OBSERVATIONS ON THE EFFECT OF VARIOUS CHEMICAL REAGENTS ON THE MORPHOLOGY OF SPIROCHAETES.

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4 Figures.

It has been stated[1] that in the stomach of the bed-bug the spirochaetes of relapsing-fever may frequently be observed to lose their normal regular curves and become more or less "worm-like." This is of interest to those who oppose the view that spirochaetes are rigid spirals, a view which, after gradual abandonment by most observers, has lately been again advanced by Novy and Knapp in their study on *S. recurrentis.*

It seems probable that the alteration in form may be due to the action of some constituent of the digestive juices of the bug. Professor Nuttall suggested to me that it might be possible to produce the same effect artificially by treating the living organism with various chemical reagents. In this attempt I was not altogether successful. Certain acids (notably formic acid) and quinine, and, in a lesser degree, glycerine, did cause a straightening out of the body and a corresponding flattening of its curves; but the appearances could scarcely be described as "worm-like." They help, however, to combat the notion that the curves are rigid.

In addition to the liquids just mentioned, and certain other drugs and acids, I subjected *S. recurrentis* to some of the reagents used by previous observers on various spirochaetes in their attempts to decide the systematic position of these organisms.

I employed *S. recurrentis* (Russian strain) in thin films of infected mouse-blood, mixed with equal parts of citrated salt solution. As a rule,

[1] Löwenthal (1905, Die Spirochaeten, *Biophysik. Centralbl.* I.) quotes Schaudinn's observations on this point. Klodnitsky (1907, *Centralbl. f. Bakt.* XLV. pp. 126–8) claims to have seen the same phenomenon. Nuttall has repeatedly observed it himself, but has expressed the opinion (1908, *Parasitology*, I. p. 144) that Klodnitsky's photographs show that that author was dealing with the spermatozoa of the bug, and not with altered spirochaetes.

the reagent was diluted (with citrated salt solution where possible) in about six graduated strengths, and these were allowed to diffuse in minute quantities below the cover-glass, the effect of each dilution being carefully watched on the living organism. Care had to be taken to prevent the diffusion currents from being too violent, as these were apt to distort the spirochaetes mechanically, and so vitiate the results.

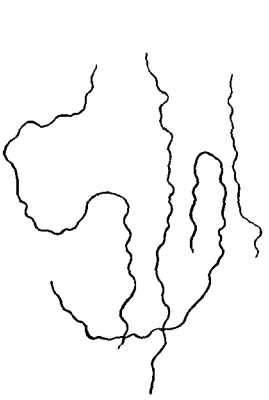

Fig. 1.

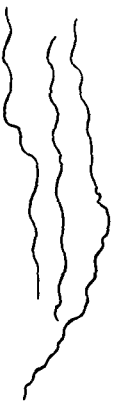

Fig. 2.

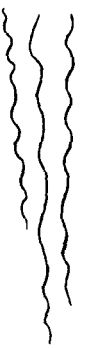

Fig. 3.

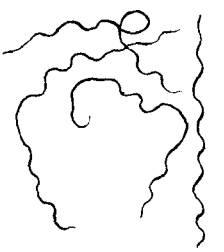

Fig. 4.

The following table gives the results of my observations:

Reagent employed	Effect on *Spirochaeta recurrentis*
1. Distilled water ...	Spirochaetes gradually immobilized—by the end of a few minutes all at rest. There was a strong tendency to collect in tangled skeins. Occasionally an individual showed flattening of its curves. There was marked blurring of outlines. Distilled water after 25 % salt solution produced no effect.
2. *Sodium chloride* (1 % to 40 %)	All solutions immobilized (in the stronger solutions, instantly), but produced no other effect beyond giving the spirochaetes a more refractive appearance. Unchanged after 16 hours.
3. Caustic potash ... (5 % to 25 %)	Majority of spirochaetes dissolved. Some remained as pale shadows.
4. *Hydrochloric acid* (·01 % to 10 %)	All dilutions produced instant immobilization. The spirochaetes had their curves much flattened out, or even reduced to minute irregular "crimping": there was also a tendency to coil at the ends, and they were often twisted together in parallel bunches. They became paler, but were not dissolved after 16 hours. (Fig. 1.)
5. Nitric acid ... (2 %)	Produced much the same effect as hydrochloric acid. On the whole, the spirochaetes seemed less inclined to curl and twist up.
6. Citric acid ... (·01 % to 10 %)	Spirochaetes at once immobilized: there was a tendency to run together in bunches: they also became paler and showed occasional flattening of curves. Undissolved after 6 hours.
7. Formic acid ... (·02 % to 40 %)	Instant immobilization: curves frequently much flattened out: occasional tendency for curling over at the ends: many become

Reagent employed	Effect on *Spirochaeta recurrentis*
	much paler. After 18 hours no further change, except that in the stronger solutions the spirochaetes often appeared very pale and shadow-like.
8. Alcohol ... (5 % to 100 %)	Immobilization instant in the stronger solutions—in solutions below 30 % this was more gradual. No appreciable change in form, beyond occasional slight flattening of curves.
9. Glycerine ... (5 % to 85 %)	Immobilization complete in about a minute or two in the weaker solutions: above 25 % it was almost instantaneous. The form was very little altered, except for occasional marked flattening of the curves. No further change after 23 hours. (Fig. 2.)
10. Quinine (sulphate) (·01 % to 10 %)	Instant immobilization. The curves became much flattened out: frequently the spirochaetes were almost straight, or minutely "crinkled." This was particularly marked in the stronger solutions, but was noticeable in all. (Fig. 3.)
11. Soamin (1 %) ...	Much the same effect as in distilled water. A few spirochaetes still moving after 10 minutes, but only very sluggishly. No alteration after 16 hours.
12. Arsacetin (5 %)...	Some immobilized at once: the majority continue to move sluggishly for a few minutes. The general appearance was much as in distilled water. No alteration after 16 hours.
13. Trypanblau (1 %)	Much the same effect as in distilled water. A stronger tendency for the spirochaetes to form intertwining bundles: frequently the individuals forming the groups were much twisted upon themselves. 20 hours later, no alteration. (Fig. 4.)
14. Trypanrot (2 %)	Same as in (13).

CONCLUSIONS.

The form of *Spirochaeta recurrentis* is not appreciably altered by immersing it in distilled water, NaCl solutions, alcohol, soamin, arsacetin, trypanblau, trypanrot. Flattening of the curves of the spiral produced by glycerine, citric acid, formic acid, nitric acid, quinine and hydrochloric acid —this effect is most marked in quinine and hydrochloric acid. In several of the reagents employed—notably quinine, HCl, trypanrot and trypanblau and in distilled water—there is a distinct tendency for the spirochaetes to curl up at their extremities, and to become intertwined in bundles. There is no evidence of plasmolysis. The spirochaetes dissolve readily in caustic potash: the acids employed tend to make them become paler. All the reagents produce rapid immobilization.

For the sake of comparison with the above, and in order to bring together into a convenient form observations scattered throughout the literature, I have drawn up a tabular summary of the results of previous experiments similar to mine[1].

[1] The list contains all the chief references, but cannot pretend to be exhaustive.

Comparative summary of observations on the effect of various chemical reagents on the morphology of Spirochaetes.

Reagent	Spirochaeta	Effect on Spirochaete	Observer
1. Distilled water	*S. balbianii*, Certes ...	Death occurs: plasma swells and bursts periplast	Perrin.
		Die quickly	Fantham.
	S. culicis, Jaffé ...	Die quickly	Jaffé.
	S. marchouxi, Nuttall (=*gallinarum*, Blanchard)	(After 10 % NaCl solution.) No bursting out of plasma through periplast	Prowazek.
	S. pallida, Schaudinn	Die quickly	Eitmer.
	S. recurrentis, Lebert	Increased activity for 6–8 hrs.: then become sluggish: after coming to rest, their form is perfectly retained: revived by 5 % NaCl solution[1]	Novy and Knapp.
2. Tap-water ...	*S. balbianii* ...	Live slightly longer than in distilled water	Fantham.
3. Sea-water ...	*S. anodontae*, Keysselitz	Lived one hour	Fantham.
4. Sodium chloride	*S. balanitidis* ...	10 % solution causes spirochaetes to appear narrower and more refractive: *no* plasmolysis	Hoffmann and Prowazek.
	S. marchouxi ...	5–10 % solutions had no effect beyond occasional production of a protoplasmic "bead"	Prowazek.
	S. pallida ...	Swell: fraying repeatedly seen, also knot-like thickenings	Siebert.
	S. vespertilionis, Novy and Knapp	2–5 % solutions produce no plasmolysis	Gonder.
	Spirochaetes of the mouth	10–12 % solutions produce shrinkage here and there, through extraction of water: tendency to form tangled skeins	Siebert.
5. Sodium carbonate	*S. buccalis* ...	In saturated solution are unaltered ...	Swellengrebel.
	Spirochaetes of the mouth (*S. buccalis*, etc.)	Become paler	Hoffmann and Prowazek.
6. Caustic potash[2]	*S. balanitidis* ...	Become paler and fewer: after 2 hours all disappeared	Hoffmann and Prowazek.
	S. balbianii ...	In 10 % solution some retained shape for a long time and were not dissolved. (Did not appear blue in iodine and sulphuric acid after caustic potash)	Fantham.
	S. buccalis ...	Dissolve	Hoffmann and Prowazek.
		In 10–20 % solutions become paler ...	Swellengrebel.
	S. culicis	Dissolve in 1 % solution	Jaffé.
	S. marchouxi ...	Die; some dissolve, others remain as pale "shadows"	Prowazek.
	S. pallida ...	Dissolve	Eitmer.
	S. vespertilionis ...	Die and are dissolved	Gonder.

[1] I fail to understand how Novy and Knapp arrived at this result. I repeatedly tried the effect of distilled water on the spirochaetes, and always found that they became immobile in a very few minutes.

[2] Thesing tried the effect of caustic potash on "various spirochaetes," and found that they remained unaltered.

Reagent	Spirochaeta	Effect on Spirochaete	Observer
7. Eau de Javelle	*S. buccalis*, etc. ...	No effect	Hoffmann and Prowazek.
	S. marchouxi ...	No effect at first, but after a time become knotted and twisted up	Prowazek.
8. Hydrochloric acid (dilute)	*S. buccalis*, etc.	Swell somewhat	Hoffmann and Prowazek.
		Become paler	Swellengrebel.
9. Nitric acid (dilute)	*S. buccalis*, etc. ...	Dissolve	Hoffmann and Prowazek.
		In 1 : 5 solution become paler ...	Swellengrebel.
10. Sulphuric acid (concentrated)	*S. balbianii* ...	Periplast dissolved after 3 hours: undulating membrane not especially soon dissolved: nuclear core remained	Fantham.
11. Acetic acid (strong)	*S. balbianii* ...	Not dissolved: retain shape for long time: a certain amount of fraying	Fantham.
12. Acid carb. liquefact	*S. marchouxi* ...	No effect. (Other acids produce no effect either)	Prowazek.
13. Iodine ...	*S. balbianii* ...	Stain brown: in iodine + concentrated sulphuric acid stain light yellow, and retain shape for long time	Fantham.
	S. marchouxi ...	Stain yellow like bacteria	Prowazek.
14. Sublimate[1], Lysol, and other antiseptics	*S. buccalis*, etc. ...	In antiseptics break up into numerous short individuals	Wechselmann and Löwenthal.
	S. pallida ...	Become immobile	Eitmer.
		In sublimate (1 : 1000) are killed, but not dissolved	Prowazek.
		In sublimate (1 : 1000) all spirochaetes in tissues are not in 10 % solution, no swelling or maceration, but become thin and strikingly paler	Siebert.
15. Alcohol ...	*S. pallida* ...	In 30 % die quickly	Eitmer.
16. Glycerine ...	*S. balanitidis* ...	Die quickly without losing curves, though these become somewhat flattened	Hoffmann and Prowazek.
		Become immobile	Müller and Scherber.
	S. marchouxi ...	In 40 % solution majority contract, some die, while others take on peculiar tangled form, but continue mobile. (Still infective after 12 hours)	Prowazek.
	S. pallida ...	Some not killed, but altered to spindle-shaped forms	Schaudinn.
		Even in 10 % solution spirochaetes stretch out quickly, lose their mobility and remain in this condition; NaCl solution fails to revive them, and they are uninfective	Eitmer.
	S. vespertilionis ...	Do not live long: stretch out, or first stretch and then contract together	Gonder.

[1] Wechselmann and Löwenthal also state that the mercury treatment causes *S. pallida* to break up into fragments.

Comparative summary, etc. (continued).

Reagent	Spirochaeta	Effect on Spirochaete	Observer
17. White of egg	*S. balbianii* ...	Die very quickly	Perrin.
		Die very quickly	Fantham.
18. Pepsin + HCl	*S. buccalis*, etc. ...	Some swell up, some are practically dissolved, others remain unaltered	Siebert.
	S. pallida ...	Contents of periplast apparently dissolved	Prowazek.
		Swollen appearance and partial paling	Siebert.
19. Sodium taurocholate	*S. buccalis*, etc. ...	Dissolve instantly	Neufeld and Prowazek.
	S. marchouxi ...	Dissolve instantly	Neufeld and Prowazek.
	S. pallida ...	Dissolve in 10 % solution	Prowazek.
		Dissolve	Neufeld and Prowazek.
		In 10 % solution first swell, then gradually dissolve	Siebert.
	S. recurrentis ...	Dissolve	Neufeld and Prowazek.
	S. schaudinni (Spirochaete of *Ulcus tropicum*)	Dissolve	Prowazek.
20. Sapotoxin ...	*S. buccalis*, etc. ...	Become immobile and die	Neufeld and Prowazek.
	S. marchouxi ...	Become immobile and die	Neufeld and Prowazek.
21 Saponin ...	*S. pallida* ...	In 10 % solution become swollen and show partial paling	Siebert.

Conclusions.

The general consensus of opinion goes to show (1) that spirochaetes are dissolved in solutions of caustic potash (5 to 25 %) and of sodium taurocholate (10 %), and possibly to a slight extent in acids; (2) that they are not plasmolyzable in the strict sense, and that plasmotypsis is rare; and (3) that certain reagents, such as glycerine, may alter the form, by flattening the curves, or by causing contraction.

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