THE LABORATORY DIAGNOSIS OF LEPTOSPIROSIS

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INTRODUCTION

SINCE the discovery by Inada and his associates (1916) and almost simultaneously by Uhlenhuth and Fromme (1916) of the causal organism of Weil's disease, a kind of spirochete for which Noguchi (1918) coined the name *Leptospira*, our knowledge of the infections caused by these microorganisms has considerably increased.

In later years, after laboratory methods for the isolation and cultivation of Leptospira had been developed, researches in several countries showed that leptospiral diseases occurred much more frequently than had formerly been realized. Besides Weil's disease, a number of other infections were detected, caused by pathogenic leptospirae which were morphologically and culturally indistinguishable from *L. icterohaemorrhagiae*, but which proved to be serologically distinct from the Weil Leptospira. It was also found that rats and other rodents, dogs, pigs and bovines could become renal carriers of leptospirae and that in certain areas these animals formed a reservoir from which human infections arose.

In medical as well as in veterinary practice, this is a field of research in which close co-operation between clinician, laboratory worker and epidemiologist has been exceedingly fruitful. Once attention was focused on the occurrence of leptospirosis in a certain area, further cases were nearly always detected when a thorough search was made by screening the patient's surroundings. Moreover, in many instances mild and even inapparent cases were then found which would have been overlooked by clinical investigations alone.

The accumulation of knowledge of the etiologic, clinical

and epidemiologic aspects of leptospirosis, combined with laboratory investigations, brought about recognition of the fact that in many instances clinical symptoms alone were of insufficient diagnostic value to differentiate leptospiral infections from other infectious diseases and to separate the various leptospiroses caused by serologically and epidemiologically different species of leptospirae. Cultural and serologic procedures became of more and more importance as a diagnostic aid.

In the early period of leptospiral research, laboratory investigations were restricted to a few laboratories, where, owing to the interest shown by some specialists in this field of research, the study of all aspects of leptospirosis was fostered.

The work of those pioneer research workers and their associates gradually created a world-wide awareness of the importance of leptospiral diseases in men and in animals. Cases of leptospirosis have now been detected in nearly every country in the world and many laboratories for clinical research apply laboratory methods for the diagnosis of leptospiral infections.

Nevertheless our knowledge of the evaluation and interpretation of the various laboratory procedures for the investigation of leptospirosis is far from complete. On the one hand, the number of antigenically different strains of leptospirae isolated in different parts of the world is continually increasing; on the other hand, divergencies in the results obtained by established laboratory methods, depending on the special technique used, have been reported (Borg Petersen-Fagraeus, 1949, Wolff, 1950).

There is a growing need for international co-operation to promote the comparison of laboratory techniques for leptospiral research, on the same lines as the co-operation

in the field of influenza and salmonella research, which has produced such excellent results.[1]

[1] International cooperation has now been inaugurated by members of the Leptospira Subcommittee of the Nomenclature Committee of the International Association of Microbiologists.

PREFACE

Most of the methods herein described were developed by Schüffner and his co-workers in the Institute of Tropical Hygiene, Amsterdam, during the period between 1923 and 1944, when this laboratory became a center of leptospiral research for the Netherlands. It has been the writer's privilege, as one of Schüffner's early assistants, to co-operate in these researches during the first years in Amsterdam and subsequently to have had an opportunity of pursuing investigations on leptospirosis for many years in various laboratories in Indonesia.

The aim of the work is to give in a concise form practical suggestions and guidance for workers who are not specialized in leptospiral research. The list of references appended is incomplete; for a survey of the various aspects of leptospiral infections the reader is referred to the excellent reviews by Walch-Sorgdrager (1939), Van Riel (1946), Van Thiel (1948), Wiesmann (1949), Gsell (1949), Rimpau (1950), Gsell (1952) and Wiesmann (1952).

It is hoped that this little work will be of value to all medical and veterinary laboratory workers who are interested in this expanding field of research.

J. W. W.

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GENERAL REMARKS

DARK-FIELD microscopy is an indispensable aid in all leptospiral investigations; no other method of observation reveals the microorganisms clearly in a fluid medium. Owing to their extreme thinness, they are practically invisible in bright-light microscopy and only slightly better results are obtained when phase contrast microscopy is applied.

Any dark-field condenser of good quality may be used with a well-focussed source of light. In field work excellent images can be obtained by using a low-voltage pointolite lamp with the beam concentrated through a small spherical glass bowl filled with distilled water and closed by a rubber stopper. The best results are obtained if slides of the exact thickness as recommended for the particular type of condenser are used.

All glassware used in dark-field work must be kept scrupulously clean; scratches, oil or dust particles on the surface of the slide spoil the dark-field image and make it difficult to detect leptospirae if present in small numbers. It is advisable to reserve a number of slides of the proper thickness for dark-field work only. These should be stored in well-stoppered glass vials in 96 per cent alcohol, removed with a clean forceps and dried with a clean linen towel or tissue paper. Finger contact with the surface must be entirely avoided.

For routine examinations of culture fluids, blood or agglutination tests, dry power objectives will give sufficient enlargement. Leptospirae can easily be distinguished with magnifications of approximately 200-300 times (e.g., Zeiss

objective C:20x, ocular 10x) in drops of fluid without using cover slips, a method which speeds up routine examinations considerably. When searching for the microorganisms in blood, peritoneal fluids, urine or any medium in which many other cell elements are present, the drops must be covered with a cover slip and higher magnifications of about 400 x are advisable. The use of an immersion oil objective is seldom necessary, unless special morphologic details in single leptospirae are to be examined.

When a great many consecutive examinations of drops of fluid must be examined, instead of immersion oil, a drop of distilled water placed between the condenser and the bottom of the slide will be found much cleaner than oil and quite suitable if dry power objectives are used.

II

MORPHOLOGY

IN THE EARLY period of leptospiral research, the morphology of these microorganisms as seen in dark-field or in stained preparations was described very accurately (Noguchi, 1918; Zuelzer, 1918; and Pettit, 1928).

Leptospirae are slender thread-like organisms without flagella. They possess very minute spiral coils having an amplitude of about 0.5μ. Most workers have stated their thickness to be about 0.25μ but recent electron-microscopic measurements suggest it may be less than 0.1μ (Babudieri, 1948). Their length is very variable and may be from 6 to 12μ but sometimes, particularly in old cultures, very long specimens of 30-40μ can be detected.

When living leptospirae in a fluid medium are examined with lower magnifications in dark-field illumination, the primary spiral coils are scarcely visible; the impression usually obtained is that of a row of small dots or a string of fine beads.

Their movements are very peculiar; the middle part is held rigid while one or both ends, bent like a coat hanger, are seen to make quick twists and whiplike darts so that rotations along a longitudinal axis occur.

Further knowledge of the internal structure of Leptospirae has been gained by electron-microscopy. Some authors believe that a rigid central filament is present (Babudieri, 1948, 1949; and Breese, *et al.* 1952), as was suggested in Zuelzer's studies (1918), while others deny its existence (Van Thiel-Van Iterson, 1947; and Morton-Anderson, 1943). Schlipköter and Grün (1952) studied the morphological changes of the aging of leptospiras in cul-