonstrated outside of syphilis.
3. Its localisation in syphilitic lesions agrees perfectly with the various pathological processes.
4. Mercurial treatment exercises a marked action on the organism.

These facts enable us to state that as far as the specificity of an organism which has never been cultured in pure cultures and inoculated into an animal, can be affirmed, we are in a position to declare the Treponema pallidum to be the causal agent of syphilis.

The microorganism of Schaudinn finds its self in the same position as the haematozoa of malaria, the spirochaete of Obermeyer, the trypanosoma of sleeping sickness, the bacillus of leprosy, organisms which have not been cultured as yet in pure cultures and yet are generally accepted as being the specific germs of those diseases.

It

It had been so often announced that the microbe of syphilis had at last been discovered, that a large amount of scepticism was but logical and natural, when Schaudinn first published his discovery of the *Spirochaeta pallida*.

"It is always extremely difficult to appreciate at its proper value the work of scientific men. We cannot help at times being struck by the divergent results obtained by different observers. It may not be out of place to examine closely the reasons which may in all good faith occasionally lead the wisest into error, and cause to be put into doubt the deductions derived from their discoveries. This little problem in scientific psychology is one of general interest.

Well may it be asked why have all these men, all these searchers of the truth thought that their discovery was the real one? By what optical intellectual error did they imagine that they held even a shred of the truth? There are many who may wish to hear their names at any price pronounced by the multitude, but we are dealing here with honest men, men of the highest scientific probity, and moreover scientists who know full well what are the exigencies of a scientific discovery and are aware of the many pitfalls and illusions which surround the search after truth.

It is here that human weakness reveals itself as the fissure through which error insinuates itself into the minds of the wisest and strongest. Consciously or unconsciously, as long as human nature is what it is, the first use that we make of our love of truth is to prove to ourselves that what we believe is true. Man is egocentric. Physiology teaches us that he does not know the reality of the external world, he only knows the picture which he has formed of it; this picture is of his own making, his also is the judgment which he expresses on it. He is the resonator on which converge all external impressions. As the noise of the ocean seems entirely confined to the solid rim which lies on its border, so is life with its multitudinous phenomena, its flux and reflux, condensed in the personality of each individual. From this arises a necessary and inevitable egotism. As a child is always beautiful to the eyes of its mother, so is a discovery always beautiful to him who makes it. He does not see its defects, or if he sees

them

them he involuntarily ~~attempts~~ <sup>attempts</sup> them., he finds at least that it has less faults than the discovery of others.

His discovery is therefore preferable, and he fondly imagines that progress has been realised; desire, pride, the joy of having vanquished some difficulty, all these help to further the impression of intellectual paternity.

Who amongst searchers has not known that ephemeral triumph, that joyful transport which increases the feeling of one's own power and personality? Who has not felt that internal pride which the external projection of every discovery creates and causes the worker as he sits at his table to see around him the smiling visions of the dreamer, and hear the chorus of eager praises, the murmur of admiration and the mention of his name in the mouths of men.

It is to this all impulsive because sentimental psychology that we owe a large number of those publications which vanish at the first breath of *the spirit of* criticism, and of which nothing remains but the astonishment that they could even for a second have been deemed worthy to attract the attention." (1)

Scepticism and criticism are one might say, essential parts of every scientific discovery without which true progress would be impossible. For it is the critical spirit which scepticism engenders, which tends to stimulate research and so helps to elucidate the truth; it induces every worker in the field of science, to try and make the structure, which he may have laboriously erected, as proof as possible against the shafts and arrows of destructive criticism. This however when carried too far, may hinder progress, and such we believe to be the case as regards the discovery of the late-lamented Schaudinn which marks a most important and useful landmark in the study of syphilis.

(1) Bayet, Conférence à l'Université libre de Bruxelles, Dec. 1906.

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ADDENDUM.

In smear preparations the demonstration of the Treponema usually entails considerable search, which greatly reduces its value for practical clinical purposes.

We have therefore tried to devise some method of staining by means of which the Treponema could be more easily demonstrated.

In conjunction with Staff Surgeon Kilroy, we have applied the Levaditi method which answers so well for sections to smear preparations.

Several smear preparations were placed in a 3% Silver Nitrate solution and kept for 4 days at a temperature varying from 100° to 108° C in a corner of the engine-room on board H.M.S. "HOGUE":

The preparations were then washed and reduced for 16 hours in a 4% Pyrogallic Acid solution.

It seemed to us that the treponema could be more easily brought into view by this method than by the stains derived from the Romanowsky method.

After further experiments <sup>we found that</sup> equally good results could be obtained by using a 10% Silver Nitrate solution for one hour at a temperature of 100°, and then reducing for 10 minutes in a 5% Pyrogallic Acid solution.

In one of the preparations as many as six treponemata could be seen under one field of the microscope, a condition rarely observed in smears obtained by the methods at present in use.

We are continuing our experiments to see whether good results cannot be obtained by using the solution of Silver Nitrate at the ordinary room temperature and by varying the strength of the *reagents* employed.

In these preparations the treponemata are stained a dark brown, the connective tissue cells

are

are stained yellow while the different histological debris are deeply coated with metallic silver

We claim that by this method the Treponema is more easily found, and that the extremities and spirals are more distinctly and clearly defined.

## R E F E R E N C E S. (contd.)

- Anonymous. The etiology of syphilis. Med. Record, 6 Jan, 1906.
- Bayet. Le Microbe de la Syphilis et les Essais de Vaccination. 1907.
- Britzke. Presence and distribution of *Sp. pallida* in macerated fetuses. Berlin Klin. Woch Jan. 11, 1906.
- Bruns. Experimental inoculation of syphilis. Berlin Klin. Woch. Nos. 48 & 49, 1906.
- Castellani. Spirochaetes in yaws. Brit. Med. Jour 18 Nove. 1905 and 25 Nov. 1905.
- do- Is Yaws syphilis? Jour. Trop. Med. 1 Jan. 1906.
- do- Note on the Spirochaete of yaws (*sp. pertenuis*) Brit. Med. Jour. Nov. 23, 1907.
- Fischer. *Sp. pallida*. Berlin Klin. Woch. Jan. 1907.
- Gaucher. Le chancre et les Syphilides. Dec. 1906.
- Hoffmann, Levaditi, Report of International Congress  
Metchnikoff, Doflein, of Hygiene. Berlin July 1907.  
Bertarelli, Land- Discussion on Spirochaetes and  
steiner, Laboratory, Syphilis.  
and others.
- Hallopeau. Sodium aniline arsenate (atoxyl) in Syphilis. Gaz. Med. de Paris, June 15. 1907.
- Hoffmann and Halle. An improved method of fixing *Sp. pallida*. Muench Med. Woch. July 31, 1906.
- Hollander. *Sp. pallida* in case of congenital syphilis. Berlin Klin. Woch. June 11, 1906.

REFERENCES. (contd.)

- Huebschmann. Sp. Pallida in congenital syphilis. Berlin Klin Woch, June 11<sup>th</sup> 1906.
- Lesser. Treatment of syphilis. Deut. Med. Woch. July 31, 1907.
- Levaditi in conjunction with Mc Intosh. An attempt to cultivate the Treponema pallidum. Ann. de l'Inst. Past. Oct. 1907.
- Lévy-Bing. Le Microorganisme de la syphilis Jan. 1907. This book contains an excellent bibliography of all the literature that has appeared on the subject until Oct. 1906.
- Maissonneuve. Experimentation sur la prophylaxie de la Syphilis July 1906. English translation by deVerteuil.
- Mc Lennan. A preliminary note on Cytorrhyses luis and Sp. pallida. Brit Med. J. Feb. 3 1906.
- do- On the Sp. pallida and its variations. Brit. Med. Jour. 12 May 1906.
- do- The place of the Sp. pallida in the diagnosis of syphilis. Nov. 23 1907.
- Metchnikoff and Roux. Études expérimentales sur la syphilis. Ann. de l'Inst. Past. Dec. 1903, Jan. 1904, Dec. 1905. Oct. 1906.

Ravant.

R E F E R E N C E S. (contd.)

- Ravant and Ponselle. A bacterological study of the lesions of the brain in new-born syphilitics. Gaz.des. Hop. July 13, 1906.
- Schaudinn. Sp. pallida. Brit. Med. Jour. Oct. 28, 1905.
- Schlimpert. Sp. Pallida in hereditary syphilis. Berlin Klin Woch, June 28, 1906.
- Schulze. The Silver Spirochaete. Berlin Klin Woch. Sept, 10, 1906.
- Shennan. Spirochaeta pallida (spironema pallidum). in syphilis. Lancet. 10 March, 1906 and 17 March 1906
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