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2. TAXONOMY

The name “spirochaete” was first given by Ehrenberg in 1838 to large, free-living, flexible organisms floating in fresh and marine water, e.g., *Spirochaeta plicatilis*. Spirally-shaped organisms were for a long time grouped under the common name of *Spirillum*, *Spirochaeta* or *Vibrio*. Later

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1 See Wilson & Miles (1955), Wenyon (1926), Jordan & Burrows (1945).
some were shown to be bacteria, and these are now grouped under *Vibrio* and the various species of *Spirillum*. The remainder are actively motile not by flagella, but by means of a screw-like rotation of the organism. All these spiralled organisms have commonly been loosely termed “the spirochaetes”. This term, however, is now strictly confined to members of the family Spirochaetaceae.

There are four main genera of “spirochaetes” in the loose usage of the word: *Spirochaeta, Cristispira, Leptospira* and *Treponema*; from the last-named *Borrelia* have now been separated. The first two genera are the Spirochaetaceae; the others form the family Treponemataceae.

**SPIROCHAETA**

The various species of this genus are large slender organisms 200μ-500μ long and 0.5μ-0.7μ thick with 100-250 spirals, and they are not capable of causing disease. The term *Saprospira* is used for smaller free-living forms which may be encountered in foraminiferous sand (Jordan & Burrows, 1945).

**CRISTISPIRA**

Members of this group are characterized by a spirally arranged veil-like membranous appendage or crista round the body of the cell. The crista may splay out to resemble the undulating membrane of a trypanosome—in fact, it was first named *Trypanosoma balbiani* (Bradfield & Cater, 1952). *Cristispira* are saprophytic in certain molluscs (e.g., *Cristispira balbiani*, found in the crystalline style of the oyster, which is 45μ-100μ long and 1μ-1.5μ thick; its body is divided into chambers by septa of thickened cytoplasm).

**LEPTOSPIRA**

Some members of this group are pathogenic to man and are the cause of Weil’s disease, or infectious jaundice, and also of certain fevers. They have a large number of closely-wound spirals, and the ends are frequently turned round at a sharp angle; at rest they are characteristically hooked. They are the thinnest of all spirochaetes, being only 0.1μ-0.2μ thick and 5μ-18μ long. Included in the group are *L.icterohaemorrhagiae*, the cause of Weil’s disease, or infectious jaundice; *L. canicola*, a pathogen of dogs; and *L. biflexa*, an easily-cultured, free-living leptospire said to inhabit ponds, gutters and dripping taps (Bradfield & Cater, 1952).

**TREPOGONEMATA**

Treponemata are widely distributed in nature. Numerous species have been described in water, in the gut of certain insects (e.g., the ant and the cockroach) and in the large gut of the toad. In man they are found in the mouth, alimentary tract, bronchi, around the urethral orifice, in certain ulcerating conditions of the skin, in condylomata, in the blood of patients with relapsing fever (although these are now classified as *Borrelia*), and in many of the lesions of syphilis. They are considerably thicker than leptospirae, are less closely wound, and vary considerably in size. *T. termitidis* is 20μ-60μ long, while *T. parum* may be only 3μ in length. A saprophytic water form, *T. elusum*, has been described.

*Borrelia*

Most treponemata have a low refractive index and stain poorly with aniline dyes. Those associated with Vincent’s angina and relapsing fever in man and with avian spirochaetosis have a refractive index like that of bacteria, and stain more readily. On these and other less distinctive grounds they are assigned by many authors to a separate genus, *Borrelia* (Wilson & Miles, 1955; Swain, 1955; Breed et al., 1948; Eagle, 1948).

Those now classified as *Borrelia* include *B. vincentii*, the cause of Vincent’s angina, *B. anserinum* which affects geese and turkeys (McNeil et al., 1949), and the relapsing fever *borreliae*. The latter include *B. recurrentis*, agent of cosmopolitan relapsing fever which is transmitted by lice and is found in Europe, Africa, Asia and South America, and other species transmitted by soft ticks (*B. duttoni*, found in Africa, *B. hispanica*, in Europe, *B. turicata, B. parkeri, B. venezuelensis, B. mazzottii* and others in the Americas). (Some authors still describe these as treponemes with the generic prefix “T.” instead of “B.”.) Some writers include also as *Borrelia* certain of the saprophytic genital treponemes.

*B. vincentii* is 7μ-18μ in length and 0.23μ-0.64μ (average 0.42μ) thick. It has 3-8 shallow spirals of varied amplitude and, unlike *B. duttoni* and *B. recurrens*, is stated to have no external amorphous layer but is enclosed by a clearly-marked cell membrane (Swain, 1955).

*B. duttoni* is 9μ-16μ thick and 0.24μ-0.60μ wide. It is tapered at the end and is not of constant thickness. Short forms with up to four spirals of inconstant amplitude and longer forms of 5-8 spirals
are encountered. There is an outer covering of structureless material. *B. recurrentis* closely resembles *B. duttoni* in size and general morphology (Swain, 1955).

**Trepomonata of man and animals**

Those remaining for classification as trepomonata include *T. cobayae*, found in the blood of guinea-pigs; *T. microdentium*, *T. macrodentium* and *T. muco-sum* (Noguchi, 1912c), which are apparently non-pathogenic and are found in the mouth of man (being smaller than the pathogenic *B. vincentii* also found in that site 1); a number of saprophytic genital treponemes found in increased numbers in septic conditions (*T. refringens*, *T. phagedenis* (*T. balanitidis*), *T. pseudopaludum*, *T. gangrenosa nosocomialis*, *T. minutum* (*T. genitalis*), and *T. calligyrum*); *T. cuniculi*, which is responsible for a venereal disease in rabbits; *T. pallidum*, the cause of human syphilis, and *T. pertenue* and *T. carateum*, the organisms responsible for yaws and pinta, respectively.

Of those infecting man, *T. microdentium*, the small mouth treponeme, resembles *T. pallidum* in morphology and is also cultivable with least difficulty. According to Rosebury & Foley (1941) it is the only member of the oral group which can be accepted without doubt as a distinct species. Of the human genital trepomonata, Schaudinn & Hoffmann (1904-05) also described *T. refringens*, at the time of their original description of *T. pallidum*, as a broader, less regular, more refractile organism, but did not decide whether it was a separate species (see also Levaditi, 1906). It has a right-handed spiral (Sequeira, 1956). *T. phagedenis*, with its variety of alternative names, was described by Noguchi (1912a), who also (1913) described *T. calligyra* (*calligyrum*) and *T. minutum* or *T. genitalis* (see also Noguchi, 1918; Noguchi & Kaliski, 1918): all may be obtained from genital sores or condylomata (see Moreau & Giuntini, 1956; Coutts et al., 1952).

Moreau & Aladame (1957), who studied three oral treponemes (including *T. microdentium*) and four genital treponemes (*T. refringens*, *T. phagedenis*, *T. calligyra* and *T. minutum*) noted that their fermentative pattern in culture differed according to species. It has been suggested from electron microscope studies of these organisms (Moreau & Giuntini, 1956) that although *T. calligyra* and *T. minutum* may be considered to be treponemes, *T. refringens* and *T. phagedenis* should be classified as *Borrelia*.

Treponemes will resist trypsin digestion for many days, but 10% bile salts (sodium taurocholate) cause complete disintegration (von Prowazek, 1907) and all, with the exception of leptospiroae (Noguchi, 1917; Wilson & Miles, 1955) are eventually broken up by 10% saponin (Jordan & Burrows, 1945).

**Relationship of treponemes to protozoa**

An argument which has recurred from time to time is whether treponemes should be classified as protozoa. McDonagh (1912) so classified spirochaetes and described an apparent life-cycle resembling that of the malaria parasite. At the same period, on the other hand, Dobell (1912), among others, pointed out that the one important feature which distinguished protozoa from bacteria was motility without flagella. When early workers with the electron microscope claimed to have found flagella on *T. pallidum* even this was in doubt (Wilson & Miles, 1955). However, it seems that the apparent "flagella" in *T. pallidum* arose from rupture of the treponeme before examination, but, even knowing this, van Thiell (1959) still inclined towards protozoa, stating that the presence of flagella could no longer be used as an argument for its relationship to bacteria: neither, he considered, did the movement of *T. pallidum* fit in with that of spirochaetes. Bessemans & de Geest (1933), pointing out the difficulty of culture of *T. pallidum in vitro*, also inclined to protozoa. Most workers, however, like to consider spirochaetes as representing a phase of life midway between the bacteria and protozoa (Jordan & Burrows, 1945; van Thiell, 1959).

**DISCOVERY OF PATHOGENIC TREPONEMES**

At least four different varieties of pathogenic organisms of the genus *Treponema* are known: *T. pallidum* (syphilis), *T. pertenue* (yaws), *T. carateum* (pinta) and *T. cuniculi* (venereal treponematosis of rabbits). They all resemble each other morphologically.

**T. pallidum**

Donné, in 1837, is stated (Campbell & Rosahn, 1950) to have been the first to describe a spiralled micro-organism in the exudate of primary syphilitic lesions of the genitalia, and he believed he had found the causative organism of syphilis. His work

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1 *T. buccalis*, *T. dentium* and *T. spirillum* have also been described—see Wilson & Miles (1955, page 2252).
was confirmed by Vanoye (1840-41), who considered that these organisms could be used in the diagnosis of syphilis.

According to Stokes & Beerman (1934), Metchnikoff stated that Bordet & Gengou in 1903 undoubtedly saw the “spirochaete” of syphilis. Klebs is also stated to have seen it in 1875-77 in syphilitic material, and also to have transmitted the disease to the monkey (see Klebs, 1932; Wilson & Miles, 1955). Credit, however, is usually given to Metchnikoff and Roux in Paris for having infected monkeys and apes experimentally with syphilis in 1903 (Metchnikoff & Roux, 1903, 1904, 1905; see Jordan & Burrows, 1945). They also showed that calomel ointment applied to chimpanzees 1-2 hours after local inoculation would prevent infection.

The final confirmation of T. pallidum as the causative agent of syphilis—indeed, its discovery—is attributed to Schaudinn & Hoffmann (1904-5, 1905a, b) (see also Hoffmann, 1905, 1906; D’Aguistino, 1957) who observed the organism in chancres of syphilitic patients. Other early workers include Mansurov (1885), who described a vegetable parasite of syphilis, and Zabotolnyj (1909), who apparently saw T. pallidum two years before Schaudinn & Hoffmann but attached no importance to his discovery. After the discovery by Schaudinn & Hoffmann, numerous confirmatory papers appeared within a very short time all over the world (including Russia—e.g., Zabotolnyj, 1905, 1906, 1909: Ivanov, 1905). See also Minouflet (1906).

The discovery of T. pallidum added enormous impetus to experimental research. The following year (1906) claims were advanced that the organism had been cultured (Volpino & Fontana, 1906; Scherschewsky, 1909a, b), although the cultures obtained were not shown to be virulent. In the same year Bertarelli (1906) showed that syphilis could be passed from the eye of one rabbit to that of another and Parodi (1907) first transmitted the disease to the testicle of a rabbit. Virulent cultures were, however, claimed by Noguchi (1911a)—but this also proved a disappointment as the virulence soon disappeared.

Around this time, too, the first diagnostic serum test for syphilis was evolved by Wassermann (Wassermann et al., 1906) and the dark-field method of diagnosis was soon introduced (Coles, 1909). Zabotolnyj & Maslakovec (1907) described the cessation of movement and the agglutination of T. pallidum under the effect of serum from a syphilitic patient. Ehrlich & Hata (1910) discovered salvarsan, or 606, to provide what was hoped to be an effective and rapid treatment for the disease (and also for yaws). From the cultured treponeme, Noguchi (1911b) prepared luetin, which was tested in many hospitals and which would produce a positive skin reaction in most cases of late syphilis (less often in early cases). Moreover, it was considered to be specific for the disease (Noguchi, 1912b).

Thus, during less than a decade before the First World War, most of the essential problems of syphilis appeared to have been solved—at least, it must have appeared so to syphilologists at the time.

T. pertenue, T. carateum and T. cuniculi

T. pertenue, the causal organism of yaws, was also discovered in 1905, by Castellani. It is morphologically indistinguishable from T. pallidum (Wilson & Miles, 1955). T. carateum, the causative organism of pinta, although suggested by Menk (1927) and by Gonzalez-Herrejon (1927) on the basis of a high incidence of positive Wassermann reactions in pinta patients (see Turner & Hollander, 1957) was first demonstrated by Saenz et al. (1938) (see Fox, 1939; Jordan & Burrows, 1945). León y Blanco (1940), who called it T. herrejoni, showed this treponeme also to be morphologically indistinguishable from T. pallidum. For further history see Schuberg & Schlossberger (1930).

Although T. pertenue was soon shown to be transferable to rabbits (Nichols, 1910), who was already working on a strain obtained from a coloured soldier returning from the Philippines), T. carateum, on the other hand, is not easily passed to animals and only one such claim has been made, that of León y Blanco & Oteiza (1945) (see Holcomb, 1942).

T. cuniculi, the cause of a venereal treponematosis in rabbits first described by Ross (1912), was identified by Bayon (1913) and is also morphologically similar to T. pallidum. It causes a complaint known as “rabbit syphilis”, or sometimes as pallidoidosis. Affected rabbits develop superficial small, scaly, eroded, sometimes crusted, lesions on the genitalia and adjacent perineal region: sometimes the nostrils and eyelids may be affected (see section 17).

EVOLUTION OF PATHOGENIC TREPONEMES

The evolution of the pathogenic treponemes can only be a matter of speculation. It is considered by some (e.g., Cockburn, 1961) that they arose aeons ago from free-living water forms which have since become adapted to their human or animal hosts,
having been subjected to all the influences concerned in natural selection. Although it is suggested that the four treponemes may have come from a single source (Hoffmann-Bonn, 1953) speculators may differ as to how far back in the evolutionary scale the separation between them occurred. Hudson (1946) considered the treponemes pathogenic to humans (T. pallidum, T. pertenue and T. carateum) all to be T. pallidum, the disease picture in man being modified by various external environmental factors and by factors within the host. This author (1963) believed that a common organism became adapted to man in Africa in palaeolithic times. Hackett (1963) suggests that the human treponematoses probably arose long ago from an animal infection. Mutations subsequently occurred—T. carateum probably being the earliest—resulting in the diseases now known as yaws, pinta and venereal syphilis. That the distribution of the disease syndromes of yaws, pinta and endemic and non-venereal syphilis conforms to some considerable extent to the environmental background is well known (Guthe & Luger, 1957; Guthe & Willcox, 1954). Willcox (1960) has indicated the evolutionary cycle which may occur in the disease syndromes caused by human treponematoses resulting from pressures in the extrinsic environment, and in the intrinsic environment of the host.

**STRAINS OF T. PALLIDUM**

Those strains of T. pallidum which have been maintained in animals have remained virulent to both animals and man. These include: the Nichols (Nichols-Hough or Nichols pathogenic), Truffi, Gand, Gent, Armi, Fan, Tho, Est, Rei and Pour strains (see section 15). Strains maintained in animals for a period of years may undergo modification (Turner & Hollander, 1957).

Although virulent treponemes have not been established in culture outside the body, there are a number of strains—usually designated T. pallidum—which have been maintained in artificial media for many years. These include the Reiter, Noguchi, Nichols non-pathogenic, Kazan, Kroó and Vásárhelyi. Details of these are given below (see section 19).

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3. MORPHOLOGY: I. METHODS OF EXAMINATION

The morphology of *T. pallidum* may be examined in slide preparations of stained smears, by dark-field under the light microscope, and by phase-contrast and electron microscopes. With the light microscope without dark-field and with the electron microscope, the treponemes are examined in the dead state: under the dark-field and phase-contrast microscopes, live organisms can be studied. Using the light microscope, the unstained organism is extremely difficult to see.

**STAINED SMEARS**

Stained smears are examined under the conventional light microscope, and two basic techniques of staining are used: (a) the treponeme is impregnated with a dye, or a metallic ion such as silver, to render the organisms visible against a pale background; or (b) the background may be stained black, leaving the unstained treponeme pale by comparison (see Bessemans et al., 1936; Yamamoto, 1929a, b). Matsumoto (1930) and Turner & Hollander (1957) succeeded in staining *T. pallidum* with no less than 402 different dyes.

**Impregnation methods**

Contrary to general belief treponemes are easily stained (Turner & Hollander, 1957; Wheeler, 1960) but they are difficult to see under the light microscope because of the small amount of protoplasm possessed by the organism and also because the suspending medium frequently contains too much tissue debris which also takes up the stain (Wheeler, 1960). Early investigators used aniline dyes or coal tar derivatives (e.g., methylene blue, azure eosinates, Victoria blue, cresyl violet, Giemsa and similar mixtures). A mordant (i.e., a protein precipitant such as phenol, tannic acid, acetic acid, phosphotungstic acid, etc.) has to be used to make the stain effective. Schaudinn & Hoffman (1904-05) employed a modified Giemsa stain (see Wilson & Miles, 1955), with which *T. pallidum* and other pathogenic treponemes stain rose-red.

Noguchi (1918) found that treponemes fixed with Fontana’s fixative and Fontana’s mordant could be stained by carbol fuchsin. This was further confirmed by DeLamater, Wiggall & Haanes (1950),

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1 See Campbell & Rosahn (1950).