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The Demonstration of Spirochæta Pallida in Lesions of Acquired Syphilis.

BY ALEXANDER A. UHLE, M.D., AND WM. H. MACKINNEY, M.D.

OF the various methods employed to establish the etiologic relation of Spirochæta pallida to syphilis, those methods dealing with its demonstration in secretions of syphilitic lesions, or in the fluids obtained from them by artificial means, have received the most attention. Schaudinn based his discovery upon positive findings in 26 cases of syphilis, the secretions being examined in a fresh state.

According to his description, the organism varies in length from 4 to 14 microns and its width does not exceed 0.5 micron. Its ends are pointed and it shows three to twelve spiral curves. It is actively motile, showing both a rotatory and longitudinal motion. Flagella have been demonstrated at either extremity.

Hoffmann, shortly after, demonstrated a spiral organism in the fluids obtained from the liver, spleen, inguinal glands, and pemphi-

goid eruption of a child dying of hereditary syphilis, which was in all respects similar to that described by Schaudinn.

Since this discovery, numerous observers have confirmed their finding by similar or slightly different technique. In all of these observations the findings were so constant that at the present time the causal factor of syphilis is conceded by many observers to be Spirochæta pallida, Treponema pallida, or Spironema pallida, as it is variously called.

The first attempts made to demonstrate spirochætæ in the syphilic tissue were made by Berterelli and Volpino, who succeeded in demonstrating the organism in the liver and spleen of children dying of hereditary syphilis. Their method consisted in treating very thin sections of tissue to a twenty-four to forty-eight hours' immersion in a 0.5 per cent. solution of silver nitrate, followed by washing in a solution of pyrogallic acid and sodium acetate. They remain in this solution for fifteen minutes and are then returned to the silver solution, in which they remain until they take on a yellowish-brown color. They do not give their method in detail.

In this manner they succeeded in demonstrating Spirochæta pallida, it taking a dark-brown, almost black stain. They guarded their findings with comparisons of normal tissue.

Levaditi modified the Ramoan y Cajal method for nerve fibers, and formulated an exact method for staining syphilitic tissue *in toto*, with a view of determining the presence or absence of spirochætæ. His method has been repeatedly employed and has given satisfactory results.

It is as follows: Small pieces of tissue, not more than 2 mm. in thickness, are hardened in 10 per cent. formalin for twenty-four hours; they are then placed in 95 per cent. alcohol for the same period, and washed in water for a few hours.

Specimens are then transferred to a freshly made solution of silver nitrate (1.5 per cent.) for three successive days, changing the solution each day, and maintaining a blood temperature. Light must be excluded. This is best accomplished by placing the bottles in the incubator.

From the silver solution they are transferred to a 2 per cent. solution of pyrogallic acid, to which is added 5 per cent. of formalin.

This is also kept in the dark, but at room temperature. They are then passed through 85 per cent., 95 per cent., and absolute alcohol, embedded in paraffin and cut at about 5 microns.

By this method he succeeded in demonstrating Spirochætæ pallida in the initial lesion of syphilis and in secondary papules. Syphilitic tissue from apes inoculated with syphilis also gave positive results, as did the organs of children dying of hereditary syphilis.

A. Buschke and W. Fischer (*Berl. klin. Woch.*) employed this method successfully in demonstrating Spirochætæ pallida in a chancre and in secondary papules, without noting any particular changes in the histologic appearance of the tissue. Their most interesting discovery, however, was the finding of this organism in the heart muscle, as well as in the remaining organs of a child dying of hereditary syphilis. In this case the spirochætæ had been found in large numbers before death in the fluid obtained from an artificial cantharidal blister.

In a second article by Levaditi and Manouélian (*Compt.-rend. Soc. de biol.*, Paris, 1906) the more exact findings regarding the occurrence of Spirochætæ pallida in the primary and secondary lesions of syphilis are described. Their deductions are drawn from a study of six doubtless syphilitic lesions. They were able to demonstrate this organism in two initial lesions and in a secondary papule from the anterior belly wall. From these studies they conclude that spirochætæ reach the deeper tissues by way of the blood and lymph channels, and their appearance in large numbers in the walls and lumen of bloodvessels explains the endarteritis and periarteritis so constant in syphilis.

In six lesions removed from apes artificially inoculated with syphilis, they report positive findings in two cases, Spirochætæ pallida being found in the periphery of the ulceration, in the skin papillæ, in the wall and lumen of the bloodvessels, and in the deeper layers of the epithelium. They were unable to demonstrate organisms in the depths of the tissue.

Burent and Vincent (*Compt.-rend. Soc. de biol.*, Paris, 1906) demonstrated Spirochætæ pallida in the chancre, finding many organisms in the papillæ, and in the subepithelial connective tissue. They occurred in lymph channels and in the thickened vessel walls.

Veiklon and Girard report the findings in the roseola of syphilis. They found large numbers of Spirochætæ pallida in the capillaries of the papillæ and in the vessels beneath. From their examinations the authors conclude that the roseola is not due to a toxic activity but to embolism in the vessels of the skin papillæ, which provokes a congestive hyperemia, and perivascular infiltration similar to the roseola of typhoid. They also report positive findings in the white pneumonias of two cases of hereditary syphilis, in the adrenals of another case, and in both the maternal and fetal portions of the placenta.

Finger reports positive findings in two chancres, one being of the gangrenous type.

Ehrmann (*Dermat. Zeitschr.*, Berlin, 1906) found Spirochætæ pallida between the epithelial cells in non-ulcerated secondary lesions. The organisms were most abundant above the stratum granulosum, although present in all layers. In the papillæ they were found in greatest number, and he offers the hypothesis that those in the epithelium are extruded from the papillary bloodvessels. They are always found between the cells and never within them. He therefore concludes that not only are moist and ulcerated lesions to be considered contagious, but the dry forms as well, and should therefore be handled with great care.

E. Hoffmann and Beer (*Deutsch. med. Woch.*, 1906) call attention to a method of staining which can be carried out in less time than the Levaditi method, but the results they have obtained are no more satisfactory than those obtained by the older method. This fact is also noted by subsequent writers who employed this method, which is the following: Fixation in formalin (10 per cent.) for twenty-four hours; alcohol (95 per cent.) for fifteen hours; immersion in a 1.5 per cent. solution of silver nitrate, to which is added 10 c.c. of pure pyridin, for three hours at the room temperature, and a further three hours in the paraffin closet at a temperature of 40° to 50° C. Light must be excluded.

They are then placed in a solution made by taking 90 c.c. of a 4 per cent. solution of pyrogallic acid, to which 10 c.c. of pure acetone is added, and to 85 c.c. of this solution 15 c.c. of pure pyridine is added. The tissue remains in this solution over night, light being excluded. The tissue is then embedded and cut. Sections can be counterstained

with polychrome methylene blue. By this method they succeeded in demonstrating Spirochætæ pallida in a syphilitic bubo during the secondary period of incubation and in an orbicular syphilide.

A. Blaschko (*Med. klin. Woch.*, 1906) used the Levaditi method in the study of four chancres and one condyloma. In all of these cases he reports positive findings, Spirochætæ pallida occurring in the epithelium, in the papillæ, and in the connective-tissue spaces. He found them in great numbers in the walls of bloodvessels and in their immediate vicinity. It is interesting to note that one of his specimens had remained in formalin for a year.

In July, 1906, Dr. Karl Reuter (*Zeit. f. Hygiene u. Infectionskr.*) reports several very interesting observations. He examined the tissue of a child dying of hereditary syphilis, the tissues having been preserved in formalin since 1903. The lesions included a diffuse white pneumonia, a gumma of the upper lobe of the right lung, and an interstitial pancreatitis. In all of the sections he had positive findings. In the gumma of the lung large numbers of Spirochætæ pallida were found at the periphery, underlying the limiting membrane. He could not demonstrate them in the central necrotic area. In the white pneumonic areas they occurred in less number, but were more evenly distributed. In the pancreas they were found in the interstitial connective tissue.

In a subsequent study of a recent gumma of the testicle he was unable to find spirochætæ, and cites as an analogy the difficulty of demonstrating tubercle bacilli in caseous lesions.

A series of experiments was carried out to determine the influence of mercury upon the numbers of Spirochætæ pallida found. With this object he studied three cases of hereditary and one case of acquired syphilis. In them he found it very difficult or impossible to demonstrate Spirochæta pallida after mercury had been ingested for a short time. In all of them large numbers were found prior to treatment, the examinations being made from smears of existing lesions.

The same author made studies to determine the presence of Spirochætæ pallida in cases of specific aortitis. In a patient dying suddenly of embolism, with no knowledge of syphilitic infection, and having taken no mercury, he was able to demonstrate Spirochætæ pallida in the wall of the aorta, particularly beneath the intima. This dis-

covery is of great value in explaining the arteritis of the Döhle-Heller type.

As to the findings in tertiary lesions they are almost entirely negative. We have been unable to find positive reports in gummatous or tuberculous syphilitic lesions.

E. Tomasczewski examined the scrapings from ten tuberculous syphilides, cover-slips being made and stained by the Giemsa stain. In five of these patients he reports the presence of Spirochætæ pallida. By this method, however, the opportunity for error is so great that they can have little positive value.

PERSONAL OBSERVATIONS. During the past four months we have been engaged in the study of syphilitic tissue, with the object of determining the presence or absence of Spirochætæ pallida in the lesions of acquired syphilis. In selecting the specimens care was taken to include with preference only non-ulcerated and non-eroded lesions. This was possible with the majority of papular syphilides, and to a limited extent with chancres. In tuberculous lesions the periphery was selected at a point showing the least ulceration, the object being to avoid contaminations of spiral organisms.

The method of Levaditi was employed, and we believe it to be the best method for staining the tissue *in toto*. The specimens were removed from twenty-four patients suffering with acquired syphilis, the lesions including chancres, various papular lesions, tuberculous syphilides, and one gumma.

As controls lesions of chancroids, chancroidal buboes, psoriasis, scabies, acne vulgaris, varicella, and venereal vegetations were studied.

Normal skin taken from fifteen individuals was stained in the same manner and studied.

The following is a report of the findings in each case.

CASE I.—J. D. Syphilis of five months' duration. Was taking potassium iodide for two months prior to examination. There is still induration present at the site of the chancre. No syphilitic lesions were present. Has an iodide acne on the back, one being removed and examined. No spirochætæ were found.

CASE II.—W. U. Chancre in sulcus. Fading, slightly pigmented macular eruption on back and chest. Macule removed from back. No spirochætæ found.

CASE III.—A. H. Chancre three months ago. Profuse general eruption of large papulosquamous syphilides. No treatment. Epithelium intact. A few spirochætæ between the epithelial cells and many in the cellular infiltrate.

CASE IV.—H. P. Eruption of five days' duration. History of sore on penis four weeks ago. Profuse maculopapular syphilide. Marked round-cell infiltration, but no spirochætæ found.

CASE V.—H. T. Chancre in sulcus. Discrete papular syphilide. No treatment. Spirochætæ pallida found in moderate numbers in the papillæ and in the connective-tissue lymph spaces. After five weeks' treatment by mercury in pill form, no spirochætæ were found in a pigmented portion of skin removed from the back, marking the site of a syphilitic papule.

CASE VI.—W. H. A. History of chancre seven months ago. Was treated at once for two months with pills, and then irregularly until five weeks ago. For the past five weeks no treatment. Now has a discrete papular syphilide on the back and mucous patches on the tongue and lip. Papule removed from back. No spirochætæ were found.

CASE VII.—A. H. Syphilis of eighteen months' duration. Has been taking mercury and potassium iodide. Has an iodide acne, one lesion of which was removed. No spirochætæ were found.

CASE VIII.—J. C. Chancre and general syphilitic macular eruption. Was circumcised and a part of the chancre, the skin of the prepuce, together with a mucous patch from the scrotum and a macule from the leg, were examined. Spirochætæ pallida found in moderate numbers in the chancre, mucous patch, and macule. Not found in the skin of the prepuce.

CASE IX.—G. A. Syphilis of three months' duration. Has neglected treatment. Took eight mercurial inunctions and then discontinued treatment until eighteen days ago, when he began to take mercury in pill form. A fading maculopapular eruption is still present, a papule being removed. No spirochætæ were found, and very slight round-cell infiltration.

CASE X.—J. Mc. Has been admitted to the Philadelphia General Hospital three times in the past eight years for the treatment of recurrent tuberculous syphilides. Has a large lesion on the left arm,