THE SPIRONEMA OF CYPRIAN RELAPSING FEVER.

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INTRODUCTION.

A series of cases of tick-borne relapsing fever among troops in Cyprus has been described by Wood and Dixon (in the press). The vector of infection was shown in all probability to be the tick Ornithodorus tholozani and the infection was generally acquired by men sleeping in earth shelters or caves. Some pathological features described in association with Cyprus cases (Dixon, 1942, in the press) are the almost general presence of urobilinuria and the fact that about 20 per cent of the cases have positive Kahn reactions.

It is stated that louse-borne relapsing fever cannot be transmitted to the guinea-pig but that the tick-borne variety can infect this animal. Accordingly, at the suggestion of Colonel J. S. K. Boyd, I attempted to infect guinea-pigs with the Cyprian spironema. Successful infection of these animals would constitute independent evidence incriminating the tick as the vector of the disease.

The present communication describes firstly the results of inoculation of guinea-pigs with the Cyprian spironema and secondly the morphology of the organism as isolated from these animals.

(1) ANIMAL INOCULATION.

An Indian soldier was admitted to hospital on November 16, 1942, complaining of vague abdominal pain. He gave a history of having spent a night in a cave while on manoeuvres about two weeks previous to admission. No lice were found on his body or clothing. On November 22, 1942, he developed fever with a temperature of 101°F, and spironema was found in his blood by Major B. J. Doran, I.M.S., under whose care he was. On November 23, 1942, temperature was 103°F, and 5 c.c. of blood were drawn into 1 c.c. of sterile citrate. The specimen was then despatched to the laboratory and 0·5 c.c. of the citrated blood was injected intraperitoneally into a guinea-pig within two hours of removal from the patient. On December 2 a few spironemas were found in a thick drop film taken from the ear of the guinea-pig. On December 3 very numerous spironemas were present. This period of gross septicemia lasted for at least three weeks. By January 3, 1943, however, no spironemas could be found in blood by microscopy and this state of affairs persisted until the animal was killed on January 7, 1943. Heart blood removed from the animal at the time of death gave a partially positive Kahn reaction, tube 1 being negative, tube 2 doubtful and tube 3 positive. The patient's blood was seropositive on the day of inoculation, being doubtful in tube 1 and positive in tubes 2 and 3. Sections of brain and spleen of guinea-pig stained by Levaditi's method failed to show any spironemas.

A second guinea-pig was infected by intraperitoneal passage of 0·04 c.c. of blood from first guinea-pig on December 14, 1942, while the blood of the former was heavily infected with the spironema. On December 15 a very few spironemas were found in blood of second guinea-pig. None however
could be found during the succeeding few days until December 21, when a few were seen. By December 23 the blood of this second guinea-pig was also swarming with the spironema. Heavy infection persisted in this animal for about three weeks, many being observed on January 11, 1943. The spironemas however had disappeared, so far as microscopy could elucidate, by January 14, 1943.

On January 15, 1943, a third guinea-pig and a rat were inoculated intraperitoneally with blood from the second guinea-pig, although no spironemas could be detected in this blood by microscopic examination. The blood however was still infected, as both the third guinea-pig and the rat subsequently became infected with the spironema. In fact three days later a few spironemas were found in the blood of the third guinea-pig and by the fourth day it was heavily infected. The rat became mildly infected in six days though for the first three days after inoculation no spironemas could be detected in its blood. The rat remained infected for at least five days but at no time did it develop the gross septicaemia characteristic of the guinea-pigs. This is in contrast to louse-borne relapsing fever in which rats are stated to become heavily infected within forty-eight hours.

(2) Morphology of the Spironema.

The spironema from the guinea-pigs was observed by two methods. Firstly thick drop blood films were stained by Field's or Leishman's stain and secondly wet films were examined by dark field illumination. The technique employed for the latter method was as follows: A drop of human serum (previously desensitized at $56^\circ$ F. for serological tests) is mixed on a slide with one fourth of its volume of 3·8 per cent sodium citrate solution. A small drop of blood is taken from the ear of the infected guinea-pig by means of a capillary pipette and is mixed with the drop of fluid on the slide. A cover slip is applied and the preparation is blotted and ringed with vaseline. By this means the corpuscles of the guinea-pig are agglutinated by the human serum leaving clear spaces in which the morphology of the spironema is readily observed in clear serum free from corpuscles.

With the above technique using blood from infected guinea-pigs suspended in human serum the following results were obtained.

After about an hour from the time of setting up the preparation the spironemas have migrated away from the clumps of agglutinated corpuscles and are seen free in the clear serum. The spironema under these conditions remains alive and actively motile for over forty-eight hours. During this time there is little infection with other microorganisms.

The spironema is a regularly coiled spirochète. Its wave-length is about $2\mu$. This agrees with measurements of other observers on the louse-borne type. It has between 3 and 11 coils. Its amplitude is about $1·5\mu$. Its wave-length is quite constant being much more regular than in stained films. It is thus clear that the loose and irregular coils seen in stained films are the result of fixation. Sometimes, however, when alive, the spironema bends into loose secondary sinuosities but at no time does it lose its fine regular wave structure. It is of interest that when similar technique is applied to the spironema of Vincent's angina, the latter is
found to be very unlike the spironema of Cyprian relapsing fever. In a serum preparation examined by dark ground illumination, *Spironema vincenti* was found to possess loose and irregular coils with a wave-length of about 6 μ and was not nearly so tightly coiled as the spironema of relapsing fever. *Sp. vincenti* has angular waves unlike the regular fine curves of *Sp. recurrentis*. It is clear that the spironema of relapsing fever is fairly close in morphology to *Treponema pallidum*.

The spironema of Cyprian relapsing fever under dark ground illumination shows a dark central core with brightly illuminated walls. Terminally these walls converge and no central dark zone can be seen. Sometimes spiral flagellae are visible as very fine bright threads attached to the end of the organism. Double forms are often encountered joined end to end by constriction without any dark core. Sometimes double forms are united by a single fine spiral thread. These double forms have been seen to divide at the constrictions.

Cog-wheel or annular forms are frequently observed in these preparations. Here the spironema forms a continuous ring. It circulates in this ring rotating to and fro. A fine thread joins the two ends so that a complete circle is produced. It appears as if these annular forms result from entanglement of the flagellae of one organism when bent on itself. These annular forms would explain the frequent occurrence of coiled rings seen in fixed films—but in fixed preparations the primary wave structure is lost.

The organism remains alive and actively motile in serum for more than two days. It appears to thrive under the anaerobic conditions present in such serum preparations without any attempt to maintain a constant temperature in its environment. Culture under such conditions would appear possible. The motility of the spironema consists of corkscrew like wave motion accompanied by spacial translation. The spironema also exhibits a lashing type of movement in which the organism bends on itself. It is sometimes contorted into loose secondary waves. Active wave motion in the fine primary coils is also seen in stationary organisms, especially in older preparations. Here, however, it is possible that the organism is stationary owing to anchorage of its flagellae on the fine threads of fibrin which tend to form in these preparations.

**Summary**:

(1) The spironema of relapsing fever contracted in Cyprus is transmissible to guinea-pigs. This substantiates the view that tick-borne relapsing fever occurs on the island since only this variety of the disease can infect the guinea-pig.

(2) The strain of spironema isolated was found to cause heavy infection of guinea-pigs four to twelve days after intraperitoneal inoculation. The heavy infection in which the blood is teeming with the spironemas persists for about three weeks. The blood, however, remains infective for a longer period as successful passage can be achieved later.
(3) The strain is infective to the rat but this animal apparently does not show heavy infection.

(4) The spironema is best studied by suspending blood from a heavily infected guinea-pig in human citrated serum and then examining by dark field illumination. Under these conditions the spironema remains alive and actively motile in vitro for at least forty-eight hours. It can readily be observed in the clear serum apart from the clumps of agglutinated corpuscles.

(5) The living spironema of Cyprian relapsing fever has a wave-length of about 2 μ. It is extremely constant in wave-length which is shorter and very much more regular than that observed in fixed films. Its amplitude is only slightly shorter than its wave-length. It has between 3 and 11 coils.

(6) The living spironema of relapsing fever is very different in structure from that of Vincent's angina. The latter when examined alive by the same technique has open irregular waves like those seen in fixed preparations and with about three times the wave-length of the living spironema of relapsing fever.

(7) Wheel-like annular forms are frequently encountered in these serum preparations of the spironema of Cyprian relapsing fever. In these the spironema forms a complete highly motile circle. A fine thread is seen to join the two ends of the spironema so that it seems likely that the annular forms are produced by entanglement of the terminal flagellae of one organism. These annular forms revolve, spinning to and fro.

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A CASE OF PNEUMOCOCCAL MENINGITIS WITH RECOVERY FOLLOWING CHEMOTHERAPY.

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Bombardier W., a previously healthy man aged 32, was admitted to hospital on the evening of August 21, 1942, with a provisional diagnosis of sandfly fever. For two days he had suffered from slight frontal headache, generalized muscular pains and sensations of heat and cold. He had, however, carried out his usual duties up to the afternoon of the day of admission to hospital.