FILTRATION EXPERIMENTS WITH *SPIROCHAETA SCHAUDINNII*

By E. C. SMITH, Sc.D. (Dublin)

From the Medical Research Institute, Lagos, W. Africa

In a paper dealing with the filtration of spirochaetes, Hindle and Elford (1933) describe the successful filtration of *S. biflexa* and *S. pallida*. Their paper includes a useful summary of experiments carried out by other workers, and it is of interest to note that the filtration of the commensal spirochaetes such as *S. vincenti* and *S. schaudinnii* has not been recorded.

The present paper is concerned with filtration experiments on *S. schaudinnii*, but since they were made with Berkefeld candles the results are not as accurate as those obtained through the use of graded collodion membranes for filtration (Elford, 1931), a method which it is hoped will be tried later.

Methods

Twenty-five filtrations were made as follows:

(*a*) In twenty, Berkefeld No. 12V filter candles were used in connection with a negative pressure corresponding to a mercury column averaging 450 mm.

(*b*) In five, Berkefeld No. 12N filter candles were employed with a negative pressure of 760 mm. of mercury in two instances and a pressure of 45 lb. per square inch in the remaining three.

A suitable case of tropical ulcer having been obtained, *i.e.* with numerous actively motile spirochaetes (*S. schaudinnii*), superficial scrapings of the ulcer base were made, after a preliminary washing with saline, and collected in about 20 c.c. of nutrient broth. After standing, to allow the larger particles to settle, a drop of the mixture was examined by the dark ground method to confirm the presence of active spirochaetes.

Approximately 10 c.c. of the fluid were then passed through the filter candle, 10 c.c. of nutrient broth being first passed through the filter and a further 10 c.c. immediately afterwards. The filtrate was then transferred by means of a sterile pipette into centrifuge tubes and centrifuged at about 4000 revolutions per minute for one hour. The supernatant fluid was aspirated off leaving a small residual quantity which was examined for spirochaetes by the dark ground method.

The usual dark ground apparatus was proved to be unsatisfactory because only a small fraction of the residual fluid could be examined at a time, and spirochaetes, if sparse, might be missed unless many examinations were made. Consequently, a Zeiss "Präparier Kondensor für schwache Objective," with a

focal length of 10 mm., was resorted to. With this apparatus it was only necessary to place a drop of the fluid to be examined on a thin oblong cover-slip and to invert this over the aperture in the stage of the microscope. Using ocular × 30 and objective 16 mm. a magnification of 300 was obtained with which the spirochaetes could be readily seen. Fusiform bacilli, motile bacteria and other organisms, if present, were also readily recognised. This method saved much time, permitted the examination of a relatively large amount of fluid and avoided the use of oil.

Observations on the filtrates

(*a*) Of the twenty filtrations made with Berkefeld 12 V candles, spirochaetes (*S. schaudinnii*) were found in the filtrate in fourteen, being actively motile in nine. Most of the spirochaetes seen were identical with those present in material taken direct from a tropical ulcer, but there were also present some very short forms suggesting a transverse fragmentation of the organism. In addition, an actively motile, minute bacillus was present in all the filtrates. Cultures of the filtrates were made in the modified Wenyon media (described by Smith, 1930) and on agar. Scanty growths of spirochaetes were obtained but twice in the modified Wenyon media, all the other cultures being heavily overgrown with *B. pyocyaneus*.

The actual number of spirochaetes present in the filtrates varied greatly; in some, every field examined showed numerous organisms, in others, only one or two spirochaetes were present per field.

It was found that filtrations made during the dry season were very unsatisfactory. Seven filtration experiments, in addition to those already described, were made during this time of the year and using Berkefeld 12 V candles. *S. schaudinnii* were found in only two of the filtrates, and in both instances the organisms were scanty and showed no motility.

Each candle served for about six filtrations and after each experiment a measured volume (500 c.c.) of distilled water was passed through the filter in the reverse direction at a constant negative pressure (500 mm. mercury). It was observed that for a new filter 3–5 min. were required for the passage of this amount of water but that after each experiment the time increased until, when five or six filtrations of spirochaetes had been made, 12–15 min. were required. When this stage was reached the filter was discarded.

(*b*) The experiments made with Berkefeld 12 N filter candles were all negative, both as regards the passage of spirochaetes and of *B. pyocyaneus*.

Inoculation experiments on volunteers (i.e. healthy male African adults)

In order to obtain some information relative to the infectivity of *S. schaudinnii* for man, it was decided to carry out inoculation experiments on volunteers.

Exp. A. Four volunteers were each inoculated intradermally in the deltoid and ankle region with 1 c.c. of a filtrate rich in actively motile *S. schaudinnii*. Result negative, only slight redness occurred in 24 hours.

Since *S. schaudinnii* exists in the lesion known as tropical ulcer in close association with *B. fusiformis* it is possible that the presence of the latter organism is necessary for infection to take place. A further series of inoculations (Exp. B) was therefore carried out on seven volunteers as outlined in Table I. All inoculations were made into the lower third of the leg (outer aspect).

Table I. *Summary of Exp.* B.

	Contents of inoculum	No. of volunteers	No. and type of inoculation in each volunteer	Total no. of inoculations	Remarks
1	*S. schaudinnii* and *B. pyocyaneus* in filtrate	2	2 intra-cutaneous 2 subcutaneous	4	No ulcer produced. Slight local reaction passing off in 24 hours
2	*S. schaudinnii* and *B. pyocyaneus* in filtrate with *B. fusiformis**	3	3 intra-cutaneous 3 subcutaneous	6	No ulcer produced. Localised abscesses developed with the subcutaneous inoculations. Examination of contents by dark ground showed scanty *B. fusiformis* and numerous *minute motile* bacilli. Bacilli gram-negative. No spirochaetes seen
3	*B. fusiformis* in broth emulsion	2	3 intra-cutaneous 1 subcutaneous	4	No ulceration produced. Transitory local reaction, more marked in the case of the subcutaneous inoculation

The strain of *B. fusiformis* used in these experiments was obtained from a case of tropical ulcer by the Krumwiede and Pratt method of plate culture. A 5-day plate growth of a first subculture which showed clusters of typical colonies was used. After a preliminary examination of a colony by the dark ground to determine the presence of actively motile forms of *B. fusiformis* and after staining with Giemsa to determine the presence of the characteristic beading, several clusters were cut out of the agar plate with a sterile knife and these were emulsified in a few c.c. of broth (Exp. B 3). Further clusters of colonies were removed in the same manner and were emulsified in a filtrate containing active *S. schaudinnii* and *B. pyocyaneus* (Exp. B 2). The emulsions were examined before and after the inoculations were made in order to confirm the presence of motile spirochaetes and of fusiform bacilli. Cultures on agar of the emulsion used in Exp. B 2 were positive for *B. pyocyaneus* within 24 hours.

Discussion of results

From a consideration of the experiments detailed, in which twenty-two inoculations were made on eleven volunteers, it is apparent that *S. schaudinnii*, neither in combination with *B. pyocyaneus* nor in combination with *B. pyocyaneus* and *B. fusiformis*, is capable of giving rise to a tropical ulcer when inoculated in the manner described. D. T. Smith (1932), working with fuso-spirochaetal pulmonary abscess, found that mixed cultures of fusiform bacilli and *S. vincenti* were entirely non-pathogenic for mice or guinea-pigs. Since *S. vincenti* is regarded by most authors as being identical with *S. schaudinnii* these findings are of interest in relation to those described in the present paper. E. C. Smith (1933) showed that pure cultures of *B. fusiformis* isolated from cases of tropical ulcer were devoid of pathogenicity when inoculated into volunteers. A combination with other organisms is apparently required to reproduce the condition known as tropical ulcer, and D. T. Smith considers that fuso-spirochaetal angina is due to the combined action of a spirochaete, a fusiform bacillus, a vibrio and a coccus. This last observation is of particular interest in view of the experimental reproduction of tropical ulcer by E. C. Smith and Elmes (1931) by using mixed cultures which contained spirochaetes

spirilla, fusiform bacilli and *B. pyocyaneus*[1]. The susceptibility of the patient no doubt plays an important part in the experimental reproduction of the condition and may indeed be related to vitamin deficiency, for D. T. Smith (1932) showed that noma could be produced by the inoculation of fuso-spirochaetal material into guinea-pigs fed upon a diet deficient in vitamin C, normal control animals being unaffected.

Summary

It has been shown that *S. schaudinnii* will easily pass through Berkefeld V filters under a negative pressure of considerably less than one atmosphere, but *B. pyocyaneus* was always found in the filtrates in association with the spirochaetes. Attempts to pass *S. schaudinnii* through Berkefeld N filters were uniformly negative.

Inoculation experiments on native volunteers made with (*a*) filtrates containing *S. schaudinnii* and *B. pyocyaneus*, (*b*) a mixture of these organisms combined with *B. fusiformis*, and (*c*) an emulsion of *B. fusiformis* in broth, did not give rise to ulceration.

[1] In these experiments two types of mixed culture were employed: (*a*) cultures in modified Wenyon media, grown aerobically and containing *S. schaudinnii* in abundance, scanty fusiform bacilli spirilla and *B. pyocyaneus*; (*b*) cultures in the same media but grown anaerobically and containing *B. fusiformis* in abundance, scanty spirilla and *S. schaudinnii* and *B. pyocyaneus*. Eight experimental inoculations were made on volunteers (adult African natives). Each volunteer received one inoculation of 0·5 c.c. of a culture (four received culture (*a*) and four received culture (*b*)) intracutaneously in the deltoid region. Severe local reaction occurred in five instances with the formation of well-marked ulceration in three of the individuals, two of whom had received culture (*a*) and one culture (*b*).

REFERENCES

Elford, W. J. (1931). A new series of graded collodion membranes suitable for general bacteriological use, especially in filterable virus studies. *J. Path. Bact.* **34**, 505.

Hindle, E. and Elford, W. J. (1933). The filtration of spirochaetes through graded collodion membranes. *Ibid.* **37**, 9.

Smith, D. T. (1932). *Fuso-Spirochaetal Disease.* London; Baillière, Tindall and Cox.

Smith, E. C. (1930). Cultivation of the spirochaetes associated with tropical ulcer. *Proc. Roy. Soc. Med.* **24**, January, No. 4.

—— (1933). Inoculation experiments with *B. fusiformis* isolated from tropical ulcer with observations on the bacillus. *J. Hygiene*, **33**, 95.

Smith, E. C. and Elmes, B. G. T. (1931). Experimental tropical ulcer. *West African Med. J.* **4**, 87.

(*MS. received for publication* 10. vii. 1934.—Ed.)