

## OBSERVATIONS ON THE DIVISION OF SPIROCHAETES.

By DORIS L. MACKINNON, B.Sc.

(*From the Quick Laboratory, Cambridge.*)

2 Text-figures.

THE morphology of the spirochaetes has proved in recent years a fruitful source of controversy. One has only to look through some of the vast literature on the subject to be convinced that the time has not yet come for drawing final conclusions, so varied and so contradictory are the observations by different well-accredited authors.

Concerning even such an elementary point as the mode of division, opinion is far from unanimous. In the well-worn dispute—are the spirochaetes protozoa or bacteria?—the supporters of the former view have almost all maintained that the division is longitudinal, while their opponents have as strongly insisted that it is transverse. One may be inclined to doubt whether the mere direction of division is really a point of very great value in such a discussion: as Schellack (1907) has recently pointed out, bacteria never divide by simple constriction as in all hitherto described cases of transverse division in spirochaetes<sup>1</sup>. Furthermore, it may be well to remember that transverse division is not the peculiar property of the bacteria but occurs among certain groups of protozoa also.

But apart altogether from systematic considerations, it is of interest to determine the mode of division in this group of organisms.

It was suggested to me that it would be useful to compile comparative summaries of the opinions of various observers on certain

<sup>1</sup> Swellengrebel (1907) described the formation of a sort of cell-wall between the dividing halves of *S. balbianii* just as in a bacterium—his observation has so far received no confirmation.

Comparative summary of evidence for longitudinal and transverse division in *spirochaetes*<sup>1</sup>.

Spirochaeta	Observers of longitudinal division	Nature of observations	Observers of transverse division	Nature of observations
<i>S. anodontae</i> , ... Keysseltz	*Fantham ... *Keysseltz ...	Longitudinal division seen <i>in vivo</i> . Observed <i>in vivo</i> . Longitudinal split divides organism into approximately equal halves, remaining at first attached throughout length by ectoplasm. Split proceeds from one end to the other as the uniting ectoplasm disappears. Separating individuals move independently. Final act of separation takes some time.	... Schellack ...	... Transverse division described and figured from stained preparations. Periplast becomes thinner at a middle point in the body—then disappears there, and spirochaete divides abruptly into two halves. There is no stage with long drawn-out protoplasmic connection, and young individuals after separation are correspondingly blunt at one end. In this respect division differs from that in blood spirochaetes <sup>2</sup> .
<i>S. balantidis</i> ...	*Hoffmann and Prowazek	Doubling of the "Periplastanhang," followed by a Y-stage, observed <i>in vivo</i> .	Schnberg ...	Records transverse division. Measurements of breadth are against possibility of longitudinal division.
<i>S. balbiani</i> , ... Certes	Certes ...	Normal method of division.	Borrel and Cer- novodeanu Certes ...	Transverse division described. Transverse division also occurs.
	*Fantham ...	Usual method of multiplication. Splitting of basal granule, then of undulating membrane, finally of body. Usually the two halves separate by wriggling till 180° apart, but may remain at 30°-40° apart for a long time, executing very rapid movements. On two occasions forms watched <i>in vivo</i> for an hour: they died before division was complete.	Fantham ...	Transverse division also occurs, but not so commonly as longitudinal. 1. Both long and short forms seen. 2. In stained preparations membrane occasionally discontinuous in centre. 3. Living specimens sometimes seen vibrating about a point possibly not central.
	Lustrac ...	Longitudinal division described and figured.	Lustrac ...	Transverse division suspected.

<sup>1</sup> Asterisks are placed before the names of such authors as state that they made observations on the living organisms.<sup>2</sup> Schellack (1909) also describes and figures similar transverse division in no less than eleven new species of spirochaetes from molluscs—*S. ostreae*, *S. chamae*, *S. spiculifera*, *S. modiolae*, *S. imae*, *S. cardii-papillosi*, *S. tapetos*, *S. acuminata*, *S. saricariae*, *S. gastrochaenae*, *S. pusilla*. The conclusions were drawn from stained specimens.

<i>S. balbiani</i> , Certes (continued)	*Perrin	...	Normal method of multiplication. Division of membrane; then longitudinal split resulting in compass-forms, making angle of 180°; final separation preceded by constriction first of periplast, then of contained plasma—separating individuals finally connected by strand of periplast only. Longitudinal splitting does not take long—this stage seldom seen: final separation takes longer—an individual at this stage watched for 40 mins., when it died.	Laveran and Mesnil	In stained preparations individuals showing great inequality in length—others in which the "gaine" was discontinuous in the middle of body, others in which body was drawn out thin in this region, and ( <i>in vivo</i> ) some united two and two by a fine thread.
				Schellack	As in <i>S. anodontae</i> ( <i>vide supra</i> ). Final stages seen <i>in vivo</i> .
				Swellengrebel...	Transverse division with a cell-wall as in <i>Spirillum gigantea</i> . Occasionally individuals seen constricted in the middle; author is doubtful whether these are dividing forms. Apparent longitudinal division simply parallel juxtaposition of two individuals: it may also be simulated by organisms bending on itself at a middle point.
<i>S. buccalis</i> , Cohn	...	...		Swellengrebel...	First sign of division is the formation in middle of cell of an abrupt curve, resembling a rupture. At this spot the cell begins to thin, and becomes paler: after formation of the two daughter-cells, the connecting filament is drawn out still finer, and finally breaks.
<i>S. culicis</i> , Jaffé	Jaffé	...	In stained preparations one individual seen that might be interpreted as beginning of longitudinal division.		...
<i>S. dentium</i> , Koch	Mühlens and Hartmann		Observed in stained specimens.	Mühlens and Hartmann. Zettnow	In stained specimens, possibly transverse division. In stained preparations, individuals seen that might be interpreted as in process of transverse division.
<i>S. auttoni</i> , Breinl	Breinl	...	Occasionally met with, especially at stage of infection when parasites are disappearing from peripheral circulation. Scantiness of dividing forms suggests that process of division is rapid.	Breinl	This the more usual mode of division. Parasites increase in length, become thinner in middle, and thinner part elongates till daughter halves separate.

Comparative summary of evidence for longitudinal and transverse division in *spirochaetes* (continued).

Spirochaeta <i>S. duttoni</i> , Breinl (continued)	Observers of longitudinal division	Nature of observations		Observers of transverse division	Nature of observations	
		Y-forms seen in stained specimens: ap- parent equal division of chromatin granules.	Stained specimens. Longitudinal division less frequent than transverse. Occurs generally towards end of attack, when parasites are disappearing from blood. Before division spirochaete increases in width, then a longitudinal split begins at one end, so that Y-forms are produced. Y-forms numerous in juice from splenic puncture at close of a fatal attack.		Records transverse division.	Stained preparations. Perhaps most usual method of multiplication. Parasite in- creases in length, becomes thinner about middle, then is drawn out at this point till halves separate.
	Carter	...		Czaplewski	...	
	Dutton & Todd			Dutton & Todd		
Mayer	...	Apparent longitudinal division forms in stained preparations: especially abun- dant in smears from lungs.		Koch	...	Longitudinal division never seen. Where there is anything that can be interpreted as division, it is transverse.
				Levaditi	...	1. Spirochaetes frequently seen joined end to end in pairs by fine filament, which breaks at final separation. 2. Extraordinary inequality in length of in- dividuals in same film.
				*Schellack	...	As in <i>S. recurrentis</i> (vide <i>infra</i> ).
				*Zettnow	...	Studied both <i>in vivo</i> and stained. A long spirochaete divides in the middle—the two halves separating as the constriction thins out.
<i>S. equi</i> , Novy and Knapp	...	...		Martin	...	Some spirochaetes in stained preparations seen to have a paler and thinner portion in the middle.
<i>S. hartmanni</i> , Gonder	Gonder	...	Observed in stained specimens.	...	...	...

<i>S. marchouzi</i> , Nuttall (= <i>gallinarum</i> , Blanchard)	*Prowazek	...	1. Earliest stages difficult to make out. Dividing spirochaetes detected by their spasmodic movements. Before division the spirochaete increases in length and breadth. Division begins at one end; the split proceeds rather slowly through the length of the individual: the whole process once observed for a considerable time <i>in vivo</i> . 2. More common to see two spirochaetes still hanging together by a thin protoplasmic bridge—these are individuals in last stage of longitudinal division. 3. Presence of an undulating membrane and movements pointing to polarity in the cell, preclude possibility of simple transverse division.	Borrel	... A constriction marks beginning of division: this is drawn out into a fine faintly-staining protoplasmic thread, uniting the two young individuals. ... A simple statement that division is transverse.
<i>S. ovina</i> , Blanchard	Martoglio and Carpano	...	AY-form figured from a stained preparation.	Martoglio and Carpano	Observed in stained specimens.
<i>S. pallida</i> , Schaudinn	*Eitmer	...	Forms seen <i>in vivo</i> that might be interpreted as longitudinal division.	*Eitmer	Form seen <i>in vivo</i> that might be interpreted as transverse division.
	Gonder	...	Observed in stained specimens.		
	Krystallowicz and Siedlecki	...	Observed in stained specimens.		
		...		Prowazek	Dividing forms found in all preparations.
		...		*Schaudinn	Doubling of flagellum followed by rapid longitudinal splitting requiring only a few seconds. Process followed through <i>in vivo</i> in three cases. During division spirochaete becomes irregular in its curves—normal form recovered only when division is almost complete, and daughter spirochaetes are hanging together by their posterior ends. Dividing forms fairly numerous in stained preparations.

## Comparative summary of evidence for longitudinal and transverse division in spirochaetes (continued).

Spirochaeta	Observers of longitudinal division	Nature of observations	Observers of transverse division	Nature of observations
<i>S. pallida</i> , Schaudinn (continued)	...	...	*Siebert	Dividing spirochaetes observed <i>in vivo</i> showed relatively rapid splitting to form Y-stage: then remained in this condition for some time. Marked tendency for rigid spiral form to be lost, curves becoming irregular till division complete, while wave-like movements over body are substituted for normal rotation round long axis.
<i>S. portenvis</i> , Castellani	Borne	Described and figured.	...	...
	Prowazek	Numerous very distinct longitudinal divisions seen.	Schellack	Transverse division as in <i>S. anodontae</i> — <i>vide supra</i> —studied on stained material.
<i>S. pinnae</i> , Gonder	Gonder	Longitudinal splitting very rapid. Begins with division of blepharoplast at anterior end. Equal division of chromatin to the two daughter cells.	Zopf	Spirochaete breaks up into fragments.
<i>S. plicatilis</i> , Ehrenberg	Schellack	Statement that the spirochaete divides longitudinally.	Laptschinsky	Spirochaete fragments transversely: observed <i>in vivo</i> .
<i>S. recurrentis</i> , Liebert (= Obermeier, Cohn)	...	...	Anastasiades ... Fränkel and Pfeiffer Norris, Pappenheimer, and Flournoy ... *Novy and Knapp	Observed in stained preparations. Shown in figures of stained preparations. Observations agree with those of Novy and Knapp. 1. In stained preparations long spirochaetes frequently show pale transverse band in middle—suggestive of cell-wall. 2. Longitudinal division looked for daily both in living and in stained spirochaetes, but never seen. 3. <i>In vivo</i> long spirochaetes frequently seen to separate in two halves transversely. (This might be result of agglutination.) 4. In earliest stages of infection—before agglutination—long forms predominate. If division were longitudinal, short thick forms might be expected at this stage.

<i>S. recurrentis</i> , Lebert (= <i>obermeieri</i> , Cohn) (continued)	...	...	*Schellack	... 1. Extraordinary rarity of Y-forms, even in stained preparations, and the impossibility of distinguishing these from closely-appeared or agglutinated individuals. 2. Great constancy in thickness of individual spirochaetes. 3. Number of transverse divisions seen is quite in accordance with rapidity of multiplication. 4. Practically all spirochaetes of maximum length show a median constriction <i>in vivo</i> . 5. In final stage of division, the daughter spirochaetes, connected by a fine protoplasmic strand, are of a very constant length.
<i>S. schaudinni</i> , Prowazek (spirochaete of <i>Utricularia tropicum</i> )	...	...	*Prowazek	... Observed repeatedly. Entire process of longitudinal splitting followed <i>in vivo</i> . During division, spirochaete becomes less active and moves spasmodically.
<i>S. vespertilionis</i> , Novy and Knapp	...	...	*Gonder	... Occurs both in blood of bat and in food-canal of tick. 1. Difficult to follow <i>in vivo</i> . A division already in Y-stage followed once for a short time. In stained preparations several division forms found. Longitudinal split begins at one end and spreads gradually to the other. 2. Not infrequently individuals seen hanging together by their ends—probably final stage of longitudinal division, but interpreted as transverse division by Nicolle and Comte. 3. Agglomeration never seen, and very rare to see spirochaetes twisted together.
<i>S. vincenti</i>	...	...	Mühlens	... "Apparently transverse."
			Nicolle & Comte	The daughter-cells remain for some time connected by their ends.

questions connected with the morphology of spirochaetes. The literature is so extensive, and the observations so scattered, that a résumé of this sort may make it easier to weigh the pros and cons. The preceding table deals thus with the evidence for longitudinal and transverse division in spirochaetes. It does not pretend to be exhaustive, but it includes the chief statements of the best-known authorities.

In comparing the results given above, I was struck by the fact that the conclusions were so seldom based on observations of the living organism. It is not always clear from authors' statements whether they confined their study to stained preparations, but, so far as I can gather, the *entire* process of longitudinal division has been observed *in vivo* only by Prowazek (*S. marchouxi*, spirochaete of *Ulcus tropicum*, *S. balanitidis*)<sup>1</sup>, Schaudinn (*S. pallida*), Siebert (*S. pallida*), and Keysselitz and Fantham (*S. anodontae*)<sup>2</sup>, while Schellack (*S. recurrentis*, *S. duttoni*), Novy and Knapp (*S. recurrentis*) and Zettnow (*S. duttoni*) are the only observers of transverse division from its first indication till the separation of the daughter parasites. This seems strange, since stained preparations are, in a case like this, notoriously unreliable: and so long as these are used as sole evidence, just so long will the supporters of the view that spirochaetes are bacteria insist on regarding the Y-forms as agglutinations, and the supporters of the view that spirochaetes are protozoa will see in the so-called transverse divisions simply the final stage of longitudinal division.

#### PERSONAL OBSERVATIONS ON THE DIVISION OF *SPIROCHAETA RECURRENTIS*.

Professor Nuttall kindly suggested that I should try to make some observations on the division of *S. recurrentis* (Russian strain) *in vivo* from the blood of infected mice. At first I examined, at room temperature, very thin films of fresh infected blood, sealed with vaseline. Later on I employed a warm stage (Nuttall thermostat), with a

<sup>1</sup> Prowazek (1908), further states, but without particulars, that he has observed longitudinal division in all the smaller spirochaetes, with the exception of *S. dentium*, *S. vincenti*, *S. recurrentis* (European variety), spirochaetes from the intestines of the dog and the cat, spirochaetes from an abscess on the lower jaw of a chimpanzee, and *S. plicatilis*. Keysselitz and Blanchard also express their opinion that the spirochaetes as a group are characterized by longitudinal division.

<sup>2</sup> Perrin and Fantham both observed longitudinal division in *S. balbianii* up to the final stage when, however, the individuals died before having separated.



temperature of 37° C., and I found it more convenient to use the serum only of citrated blood<sup>1</sup>.

For the first half hour or so after being put into the warm stage, the spirochaetes were so active that it was impossible to follow any one individual. Gradually they slowed down somewhat, and it was at this stage that I made my observations. Later on, when they became still more sluggish, agglutination produced appearances often deceptively like divisions, which had to be discounted. Even when it was not a question of actual agglutination, the tendency of the spirochaetes to twist together for a short time in groups of two or three, made it very difficult to keep any one separately in view. At first also I lost many among blood-corpuscles.

I. *Longitudinal Division.* On two occasions only did I see what I felt convinced were spirochaetes in process of longitudinal division.

(1) The first was an individual that caught my attention by the character of its movement, which was slower and more spasmodic than that of the other spirochaetes in the same field. I then saw that it was apparently splitting longitudinally, and had already reached the Y-stage. The splitting portions were connected through the first two curves of their length, and then diverged at an acute angle: one individual had about seven curves free, the other was rather shorter. A certain amount of independent movement was shown in the two halves, one moving more rapidly or more slowly than the other; frequently they lay parallel, and twisted round one another, their curves coinciding or opposite. During the first fifteen minutes the spirochaete showed scarcely any appreciable forward movement, then it moved across the field, unsplit end first. Shortly before it was finally lost to sight—after having been watched for nearly 40 minutes—the split appeared to have extended over another curve. (Fig. 1 *a, b, c, d.*)

(2) An individual, apparently in an advanced Y-stage of longitudinal division, was kept in sight for seven minutes. The two halves

<sup>1</sup> A simple method of obtaining small quantities of serum full of spirochaetes but almost free of blood corpuscles, is the following:—Draw up into a fine pipette, *with an opening narrower than the general lumen of the tube*, a few drops of citrated blood. Lay the pipette horizontal for a short time; the blood corpuscles will settle in a layer in the under half of the lumen of the tube below the level of the opening, while numerous spirochaetes remain in the clear serum above. Then, still keeping the pipette nearly horizontal, blow out a minute drop of the serum on to a slide. This method has the advantage of avoiding possible injury to the organisms through centrifugalizing, and makes it easy to employ very small quantities of blood at a time.

were in this case approximately equal, and the movement, though rather spasmodic, was more rapid than in (1).

II. *Transverse Division.* I was struck by the fact that in most spirochaetes that had attained considerable length—9 to 14 curves—there appeared a weak point, about half-way along the organism, at which bending occurred, as the arm bends at the elbow-joint. In some cases there seemed to be a distinct constriction at this point.

On eleven occasions I saw what I should interpret as transverse division.

1. An individual with a thin drawn-out portion in the middle was seen to separate into two at that point.

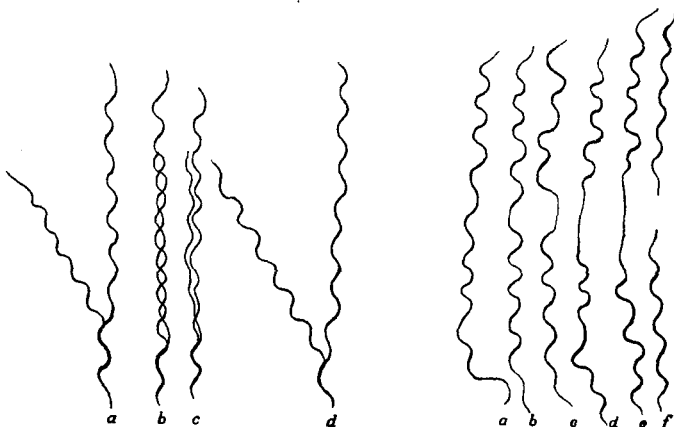


Fig. 1.

Fig. 2.

2. Two short spirochaetes joined only by a fine protoplasmic bridge were watched for some time, during which the connection thinned out till they separated. This appearance has been aptly likened by Schilling to the drawing out of a glass tube in a flame.

3. A long individual, with approximately 14 curves, showed a thin zone half-way along its length. It was watched for 20 minutes. During this time the thin portion became more and more drawn out and the two halves separated, after spasmodic and "purposeful" wrenching movements.

4. Two short individuals—one rather longer than the other—connected by a fine strand, separated after having been watched for several minutes.

5. A long individual (about 12—14 curves), with a thinner median portion was watched for 20 minutes. Its movements were agitated and

jerky, but it did not change its position much. The thin portion became finer and finer, till its presence could scarcely be suspected, but for the simultaneous tugging movements of the separating halves. Finally the connecting thread broke, and the daughter-spirochaetes departed in different directions.

6. A long spirochaete was seen, in which the two halves were separated by a finely drawn out middle portion.

7. A spirochaete with a thinner median portion was observed for 20 minutes. During that time the middle portion became longer and finer till scarcely visible. Death occurred before complete separation took place.

8. A long spirochaete was noticed that showed very marked bending at a slightly narrower portion half-way along its body. This portion was watched thinning out till the connection between the daughter halves became scarcely perceptible: they died after 20 minutes, and before they had completely separated.

9. A long spirochaete with a weak central point was followed for about 10 minutes, during which time the median portion had thinned out considerably.

10. A long spirochaete—about 14 curves—was followed for 30 minutes. During that time the rather narrower median portion became still thinner, till the two halves were widely separated, though still attached by a fine protoplasmic bridge. At this point the spirochaete became gradually immobile and died.

11. A long individual was kept in sight for about 25 minutes. Shortly after the beginning of the observation, a median thin portion became visible: division at this point went forward rather rapidly and the two halves separated. (Fig. 2 *a, b, c, d, e, f.*)

#### CONCLUSIONS.

In two cases apparent longitudinal division was seen in *Spirochaeta recurrentis*: in eleven cases the division was apparently transverse. It is not possible to say with absolute certainty, except in one instance, that these transverse divisions were not the final act in a longitudinal division. I am nevertheless inclined to think *that both forms of division may take place, transverse being the more common*. Breinl, and Dutton and Todd have expressed the same opinion with regard to *S. duttoni*, and Fantham also states that in *S. balbianii* both modes of division occur.

I should like to express my thanks to Professor Nuttall for his kind suggestions and help, and to the Carnegie Trust for the grant which has made my work possible.

## REFERENCES.

- ANASTASIADIS, S. J. (1908). Ein Fall von Febris recurrens. *Centralbl. f. Bakt.* XLVII. pp. 466-71. 1 text-fig.
- BLANCHARD, R. (1906). Spirilles, Spirochètes, et autres micro-organismes à corps spiralé. *Semaine médicale*, Paris, Jan. 3, pp. 1-5.
- BORREL, A. (1906). Cils et divisions transversales chez le spirille de la poule. *Compt. rend. Soc. Biol.* LX. pp. 138-41. 2 figs.
- and CERNOVODEANU (1907). Membrane ondulante de *Spirochaeta balbianii*. *Ibid.* XLII. p. 1102. 1 fig.
- BREINL, A. (1907). On the morphology and life-history of *Spirochaeta duttoni*. *Ann. Trop. Med. Liverpool*, I. pp. 435-8.
- and KINGHORN, A. (1906). An experimental study of the parasite of African Tick-fever (*Sp. duttoni*). *Liverpool School of Trop. Med.* Mem. XXI.
- CERTES, A. (1882). Note sur les parasites et les commensaux de l'huître. *Bull. Soc. Zool. de France*, VII. pp. 347-53. 4 figs.
- (1891). Sur le *Trypanosome balbianii*. *Bull. Soc. Zool. de France*, XVI. pp. 95-130.
- CZAPLEWSKI (1908). Tagung der freien Verein. d. Mikrobiol. *Centralbl. f. Bakt.* (Beilage).
- DUTTON, J. E. and TODD, J. L. (1907). A note on the morphology of *Spirochaeta duttoni*. *Lancet*, Nov. 30, pp. 1523-5.
- EITMER, E. (1907). Über Beobachtungen an der lebenden *Spirochaeta pallida*. *Münch. med. Wochenschr.* LII. pp. 770-3.
- FANTHAM, H. B. (1908). *Spirochaeta (Trypanosoma) balbianii* (Certes), and *Spirochaeta anodontae* (Keysseltz). *Quart. Journ. Microsc. Sci.* LII. pp. 1-73. 3 plates and 11 text-figs.
- (1908). The Spirochaetes: a review of some borderline organisms between animals and plants. *Science Progress*, No. 9, July, pp. 148-62. 4 figs.
- FRAENKEL, C. (1907). Über die Spirillen des Zeckenfiebers. *Münch. med. Wochenschr.* p. 201.
- and PFEIFFER, R. (1892). Mikrophotographischer Atlas der Bakterienkunde, pl. LXVI.
- GONDER, R. (1907). Studien über die Spirochaete aus dem Blute von *Vesperugo Kuhl.*. *Arbeit. a. d. Kaiserl. Gesundh.* XXVII. pp. 406-13. 1 plate.
- (1908). Spirochaeten aus dem Darmtraktus von *Pinna*: *Spirochaeta pinnae*, nov. spec. und *Spirochaeta Hartmanni*, nov. spec. *Centralbl. f. Bakt.* XLVII. pp. 491-4. 1 plate.
- (1909). Die Stellung der Spirochaeten unter den Protisten. *Centralbl. f. Bakt.* XLIX. pp. 190-6. 2 plates.
- HOFFMANN, E. and PROWAZEK, S. v. (1906). Untersuchungen über die Balanitis- und Mund-Spirochaeten. *Centralbl. f. Bakt.* XLI. pp. 741-4 and 817-21. 1 plate.

- JAFFÉ, J. (1907). *Spirochaeta culicis*, nov. spec. *Archiv f. Protistenkunde*, ix. pp. 100-7. 1 plate, 2 text-figs.
- KEYSELITZ, G. (1906). *Spirochaeta anodontae*, n. sp. *Arbeit. a. d. Kaiserl. Gesundh.* xxiii. pp. 566-7. 6 figs.
- (1907). Über die undulierende Membran bei Trypanosomen und Spirochaeten. *Archiv f. Protistenkunde*, pp. 127-8. 1 plate.
- KOCH, R. (1904). Über afrikanischen Recurrens. *Berl. Klin. Wochenschr.* Feb. 12, pp. 185-94. 10 figs.
- KRYSZTAŁOWICZ, FR. and SIEDLECKI, M. (1905). Contribution à l'étude de la structure et du cycle évolutif de *Spirochaeta pallida*, Schaudinn. *Bull. Acad. Cracovie*, Nov.
- LAPTSCHINSKY (1880). Zur Kenntnis der Spirochaeten. *Centralbl. f. med. Wissenschaft.* xviii.
- LAVERAN, A. and MESNIL, F. (1901). Sur la nature bactérienne du prétendu trypanosome des huîtres (*Trypanosoma balbianii*, Certes). *Compt. rend. Soc. Biol.* liii. pp. 883-5.
- LEVADITI, M. C. (1904). Contribution à l'étude de la spirillose des poules. *Ann. Inst. Past.* xviii. mars, pp. 129-49.
- (1906). Culture du spirille de la fièvre recurrenente africaine de l'homme (Tick-fever). *Compt. rend. Acad. Sci.* 14 mai, pp. 1099-1100.
- LÖWENTHAL, W. (1905). Die Spirochaeten. *Biophysik. Centralbl.* i.
- LUSTRAC, A. de (1896). *Trypanosoma balbianii*, Certes. *Actes Soc. Linn. Bordeaux*, 5<sup>e</sup> sér. x. pp. 265-75. 2 plates.
- MARTIN, G. (1906). Sur un cas de Spirillose du cheval observé en Guinée française. *Compt. rend. Soc. Biol.* lx. pp. 124-6. 1 fig.
- MARTOGLIO, T. and CARPANO, M. (1904). Spirillosi ovina (Nota preventiva). *Ann. d'igiene sper.* xiv. pp. 577-82. 1 plate.
- MAYER, M. (1901). Beiträge zur Morphologie der Spirochaeten (*Spirochaeta duttoni*). *Beiheft zu Archiv f. Schiffs- u. Trop.-Hyg.* xii.
- MÜHLENS, P. (1907). Vergleichende Spirochaetenstudien. *Zeitschr. f. Hyg. u. Infektionskr.* lvii. pp. 405-16. 2 plates.
- and HARTMANN (1906). Über *Bacillus fusiformis* und *Spirochaeta dentium*. *Zeitschr. f. Hyg. u. Infektionskr.* lv.
- NORRIS, PAPPENHEIMER and FLOURNOY (1906). Study of a Spirochaete obtained from a case of relapsing-fever in man, with notes on morphology, etc. *Journ. Infect. Dis.* iii. p. 266.
- NOVY, F. G. and KNAPP, R. E. (1906). Studies on *Spirillum obermeieri* and related organisms. *Journ. Infect. Dis.* iii. pp. 291-3.
- PERRIN, W. S. (1906). Researches on the Life-history of *Trypanosoma balbianii*. *Archiv f. Protistenk.* vii. pp. 131-51. 2 plates, 26 text-figs.
- PROWAZEK, S. v. (1906). Morphologische und entwicklungsgeschichtliche Untersuchungen über Hühnerspirochaeten. *Arbeit. a. d. Kaiserl. Gesundh.* xxiii. pp. 554-69. 2 plates.
- (1907). Vergleichende Spirochaetenuntersuchungen. *Arbeit. a. d. Kaiserl. Gesundh.* xxvi. pp. 23-31. 1 plate.
- (1908). Bemerkungen zur Spirochaeten und Vaccinefrage. *Centralbl. f. Bakt.* xlvi. pp. 229-31

- SCHAUDINN, F. (1905). Zur Kenntnis der *Spirochaete pallida*. *Deutsche med. Wochenschr.* pp. 1665-7.
- (1907). Zur Kenntnis der *Spirochaeta pallida* und anderer Spirochaeten. *Arbeit. a. d. Kaiserl. Gesundh.* xxvi. pp. 11-22. 2 plates.
- SCHELLACK, C. (1907). Morphologische Beiträge zur Kenntnis der europäischen, amerikanischen, und afrikanischen Rekurrensspirochaeten. *Arbeit. a. d. Kaiserl. Gesundh.* xxvii. pp. 364-87. 1 plate, 2 text-figs.
- (1909). Studien zur Morphologie und Systematik der Spirochaeten aus Muscheln. *Ibid.* xxx. pp. 379-428. 6 plates.
- SCHILLING, C. (1906). Rückfallfieber. In Mense's *Handb. d. Tropenkr.* III. pp. 668-88. 2 figs.
- SCHUBERG (1908). Tagung der freien Verein. d. Mikrobiol. *Centralbl. f. Bakt.* (Beilage).
- SIEBERT, W. (1908). Studien über Spirochaeten und Trypanosomen. *Archiv f. Protistenkunde*, xi. pp. 363-71. 4 text-figs.
- SWELLENGREBEL, N. H. (1907). Sur la cytologie comparée des Spirochètes et des Spirilles. *Ann. Inst. Pasteur*, xxi. pp. 448-65 and 562-86. 2 plates, 3 text-figs.
- (1909). Vergleichende Cytologie der Spirillen und Spirochaeten. *Centralbl. f. Bakt.* XLIX. pp. 529-49. 2 plates and 4 text-figs.
- ZETTNOW (1906). Färbung und Teilung bei Spirochaeten. *Zeitschr. f. Hyg. u. Infektionskr.* LII. pp. 485-94. 1 plate.
- ZOPF, W. (1882). Zur Morphologie der Spaltpflanzen. Leipzig. 74 pages, 7 plates.