SPIROCHAETES IN BALTIMORE MARKET OYSTERS[1]

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INTRODUCTION

Several investigators in different parts of the world have reported the presence of large, coarse spirochaetes living in the digestive tract of oysters and other shell fish. Certes, Laveran, Mesnil, and Perrin named these spirochaetes *Trypanosoma Balbianii*, while Keysselitz, Fantham and Schellack named them *Spirochaeta Balbianii*. Gross has proposed the generic term *Cristispira* for the large spirochaetes found in the digestive tract of the lamellibranchs.

The present investigation was undertaken, in the first place, to discover whether spirochaetes are found in market oysters in Baltimore; secondly to determine what percentage of the market oysters are infected with spirochaetes; in the third place, to determine the species of spirochaetes found.

REVIEW OF LITERATURE

Certes (1882) first discovered and described a spiral organism which he found in the stomach of the oyster. He believed that this organism was a trypanosome therefore he named it *Trypanosoma Balbianii*, in honor of his teacher and colleague in the College de France.

Laveran and Mesnil (1901) repeated Certes' work and described *Trypanosoma Balbianii* as resembling the spirilla in form and movements, but as differing from the spirilla in the width of the cell body and in the possession of an undulating membrane.

[1] Thesis presented in partial fulfilment of the requirements for the degree of Doctor of Science in Hygiene in the Department of Bacteriology in the School of Hygiene and Public Health, the Johns Hopkins University, June, 1925.

They concluded that *Trypanosoma Balbianii* is more closely related to the bacteria than to the trypanosomes.

Perrin (1906) found *Trypanosoma Balbianii* in the crystalline style of almost every oyster which he took from the sea and examined immediately.

Keysselitz (1906) described a new species, *Spirochaeta anodontae*, which he found in the crystalline style of *Anodonta mutabilis*, a fresh water mussel.

Fantham (1908) found *Spirochaeta (Trypanosoma) Balbianii (Certes)* in the crystalline styles of the oysters which he purchased in the markets of London. He also found these spirochaetes less frequently in the gut, in the stomach, in the caecum, and in the liver bordering on the stomach. He found *Spirochaeta anodontae* in the crystalline style of *Anodonta mutabilis*.

Schellack (1909) found larger spirochaetes in the inner layer of the crystalline style, while smaller spirochaetes were found between the outer and the inner layer of the style. He is of the opinion that many spirochaetes swim out from the style into the gut.

Gross (1910) working in the Zoological Station at Naples, found two new species, *Cristispira pectinis* and *Cristispira interrogationis*, embedded in the walls of the stomach, in the walls of the intestine, and occasionally in the crystalline style of *Pecten Jacobeus* (scallop). In the same year Gross described two new species, *Saprospira grandis* and *Saprospira nana*.

Bosanquet (1910) reported observations on *Spirochaeta anodontae* and concluded that these spiral organisms are bacteria.

Dobell (1910) found a large species of *Cristispira* in the crystalline style of *Venus (Meretrix) casta Chem.*, the clam, obtained from the salt water lake Tamblegan in the island of Ceylon.

During the summer of 1916 Noguchi studied the spirochaetes found in shell fish in the vicinity of Woods Hole, Massachusetts, and in Long Island Sound. He found the cristispiras most frequently in the styles of oysters, next in clams, and then in the mussels. No other species examined showed any cristispiras.

EXPERIMENTAL

a. Materials and methods

The spirochaetes here described were obtained from oysters purchased in the markets of Baltimore. There were two varieties of oysters. One variety apparently came from muddy oyster beds, and still had fine silt adhering to the shells. The other type was comparatively free from mud and apparently came from sandy or gravelly oyster beds. The muddy oysters were more highly infected with spirochaetes than the clean specimens.

The oysters were opened by inserting an oyster knife between the lips of the shells, cutting the abductor muscle, removing the right shell, and draining off the liquor. Care was taken to avoid cutting into the body of the oyster. The gills were pulled dorsally in order to expose the intestine, the caecum, and a portion of the liver.

During the first observations the intestine was split longitudinally in order to locate the crystalline style lying in the lumen of the gut near the liver. Fragments of the crystalline style were found only in one oyster, because the market oysters are usually not fresh, and the crystalline style disappears in two hours after the removal of the oyster from its natural habitat. Since no crystalline styles were found, specimens for microscopic examination were taken from the stomach and from the intestine by inserting the point of a Pasteur pipette through the intestinal wall into the lumen and sucking up some of the liquid.

A hanging drop preparation was made and examined microscopically in order to determine whether spirochaetes were present in the oyster before making stained preparations. If no spirochaetes were found in the intestine, the contents of the stomach were examined for spirochaetes in the same way. Often the spirochaetes were not found in the intestine, while they were found in the contents of the stomach. This was especially the case with oysters that had been kept in the ice box for several days. It appeared that the spirochaetes migrate towards the stomach as the oyster slowly dries up (table 1). In other cases the spirochaetes were just as numerous in the intestine as they were in the stomach after storage in the ice box for seventeen days.

There was no way to determine how long the oysters had been out of water before they were brought into the laboratory. It is safe to assume, however, that they had been out of water at least one week when purchased in the market. They were then stored in the ice box from one to seventeen days before they were examined for spirochaetes. Table 1 shows that living spirochaetes were

TABLE 1

Table showing the infection of oysters by spirochaetes

NUMBER OF DAYS OYSTERS WERE KEPT IN ICE BOX BEFORE EXAMINED	NUMBER OF OYSTERS EXAMINED	NUMBER OF OYSTERS		SPIROCHAETES FOUND IN	
		Negative	Positive	Intestine	Stomach
0	17	3	14	13	14
2	5	1	4	1	4
2	4	0	4	4	4
3	17	2	15	13	15
4	6	0	6	5	6
5	3	1	2	2	2
6	7	0	7	7	7
7	1	0	1	1	1
8	2	0	2	2	2
9	9	0	9	9	9
12	6	0	6	6	6
15	1	0	1	1	1
16	1	0	1	1	1
17	2	0	2	2	2
Totals........	81	7	74	67	74
Percentage...	100	9	91	83	91

observed in the digestive tract of the oyster during this whole period.

Analysis of table 1 shows that out of 81 oysters examined only 7, or 9 per cent, did not contain spirochaetes. The table further shows that the spirochaetes were found more frequently in the stomach (91 per cent) than in the intestine (83 per cent). It is also evident that the oysters which were kept in the ice box for seventeen days showed sporochaetes both in the stomach and in the intestine. As long as the oyster retains some of its liquid in the digestive tract the spirochaetes are apparently able to survive. These spirochaetes have no pathological significance for man whatsoever. They simply are the normal inhabitants of the shellfish.

The ability of the spirochaetes to live in the drying oyster was further tested by opening the oyster and removing the right shell. The gills were pulled back, exposing the digestive tract. The presence of spirochaetes was determined by microscopic examination of hanging drop preparations. If the oyster contained spirochaetes it was put into an open Petri dish and set in the ice box. Daily microscopic examinations were made as long as the spirochaetes were motile and the results are shown in table 2.

TABLE 2

Record of spirochaetes found in the digestive tract of oysters after being open for several days

EXPERIMENT	SPIROCHAETES FOUND IN THE DIGESTIVE TRACT OF OYSTERS AFTER BEING OPEN FOR							
	One day	Two days	Three days	Four days	Five days	Six days	Seven days	Eight days
I	+	+	+	+	+	+	+	0
II	+	+	+	+	+	+	0	
III	+	+	+	0				
IV	+	0						
V	+	+	+	+	+	0		

+ = spirochaetes found; 0 = spirochaetes not found.

Table 2 shows that the spirochaetes remained alive for some time in the open oyster. In observation I the spirochaetes were found on the seventh day, while in IV they were found only on the first day. The spirochaetes survived as long as there was some fluid in the lumen of the digestive tract. As the intestine began to disintegrate the number of motile spirochaetes decreased. Gradually the gut became a soft, pulpy mass, and with this decomposition the motile spirochaetes disappeared entirely.

The spirochaetes were stained by various methods which are described as follows:

Air-dried films were fixed in absolute alcohol for thirty minutes, washed in water, and stained in Giemsa's solution overnight (figs. 1 to 6, 10, plate 1; fig. 13, plate 2; figs. 20 and 24, plate 3).

Air-dried films were fixed by heat and stained with Giemsa's solution overnight (figs 8, 9 and 15, plate 2).

The moist films were exposed to osmic acid vapor for one minute, fixed in absolute alcohol for thirty minutes, washed in water, covered with Lugol's solution for five minutes, washed in water, flooded with 0.5 per cent sodium thiosulphate for five minutes, washed in water, and stained in Giemsa's solution overnight (fig. 18, plate 2; figs. 21, 23, 25 and 26, plate 3; figs. 27, 32, 33 and 37, plate 4).

The moist (wet) films were exposed to osmic acid vapor for one minute, fixed in absolute alcohol for thirty minutes, washed in water, immersed in 5 per cent picric acid for thirty minutes, washed in water, and stained in Giemsa's solution overnight (figs. 7, 10a and 11, plate 1; figs. 14, 16 and 19, plate 2; figs. 28, 29, 31, 34, 35 and 36, plate 4).

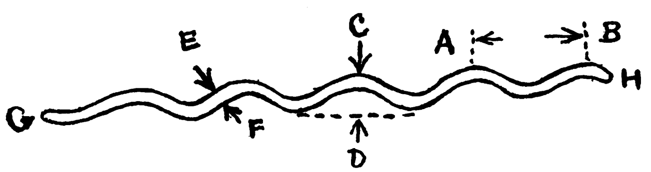
The moist films were exposed to osmic acid vapor for one minute, immersed in 5 per cent picric acid for thirty minutes, allowed to remain in a dish of clean water for ten minutes in order to wash out the picric acid, rinsed thoroughly in fresh water, stained in Giemsa's solution (1 drop of the stain to 1 cc. distilled water and 1 drop of potassium carbonate to each 10 cc. of the stain) for three days. The preparations were then allowed to remain in a dish of clean water for thirty minutes in order to wash out all superfluous stain, rinsed in fresh water, air-dried and examined. This method is most affective in staining the crista although there is a tendency to overstain and thus lose to a certain degree the clearness of the internal structure. Experience with the reagents shows the best technique for good results. With this method the spirochaetes stain dark purple and the crista stains pink (figs. 38 to 58, plates 5 and 6).

The wet films were fixed in Fontana's solution no. 1 (acetic acid, one part; formalin 2 parts; distilled water, 100 parts) for two minutes, washed in water, immersed in absolute alcohol for thirty minutes, washed in water and stained in Giemsa's solution for one hour (fig. 30, plate 4).

The wet film was exposed to formaldehyde gas for five minutes, allowed to dry, then immersed in absolute alcohol for thirty minutes, washed in water, and stained with Giemsa's solution for two hours (figs. 12 and 14, plate 2).

b. Definition of terms

The definition of a Spirochaete according to Swellengrebel is as follows: Spirochaetes are spiral microörganisms in which the individual cell occupies one or more complete spiral turns. The cell body is made up of an axial filament surrounded by periplast or various modifications of this structure. Terminal prolongations of periplast or of axial filament are present in at least one genus. A chambered or honey-combed arrangement is present in two genera. They possess no true bacterial flagella. Volutin granules are present in nearly all forms of spirochaetes and chromidial granules are found in many species. It is probable that



TEXT-FIG. 1. SHOWING METHODS OF MEASURING SPIROCHAETES

the spirochaetes have no true chromatin.[2] The cell bodies are always elastic. The spirochaetes are free living, saprophytic, or parasitic, pathogenic and non-pathogenic microörganisms.

The terms used in this paper to describe the measurements of the spirochaetes are those used in the literature dealing with this subject. For the sake of clearness they are stated as follows:

The spiral amplitude (wave length of the spiral) is the length of one complete spiral turn, measured from the apex of one crest to the apex of the next crest as is indicated by *AB* in text-figure 1.

The spiral depth (width of the spiral) is the diameter of the spiral measured transversely on the outside of two opposite apices of the spiral as is indicated by *CD* in text-figure 1.

[2] The term chromatin as used in this discussion is not limited strictly to the nuclear matter of the cell, but includes the elements in the protoplasm that take the chromatin stain.

The width of the cell body is the transverse distance of the cell body measured perpendicularly to the long axis as is shown by *EF* in text-figure 1.

The length of the cell body is the distance between the two poles as is indicated by *GH* in text-figure 1.

The spirochaetes found in the oysters and described in this paper are classified according to whether they possess or do not possess crista. Those sporichaetes that do not possess crista are grouped under the genus *Saprospira Gross.* This genus is further subdivided into two divisions. One division includes the saprospiras having pointed ends, while the other division includes the saprospiras having rounded ends. All the species of saprospiras are classified under these two divisions, and then identified by their morphological characteristics.

The oyster spirochaetes having crista are classified under the genus *Cristispira Gross.* This genus is further subdivided into two divisions. One division includes the cristispiras having pointed ends, while the second division includes the cristispiras having rounded ends. All species of cristispiras are classified under these two divisions and then identified by their morphological characteristics.

c. Descriptions of species

Four different types of saprospira were found and investigated. One of these species proved to be *Saprospira grandis*, two types of *Saprospira lepta* and one a type of *Saprospira puncta.* Seven species of cristispira, *Cristispira Balbianii*, *Cristispira anodontae*, *Cristispira spiculifera*, *Cristispira modiolae*, *Cristispira modiolae var A* nov. var., *Cristispira mina* nov. spec., *Cristispira tenua* nov. spec. and *Spirillum ostrae* were also found and described.

1. *Saprospira grandis* Gross. *Saprospira grandis* was observed in four stained preparations. It was found in the digestive tract of the oyster less frequently than *Cristispira Balbianii.* It possesses a chambered structure, but no crista was observed on any of the specimens that were studied (figs. 20 to 26, plate 3).

The fully grown *Saprospira grandis* averages about 80 micra in length by 1.2 micron in width, while the extremes are 52 to 90 micra for the length and 1.2 to 1.4 micron for the width. The average spiral amplitude is 24 micra, while the extremes are 20 to 28 micra. The number of turns varies from 3 to $4\frac{1}{2}$. The wide variations in the wave length and in the width of the spiral are due to the different methods of fixing and staining.

The chambered structure found in all specimens examined was the same, irrespective of whether the spirochaetes were stained in dry or in wet films. The chromatin appeared in narrow transverse bars equidistant from each other.

Gross has described the heavily stained areas found at the tips of the poles both in saprospiras and in cristispiras as "Polkappen" or polar caps. These structures were present in nearly all the specimens of *Saprospira grandis* observed. They appeared to be nothing more than chromatin material segregated at the end portions of the organisms.

A heavy border line surrounding the entire cell body was observed in stained preparations. In hanging drop preparations a distinct, shadow-like margin was seen about the cell body of this species. This apparently represents the cell membrane.

These organisms move like a corkscrew rotating through the medium. When the motion slows down, the moving saprospira gives the appearance of a chain made up of a figure 8, due to the spirals rotating on the long axis. Frequently, contracting and stretching motions can be observed, simultanuous with the rotating movements. The forward movement of the saprospira appears to be due to waves of peristalsis-like contractions of the protoplasm or of the elastic cell membrane. According to Fantham these impulses are due to contractile myonemes or muscular fibrils, embedded in the periplast at right angles to the long axis. He has described 4 to 6 successive impulses passing simultaneously over the organism. In this investigation only one impulse was observed at any time. It is possible that the motility is caused by a motile "posterior" end portion. This is suggested by the observations on specimens having a retarded movement.

The method of multiplication was not observed in the living specimens, although one stained preparation (fig. 17, plate 2) shows that multiplication takes place by a kind of transverse division, corresponding to what Gross called "incurvation." This he described as follows: As the cristispira approaches the stage of cell division it bends (incurvates) a short distance from the middle. During this incurvation the two halves beat and lash violently, then the lashing ceases for intervals of one-half of one second. The end of the unbent half glides over the other half until both ends come together, then the two halves intertwine and simulate a stage of longitudinal division as is illustrated by figure 16, plate 2. At this time, constriction of the cell body begins at the point of incurvation and the process of division is completed in thirty to sixty minutes. After apparently complete constriction the new daughter cells do not separate but seem to remain attached, whipping and lashing movements taking place as if the two halves were struggling to detach themselves from each other.

2. *Saprospira lepta* nov. spec. This organism was observed in two stained preparations, taken from two different oysters on two different occasions. Whenever it is seen in stained preparations it is very characteristic because it appears coiled and twisted into knots of various designs. Often one end bends and forms a loop while the remainder of the cell body is straight (figs. 7 and 11, plate 1). The chromatin is segregated in wide blocks, although narrow transverse bars are also found with clear, circular, unstained areas between each block of chromatin material. Slight bulging is evident at the circular, unstained areas and occasionally the ends show bulging if there are unstained portions there. This spirochaete looks like a chain with long narrow solid links connected by larger, circular links. The ends taper down to sharp points, although individuals are found with ends less pointed, and occasionally one end is sharp while the other tapers down to a blunt point.

Accurate measurements of the spiral amplitude and the spiral width are impossible on account of the irregular spirals. The width and the length of the cell body can be determined however. The average spirochaete is 70 micra long by 0.5 micron wide, with extremes of 54 to 92 micra for the length, while the width of the cell body is constant. The average spiral amplitude is 8 micra with extremes of 5 to 13 micra. The average spiral width is 1.6 micron with extremes of 1.6 to 4.8 micra. The average number of turns is 6 with extremes of $3\frac{1}{2}$ to 10 turns. The longest individual observed measured 92 micra.

A crista was never found on this spirochaete, although crista were observed on other species in the same film (fig. 19, plate 2, and fig. 36, plate 4).

This spirochaete was never seen in the living condition so that its manner of motility could not be determined.

The process of division was never observed either in the living or in stained specimens. On the basis of mathematical measurements multiplication probably takes place by transverse division. Spirochaetes 92 micra in length, nearly twice as long as the shortest specimens, 54 micra long, were found, but no specimens were observed that were twice as wide as the narrowest individual. The width of the cell body is constant, i.e., it is 0.5 micron. It is possible that the coiling and twisting are stages of incurvation in the process of transverse division, but no constriction of the cell body were observed on any specimens.

In stained preparations this spirochaete shows what Perrin has described in *Trypanosoma Balbianii* as coiling and encystment. In *Saprospira lepta* the coiling was undoubtedly due to unfavorable conditions during the fixation and the staining of the films. This spirochaete is a type of saprospira, although it differs from *Saprospira grandis* Gross in having pointed ends, in the distribution of the chromatin, and in the irregularity of the spirals. This species has not been described in the literature, therefore the name *Saprospira lepta* (a thin or fine saprospira) is suggested, to distinguish it from *Saprospira grandis* and *Saprospira nana* Gross.

3. *Saprospira lepta var. A* nov. var. This spirochaete (figs. 10 and 10a, plate 1) and *Saprospira lepta* were observed in the same stained preparations. They have many characteristics in common but they differ in the shape of the ends thus necessitating separate descriptions.

Saprospira lepta var. A is found coiled and twisted in knots of various designs, and in a straight position. The spirals are irregular and the crests form corners rather than smooth curves. The cytoplasm coagulates in solid, long blocks, with circular, clear areas between them (fig. 10a, plate 1). Some specimens have the cytoplasm in long and short blocks, in transverse bars, or in narrow transverse lines with the typical clear, circular areas between each block. A slight bulging is noticeable at each unstained area and occasionally the terminal portions show bulging (fig. 10, plate 1). These appearances may have been caused by the heating during the process of staining.

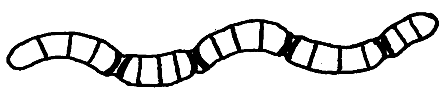
Accurate measurements of these spirochaetes is impossible on account of their irregular spirals. The average length of the cell body is 60 micra, with extremes of 45 to 72 micra. The length of the spiral is 20 micra. The width of the spiral is 4 micra with extremes of 3.2 to 5.2 micra. The number of turns is 2 to 4. averaging about 3 turns for the fully grown individual.

Saprospira nana Gross and *Saprospira lepta var. A* are similar in having thin (narrow) cell bodies, but they differ in all other points. *Saprospira lepta var. A* and *Saprospira lepta* are similar in having the cytoplasm in wide blocks; in having clear circular areas between the dark stained portions; in forming coils and twisted knots; in having thin (narrow) cell bodies. They differ, however, in the shape of the ends, in the wave length and the width of the spiral, and in the number of turns. Since this spirochaete corresponds to neither of the spirochaetes that have been described it is regarded as a new variety and named *Saprospira lepta var. A* nov. var.

4. *Saprospira puncta* nov. spec. *Saprospira puncta* is similar to *Saprospira grandis* except that its ends are pointed and the chromatin is arranged in transverse bars farther apart. It is the longest oyster spirochaete which we found and was present in five different stained preparations. In one of these preparations it was observed in considerable numbers.

The contours of the cell body are parallel except that the ends taper down to sharp points. In some specimens the spirals are regular and deep. In other specimens a portion of the cell body forms regular spirals, while the remainder may be straight. Specimens that have lost their spiral shape are twisted into s-shape, into c-shape, etc. The terminal portions are straight, pointing in the direction of the long axis.

The chromatin material (dark stained areas) segregates either in narrow transverse bars or in wide blocks, which are farther apart than the transverse bars in *Saprospira grandis* or in cristispiras. Chromatin is found in the terminal points, thus giving them the appearance of the head of a javelin. Constrictions in the cell membrane were observed wherever the chromatin formed wide blocks or bands. Figure 12, plate 2, shows such a constriction near the middle. It certainly suggests simple transverse division. Figure 13 shows constrictions at three points, while Figure 14 shows four such constictions. Figure 15 shows five constrictions and the chromatin between them appears porous, due to the plasmolized condition of the spirochaete, as is indicated by the wide portions between the constricted points. It is possible that these numerous constrictions indicate multiple transverse fission.



TEXT-FIG. 1a. SHOWING PAIRS OF THICK TRANSVERSE BARS AT SHORT INTERVALS

The transverse bars in one specimen, 86 micra long and 1 micron wide, shows an unusual arrangement. At short intervals a pair of transverse, crescent-shape, thick bars is found close to each other, while two or three bars are present in the cytoplasm between each set of these double bars (text-fig. 1a). A constriction of the cell membrane can be seen opposite each pair of double bars. This appearance is present only in the middle portion of the cell body, but the regular transverse bars are closer together towards the extremities than they are in the middle portion. This arrangement is similar to the multiple transverse fission described by Gross in *Saprospira grandis*. It also resembles the constrictions of the cell body described in the preceding paragraph.

The presence of chromatin spirals in the cytoplasm of the oyster spirochaetes has been observed on several occasions but never with any degree of clearness. In this specimen zigzag lines were observed running through the cytoplasm. One line was wider and approximately at right angles to the main axis, the other line was narrower and diagonal to the main axis. Chromatin granules were observed at the junction of these lines and the cell membrane. This structure was best observed in spirochaetes having lightly stained portions against a back ground free from debris. It is possible that these zigzag lines are part of the normal structure of oyster spirochaetes but they cannot be demonstrated by the present methods of staining with sufficient clearness to justify any conclusion.

The measurements were made on specimens having comparatively normal spirals. The longest undivided spirochaete is 100 micra long. The average *Saprospira puncta* is 80 micra long with extremes of 60 to 100 micra. The width of the cell body is 1 micron with extremes of 0.9 to 1.2 micron. The spiral amplitude is 6 micra with extremes of 4 to 8 micra. The average number of turns is 3 with extremes of $2\frac{1}{2}$ to 6 turns.

The process of multiplication was never observed in the living spirochaetes. Stained specimens showed typical incurvation which suggests that multiplication takes place by transverse division. No specimens were found that were twice as wide as the narrowest spirochaete. The widest saprospira observed was 1.2 micron and the narrowest 0.9 micron. On the other hand organisms nearly twice as long as the shortest were numerous. The longest spirochaete observed measured 100 micra and the shortest 60 micra.

The width and the wave length of the spirals in this species are similar to those of the larger type of *Saprospiras* (figs. 12 and 13, plate 2), but the transverse bars are farther apart and the ends are pointed. Morphologically this spirochaete is similar to Schellack's *Spirochaeta tapetos* with the exception of the pointed ends and the absence of the crista. This spirochaete resembles Keysselitz's *Cristispira anodontae* except that it has no crista. It also resembles Schellack's *Spirochaeta ostrae* (a cristispira) in the shape of the ends, but it has no crista and the spiral amplitude is very long. It seems clear that this spirochaete belongs to the genus *Saprospira Gross*. The name *Saprospira puncta*, (puncta = pointed) is suggested as descriptive of its characteristics.

5. *Cristispira Balbianii* (Certes) Gross. This species of cristispira was most common in the specimens taken from the intestinal tract of the oyster. Figures 16, and 19, plate 2; figures 27, 33, 36, and 37, plate 4; figures 40 to 43 and 46, plate 5; figures 51 to 55, plate 6, are types of *Cristispira Balbianii*, although they show differences which were caused by the methods of fixation and staining.

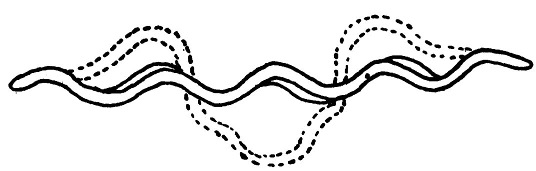
The average *Cristispira Balbianii* measures 60 micra in length and 0.8 micron in width, with extremes of 39 to 78 micra for the length and 0.8 to 1 micron for the width. The average spiral amplitude is 8 micra, with extremes of 6 to 8 micra. The average width of the spiral is 1.6 micron with extremes of 1.2 to 2.4 micra. Slight variations in dimensions were observed and probably can be traced to different methods of staining.

The cell body of *Cristispira Balbianii* is spiral, flexuous, with a crista running obliquely to the main axis. The average number of complete turns is 7, with extremes of $4\frac{1}{2}$ to 11 turns. The contours of the cell body are parallel, although the ends are rounded.

The chambered structure of *Cristispira Balbianii* is most evident in stained preparations, although in some specimens the chromatin is segregated along the cell membrane, as is shown in figure 18, plate 2; figure 36, plate 4. The chromatin is also arranged in wide transverse bars, as is shown in figures 28, 32 and 37, plate 4. In several specimens it was present in a series of broken lines running longitudinally through the middle of the cytoplasm. Specimens that stained solidly were also observed (fig. 16, plate, 2; figs. 31 and 33, plate 4).

These cristispiras move so rapidly that they cannot be seen easily under the low power objective, but their presence is detected by the disturbance of the medium caused by their motion. They move across the microscopic field with lightning speed, dashing in one direction and suddenly coming to a dead stop, then immediately darting in the opposite direction. Their spiral form can be observed during this momentary stop. Careful observations on their manner of motility are not possible, except in specimens with retarded movement due to exhaustion and to the drying of the medium. As the motility becomes retarded the cristispiras show a serpentine twisting. In addition to their regular spirals the cristispiras form a secondary spiral of wider and greater amplitude as is indicated by the dotted lines in text-figure 2. The secondary spiral disappears and the motility of the cristispiras gradually ceases. At this time the cristispiras begin to vibrate apparently from the peristalsis-like contractions of the elastic cell body. During the vibrating the normal spiral is distorted and appears irregular. When the cristispiras cease to move they take the true spiral position as shown in plate 4, which approximates the position of the living organism.

The cristispiras move with equal velocity in either direction and have no anterior-posterior polarity. They show rotation on their

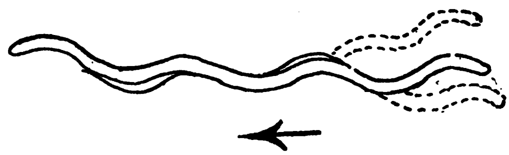


TEXT-FIG. 2

The dotted lines indicate the position of the secondary spiral. The solid lines indicate the normal spiral.

long axis, boring through the medium like a cork screw. During the normal spiral movement the first spiral follows in the path of the one end which may be called the "anterior pole," the second spiral follows in the path of the first spiral, the third spiral follows the second spiral, etc. In other instances the normal spiral follows the path of the secondary spiral and irregular bendings and twistings of the organism are frequent. The direction of motion is reversed by a reversal of the direction of the spiral rotation. In other words the movement in one direction is accomplished with the spirals rotating clockwise, while the movement in the opposite direction is accomplished with the spirals rotating the other way.

The cristispiras also show a rotating and lashing movement when they meet resistance or become fixed in the drying margin of the film. The spiral movement begins from the fixed end and is transmitted through the entire organism. The spirochaete rotates on its long axis and at the same time lashing movements of the "posterior" third of the cell body are seen as is shown by the dotted lines in text-figure 3. Similar rotating and lashing movements are observed in freely moving cristispiras. One-third of the "posterior" cell body flaps like a fish tail, while the second half of the last "posterior" turn appears slightly "hooked" giving the appearance of an ellipse as the cristispira rotates on its long axis (text-fig. 4). The rotating "posterior" spiral resembles the appearance



TEXT-FIG. 3

The dotted lines indicate the rotating-lashing movements of the cristispira. The arrow indicates the direction of motion.

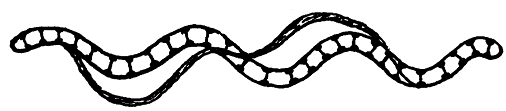
TEXT-FIG. 4

The arrow shows the direction of the motion. The dotted lines indicate the path of the "hooked" end portion.

of the "hooked" ends of a rapidly moving leptospira. When the direction of motion is reversed the "posterior" portion does the flapping while the "anterior" becomes quiescent. The lashing "posterior" ends of the cristispira are similar to what Noguchi (1918) calls "motile end portions" in spirochaetes. It is possible that the forward movement of the cristispira is caused by the rotating and the flapping movements of the "posterior" motile end portions of the cell body. This flapping is similar in execution and in effect to the lateral flappings of a fish tail which forces the fish forward. It further resembles the tail movements of a moving eel or of a swimming water snake.

Vital staining. A hanging drop preparation was made from the liquid in the intestine or in the stomach and was examined microscopically. If cristispiras were present a small amount (equal to half the volume of the hanging drop) of 1 per cent aqueous solution of the dye was mixed with the hanging drop directly on the same cover glass. This was then inverted, placed on a clean glass slide, and examined under the oil immersion objective. It was necessary to make the film very thin because it was impossible to focus accurately on the spirochaetes through a thick film. The following dyes were used: melachite green, safranin, Bismark brown, and neutral red.

A 0.3 per cent aqueous solution of melachite green does not stain the cristispiras, while 1 per cent aqueous solution stains them uniformly green. The cell membrane stains darker green than the cytoplasm. The transverse septa (bars) are very distinct,



TEXT-FIG. 5. TRANSVERSE SEPTA AS THEY ARE SHOWN BY INTRA VITAM METHOD OF STAINING

although five minutes passed before they took the stain. The septa are very delicate, transverse partitions distributed equidistantly in the cytoplasm as is shown in text-figure 5. Chromatin granules can be observed at the junction of the septa and the cell membrane. Unfortunately the spirochaetes die immediately upon coming in contact with the dye and exhaustive study of the cytoplasm is not possible. The outer margin of the crista stains green while the area between this margin and the cell membrane does not take the stain. The complete course of the crista could not be followed because it was not stained sufficiently.

A 1 per cent aqueous solution of safranin stains the cristispiras lightly at first but the staining becomes more intense in the course of fifteen minutes. The cell membrane (dark border line) stains darker than the cytoplasm, while the transverse septa appear as thin lines. No granules at the junction of the septa and the cell membrane are visible at first but they appear after ten to fifteen minutes of staining (text-fig. 5). The crista appears narrow, i.e., no wide portions are visible, and its course can be accurately traced. The outer margin stains moderately red, while the area between it and the cell membrane does not stain. The cristispiras die as they come in contact with the stain so that the staining reaction of the living organism could not be observed.

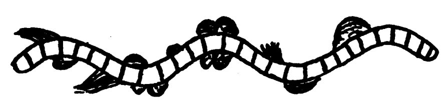
A 1 per cent aqueous solution of Bismarck brown stains the cristispiras evenly, giving them a delicate brown tinge. The transverse septa and the chromatin granules are not as pronounced as they are with melachite green, safranin, or neutral red. The outer margin of the crista is marked, as a light brown line running diagonally to the main axis of the cristispira.

A 1 per cent aqueous solution of neutral red stains the cristispira evenly red, but they die immediately upon coming in contact with the stain. The transverse septa are delicate but darker red than the cytoplasm, while the cell membrane stains dark red. The granules at the junction of the transverse septa and cell membrane are very marked and stain dark red. The crista is wider and its outer margin stains heavily, while the space between this margin and the cell membrane does not take the dye. The crista stains deeply so that its course can easily be traced.

The crista. The crista is a keel-like ridge running spirally on the outside of the organism, making one complete spiral turn to two complete turns of the cell body. The outer margin of the crista is thicker and can be observed in the living organism, while the margin attached to the cell body is thin and cannot be observed clearly. The crista merges with the cell membrane before it reaches the poles. In good preparations the crista shows a fibrillar structure (figs. 27, and 28, plate 4). Observations on the motility of these forms suggest that the crista serves as a rudder to impart the spiral movement to the cristispira. No independent movement of the crista can be observed in the living specimens. The crista stains light pink with Giemsa's solution as contrasted to the purple cell body.

Further observations on the crista revealed a change similar to plasmolysis. In a preparation exposed to osmic acid vapor for one minute and put directly into Giemsa's solution the crista appeared much wider, as if pulled away from the cell body; otherwise the cristispiras appeared normal. In some specimens the crista was frayed out and appeared fibrillar. Stained preparations fixed in picric acid showed many cristispiras whose cristas appeared like a series of semicircles adhering to the side of the cell body. At several points the crista showed a ragged appearance indicating that it had been torn from the cell body (text-fig. 6). The margins of these semicircles were stained dark red, while the enclosed areas stained a light pink.

Figures 16, 18 and 19, plate 2 and figures 51, 53 and 54, plate 6, illustrate the process of incurvation during transverse division. Figure 51 shows that transverse division does not always take place at the middle of the spirochaete. One daughter cell is 42 micra long while the other is 48 micra long. Figure 44, plate 5, represents a young individual of *Cristispira Balbianii* 39 micra



TEXT-FIG. 6. THE SHADED AREAS INDICATE THE PLASMOLYSIS OF THE CRISTA

long, while figure 41 represents a fully grown individual, 75 micra long, before a transverse division.

Certes described *Trypanosoma Balbianii* as 100 to 120 micra long by 3 to 5 micra wide with slightly tapering terminal portions, and bluntly rounded ends. Perrin described it as from 26 to 100 micra long and 5.3 micra wide. The cell body was parallel throughout, possessed 3 to 8 spiral turns and the ends were rounded. Fantham described *Spirochaeta Balbianii* as a long, sinuous, thread-like organism 50 to 150 micra long by 2 to 3 micra wide, with rounded ends. Schellack described it as averaging 39 micra long by 1.3 micron wide with extremes of 35 to 42 micra for the length and 1.1 to 1.5 micron for the width, with rounded ends, but no terminal appendages. Noguchi described *Cristispira Balbianii* as "a long, flexible cylinder, with blunt extremities towards which the diameter gradually diminishes." Gross described *Cristispira pectinis* as 72 micra long by 1.5 micron wide, the width of the spiral 4 micra, the terminal portions tapering slightly and the ends bluntly rounded without terminal filaments. Dobell described *Cristispira veneris* as averaging 50 to 60 micra long by 1.3 micron wide with extremes of 1 to 1.9 micron for the width. The terminal portions taper slightly, the ends are blunt without polar caps and without terminal filaments. The *Cristispira Balbianii*, described in this paper, does not correspond to any of the descriptions given by other investigators in respect to the width and the length of the cell body which is described as 1.1 to 5.3 micra wide by 26 to 150 micra long. In other respects the description and the drawings of the spirochaete presented in this paper coincide with the descriptions, drawings, and microphotographs of *Cristispira Balbianii* as presented by each previous investigator. On the basis of similarity in descriptions and drawings the spirochaete described in this investigation is identified as *Cristispira Balbianii.*

6. *Cristispira anodontae* Keysselitz. This organism (figs. 34 and 35, plate 4; figs. 38 and 39, plate 5) was found but a few times in the gut of the oyster. The average fully grown individual measures 70 micra in length with extremes of 44 to 88 micra. The average spirochaete measures 0.8 micron wide with extremes of 0.8 to 1.2 micron. The young individuals are about 44 micra long by 0.8 micron wide (fig. 35). The average wave length of the spiral is 8 micra with the extremes 6 to 8 micra. The average width of the spiral is 2 micra, with extremes 1.8 to 2.4 micra. These dimensions vary slightly, according to the method used in fixing and staining. The average number of complete turns is 9, with extremes of 5 to 11.

The contours of the cell body are parallel except that the ends taper to sharp points, but no terminal filaments are observed. The chromatin material is arranged in transverse bars equidistant from each other and no specimens were observed in which the chromatin had segregated in wide bars (figs. 34 and 35, plate 4; figs. 38 and 39, plate 5). No polar granules were observed in any of the specimens.

The crista is a narrow, ribbon-like band winding obliquely on the outside of the spirochaete. The margin attached to the cell body is thinner, while the free margin is thicker, as is indicated by the shaded edges in figure 34, plate 4. The crista possesses fine fibrils, which are seen only at the widest portion. In structure the crista resembles the dorsal fin of an eel. The crista in *Cristispira anodontae* has almost as many turns as the cell body, as is shown by the number of visible portions (fig. 34, plate 4). This feature aids in differentiating *Cristispira anodontae* from *Cristispira Balbianii* whose crista has fewer spirals. The crista appears to arise from the cell membrane. It does not reach to the end of the spirochaete, but merges with the cell membrane some distance from the poles.

Cristispira anodontae moves with greater speed than does *Cristispira Balbianii*, exhibiting typical cork screw movements. During retarded motility the spirochaete can be seen rotating on its long axis, although no serpentine contractions and stretchings were observed. Specimens having one end anchored show various twisting and bending movements. *Cristispira anodontae* has no antero-posterior polarity, but move in either direction with equal rapidity.

The method of multiplication was never observed, either in the living or in the stained specimens. On the basis of measurements of the width and length of the cell body, multiplication must take place by transverse division. No specimens were found that were twice as wide as the narrowest spirochaete, while spirochaetes twice as long as the shortest specimens were found. The widest specimen found was one micron wide, while the narrowest was 0.8 micron wide. The longest specimen observed was 88 micra long while the shortest specimen was 44 micra long.

The descriptions of *Cristispira anodontae* vary considerably. Keysselitz described it as having a chambered cell body and pointed ends, but measurements were not given. Fantham described it as 40 micra long by 0.7 micron wide, with extremes of 30 to 60 micra by 0.7 to 1 micron wide, and pointed ends. Schellack described "anodontae" as having rounded ends, the cell body averaging 46 micra by 1 micron with extremes of 39 to 50 micra and 0.9 to 1.2 micron. Schellack has also described another species, *Spirochaeta ostrae*, as averaging 41.5 by 1.1 micra, with extremes 38 to 42 micra long by 1 to 1.3 micron wide. The differences between *Cristispira anodontae* and *Cristispira ostrae* are not sufficient to create separate species. The observations were made by different investigators under different conditions, which may account for the minor variations that are recorded. From these observations and from comparison with the descriptions of similar species, the spirochaete described in this paper resembles Keysselitz's *Spirochaeta anodontae* in the shape of the terminal portions, although differing in the width of the cell body. This spirochaete also resembles Schellack's *Spirochaeta ostrae* in being 0.8 micron wide, but differs in the length of the cell body. Both specimens have a chambered structure and both are found in the intestine of the oyster. It seems clear that this spirochaete belongs to the cristispiras having pointed ends. It is identified as *Cristispira anodontae*.

7. *Cristispira spiculifera* Schellack. This organism was the smallest spirochaete observed during this investigation, and was found only once in an air-dried preparation stained with Giemsa's solution (figs. 8 and 9, plate 1). The contours of the cell body taper down to fine terminal filaments on both ends. Its chromatin material (dark stained portions) is distributed in wide transverse bands. This spirochaete measures 28 to 35 micra long by 0.7 micron wide. The spiral amplitude is 6.4 micra and the width of the spiral is 2 micra.

Since no living specimens were observed, its motility and mode of multiplication were not determined.

While no crista was observed this spirochaete corresponds to what Schellack has named *Spirochaeta spiculifera*, which he found in the crystalline style of *Anodonta mutabilis*. The crista may have been present and failed to take the stain, since no crista was observed on any of the specimens on the same slide.

8. *Cristispira modiolae* Schellack. A spirochaete was found in large numbers in two stained preparations taken from two different oysters. Its outstanding characteristics are: a long spiral amplitude, a wide spiral (figs. 47 to 50); chromatin material shows long dark stained blocks, but generally it is in regular transverse bars: the crista is very narrow and stains light pink, showing no

fibrillar structure. Figures 49 and 50 show normal spirals while figures 47 and 48 show distorted spirals.

The average individual is 58 micra long with extremes of 36 to 78 micra. The width of the cell body is 0.8 micron and occasionally individuals show a width of one micron. The spiral amplitude is 20 micra with the extreme of 16 to 21 micra. The width of the spiral is 5 micra with extremes of 4 to 6.8 micra. The average number of turns is 3 with extremes of 2 to 4 turns. The ends are rounded or bluntly pointed.

This spirochaete corresponds to Schellack's *Spirochaeta modiolae* in having the same width of cell body, rounded ends, no terminal appendages, and narrow crista, but differs in the length of the cell bodies. This spirochaete also resembles, in shape, Schellack's *Spirochaeta tapetos,* but differs in all other points. Had Schellack given measurements of the spiral amplitude, spiral depth, and the number of turns for each organism the comparison would be facilitated. The final classification of this spirochaete may be altered with the accumulation of more data.

9. *Cristispira modiolae* Schellack var. A. nov. var. This spirochaete is similar to *Cristispira modiolae* Schellack, yet the two organisms differ sufficiently to be considered as two varieties. Its outstanding characteristics are a long spiral amplitude, deep spirals, pointed ends, and wide prominent crista (figs. 45 and 46, plate 5). The chromatin is regular, in transverse bars, and polar caps are present.

The average individual is 63 micra long with extremes of 48 to 80 micra for the length. The width of the cell body is 0.8 micron and is constant. The spiral amplitude is 20 micra with extremes of 19 to 24 micra. The width of the spiral is 6 micra with extremes of 4 to 8 micra. The average number of turns is 3 with extremes of 2 to 4 turns.

This sprochaete is similar to *Cristispira modiolae* except that its ends are pointed and its crista is wide and prominent. It further resembles Schellack's *Spirochaeta chamae* but differs from it in length and width of the cell body. There is no spirochaete described that corresponds to this form. It is possible that this spirochaete is the same species as *Cristispira modiolae* Schellack and that the differences may have been due to the long time that the oysters had been out of water when the microscopic observation of these organisms was made.

10. *Cristispira mina* nov. spec. This spirochaete is similar to *Cristispira modiolae* in the form of its cell body, yet its morphological characteristics are half way between those of the cristispiras having narrow spirals and those having wide spirals. *Cristispira mina* (fig. 56, plate 6) was found on two occasions in stained preparations. It has a regular deep spiral with amplitude of medium length which is its chief characteristic. The chromatin material is arranged in regular transverse bars, but no polar caps were observed. The ends taper down to blunt points without terminal appendages. The crista is of medium width, visible at two or three places, showing a fibrillar structure and staining pink with Giemsa's solution.

The average individual measures 54 micra in length with extremes of 38 to 76 micra. The width of the cell body is 0.6 micron with extremes of 0.6 to 0.8 micron. The spiral amplitude is 12 micra with extremes of 9.6 to 12.8 micra. The width of the spiral is 4 micra with extremes of 3.2 to 5.6 micra. The average number of turns is 4 with extremes of 3 to 7 turns.

The mode of motility was not determined because no living specimens were observed. It is probable that *Cristispira mina* moves by rotating on its long axis in a similar manner to that described for *Cristispira Balbianii.*

Multiplication takes place by transverse division as is indicated by the extremes in length of 76 micra for the longest individual and 38 micra for the shortest. No specimens in the process of cell division were observed either living or in stained preparations.

Cristispira mina does not correspond to any other spirochaete that has been described. Its cell body is narrower than that of the other cristispiras, while its spiral amplitude is midway between that of the cristispiras having a long spiral amplitude and that of those possessing a short spiral amplitude. The width of its spiral is half way between the wide and narrow spirals. On the basis of these characteristics the name *Cristispira mina* (a cristispira having a medium spiral amplitude, a medium spiral width, and a narrow cell body) is suggested as descriptive of this spirochaete.

11. *Cristispira tenua* nov. spec. *Cristispira tenua* (figs. 57 and 58, plate 6) is unique in its morphology, resembling *Saprospira lepta* in its terminal portions, but differing from it in possessing a crista. It was found only in one stained preparation. Its cell body is narrow (thin) possessing a short, spiral amplitude, and shallow but regular spirals although often the spirals are distorted as is shown by the middle portion in figure 58. The ends taper down to sharp points, although frequently specimens with blunt points are observed without terminal filaments. The chromatin material shows a tendency to segregate in wide bands, although narrow transverse bars are common. The crista appears to follow the spirals of the cell body, but does not extend to the ends, and the fibrillar structure is not clearly seen.

The average individual is 54 micra long and 0.7 micron wide. The spiral amplitude is 8 micra while the spiral width is 2.4 micra. Since only two organisms were observed the extremes of length and width of the cell body, the spiral amplitude and spiral width could not be determined.

No spirochaete similar to this has been described. On the basis of its narrow cell body, regular spiral, prominent crista, and pointed ends the name *Cristispira tenua* (a thin, narrow cristispira) is suggested as descriptive of this species.

12. *Spirillum ostrae* Noguchi. A slender spiral organism was found in a film from the gut contents of the oyster, in several preparation on several different occasions. Because of its size one is quite apt to overlook this spirillum among the debris generally present in these preparations. This spiral organism possesses 3 to 8 shallow spirals, with the ends tapering to fine filaments (figs. 1 to 6, plate 1). The average spirillum is about 10 micra long with extremes of 7 to 21 micra. The average width of the spiral is about 1 micron. It corresponds to Noguchi's *Spirillum ostrae* which he found in the crystalline style of the oyster.

TABLE 3

Morphological characteristics of spirochaetes and Spirillum ostrae

NAME	SOURCE	LENGTH OF CELL BODY		WIDTH OF CELL BODY		SPIRAL AMPLITUDE		WIDTH OF THE SPIRALS		NUMBER OF TURNS		ENDS	CRISTA
		Average	Extremes	Average	Extremes	Average	Extremes	Average	Extremes	Average	Extremes		
Saprospira lepta	*Ostra virginiana*	70μ	54–92μ	0.5μ	Constant	8μ	5–13μ	1.6μ	1.6–4.8μ	5	3½–10	Pointed	Not present
Saprospira lepta var. A.	*Ostra virginiana*	60μ	45–72μ	0.7μ	Constant	20μ	20–24μ	4μ	3.2–5.2μ	3	2–4	Sharp	Not present
Saprospira puncta	*Ostra virginiana*	80μ	60–100μ	1.0μ	0.9–1.2μ	20μ	16–24μ	6μ	4–8μ	3	2½–6	Pointed	Not present
Saprospira grandis	*Ostra virginiana*	80μ	52–90μ	1.2μ	1.2–1.4μ	24μ	20–28μ	7μ	5–9μ	3½	3–4½	Rounded	Not present
Cristispira spiculifera	*Ostra virginiana*	30μ	28–35μ	0.7μ	0.7–1.0μ	6.4μ	6–7μ	2μ	1.6–3μ	Not determined	Not determined	Pointed filament	Present
Cristispira anodontae	*Ostra virgiana*	70μ	44–88μ	0.8μ	0.8–1.2μ	8μ	6–8μ	2μ	1.8–2.4μ	8	5–11	Pointed	Present
Cristispira Balbianii	*Ostra virginiana*	60μ	39–78μ	0.8μ	0.8–1.0μ	8μ	6–8μ	1.6μ	1.2–2.4μ	7	4½–11	Rounded	Present
Cristispira modiolae	*Ostra virginiana*	58μ	36–78μ	0.8μ	Constant	20μ	16–21μ	5μ	4–6.8μ	3	2–4	Rounded	Present
Cristispira modiolae var. A.	*Ostra virginiana*	63μ	48–80μ	0.8μ	Constant	20μ	19–24μ	6μ	4–8μ	3	2–4	Pointed	Present
Cristispira mina	*Ostra virginiana*	54μ	38–76μ	0.6μ	0.6–0.8μ	12μ	9.6–12.8μ	4μ	3.2–5.6μ	4	3–7	Bluntly pointed	Present
Cristispira tenua	*Ostra virginiana*	54μ	Not determined	0.7μ	Not determined	8μ	Not determined	2.4μ	Not determined	5	4–9	Pointed	Present
Spirillum ostrae	*Ostra virginiana*	10μ	7–21μ	0.4μ	Constant	1.5μ	Not determined	6μ	Not determined	4	3–8	Pointed filament	Not present

DISCUSSION OF SPECIAL POINTS

It has been shown that the apparent morphology of the oyster spirochaetes is influenced by the method of fixation and staining. The best material for the study of morphology was obtained with the wet film preparations.

1. *Meaning of the chambered structure.* The appearance of the chambered structure in *Saprospira Gross* was constant in all the specimens that were examined regardless of the method of preparation. On the other hand the chambered structure in the *Cristispiras* varied with the method of fixation and staining. This variation suggested that the so-called chambered structure was due to coagulation of the cytoplasm into various designs. Hogue (1922) holds a similar opinion in regard to the chambered structure in *Spirochaeta eurygyrata.* Bosanquet (1911) has reached a similar conclusion in his observations on *Spirochaeta anodontae.* Noguchi (1921) is inclined to regard the chambered structure of these spirochaetes as due to the coagulation of the cytoplasm. Gross (1910) interprets the chambered structure of *Cristispira pectinis* as an artefact, resulting from fixation, and he further adds that the cytoplasm of this species is structureless. This view is also supported by the observations in hanging-drop preparations, and dark field illumination.

On the other hand Schellack believes that the chromatin granules are scattered along the inner side of the cell membrane. Dobell is of the opinion that the chambered structure in *Cristispira veneris* is due to transverse chromatin disks, about which the nuclear chromatin is clustered. He compares *Cristispira veneris* to a bamboo pole having the septa at each node.

The correct interpretation of the chambered structure in the oyster spirochaetes probably can not be obtained from stained preparations alone. Experiments with *intra vitam* staining leave no room to doubt the existence of delicate transverse septa in these spirochaetes. It is also evident that the transverse septa, as observed in *intra vitam* preparations, are very thin (narrow), while the transverse bars found in permanent preparations are several times wider than the transverse septa as observed in *intra vitam* staining. The distance between the transverse septa and between the transverse bars is the same as is demonstrated by measurements. It is probable that the transverse septa are the basis (nuclei) about which the transverse bars are built during the staining process. It is not clear whether the transverse bars are made up of the coagulated cytoplasm, whether they consist of chromatin material; or whether they are simply the fixed and stained transverse septa. Dobell's interpretation of the chambered structure, i.e., chromatin granules embedded in the transverse septa, seems most plausible, but he accounts neither for the appearance of the cytoplasm nor for the wide transverse bars in the fixed preparation. The difference in the width between the transverse septa and the transverse bars appears to be due to the cytoplasm adhering to and coagulating about the transverse septa. In imperfectly fixed specimens the chambered structure is irregular as is shown by long blocks of heavily stained areas and wide unstained areas (fig. 7 to 11, plate 1; figs. 13, 15 and 18, plate 2; figs. 28, 32, 36 and 37, plate 4).

2. The crista

The most unique feature of the genus *Cristispira* is the ridge which extends spirally along the cell body but does not reach the poles. This ridge (often called undulating membrane) lead Certes and Perrin to consider these spirochaetes as trypanosomes. Fantham succeeded in demonstrating the fibrillar structure of the crista, while Schellack advanced evidence that the crista is not an undulating membrane, but that it is a true periplast traversed by numerous fibrils. Gross has interpreted the crista not as an undulating membrane but as a ridge winding obliquely to the spirals on the outside of the spirochaete. This interpretation is accepted by the majority of investigators.

The crista is plainly seen in the living cristispira, and its relation to the cell body can be observed. It appears rigid, like a keel, winding spirally about the spirochaete. It serves as a rudder to impart the screw-like movements to the spirochaete. It is very difficult to stain, especially in species other than *Cristispira anodontae* and *Cristispira Balbianii*. The spirochaetes that at present are called saprospira may prove to have cristae with the development of more affective methods of staining.

SUMMARY AND CONCLUSIONS

1. Spirochaetes were found in the digestive tract of 91 per cent of the oysters sold in the markets of Baltimore.

2. These spirochaetes are parasites in the gut of the oyster, and they survive as long as any liquid remains in the digestive tract of the oyster.

3. It is suggested that the classification of the spirochaetes found in the alimentary tract of shell fish be determined according to the presence or absence of crista, the shape of the ends, the width and length of the cell body, the spiral amplitude and the width of the spiral.

4. A spirochaete corresponding to *Saprospira grandis* was identified.

5. Two new species, *Saprospira lepta* nov. spec., and *Saprospira puncta* nov. spec., were found and described.

6. Another saprospira was found closely similar to *Saprospira lepta* and was considered a new variety, *Saprospira lepta var. A.* nov. var.

7. One species of cristispira described in this paper was identified as *Cristispira Balbianii*. The question is raised whether the three species, *Cristispira Balbianii*, *Cristispira pectinis*, and *Cristispira veneris* are not identical. It is suggested that they be considered as one species, *Cristispira Balbianii*.

8. One species of cristispira was identified as *Cristispira anodontae*. The question is raised whether *Cristispira ostrae* and *Cristispira anodontae* are not identical and it is believed that they could be included under the species *Cristispira anodontae*.

9. A small cristispira was identified as *Cristispira spiculifera*.

10. One species of cristispira was identified as *Cristispira modiolae*, and a similar spirochaete was considered as *Cristispira modiolae var A.* nov. var.

11. Two rather thin cristispiras were considered to be new species and were named *Cristispira tenua* nov. spec. and *Cristispira mina* nov. spec.

12. A slender spiral organism was found in the gut of the oyster and was identified as *Spirillum ostrae*.

13. The opinion is expressed that the appearance of the chambered structure in oyster spirochaetes is due to the coagulation

of the protoplasm about the transverse septa during the fixation and the staining process. The exact interpretation of the chambered structure of these spirochaetes is not at all settled.

14. Evidence is presented to show that the motility of the oyster spirochaetes is caused by the motile "posterior" end portion.

15. Certain observations on the crista suggest that it is a fibrillar structure winding spirally on the outside of the cristispira. It is difficult to stain and shows no independent movement.

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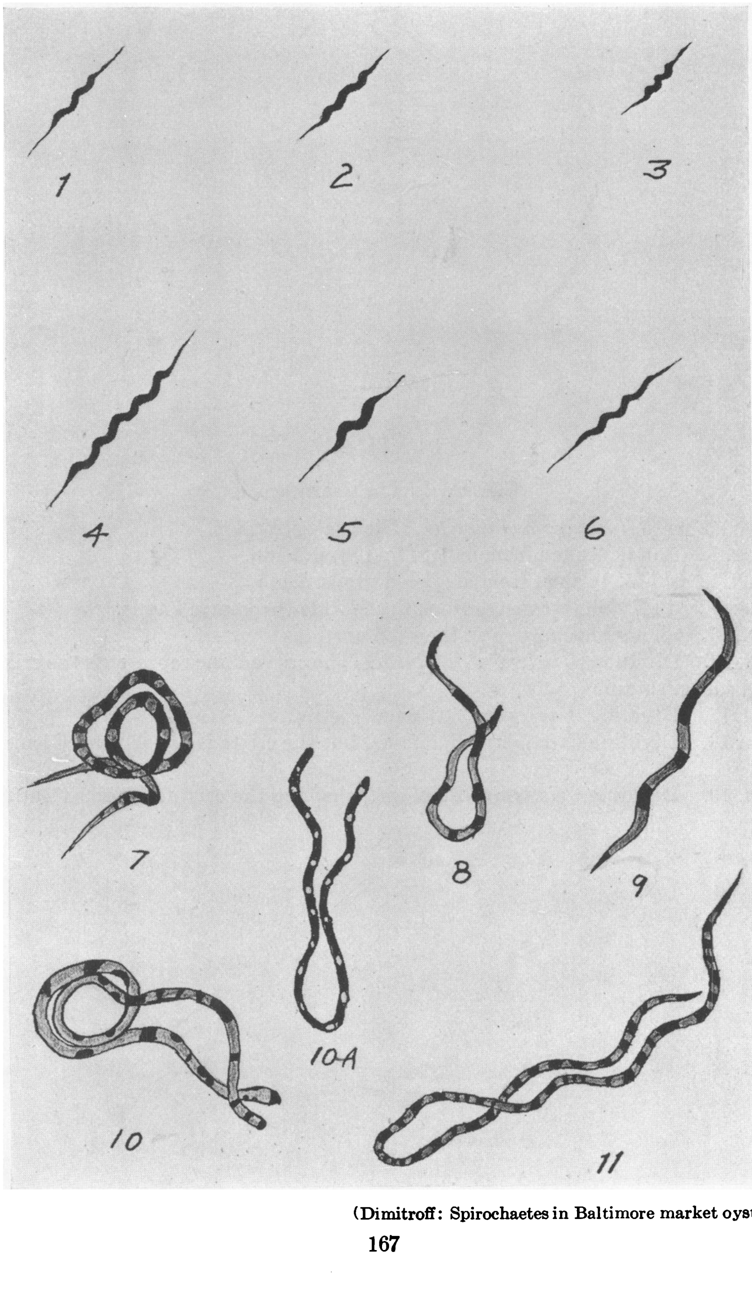
PLATE 1

Camera lucida drawings

FIGS. 1 TO 6. *Spirillum ostrae*. Giemsa's stain.
FIGS. 7 AND 11. *Saprospira lepta*. Giemsa's stain.
FIGS. 10 AND 10a. *Saprospira lepta var. A*. Giemsa's stain.
FIGS. 8 AND 9. *Cristispira spiculifera*. Giemsa's stain.



(Dimitroff: Spirochaetes in Baltimore market oysters)

PLATE 2

Camera lucida drawings

FIGS. 12 TO 15. *Saprospira puncta.* Giemsa's stain.

FIGS. 12 AND 14. Taken from moist film preparation.

FIGS. 13 AND 15. Taken from air-dried preparation.

FIGS. 12 TO 14. Show constrictions at the wide chromatin bands.

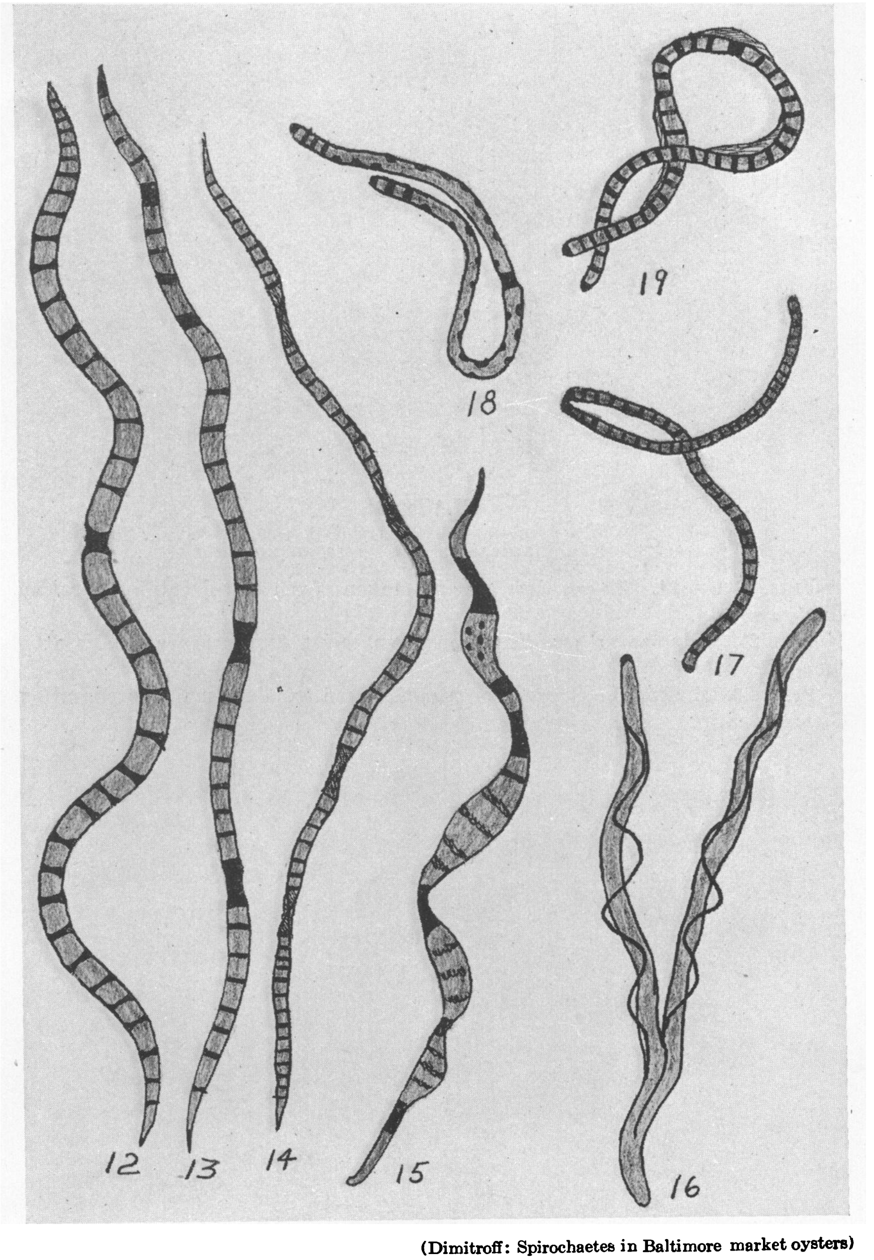
FIG. 15. Shows plasmolysis at the wide portions.

FIG. 16. Dividing *Cristispira Balbianii.* The curved line represents the crista. Carbol fuchsin stain.

FIG. 17. Dividing Saprospira. Giemsa's stain.

FIG. 18. Dividing *Cristispira Balbianii,* but the crista is not shown. Giemsa's stain.

FIG. 19. Dividing *Cristispira Balbianii,* showing the crista. Giemsa's stain.



(Dimitroff: Spirochaetes in Baltimore market oysters)

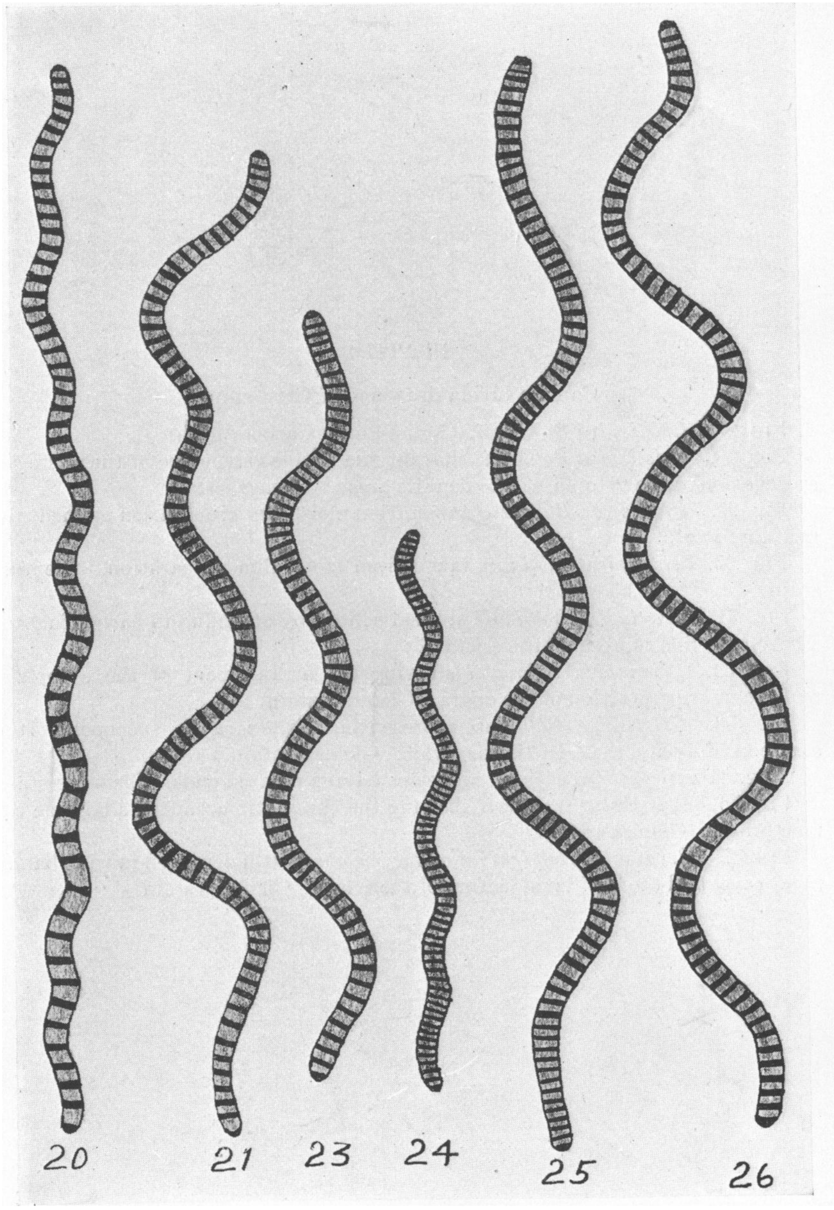
PLATE 3

Camera lucida drawings of *Saprospira grandis*

FIGS. 20 AND 24. *Saprospira grandis* taken from air-dried preparation. Giemsa's stain.

FIG. 21. *Saprospira grandis* taken from moist film preparation. Carbol fuchsin.

FIGS. 23, 25 AND 26. *Saprospira grandis* taken from moist film preparation. Giemsa's stain.



(Dimitroff: Spirochaetes in Baltimore market oysters)

PLATE 4

Camera lucida drawings of Cristispira

FIG. 27. *Cristispira Balbianii.* Moist film. Carbol fuchsin.

FIG. 28. *Cristispira Balbianii* showing the fibrillar structure of the crista and segregation of the cytoplasm into definite areas. Giemsa's stain.

FIG. 29. *Cristispira Balbianii* taken from moist film preparation stained with gentian violet.

FIG. 30. *Cristispira Balbianii* taken from moist film preparation. Giemsa's stain.

FIG. 31. *Cristispira Balbianii* stained solidly by steaming in carbol fuchsin. The curved line represents the crista.

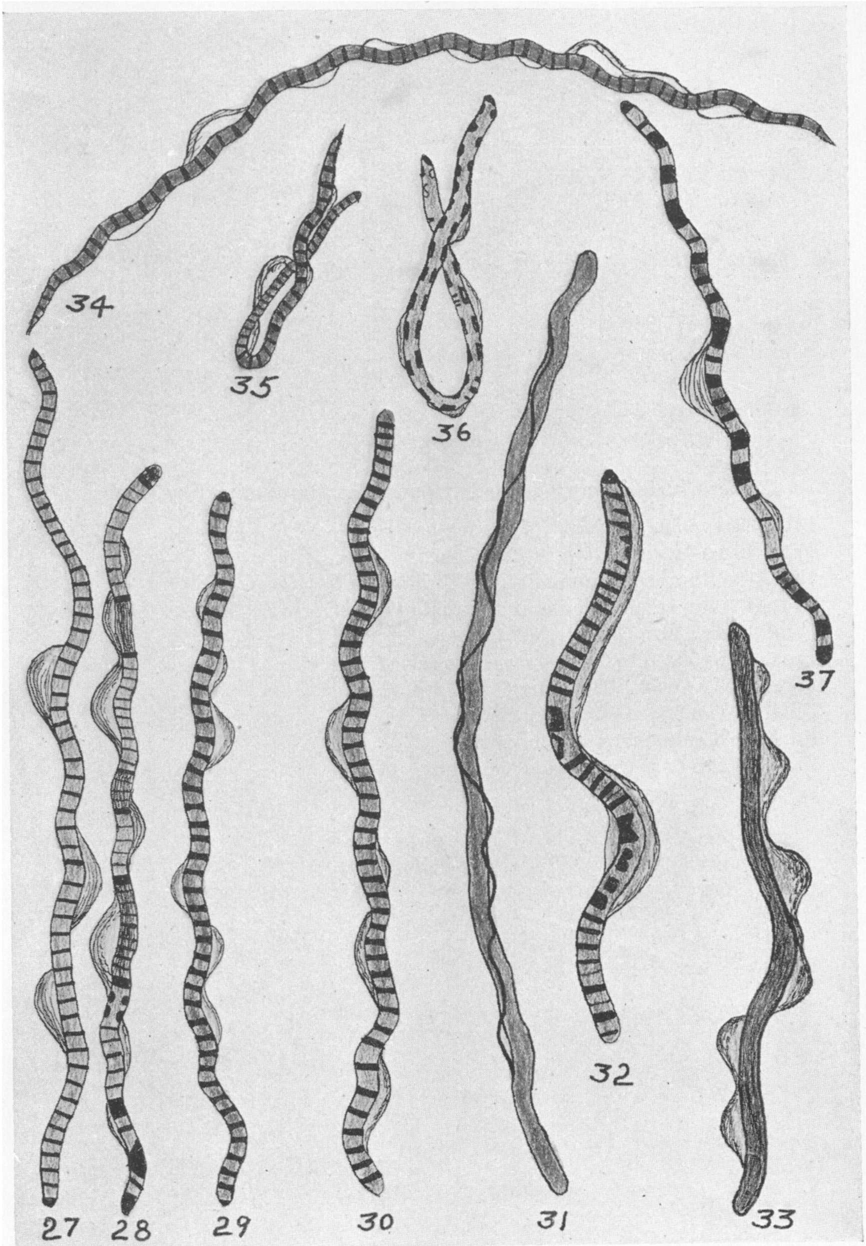
FIG. 32. *Cristispira Balbianii* showing the arrangement of the cytoplasm and the unusual position of the crista. Giemsa's stain.

FIG. 33. *Cristispira Balbianii* representing disintegrating specimen. It is stained solidly and the crista is very wide. Giemsa's stain.

FIGS. 34 AND 35. *Cristispira anodontae* having pointed ends. Giemsa's stain.

FIG. 36. *Cristispira Balbianii* showing the chromatin arranged along the cell membrane. Giemsa's stain.

FIG. 37. *Cristispira Balbianii* showing the chromatin arranged in wide bands, polar caps, and the fibrillar structure of the crista. Giemsa's stain.

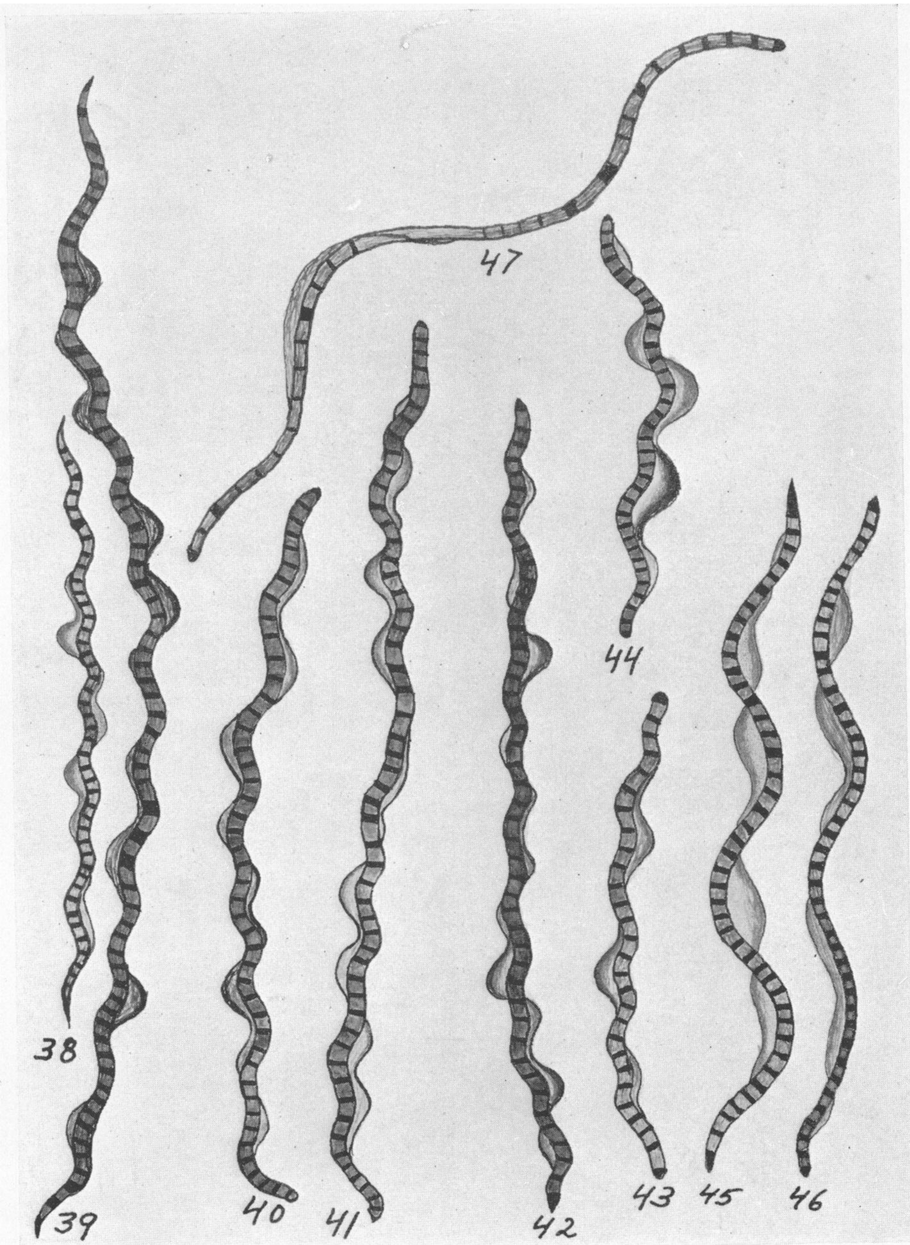


(Dimitroff: Spirochaetes in Baltimore market oysters)

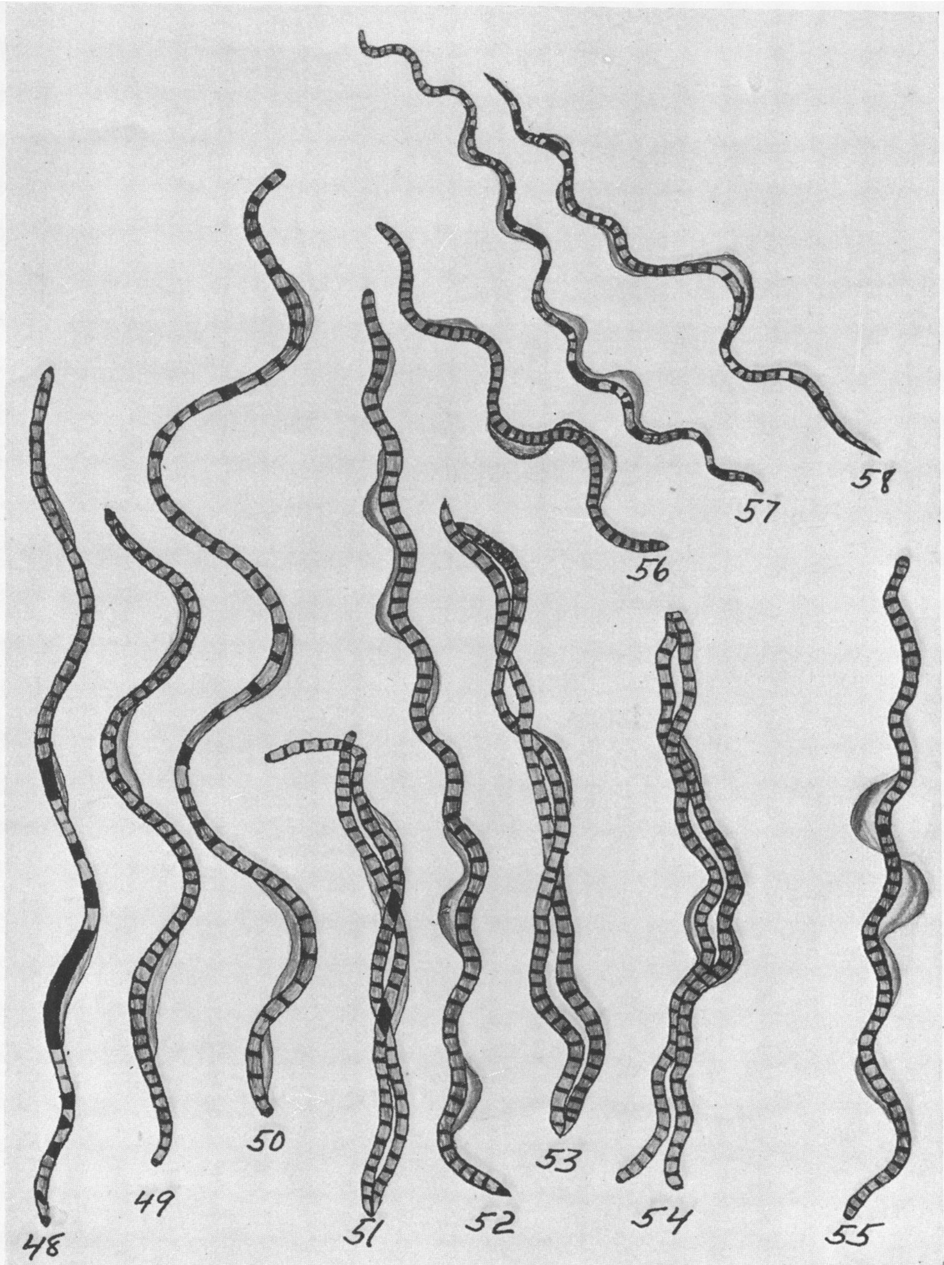
PLATES 5 AND 6

Camera lucida drawings made from moist films stained by Giemsa

FIGS. 38 AND 39. *Cristispira anodontae.*
FIGS. 40 TO 44. *Cristispira Balbianii.*
FIG. 41. Represents an undivided *Cristispira Balbianii.*
FIG. 44. Represents young individual of *Cristispira Balbianii.*
FIGS. 45 AND 46. *Cristispira modiolae.*
FIGS. 47 TO 50. *Cristispira modiolae var. A.*
FIG. 51, 53 AND 54. Dividing *Cristispira Balbianii.*
FIGS. 52 AND 55. *Cristispira Balbianii.*
FIG. 56. *Cristispira mina* nov. spec.
FIGS. 57 AND 58. *Cristispira tenua* nov. spec.



(Dimitroff: Spirochaetes in Baltimore market oysters)



(Dimitroff; Spirochaetes in Baltimore market oysters)