4501

In Vitro Cultivation of a New Spirochete From Human Source.

JOHN A. LANFORD. (Introduced by C. W. Duval.)

From the Department of Pathology, Tulane University School of Medicine.

A spirochaetal organism in pure culture has been recovered from the pleural effusion of a human case of unresolved lobar pneumonia due to type IV pneumococcus.

The organism morphologically resembles very closely the *Spirochete bronchialis* of Castellani[1] which has been demonstrated in human pulmonary lesions; however, none of the workers have successfully cultivated it and in practically all of the reported instances of this infection the spirochaete was found in the sputum. Only in cases reported by Mason[2] and Lancereaux[3] were spirochetes found in the pleural effusion and not in the pulmonary secretion. Efforts to cultivate the organism and to induce lesions in lower animals were unsuccessful.

Morphology: This organism which from the classification of Noguchi might be called a spironema is variable in its morphology, even in material fresh from the human body. The length varies from 5 to 15 microns and the width from 0.2 to 0.4 micron. The spirals are not as acute as in *Treponema pallida* and there is marked irregularity in stained preparations characterized by the presence of deeply stained granules and areas faintly stained. This irregularity may be due to 2 things: a condensation of the chromatin with the appearance of small granules or to a thinning out of the cytoplasm preparatory to transverse division. The curves vary from 2 to 6 in fresh preparations and in cultures as many as 15 or more have been noted in a single spiral. No undulating membrane has been demonstrated in fresh or stained preparation. Whereas each extremity tapers to a point, which also varies in its acuteness, no flagella have been demonstrated. These spiral organisms are very motile, moving forward and turning on the long axis. There is an undulating flexion of the body together with a corkscrew like movement taking place at the same time. In old and dying preparations the curves are less acute and the motility is very sluggish and at times absent. They divide by transverse fission.

The organisms are stained best by the Romanowsky stains which bring out quite strikingly the chromatin granules, but most simple

[1] Castellani, *Lancet*, 1906, i, 1384; *Brit. Med. J.*, 1909, ii, 782.

[2] Mason, *Johns Hopkins Hosp. Bull.*, 1920, xxxi, 435.

[3] Lancereaux, *Presse med.*, 1909, xxvii, 556.

stains easily demonstrate them. They are negative to Gram's and non-acid fast.

Fantham,[4] studying the *Spirochete bronchialis,* describes a "granule phase," apparently a progressive concentration of the cytoplasm, forming chromatin bars or granules, and this concentration ultimately forms a series of coccoid bodies in the peripheral sheath of the organism. The sheath ruptures and sets free this coccoid body which elongates and small spirochetes emerge from them. I have not yet been able to demonstrate this phase in this particular organism.

Cultivation: The organism is easily cultivated under aerobic conditions but when freshly isolated from the human case it grew more abundantly under partial anerobiasis. The first isolations were on blood agar and dextrose bouillon containing blood. Twenty-four hours after planting, the bouillon was cloudy and rich in actively motile spiral organisms. After the culture became older the organisms became longer. After several generations the organism becomes viable on plain agar but loses most of its motility. On solid media they grow as very minute colonies even smaller than the colonies of the influenza bacillus. They have no demonstrable hemolytic action on blood agar plates but in blood dextrose bouillon they cause a very slight splitting of the blood cells. When planted on serum sugar waters there is no gas formed in any of the cultures and no change demonstrated by Andrade indicator. However, using phenol red as an indicator, lactose, sacchrose, mannite, maltose and dextrine serum waters showed slightly more alkaline. Xylose and dextrose are not changed.

Animal Experiments: Three guinea pigs were inoculated with fresh material from the human case; one intradermally, one in the groin and one intraperitoneally without any effect other than the formation of a local inflammatory mass. This mass persisted for a number of days and disappeared without producing any apparent harm. Two rabbits were injected intratracheally and into the testicle with 1 cc. of the effusion fluid from the human case. These animals manifested no evidences of infection and after the first inflammatory reaction in the testicle lasting 6 to 7 days there was no demonstrable effect from the injections. Four mice were injected, 2 subcutaneously and 2 intraperitoneally with ½ cc. of the fresh effusion without any apparent effect on the mice.

A study of the sera of one guinea pig and 2 rabbits, 6 weeks after inoculation gave interesting results. The guinea pig showed no immune bodies, but the sera of both rabbits gave positive agglutina-

[4] Fantham, *Ann. Trop. Med. and Parasitol.,* 1915, ix, 391.

tion tests against the organism in dilution of 1-120 and positive complement fixation reactions. The serum of the patient showed positive agglutination in 1-120 dilution and a strongly positive complement fixation reaction. Sera from healthy human beings and also of patients giving positive Wasserman reactions showed no specific immune bodies.

Neo-salvarsan, intravenously, cured the patient.

This organism resembles the description of the *Spirochete bronchialis* of Castellani but differs in the absence of a demonstrable granule phase and the ease with which it is cultivated on ordinary laboratory media. It differs, too, in that all the reported cases of the *Spirochete bronchialis* have been in individuals with pulmonary lesions opening into the bronchi. It differs very markedly from all of the other known varieties of spirochetes and apparently represents a new variety which is pathogenic for man.