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# Oral Spirochetes, their Occurrence in Diseases of the Oral Cavity, and a Simple Method of Pure Cultivation.

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There is at present neither clinical nor experimental proof that the oral spirochetes alone can give rise to diseases in man. On the other hand the majority of research workers in this field agree that these spirochetes, in symbiosis with certain other micro-organisms (fusiform bacilli, vibriones and cocci), may give rise to both acute and chronic morbid conditions in the oral cavity. *Angina Vincenti* may be adduced as an example. I quote SMITH, who aptly describes the possibilities of oral spirochetes attacking parts of the human organism: "Situated in the tonsils and about the teeth they are in a strategic position to initiate disease locally in the tonsils, gums, cheeks, tongue, pharynx and larynx; or to invade the eyes, ears and sinuses. They may be aspirated and infect the trachea, bronchi or lungs; or may be swallowed and cause disease in the oesophagus, appendix or colon".

The name spirochete first appears in the literature in 1833 and was then given by EHRENBERG to a fairly large organism (spirocheta plicatilis), which lives in water and transports itself by means of flexile movement. According to TOPLEY and WILSON, the name is used generally for all elongated, motile flexible organisms which are twisted spirally round their long axis. Although the spirochetes vary very considerably in size, they all possess certain characteristics in common. Thus, they can move both forwards and backwards. They lack flagellae and also

localised nuclei. They divide transversally, division being either simple or multiple. No one has yet succeeded in proving any sexual reproduction. All these characteristics place them nearer bacteria than protozoa, for as DOBBEL pointed out in 1912, the only thing that distinguishes them from bacteria is the characteristic of being able to move without flagellae.

TOPLEY and WILSON divide the spirochetes into four groups: *Spirocheta, Cristispira, Treponema* and *Leptospira* It would carry us too far to go into the various groups here. The only ones which are pathogenetic to man are *Leptospira*, of which the species *Leptospira icterohaemoragiae*, causes Weil's disease (in the oral cavity a *Leptospira trimerodonta* HOFFMAN is sometimes met with, as the author was able to establish in one case; it is probably harmless), and *Treponema*, which is the most important group for us.

The micro-organisms belonging to the *Treponema* group have neither axial filaments nor crista. They have a number of primary, more thinly or closely twined spirals. When they are moving, secondary coils may also appear. The terminals may be rounded or pointed, or, as in some species, drawn out into thin filaments. The organisms of the group are widely scattered. The free-living species are met with in both fresh and salt water, but preferably in stagnant, slimy water, or in water which has been polluted by manure or sewer water. They are also present in the saliva of certain insects (fleas, cockroaches and others).

In man they appear — apart from in cavum oris — in the alimentary canal, the bronchi, the mouth of the urethra, in certain ulcerating affections of the skin, in condyloma, and last but not least in various syphilitic morbid processes. The size of these *treponemata* varies between 3 and 60 $\gamma$.

As the systemization and designation of micro-organisms belonging to this category are somewhat indefinite, the present author gives a simplified classification of the most important treponemata occurring in the human oral cavity. *Treponema microdentium* (NOGUCHI), *Treponema macrodentium* (NOGUCHI), *Treponema Vincenti* (BLANCHARD), *Treponema buccale* (DOBBEL).

*Treponema microdentium* is one of the smallest spirochetes met with in the human oral cavity. Its length varies between 3 and 12 $\gamma$ and its breadth between 0.2 and 0.25 $\gamma$. The number of coils varies with length and age. Young forms have from 4 to 6, older ones up to 20. Its coils are regular and strongly marked. There are

no undulating membranes or cilia (DOBBEL). The cytoplasm contains granules of chromatin (MÜHLENS). Its movements are slow and consist partly of rotation round its own axis, and partly of longitudinal movements which take place equally easily backwards and forwards. Division takes place transversally, single or multiple.

*Treponema macrodentium* is, as the name indicates, larger than the micro-organism described above and measures between 4 and

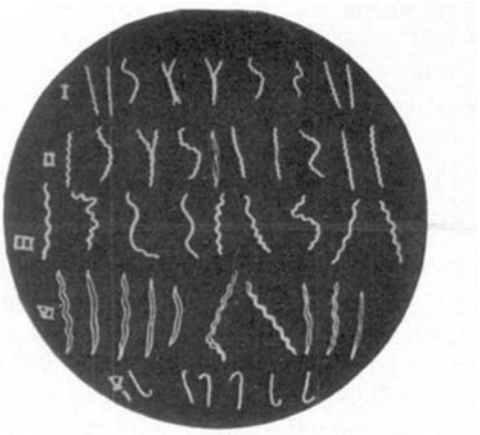


Fig. 4. Drawing (X 1,000) of organism from the mouth. I. Various appearances of *T. microdentium* due to activity. The pseudo-longitudinal division seen in the two central organisms is the result of the fact that two organisms are wrapped together. II. Variations in *T. macrodentium* due to activity; the pseudofusiform bacillus seen in the centre is due to rapid vibration of the organism about a fixed point in the center. III. Variations in *T. vincenti* due to activity. IV. Variations in *T. buccale* due to activity. V. *Leptospira* from the mouth.

Illustration 1. The oral spirochetes described above. After Smith.

15 $\gamma$ in length and 0.3 to 0.5 $\gamma$ in breadth. It has considerably coarser coils than microdentium, is considerably quicker in its movements, but for the rest has the same characteristics.

*Treponema Vincenti* is slightly larger than macrodentium but has very irregular coils. Its length caries between 5 and 20 $\gamma$ and its breadth between 0.3 and 0.5 $\gamma$. It has no cilia and moves with rapid, eel-like movements. Division takes place transversally, single or multiple.

*Treponema buccale* is the largest of the oral spirochetes, 3 to 25 $\gamma$ in length and from 0.6 to 0.8 $\gamma$ in breadth. Seen in the dark-field microscope it moves like an earth-worm. As, owing to its thickness, it appears to be double-contoured, it is the easiest

of the spirochetes to recognize. It has no cilia and divides transversally.

Historically it may be mentioned that in 1875 COHN described a spirochete which he called spirocheta buccalis, and that in 1877 KOCH discovered spir. dentium. MILLER, who is rightly called the father of oral bacteriology, was engaged for more than 35 years in investigations of the bacterial flora of the oral cavity. However, he never succeded in cultivating the oral spirochetes, all of which he assembled under the name spir. dentium.

It was in 1867 that the first suspicions of the pathogenicity of the oral spirochetes were aroused, for then LEYDEN and JAFFE found spirochetes in the lung and saliva in the case of a typical pulmonary abscess. Subsequently, in 1894, VEILLON made the observation that, together with fusiform bacilli, spirochetes formed the typical picture in cases of tonsillary angina. This was confirmed by PLAUT in 1894, by BAREGGI in 1895, and by BERNHEIM in 1898. In 1896 and 1898 VINCENT also found spirochetes plus fusiform bacilli in cases of hospital gangrene and in angina in the pharynx. Neither PLAUT, VINCENT nor their immediate successors could cultivate oral spirochetes, however, This was first done successfully in 1906, when MÜHLENS and HOFFMAN managed to obtain what they called *spir. dentium* in pure culture Since then, NOGUCHI, SCHMAMIN, KRITSCHEWSKI and SEGUIN, WILLIAMS, SMITH, VINCENT, DUFRESNE and the present author, *inter al.*, have succeeded with the pure culture of various types of spirochetes.

We will now consider the diseases of the mouth. In the case of inflammations of the oral mucous membrane we distinguish, as is well known, between different morbid conditions, all according to the parts which are attacked. Thus we say that there is a gingivitis if the inflammation is located on the border of the oral mucous membrane and the interdental papillae; if it has attacked above these limits we speak of stomatitis; and if it attacks the tonsils and the adjacent areas it is designated angina or tonsillitis.

SMITH, who devoted a lot of work to investigating what he calls fuso-spirochetal diseases and whose presentation I shall follow below, made a summary of the various diseases met with in the oral cavity in which fusiform bacilli and spirochetes occur. He then found this flora not only in practically all diseases of the gingiva but also in morbid conditions in the ear, nose, trachea, lungs, nay, even in the appendix.

VINCENT'S *angina* will be considered first. This acute infection disease occurs in both sexes at all ages, but children between 2 and 10 years and young people between 18 and 25 years appear to be especially susceptible. It is extremely probable that in the majority of cases the infection begins in the gingiva and subsequently spreads to the tonsils, as it has proved that a large percentage of these cases have either severe gingivitis or chronic pyorrhea. The disease is infectious and can be transmitted from one individual to another by way of towels, pens, toothbrushes, pipes, kisses, etc.

Bacteriologically fusiform bacilli and *trep. vincenti* predominate, and *trep. micro- and macro-dentium* are also met with. Other microorganisms are also present, but the picture of the fusiform bacilli and trep. vincenti is so typical that the disease has received its name from them.

In patho-histological sections from the typical ulcerating progressive form of this disease three sharply delimited zones can be distinguished. The outermost necrotic zone, consisting of dead tissue with quantities of all kinds of bacteria; the middle zone, which is only dead in parts with preponderatingly fusiform bacilli, which are often met with in pallisade formations and in enormous quantities; in the third zone, which thus lies nearest to the healthy tissues, the spirochetes are almost completely predominant, and apart from their presence there is nothing provably morbid in this zone. This fact that the spirochetes play the chief rôle in the infection is considered by ZINSERLING to be ample proof that spirochetes are the most active factor in this anaerobic symbiosis between fusiform bacilli and spirochetes.

Therapeutically intravenous injections of the compounds of arsenic, arsphenamine, neoarsphenamine or sulpharsphenamine, and local applications of iodine in association with supplies of vitamin C have proved satisfactory.

*The necrotic gingivitis*, also called ***Trench Mouth***, was a dreaded and common disease of the mouth during the first Great War, affecting soldiers who were in the trenches for a considerable time. In the main the disease is the same as VINCENT'S *angina*, with the difference that it is confined to the gingiva. It also occurs among civilians, both during war and peace, and may attack people of all ages. Children between 2 and 5 years and young people between 18 and 25 years are particularly liable to it. It often occurs in persons with dental caries, but may appear indepen-

dently of this disease. It also appears to be more usual in undernourished children than in others. The necrotic gingivitis may also be spread from individual to individual in the same way as Vincent's angina.

The symptoms are the following: The disease usually begins suddenly with lack of appetite and general depression, and the temperature rises to between 38 and 39°. Excessive salivation is present, and the patient also has a sensation that the teeth are woody or dead, which will probably be due to an anaesthesia of the peridentium. In addition the breath has a strange characteristic penetrating odour, and the patient has a metallic taste in the mouth. The tongue may swell, and the glands become enlarged and tender. Sometimes an exanthema resembling measles appears. As the disease progresses, pseudomembranous formations gradually appear on the gingiva. The membrane is grey or greenish-grey in colour, and if it is removed a sensitive, bleeding surface remains. These ulcers are very sharply delimited and look as if they were stamped out. They contain the usual fusospirochetal flora, the spirochetes appearing nearest to the healthy tissue as in VINCENT'S *angina*.

According to SMITH, in cases of necrotic gingivitis one should always consider the possibility of certain general injuries as the basis of the disease. Among these may be included certain poisonings with quicksilver, bismuth and other metals, and benzine and radium poisoning. Both lymphatic and myeloid leukemia may also be the basic cause of the disease, not to mention scurvy and other deficiency diseases.

At the beginning of the treatment, still according to SMITH, one should avoid scraping away the tartar and methods of treatment which demand anaesthesia. Painting with spirits of iodine is said to have a very good effect at this stage of the treatment. ROGER was able to show good results after local treatments with arsphenamine. During the last Great War arsphenamine in glycerine solution was regularly used for local applications. In obstinate cases neoarsphenamine or sulpharsphenamine should be given intravenously at the same time as the local treatment.

*Noma, cancrum oris* or *gangrenous stomatitis* is undoubtedly the severest disease of the oral cavity, the mortality being from 70 up to 100 %. Although the disease may occur at all ages, it is, however, most frequently found in children under 5 years. Formerly it often appeared in children's homes, in association with

measles, diphtheria, scarlet fever, typhus and whooping-cough. It is infectious. There are always three cooperating basic factors in this disease. The first is a C-avitaminosis and possibly also an A-avitaminosis. The second is a reduced power of resistance in the organism, owing to some acute illness. The third, finally, is an

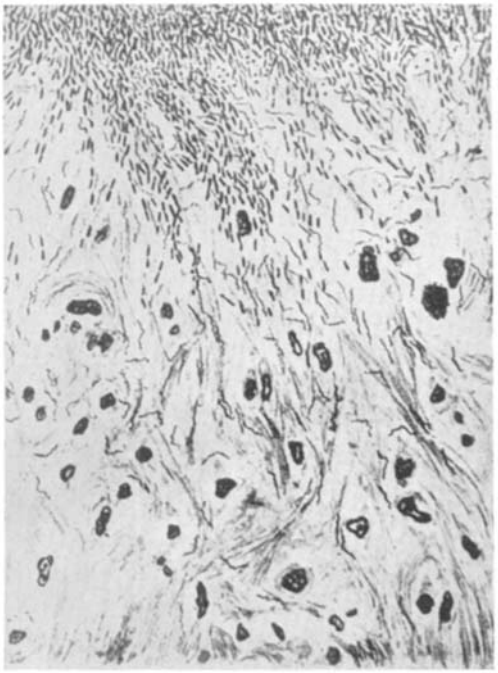


Illustration 2. Section of noma, showing the invasion of the spirochetes into the healthy tissue. After SMITH.

infection with a special group of anaerobic micro-organisms identical with those met with in VINCENT'S *angina*.

The symptoms are the following: The initial stage runs about as in an ordinary gingivitis and angina, but as soon as a noma has developed, the general symptoms become serious. Severe prostration, a rise in temperature up to 41°, apathy, weak pulse and often diarrhoea. The temperature may become subnormal before death.

At oral inspection a dull, dark-green area is usually found on the jaw and the opposite bucca. The surrounding tissue is reddened and oedematous, so that the bucca or lip may be three times

as large as normally. At this stage the disease spreads very rapidly, the jaws are destroyed, and the teeth fall out, the cheek is broken through, and the destruction attacks the whole face. Inconsiderable pain and few hemorrhages are met with. The illness lasts between five and ten days. The horrible fetid smell is characteristic and may fill a whole room, nay, sometimes a whole hospital department. If the patient survives the illness, a line of demarcation appears and the crust falls off.

The bacterical flora is the same here also as in VINCENT's *angina*. In sections from noma foci exactly the same three zones are found as were mentioned previously in connection with VINCENT's *angina*, and in the zone which lies adjacent to the healthy tissue practically pure *spirochete* cultures are found. See illustration 2.

According to SMITH and others, noma only appear on the basis of a previously present gingivitis simultaneous with a C-avitaminosis. The earlier the diagnosis is established the more favourable will the results probably be. Local intramuscular and intravenous injections of neoarsphenamine or sulpharsphenamine, and simultaneous large daily doses of orange juice or tomato juice have proved beneficial. The gangrenous parts may be removed by thermocautery, care being taken to operate in healthy tissue. NETTER, BECK and STULIK have obtained good results with local applications of arsphenamine and sulpharsphenamine with simultaneous vitamin treatment.

From what has been said above, it appears very clearly that in a number of severe infection diseases in the oral cavity, which develop on the basis of an existing gingivitis and an already present avitaminosis, there are throughout certain features in common. Firstly: Fusiform bacilli and spirochetes are always present. Secondly: In histo-pathological sections from the affected parts the spirochetes always appear adjacent to the healthy tissue. Thirdly: With local or intravenous treatment with preparations of arsenic, which are specific spirochete poisons, the spirochetes disappear, and in the majority of cases the disease is cured.

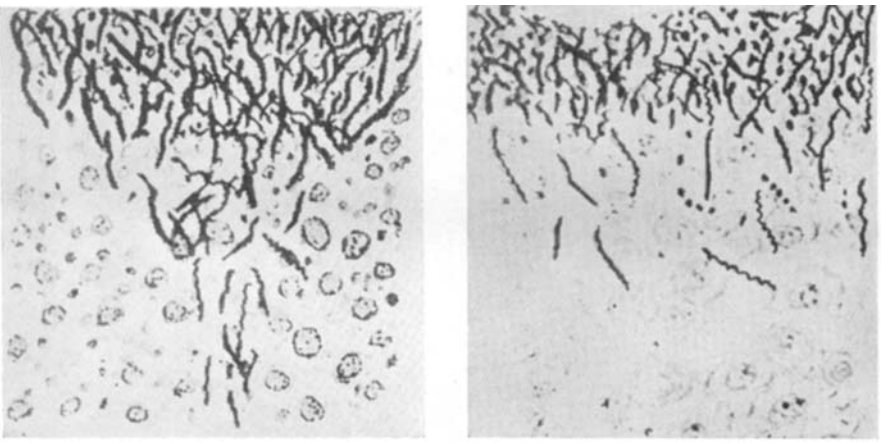
It will then readily suggest itself to assume that the spirochetes have a certain causal significance in the course of events. In spite of this there are extremely few investigations of the oral spirochetes and their significance for oral diseases. That this should be the case will probably be due in part to the difficulty of judging

as to what degree the micro-organisms provable in the tissues attacked have a pathogenic effect or are only secondary to the normal flora of the oral cavity, and in part to the technical difficulties presented by the pure cultivation of spirochetes.

Illustration 3. From the wall of an abscess from the lung of a six-year-old boy who died two months after tonsillectomy. Spir. penetrate into the healthy tissue. After SMITH.

For the present author, who had set himself the task of investigating the significance of the oral spirochetes for the appearance and development of diseases of the gingiva, it was therefore necessary first and foremost to find a simple and reliable method of obtaining pure cultures of these micro-organisms.

The first to succeed in making pure cultures of oral spirochetes were MÜHLENS and HARTMAN. For substrate they used horse serum gelatine, and the cultivated micro-organism was called *Spirocheta dentium*. NOGUCHI cultivated the same spirochete, but he designated it *Treponema microdentium*, and a larger one,



Illustrations 4 and 5. No. 4 from the lung of a rabbit which died of pulmonary gangrene eight days after intratracheal inj. of material from a pyorrhea patient. No. 5. From an abscess in the lung of a guineapig which died 2 months after intratracheal inj. of pyorrhea material. After SMITH.

which he called *Treponema macrodentium*. He used sheep serum with the addition of small pieces of fresh testicle or kidney from sheep or rabbits at 37° anaerobically. He effected the sowing by means of a stab in the centre of a tall pillar of agar in an ordinary test tube. On the basis of his own experiments, however, the present author must agree with SMITH when he says that the method is both troublesome and unreliable, for to obtain a pure culture it is necessary to inoculate 10 to 20 tubes, as gas-forming micro-organisms, which are in the mixed culture, often burst the agar pillar and thereby render it impossible to distinguish the various microbes from each other.

SMITH proceeded in the following manner. 2 % bouillon agar was mixed with ascites or pleural fluid in the proportion of 2 to 1, poured into ordniary test tubes to a heigh tof 6 to 8 cm. and then allowed to set. With a stiff platinum wire the culture material was inoculated centrally and deep down in the agar. Three to five tubes are sown without the material being renewed, so that there is at the same time a dilution of the sowing. After six to fifteen days a thin misty growth can be observed, which spreads from the stab channels out towards the wall of the tubes. When the growth is half completed a score is made round the glass

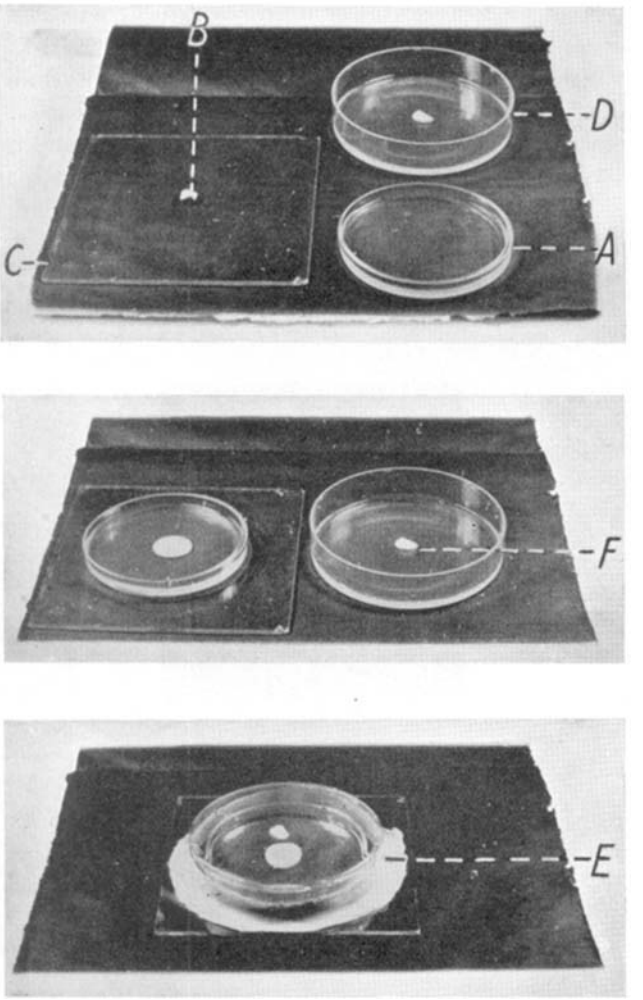



Illustration 6. The author's modified Fortner method.

tube with a sharp file on a level with the middle of the agar pillar, after which the tube is broken off. The agar is shaken out into a sterile Petri dish, and cut longitudinally with a sterile knife into thin slices. After a few slices have been removed the spirochete growth is perhaps found free from other colonies of bacteria. If, after examination in the dark field microscope, the growth is found to be pure, secondary cultures are placed in fresh

tubes, which must, however, contain pieces of fresh tissue. SMITH used pieces of rabbit kidney.

The methods described above are of course very tedious and take a long time, besides which they give uneven results. FORTNER's plate method for anaerobic culture was an improvement.

After the present author had been unsuccessful with methods previously described, the Fortner plate was employed. The procedure is as follows. Agar is left to set in a low Petri dish, after which a 2—3 mm. agar lamella is cut out in the middle. One half of the lamella is sown with the investigation material and the other with *bacillus prodigiosus*. The Petri dish is then placed

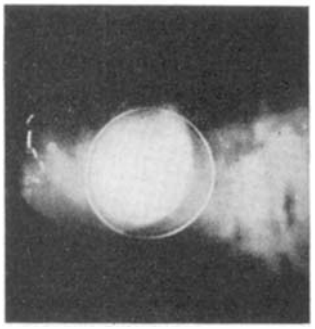


Illustration 7. The sharply defined ring formation.

with the opening downwards on a square glass plate and made airtight with plasticine. *Bacillus prodigiosus* uses up the oxygen which is enclosed in the dish, and thus an anaerobic condition is simply obtained. In its original form this method, however, suffers from certain disadvantages. Thus, it is difficult effectively to keep the half of the substrate which is sown with *bacillus prodigiosus* separate from the other half intended for the investigation material. Growth of *prodigiosus* over on to the latter half therefore often takes place, either owing to the two halves of the substrate coming into contact with each other, or to the growth being carried over by damp. Nor do optimal conditions for spirochete growth appear to be secured by this method. Thus, the present author only succeeded in obtaining spirochete growths on about every tenth dish, and it was impossible to get any secondary culture by this method.

However, the results were better when the present author

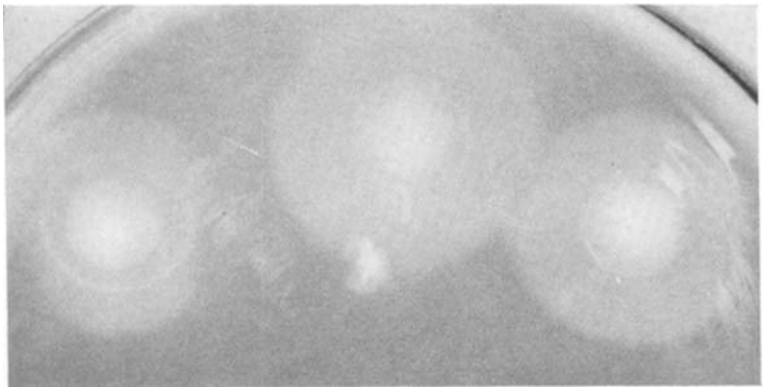


Illustration 8. The misty growth spreads outside the opaque ring. The concentric mode of growth is the general rule.

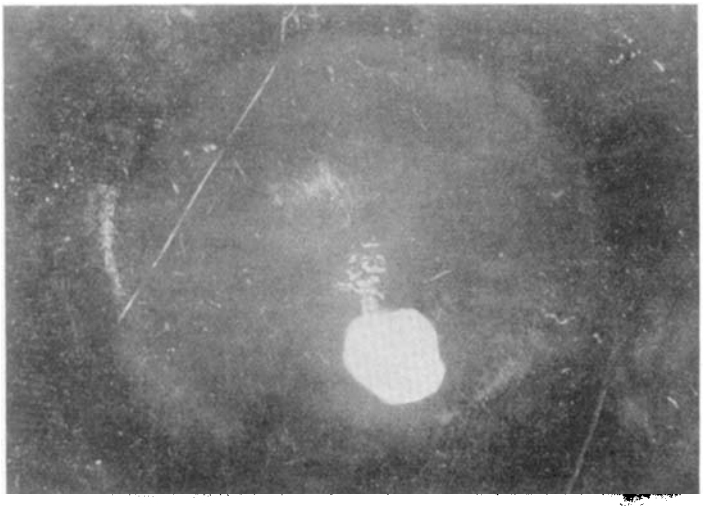


Illustration 9. The mode of growth of the pure spirochete culture.

modified FORTNER's method in the following way. In a small Petri dish with a low edge (A) containing a thin layer of ordinary agar, *prodigiosus* was sown, after which it was fastened on the glass plate C by means of a plasticine pellet B. The large Petri dish, which contained a substrate consisting of 2 parts of 2 % agar + 1 part of bouillon + 1 part of inactivated horse serum, could now be wholly sown with primary material, after which

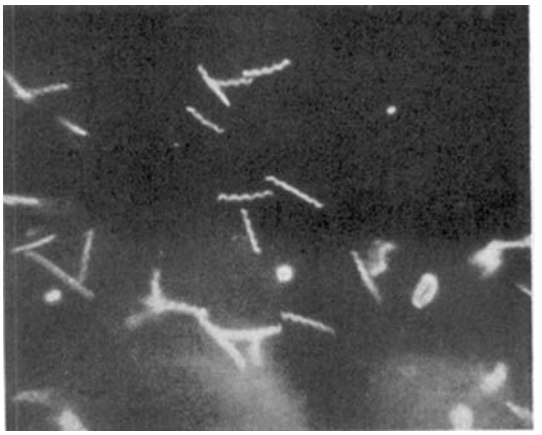


Illustration 10. *Treponema microdentium* in pure culture.

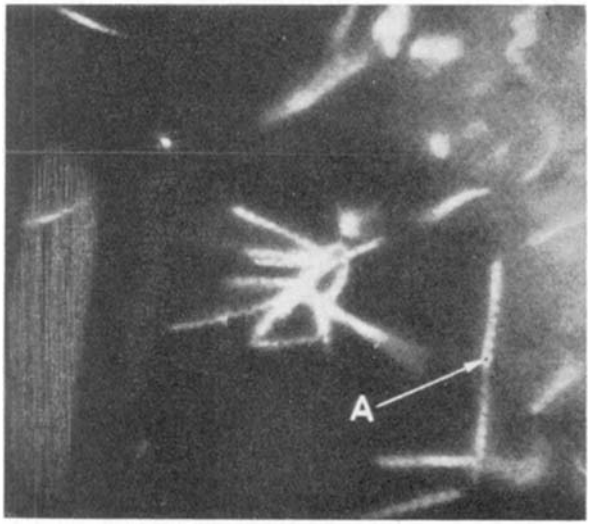



Illustration 11. *Treponema macrodentium* in pure culture. At A division.

it was placed with the surface of the substrate downwards over the smaller dish on the glass plate. It was then made airtight with plasticine E. Incubation took place during 48 hours in a thermostate at 37°, and subsequently at 30°. It was now considerably easier to get spirochetes from material from gingiva pockets to grow on the surface, but they were met with for the most

part in mixed cultures and still would not grow on secondary plates.

Bearing in mind that the oral spirochetes thrive best in gingiva pockets and cracks in the mucous membrane, the present author next made a deep sowing down in the substrate with the same original material, so that to a certain extent an artificial pocket was formed. For the rest the procedure was as above. After 24 hours at 37° there was now growth on practically every plate, firstly a surface growth, which the dark-field microscope showed to be a mixed culture containing also spirochetes, secondly, a growth within the substrate.

The growth within the substrate had a very characteristic appearance. Already after 24 hours a ring of about 3 mm. in diameter growing horizontally and sharply delimited from all other growth was observed. 0.5 of the periphery was of an opaque white colour, while the growth within the circle was of a more misty transparent character. See illustration 7.

At first the diameter increased about 2 mm. every 24 hours and after the sixth and seventh days increased by 3 mm. in 24 hours. On the eight day the misty growth began to spread outside the opaque ring as in illustration 8.

If the surface layer is removed and specimens from the edge of the growth studied in the dark-field microscope, occasional active spirochetes will be found, while specimens taken further in towards the centre prove to contain masses of spirochetes, which are not quite so active as those in the peripheral growth. Still further in towards the centre bacteria are met with wnich, in symbiosis with spirochetes of different kinds, form the central mass.

Pure cultures of spirochetes can be obtained if secondary cultures are made from the peripheral growth. The pure culture is characterized by the absence of ring formation, and by the growth appearing like a homogeneous mist formation, which migrates towards the periphery of the substrate. Secondary cultures should be incubated for 4 days and nights at 37°, and not until after that should the temperature be reduced to 30°. Fresh animal tissue must be present in the substrate for growths of spirochetes to appear in the secondary cultures. The present author uses pieces of guinea-pig testicle taken from a freshly killed animal. The pieces are transferred in a sterile condition to the Petri dishes, one piece in each. (Illustration 6, F.) The agar is poured on so

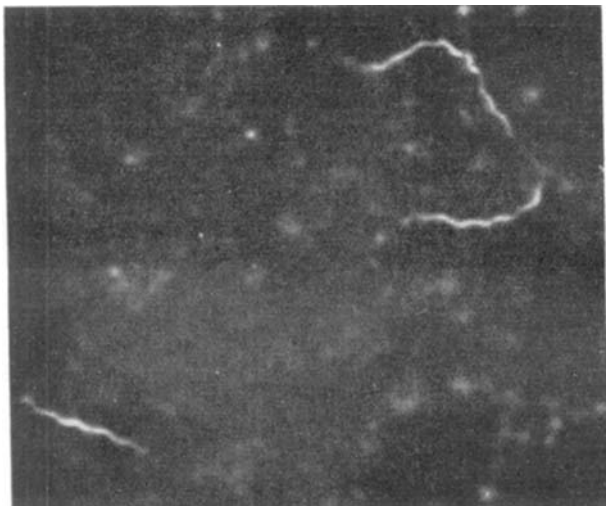


Illustration 12. *Treponema vincenti* in pure culture.

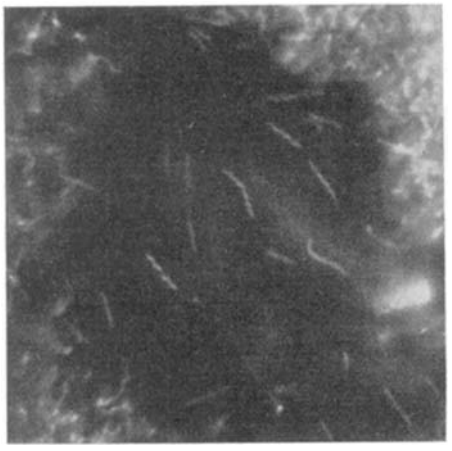


Illustration 13. *Treponema buccale* in mixed culture.

that the pieces of testicle are entirely covered by substrate. The plates can be kept in a refrigerator, but preferably not for a longer period than two weeks, as even in cold-storage the substrate is apt to become infected with mould.

With the method described above the present author has suc-

ceeded in obtaining pure cultures of *Treponema micro-* and *macrodentium*, as well as *Treponema vincenti*. *Treponema buccale* can also be cultivated by the same method, though no research worker has yet succeded in getting a *pure* culture.

By way of summary it may be said that, by means of a modification of the Fortner plate and deep sowing in serum plates with low agar content (c. 1 %), the present author succeeded in making a pure culture of oral spirochetes from material taken from gingiva pockets. The method indicated is a material simplification of earlier techniques. The investigations are being continued.

Finally I wish to express to Professor C. KLING, Docent G. OLIN and Phil. Lic. J. FÅHRAEUS my warm thanks for all the ready support I have received.

## Summary.

Through the modification of the Fortnerplate and through deep sowing in serumplates with low content of agar (1 %) the author has succeeded in cultivating pure cultures of oral spirochetes out of material from pockets in the gum. This method means a great simplifying of earlier methods.

## Zusammenfassung.

Durch eine Modifikation der Fortnerschen Plattenmethode und durch tiefe Versenkung der Aussaat in Serumagarplatten mit geringem Gehalt an Agar (za. 1 %) ist es dem Verf. gelungen, aus Material von Zahnfleischtaschen her, Mundspirochäten reinzuzüchten. Das angegebene Verfahren bedeutet eine wesentliche Vereinfachung der früheren Technik.

## Résumé.

Par une modification de la méthode des plaques de Fortner et en implantant profondément les semis dans des plaques de sérum-agar à faible teneur d'agar (environ 1 %) l'auteur a réussi à obtenir des cultures pures des spirochètes buccaux à partir

des matières provenant de poches gingivales. Le procédé indiqué représente une simplification importante de la technique ancienne.

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