# THE MORPHOLOGY OF THE GENUS *LEPTOSPIRA* AS SHOWN BY THE ELECTRON MICROSCOPE

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(With Plates 10 and 11)

The morphology of the Leptospiras has been for some time the subject of many researches (see Babudieri, 1948), but the remarkably tiny dimensions and slenderness of this micro-organism have made it impossible previously to determine precisely and with certainty its structure. Our notions on the very existence of a membrane, an axistyle, flagella, granules or spherules are very poor and contradictory.

The electron microscope has been used recently for the study of Leptospiras, but up to the present without giving great help. Apart from a photograph of *Leptospira* published incidentally by Ruska (1941), which is not accompanied by any description, the first study on this subject was due to Morton & Anderson (1943). But these two authors had not fixed their preparations so that the photographs they have published have but little demonstrative value. They do not see in the Leptospiras any special structures and doubt the existence of an enveloping membrane.

Some time afterwards, Jakob (1947, 1949) published two papers on the subject. He gave no special details on the morphology of the spirochaeta, but insisted on the presence of some special small S-shaped oval bodies which he considers as a particular life stage of *Leptospira*. He also describes other tiny granules, often provided with a thin filament, and believes them also to be a particular form in the life history of the Leptospiras.

Finally, Van Thiel (1948), in his recent book on the Leptospiras, publishes some photographs obtained through an electron microscope. But this author also neglected to fix the spirochaetes so that it is impossible to see the axistyle or the membrane. Last year I published, in an Italian review (1948), a long study describing with all possible details the morphology of the Leptospiras in normal conditions and after different treatments. I wish here to review my description together with the results of further research.

The Leptospiras I have been observing belong to numerous strains of the following species: *L. icterohaemorrhagiae*, *L. cancicola*, *L. pomonae*, *L. bataviae*, *L. Sejroe*, *L. australis*, *L. Fledermaus* 90C type, *L. acquicola*.

The first thing that has to be said is that from the morphological point of view, there appear to be absolutely no differences between these different strains.

My research has been done on Leptospiras cultivated on Korthof medium, fixed with some drops of 2 % osmic acid solution and afterwards repeatedly washed by means of centrifugation. The first point is that the transverse diameter of the Leptospiras is smaller than previously supposed. It does not reach 0·1 $\mu$ and this explains why *Leptospira*, when it is not stained, cannot be seen in the normal optical microscope, not because, as is generally thought, the refractive index of its protoplasm is equal to that of water, but because its transverse diameter is smaller than half the average length of the light wave (0·5 $\mu$), and therefore is beyond the limits of resolution of the optical microscope.

As the photographs here published show, the *Leptospira* consists of a cylinder of homogeneous protoplasm, enveloped by a very thin anistic membrane, wrapped around a rigid central filament, the axistyle. The axistyle is specially visible in spirochaetes that have undergone some autolysis when the protoplasm is wearing out. In old cultures of Leptospiras one can constantly observe together with masses of residual substance, thin filaments which evidently are axistyles become free through the destruction of the membrane and of the protoplasm of the spirochaetes. I interpret in this way the vibratile forms described by Jakob (1949) under the name of 'Leptospirogene'. That these are not involution forms but dead degenerate forms can be clearly demonstrated by the fact that they are to be found in old cultures the transplanting of which is constantly negative. The small granules that have sometimes been described in the bodies of the Leptospiras, must also be regarded as products of degeneration, as they appear only in old cultures or in cultures that have been artificially treated.

The membrane which envelopes the whole body of the spirochaetes has a tendency to separate from

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the protoplasm probably as an effect of drying and does not show a fibrous constitution even if artificially treated.

A strange phenomenon can be observed on the membrane when the Leptospiras, before being fixed, are suspended in hypertonic solutions of sodium chloride. After about 30 sec., in many Leptospiras, a little swelling of the membrane appears generally near one of the ends. This swelling gradually increases; the body of the *Leptospira* folds itself at an acute angle, and the protoplasm has a tendency to roll up upon itself and seems to be enclosed by the bubble the membrane is forming. Finally, in some cases, the whole body of the *Leptospira* appears to be rolled up on itself and contained in the said bubble. It is probable that appearances of this kind have been observed by other workers in preparations stained with silver salts and described by them as spherules or involution forms of the Leptospiras.

In regard to the so-said 'S forms' described by Jakob (1947), that author kindly provided me with the culture of spirochaetes on which they were observed. In my opinion these forms have nothing to do with the Leptospiras, but are micro-organisms of another nature, probably a mould which has contaminated a culture of Leptospiras. I have even been able to separate it from the Leptospiras and to obtain it in a pure culture, and have also been able to cultivate the *Leptospira* themselves in pure culture. I have also studied the effects of treating Leptospiras with different physical and chemical agents.

In distilled water the Leptospiras appear normal for many hours. Evident phenomena of degeneration and lysis appear after some days. Strong alkalis rapidly destroy the Leptospiras; acids on the contrary harm them less, but cause alteration in their membranes. Freezing brings about fragmentation and makes the Leptospiras look diaphanous. The membrane can no longer be seen, but the axistyle remains clearly visible.

Pepsin and papaine dissolve the Leptospiras rapidly but do not affect the axistyles. Important alterations are caused by 10 % sodium taurocholate. Saponin also causes alterations which are not constantly evident.

Penicillin in bactericidal doses (about 0·1 units per ml.) dissolves the Leptospiras rapidly and nearly completely. Non-lethal doses of this antibiotic do not produce giant or monstruous forms. Anti-*Leptospira* serum produces agglutination of the spirochaetes. Their bodies come close together longitudinally and appear thicker, perhaps due to imbibition of liquid by part of the protoplasm, perhaps due to the deposition of antibody globulin on their surface.

Unless the Leptospiras are fixed they appear through the electron microscope to be altered a good deal. The spirals relax, the membrane and the axistyle are no more visible or are visible only with great difficulty.

Formaldehyde is a bad fixing agent, and Leptospiras treated with it do not show the axistyle, and their membrane is only seen with difficulty. Similar bad results follow the use of absolute alcohol and of Zenker's or Bouin's liquids. The best fixing agent remains therefore osmic acid.

These microscope studies have revealed the exact morphology of the Leptospiras, and may make possible a new and more precise systematic analysis of this group.

## SUMMARY

Several species of *Leptospira* have been studied by means of the electron microscope. Their morphology is described. The *Leptospira* consists in a long homogeneous cylinder of protoplasm, enveloped in a very thin anistic membrane and wrapped around a slender axistyle. Vibratile structures and granules generally are lacking.

The appearance of Leptospiras differs according to different methods of fixation used and according to different physical or chemical treatments to which they are subjected.

These observations have been made using the electron microscope constructed by Prof. Trabacchi and Prof. Bocciarelli, and with the technical assistance of Dr C. Castagnoli whom I have to thank.

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## EXPLANATION OF PLATES 10 and 11

### PLATE 10

Fig. 1. *L. ictero-haemorrhagiae.*
Fig. 2. *L. ictero-haemorrhagiae.* Shadowed with chromium.
Fig. 3. *L. Fledermaus* 90 C. Shadowed with chromium.
Fig. 4. *L. acquicola.* Shadowed with chromium. Partial autolysis of the protoplasm.

### PLATE 11

Fig. 5. *L. ictero-haemorrhagiae* in hypertonic solution after 1 min.
Fig. 6. *L. ictero-haemorrhagiae* in hypertonic solution after 6 hr.
Fig. 7. *L. pomonae.* Agglutination with specific serum.

All reproductions are enlarged, × 18,000.

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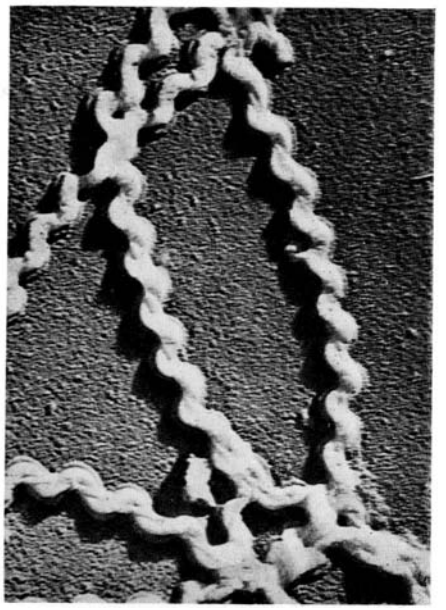
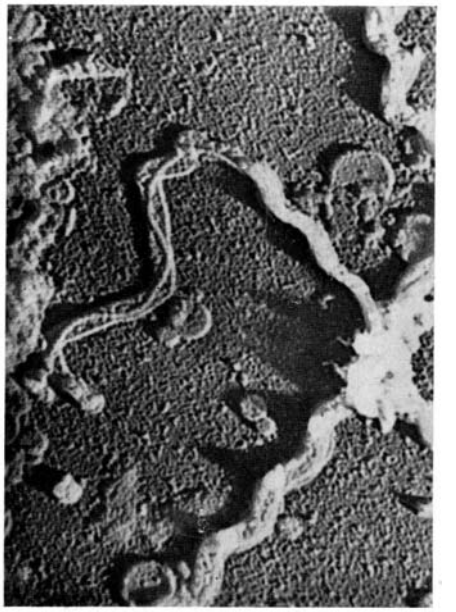


Fig. 2



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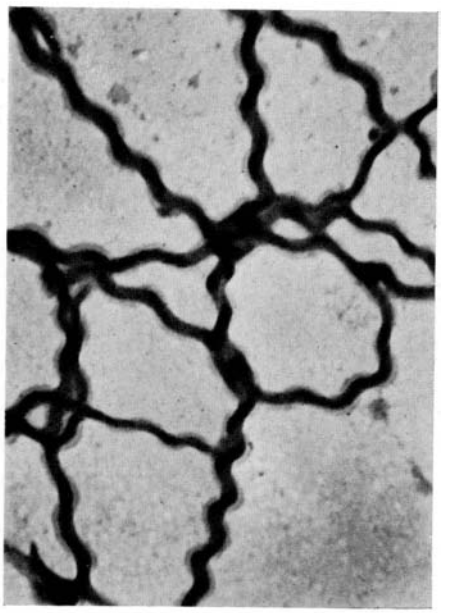

Fig. 1

Fig. 3

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Fig. 6

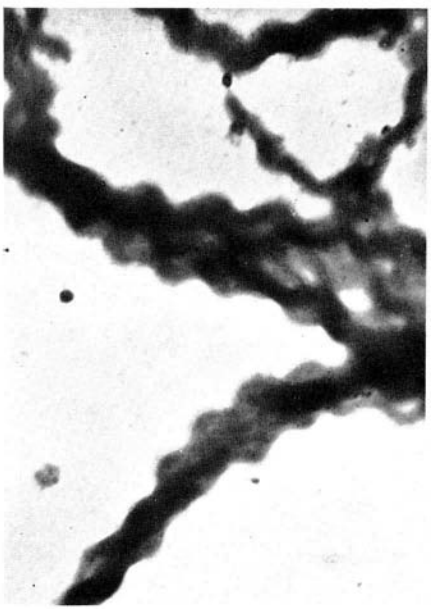

Fig. 7

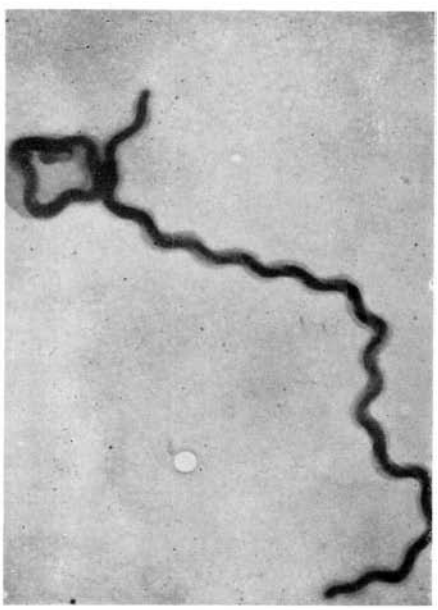

Fig. 5

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