AN EASY METHOD OF DETECTING *S. PALLIDA* AND OTHER SPIROCHAETES.

By ALFRED C. COLES, M.D., D.Sc., F.R.S. EDIN.,
M.R.C.P. LOND.,
PHYSICIAN TO THE ROYAL NATIONAL SANATORIUM, BOURNEMOUTH.

At the present time three methods are in general use for the detection of *S. pallida*: (1) The examination of fresh cover-glass preparations by means of dark-ground illumination obtained by means of a special oil immersion dark-ground condenser; (2) the examination of dry films stained with Giemsa or other stains; (3) the examination of films made by Burri's Indian ink method.

I have been in the habit of using each of these methods on every case in which I have had to make a diagnosis, but in some cases in which the spirochaetes are present in very small numbers, especially those in which the primary sore has been previously treated with antiseptics, in which healing is taking place, the hunt for a single spirochaete is very trying and requires a considerable expenditure of time.

The following method which I have devised renders the detection of *S. pallida* or any other spirochaete as easy as that of the tubercle bacillus in sputum. It depends on the following principles:

1. That structures which are coloured with a fluorescent dye—for example, eosin, fuchsin, fluorescein, etc.—when examined on a dark ground, stand out very prominently, whilst blue-stained structures are very inconspicuous.

2. That if the medium in which the red stained organisms lie be of very low refractive index, they are exceedingly conspicuous by dark-ground illumination, and can be detected with a comparatively low power, but if mounted in balsam, cedar oil, parolein, or water, they are only seen with the very greatest difficulty. The method briefly consists in examining a film containing spirochaetes, stained in the usual way with Giemsa or Leishman's stain, dry, not mounted in any medium with dark-ground illumination, using a dry lens of medium power. There are, however, some details which considerably simplify this problem.

Method.

It is not necessary to stain with Giemsa, ordinary carbol-fuchsin would answer the purpose, but if the film is subsequently to be examined with the oil immersion $\frac{1}{2}$ objective it is very advisable to stain with Giemsa or its equivalent. Previous fixation of the film with absolute alcohol for ten to fifteen minutes, and subsequent staining with dilute Giemsa as is usually done, requires too much time, and never gives the same intensity of staining as the following:

The stock solution of Giemsa is diluted with an equal part of pure methylic alcohol or acetone.

Films are made from the serum which exudes after cleaning and rubbing or scraping the margins of the primary sore in the usual way. When dry, a few drops of the diluted Giemsa stain are dropped on to the surface of the film, and immediately (the quicker the deeper the staining) ten times as much distilled water is added. This will probably be more than the slide will hold; it is, therefore, advisable to add as much water as will lie on the film, and then flood this off with more water on to the surface of a piece of curved glass plate, and place the slide film downwards in this diluted stain.

Stain for not less than ten to fifteen minutes, then wash off the stain with distilled water; dry, but do not mount.

Dark-ground Illumination.—The special dark-ground condensers in use—for example, those of Leitz, Zeiss, Baker, etc.—have two disadvantages for this examination, as, first, they all require to be in oil contact with the under surface of the slide, with the result that one cannot examine a large area of the film without the oil running on to the surface of the stage or its being used up; and, secondly, they do not allow the change from dark-ground to ordinary illumination being made. To overcome these disadvantages, and to do away with the necessity of using oil I use an ordinary achromatic condenser, used dry, without oil, provided with a Travis's expanding stop below it. A simple round stop, as long as it be of the right size, could be used; but the expanding stop is much more convenient. Any of the microscopic agents would fit either

of these, and, if necessary, explain how a dark ground is obtained. An ordinary paraffin microscopic lamp, used with or without a bull's-eye condenser, is ample illumination.

The objective I use is an 8 mm., or $\frac{1}{2}$ in., of Zeiss and Leitz, and a compensating ocular of 8, 12, or 18, or an ordinary No. 4 or 5 eyepiece. I can see *S. pallida* with a $\frac{3}{4}$ in. objective with 18 ocular, but I could not pick them out with this low power. I find I cannot use a $\frac{1}{2}$ in. objective on an uncovered objective, and its NA is too large to obtain a good dark ground with this method.

The stained film must not be mounted in balsam, oil, water, or any other medium; it must be examined dry in air. The easiest and best result is obtained by examining it without a cover-glass, but if such an uncovered film be examined with a dry lens at the correct tube length of that lens for a covered object, the image will be very imperfect and practically useless, but if the tube of the microscope be lengthened to the right height—this can only be found by trial, but when found will be practically constant for that objective—the definition is perfect.

My Zeiss 8 mm. normally working at a tube length of 160 mm. requires to be lengthened to about 195 mm., and the Leitz 8 mm. objective from 170 mm. to 240 mm. The latter length cannot be obtained on an ordinary short-tube microscope without the addition of a cardboard tube. Of course, the inconvenience of lengthening the tube may be overcome by laying a cover-glass on the film, when the objective will be used at about the normal tube length and the definition will be excellent. This, however, has the disadvantage that if a spirochaete be found by dark-ground illumination, and one wishes to examine it with ordinary illumination by removing the stop under the condenser and turning on the $\frac{1}{2}$ oil immersion lens, the spirochaete will not be seen. On the other hand, if there is no cover-glass, the $\frac{1}{2}$ lens oiled will enable it to be seen at once.

In a stained film examined on a dark ground without any mounting medium *S. pallida* can be easily detected with such a moderate power as a $\frac{1}{2}$ -in. objective. It stands out as a beautiful golden spiral, which cannot be missed by the merest tyro.

The large field of vision, the ease with which a slide can be thoroughly examined, the absence of any eye strain, and above all the remarkable way in which the spirochaete strikes the eye, have only to be seen to be appreciated.

I have now used this method for some years, and am convinced that I should find no difficulty in finding a spirochaete even if only two or three were present in a film the size of a postage stamp. Can the same be said of any other method now in vogue for the detection of these illusive organisms?

It seems to me that it would be invaluable to those who are searching for *S. pallida* in films from the brain in cases of general paralysis of the insane, etc.

It is, of course, equally serviceable in examining the blood for any of the spirochaetes, provided these are coloured a reddish tint with Giemsa, or if stained with eosin or fuchsin. Blue-stained objects are very inconspicuous.

Some few years ago whilst examining a Giemsa-stained film with the $\frac{1}{2}$ oil immersion lens, I found a spirochaete in the blood of a bat, which I provisionally named *S. vesperuginis*. This spirochaete, very slightly larger than the *S. pallida*, was only present in extremely small numbers. It would have been well-nigh impossible till have found another with the $\frac{1}{2}$ objective, but in the remaining 15 films which were not mounted, I had no difficulty in detecting and marking these spirochaetes by examining the films dry on a dark ground, although in many films there was only a single spirochaete present.

I shall be very pleased to demonstrate the extreme ease with which the method is carried out, or to answer any question thereon.

The next award of the Alvarenga Prize of the Philadelphia College of Physicians, amounting to about £50, will be made on July 14th, 1916. Essays, which may deal with any subject in medicine, must be received on or before May 1st, 1916, by the Secretary of the College, Dr. Francis R. Packard, 19, South 22nd Street, Philadelphia, Pa., U.S.A., to whom intending competitors should write for full particulars. The Alvarenga Prize for 1915 has been awarded to Dr. J. E. Sweet, Philadelphia, for his essay entitled "The Surgery of the Pancreas."