

A careful examination of the morphological and physiological behavior of the 10 irradiated generations did not show the least difference between them and the control cultures. Attention has been paid especially, besides the morphological characters described by systematists, to the germination power, the rate of growth, and the sexual behavior.

In order to have my conclusion controlled by a specialist, I sent a complete set of cultures to Miss Satina of the Carnegie Institution, who was kind enough to examine the irradiated cultures. Her conclusions were in exact keeping with mine.

In connection with these negative results I should like to emphasize a fact which, although well known, is not given sufficient attention by biologists; that is, the distance between the wave lengths of ultraviolet and X-rays is proportionately greater than that separating ultraviolet and short electric waves, and one does not usually expect the same results on living organisms from electric waves and ultraviolet radiations.

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**Spirocheta Hispanicum (Variety Marocanum). Application in
Fever Therapy and Diseases of Central Nervous System.***

FREDERICK EBERSON AND WILLIAM G. MOSSMAN.

*From the Clinical and Research Laboratories, Mount Zion Hospital, San Francisco,
California.*

The objectionable features of malaria therapy in general paresis and neurosyphilis have suggested the use of an organism that will produce a self-limited infection for which no specific therapy is needed to cure the disease induced for therapeutic purposes. In other affections of the central nervous system, such as multiple sclerosis, dementia precox, and the like, inoculations with malaria-infected blood from another syphilitic patient are not desirable.

In the treatment of the diseases mentioned, Remlinger and Bailly,¹ Nicolle and Anderson,² and others have used the *Spirocheta hispani-*

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¹ Remlinger, P., and Bailly, J., *Paris Médical*, 1930, 1, 447.

² Nicolle, C., and Anderson, C., *Arch. Inst. Pasteur de Tunis*, 1930, 19, 27.

cum (variety *marocanum*) with success in the production of a form of recurrent fever. The tick, *Ornithodoros marocanus*, carries a type of spirochete that is pathogenic for guinea pigs. These pigs can be readily infected by means of blood obtained from the tick, and a few drops of blood from the guinea pig in turn used to inoculate the patient by instillation into the conjunctiva or the nasal fossae.

An obvious practical advantage in artificial pyrotherapy would be gained by the use of a pure culture of a suitable infecting agent such as the *Spirocheta hispanicum*. To this end we have attempted to cultivate the organism in artificial media.

A number of ticks (*Ornithodoros marocanus*) were obtained through the courtesy of Doctors Bailly and Remlinger of the Pasteur Institute of Tunis. Various kinds of culture media were inoculated with blood from guinea pigs that had been infected with material obtained from crushed ticks and showed abundant spirochetes in blood smears stained by the Wright, Giemsa, and Romanowsky methods. The best growth was obtained in hormone broth containing brain mash prepared as follows: 1,000 gm. of fresh beef brain (sheep or calf brain may be substituted), from which blood and membranes had been removed previously, were boiled with 1,000 cc. of veal infusion, pH 8.0. The material was passed through a fine meat grinder, 0.2% glucose added, tubed and autoclaved at 15 pounds pressure for 15 minutes or sterilized in the Arnold steam sterilizer.

After 7 to 10 days' incubation at 38°C, the *Spirocheta hispanicum* was found in great numbers. Transplants to the same media resulted uniformly in excellent growth after an average incubation period of 7 days. The addition of normal human, rabbit, or guinea pig blood with or without isotonic dextrose and sodium citrate (Rous and Robertson method) was found especially desirable provided brain tissue was also present. For such experiments we employed one part minced brain media, one part isotonic dextrose (5.4% dextrose in water), one part isotonic sodium citrate (3.8% sodium citrate in water), and one part of whole blood. The organisms appeared to have a special predilection for brain tissue in keeping with a marked neurotropism that was also demonstrated by the abundance of spirochetes in the brain of inoculated guinea pigs.

Experiments thus far indicate that the organisms retain their invasive property following subculture, and, the blood of guinea pigs shows abundant spirochetes one week after inoculation with material taken from a culture. Remissions and relapses occur at intervals of 7 to 14 days in the animals and stained blood smears re-

veal the spirochetes during the relapsing stage of infection. In one animal the organisms were present in the blood stream for 7 days, absent for 2 days thereafter, and again present in large numbers for 4 successive days.

To date 18 guinea pigs and one *M. rhesus* monkey have been used in the study of the behavior of *Spirocheta hispanicum*. Blood smears have been made daily and when the organism could be demonstrated, subinoculations and cultures were attempted. The results have been uniformly successful.

In artificial culture media the spirochetes were still alive after more than 2 months' incubation at body temperature and retained the capacity to infect guinea pigs. A supply of culture material for clinical use was thus made available in the laboratory. Such specimens, if desired, can be shipped in ordinary test tubes containing the special media or in the form of capillary containers with similar material or infected blood sufficient for single or multiple inoculations.

Studies are in progress in a fairly extensive series of patients with a view to evaluate the possibilities of a wider application of the method described.

The life cycle of *Spirocheta hispanicum* has been studied and observed microscopically in the hanging drop, using blood from guinea pigs inoculated with culture or with blood from previously infected animals. The red blood corpuscles have been observed to undergo peculiar granulation changes and metamorphosis prior to the appearance of spirochetes. The organisms appeared to develop from these granules as extrusions, drawing the corpuscles along and finally breaking away from them with a rapid spinning motion and rotation along the spiral axes. In the course of one hour all stages of development could be seen by careful examination of isolated corpuscles in the field. In preparations from the blood stained by the Giemsa or Wright method the earliest changes observed were characterized by polychromatophilia and a peculiar violet or purple tinting of the blood corpuscles. This was followed by granulation with an appearance of fine or coarse stippling which in later stages showed large and coarse purple granules. These rounded bodies varied in number and size and were frequently seen to be attached to the spirochetes at different points along the axis. In the fresh as well as in the stained specimens of blood these phenomena could be observed regularly.

Remlinger and Bailly³ have commented upon the latent period

³ Remlinger, P., and Bailly, J., *Compt. rend. Soc. de Biol.*, 1930, 105, 433.

following inoculation of refractory species of animals with brain emulsions obtained from infected guinea pigs. Their explanation for this prolonged incubation period was a cycle of development similar to that of rabies. Studies were not made with hanging drop preparations or stained smears. Another report of interest in connection with the life cycle is that of Hatt,⁴ who studied the intestinal epithelium of *Ornithodoros* ticks engorged with blood from infected animals. Spirochetes with granules attached to the bodies were observed and said to be the result of fragmentation of the organisms. Such granules, it was stated, were absent on the first day and were, according to the author, due to a stage of evolution resulting from unfavorable environmental factors.

Our observations have led us to believe that the *Spirocheta hispanicum* develops in accordance with a definite life cycle in the infected red blood corpuscle, passing from a granular stage to that of the matured and typical spirochete form. We have been impressed by the added observation that fresh normal blood corpuscles, when added to artificial culture media containing the spirochetes, appeared to be infected *de novo*. These corpuscles underwent the same type of changes and cycle of spirochete development that was observed in the original preparations made with infected blood.

Further studies are in progress and cinematographic records are being prepared of the entire life cycle of this organism.

⁴ Hatt, P., *Arch. Inst. Pasteur de Tunis*, 1929, **18**, 258.