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CRISTISPIRA IN NORTH AMERICAN SHELLFISH. A NOTE ON A SPIRILLUM FOUND IN OYSTERS.

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PLATES 21 TO 24.

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In the European lamellibranchiata there have been found a large number of comparatively coarse forms of spiral organisms, provided with a broad, band-like membrane extending from one extremity to the other and winding itself obliquely around the body, one edge being attached to the body, the other free. The organism was first discovered in oysters by Certes (1), who regarded it as akin to the trypanosome and called it *Trypanosoma balbianii*. Laveran and Mesnil (2) considered it a bacterium, while Perrin (3), working under Schaudinn, thought it a protozoon. At that time Schaudinn (4) held the view that the spirochete represented a phase of the trypanosome and that both belonged to the protozoa. The peculiar structure of the organism, as brought out by the technique employed by Perrin, suggested the mitotic and other nuclear figures to such an extent that the organism was accepted as a protozoon by certain protozoologists (5–8). The investigations by Swellengrebel (9) and later by Gross (10) led the latter to place it in the class of *Cristispira*, a terminology accepted by Dobell (11) and Zuelzer (12). The points in dispute are: (1) the interpretation of the membrane; (2) the presence or absence of a periplast; and (3) the presence or absence of a nuclear apparatus, especially the significance of the chambered structure of the body. These questions, which had never been definitely settled, led the writer to undertake examinations of American shellfish. Furthermore, so many species have been described recently in the European shellfish that a comparison from the standpoint of geographic distribution of these cristispiras seemed desirable.

TABLE I.

Spirochæta (Schellack).

Name.	Source.	Length.		Breadth.		Ends.
		Average.	Extremes.	Average.	Extremes.	
Spirochæta balbianii.	*Ostrea edulis.*	39μ	35–42μ	1.3μ	1.1–1.5μ	Rounded, no terminal appendage.
" *ostreæ.*	" "	41.5μ	38–42.5μ	1.1μ	1.0–1.3μ	Sharp, " " "
" *chamæ.*	*Chama gryphoides.* " *sinistrorsa.*	45.6μ	45–46.5μ	1.4μ	1.3–1.5μ	Rounded, " " "
" *anodontæ.*	*Anodonta mutabilis.*	46μ	39–50.5μ	1.0μ	0.9–1.2μ	" " " "
" *spiculifera.*	"	33μ	28–36.5μ	0.9μ	0.7–1.1μ	Pointed, terminal filament.
Spirochæta modiolæ.	*M. barbata.*	37.5μ	36–40μ	0.8μ	0.7–0.9μ	Rounded, no terminal appendage.
" *pinnæ.*	*P. nobilis.*	30.4μ	29–31μ	1.0μ	0.8–1.1μ	" " " "
" *limæ.*	*L. inflata, L. hians.*	37μ	35–41μ	1.4μ	1.0–1.8μ	" " " "
" *cardii papillos.*	*C. papillosum.*	19.1μ	18.5–20μ	1.2μ	1.1–1.4μ	" " " "
Spirochæta tapetos.	*T. decussata.*	34.5μ	29–35μ	1.3μ	1.1–1.4μ	Rounded, occasionally terminal appendage.
" *acuminata.*	*Tapes læta.*	47μ	43.5–49.5μ	1.0μ	0.9–1.1μ	Pointed, no terminal appendage.
Spirochæta saxicavæ.	*Sax. arctica.*	31μ	30–32μ	1.7μ	1.6–1.8μ	Rounded, " " "
" *gastrochænæ.*	*G. dubia.*	29μ	Constant.	1.2μ	1.1–1.3μ	One end blunt, one sharp, no terminal appendage.
*Spirochæta pusilla.**	*Anodonta, Unio, Lima, Tapes, etc.*	13μ	12–14μ		0.3–0.4μ	Sharp, pointed.

* Bosanquet found a spirochete 10 to 12 μ in length which he thinks may be identical with *Spirochæta hartmanni* of Gonder or with *Spirochæta pusilla* of Schellack. (No crista?)

Schellack (13), Keysselitz (5), and Gross (10, 14) created a large number of species with only slight morphological variations. To quote an example, Schellack set up thirteen species with certain characteristics, as shown in Table I.

As will be pointed out later, the method of classifying these coarse organisms by their morphological features is subject to error because of the great variations in the terminal portions and in the thickness of body according to the degree of fixation, dehydration, or staining; a difference in these factors may cause the same organism to assume a totally different aspect. Hence the writer has studied these organisms not only in stained preparations but also under the dark-field microscope.

TABLE II.

Occurrence of Cristispira in the Styles of North American Shellfish.

Shellfish.	No. of specimens examined.	Styles present.	*Cristispira* present.
Ostrea virginiana	298	128	99 (40 degenerated).
Venus mercenaria	110	70	8 (3 ").
Modiola modiolus	97	73	4
Ensis americana	24	20	0
Mya arenaria	24	24 (hard).	0
Mactra solidissima	12 (dead?).	1	0
Mytilus edulis	42	34	0
Pecten irradians	39	34 (hard).	0
Fulgur canaliculatus	8	2 (?)	0
Nassa obsoleta	30	4	0

The present study deals only with salt water shellfish caught in the neighborhood of Woods Hole during the month of August, 1916, and includes *Ostrea virginiana* (oysters), *Venus mercenaria* (quahaugs), *Mya arenaria* (long neck clams), *Ensis americana* (razor clams), *Mactra solidissima* (large sea clams), *Pecten irradians* (scallops), *Mytilus edulis* (mussels), *Modiola modiolus* (mussels), *Fulgur canaliculatus* (winkles, whelks), and *Nassa obsoleta*. The number of specimens of each species examined and the positive findings of *Cristispira* and styles are shown in Table II.

As the tabulation shows, *Cristispira* was found most frequently in the styles of oysters, next in clams, and then in modiolas. No other species examined showed any *Cristispira*. In handling the

styles of these shellfish it was observed that those of oysters, although moderately solid, became liquefied into a viscid fluid upon standing for half an hour at room temperature in a small amount of sea water after removal. The styles of modiolas are much smaller than those of oysters and somewhat firmer; those of clams are much more solid and elastic than either of the others and did not become liquefied on exposure to air and sea water at room temperature. Scallops have large and firm styles which remain little changed after removal. Styles from the other varieties are very small and solid. The physical properties of the styles may be partly or chiefly responsible for the presence or absence of *Cristispiræ* in these shellfish; apparently the consistency of the styles of oysters offers the optimum conditions for the habitation of the organism.

Another striking fact is that, at least in the case of the oysters, the longer they were kept in a tank (eel pond) or the car (sea) after collection from their original beds, the less frequently they contained styles. In such specimens the presence of *Cristispira* is also less frequent, and in many of them only degenerated forms may be found. It is rather easy to determine the freshness of oysters by examining them for styles and the activity of the cristispira contained in them; even freshly collected oysters soon lose the styles if they are not extracted immediately. In one instance 48 oysters which had been kept in the eel pond 24 hours after collection were opened one after another and left at room temperature for about 30 minutes, when they were searched for styles, but unsuccessfully. Six more oysters from the same lot which had not been opened all had perfect styles, and five contained *Cristispira*. These external factors must be taken into consideration in making a survey as to the frequency of *Cristispira* infection of oysters. This rule does not apply to those shellfish whose styles are firm and resistant to manipulation.

The main object of the present work was to study the conditions under which these coarse spiral organisms can be kept alive or cultivated on artificial media, in order that their biological and morphological characteristics might be better studied. Experiments were also made in which the organisms were subjected to the action of various salts, acids, and alkalies in order to obtain an insight into the finer

structure of the organism. Vital stainings with neutral red, crystal violet, brilliant cresyl blue, methyl green, methyl orange, Bismarck brown, and Janus green were also applied.

Dark-Field Examination of Styles.

Oysters.—The style from the oyster is a glassy, somewhat elastic, cylindrical body of about 2 to 3 cm. long and about 3 mm. in diameter at the thickest portion, tapering to a point at the end. When placed in a Petri dish it becomes a sticky viscid fluid; it dissolves in sea water. When a drop of the liquefied style is examined under the dark-field microscope there are observed in many instances violently motile organisms of large size moving through the field with great velocity. They pass out of the field so rapidly that their exact form is not at once recognizable; they suggest butterflies constantly flapping luminous wings; their movements are backward as well as forward. They become sluggish within a short time and come to rest either in a tortuous form or stretched out. The body is a long cylinder with blunt ends. The end portion is slightly thinner than the rest, but not sharply drawn. There is a thick refractive margin all along the body, indicating the presence of definite peripheral concentration of the protoplasma (perhaps a cell membrane). The inner part of the body seems to present no distinct transverse partitions (so called chambered structure), but there are rows of refractive projections from both sides of the wall, point to point, as if they were imperfect cross-bars; the intervals between the bars are about 3 μ. Along the entire body runs a peculiar undulatory narrow membranous structure coiling obliquely and spirally, showing a free edge here and there (known as the crista, or ridge, of the cristispira). It follows a wavy course, and at each wave summit the light is highly refracted. From that point innumerable fibrillar, fan-like radiations run into the body of the organism upon which the other edge of the membranous structure rests or is in connection (Figs. 1 to 3). The fibrils along the free edge of the crista seem to be so arranged as to form a more compact structure than the portion which stretches between the edge and body of the organism. Locomotion appears to be accomplished by alternate bending and relaxation of the undulatory, fibrillar, highly elastic membrane, as is easily demonstrated in a specimen whose

motility is slackening because of exhaustion. In a later period of exhaustion the organism becomes immotile, and the elastic wavy frame of the membrane assumes its full length and shape, causing the body to conform to its spirals (Fig. 8). Only active specimens take the shape of a snake (Figs. 1 to 3). In a freshly extracted style which is still unsoftened by exposure to the air we often find a nest of organisms in one field (Figs. 4 and 5), some assuming positions like coiled snakes, and some rather regularly waved and stretched out. In a few moments the former move rapidly from their resting places with a violent locomotion and swim away; the latter, which represent skeletons or elastic frames (Fig. 8), are degenerated and remain immotile. When a liquefied style containing active specimens of the organism is left for several hours under unfavorable conditions (room) most of the organisms undergo degeneration and are found in various stages from merely stretched out, regularly wavy organisms to skeletons without the body (Figs. 6, 7, and 9). The disintegration of the body is shown by an irregular contour and various degrees of thickness of the body substance. The skeleton often shows clearly its component elastic fibrils, some of which may take the shape of a bushy horse tail (Fig. 6). The gradual overpowering of the body by the elastic membrane in a dying specimen is shown in Figs. 7 and 9.

Clams.—The styles of clams are thicker, firmer, and somewhat longer than those of oysters, faintly yellowish, shiny, somewhat opaque, and do not undergo liquefaction in sea water when removed to a Petri dish. For dark-field examination it is necessary to macerate them in a mortar in order to obtain an emulsion. Five only, of 110 styles examined, showed the presence of active *Cristispiræ*; 3 others contained degenerated specimens. The dark-field appearance of the cristispiras found in clams was identical with that of the cristispiras in oysters (Fig. 10).

Modiolas.—The modiolas are small mussels, with proportionately small vitreous styles, not longer than 1 cm.; the consistency of the styles is softer than that of clams but firmer than that of oysters. For dark-field examination they can be easily macerated on the slide by pressing under a cover-glass. *Cristispiræ* were present in 4 of 97 styles studied. The organism showed microscopically no distinctive features except that of exceptional length (Fig. 11).

Examination by Means of Vital Staining.

The cristispiras from oysters were used for this purpose.

Neutral Red.—Within a few minutes the body showed a general brownish yellow coloration, with numerous paired yellowish granules scattered with fair regularity along the entire wall. The membrane, or crista, appeared as bushy brownish fibrils projecting in all directions. The color of the body disappeared in 20 minutes, that of the fibrils in 6 hours. Coiled specimens did not take the stain.

Crystal Violet.—The body took a rather deep purple stain, the crista remaining uncolored. Fine protoplasmic reticula with intersecting granules were seen. In some specimens vacuolation of the cell contents occurred, a large mass of substance occasionally protruding into the side (plasmolysis). The deep purple color was still present at the end of 6 hours. There was no chambered appearance of the body.

Brilliant Cresyl Blue.—The body took up in mottled fashion a light bluish lavender color. The cross-bars (bluish) of the body were distinctly brought out in a few minutes; the crista remained practically unstained. Complete plasmolysis and decoloration occurred within 6 hours.

Methyl Green.—The body stained faint pink without showing any structure; the crista was not stained. Decoloration occurred in 6 hours.

Methyl Orange.—There was no coloration. The organisms remained active.

Bismarck Brown.—The body became distinctly brownish, with more deeply stained granules throughout the entire length. These granules sometimes occurred in a line along one or both sides of the wall. Degenerated specimens were not well stained. The specimens were practically decolorized in 6 hours.

Janus Green.—The body took a deep, dark bluish color, with reticular mottles, the crista a dark blue on its free margin but only a faint blue elsewhere. In some specimens comparatively large round granules of varying size, stained dark bluish, were seen scattered about in several parts of the body. Degenerated organisms did not take the stain. Within 6 hours the color gradually faded.

In general, then, the body of *Cristispira balbianii* took the vital staining, while the crista stained only slightly with some of the dyes and not at all with others. The phenomenon common to staining with neutral red, brilliant cresyl blue, Bismarck brown, and Janus green was the fact that within the first 5 minutes the body of the organism took the stains in such a way as to show the presence of chromophil granules more or less regularly and diametrically paired on both sides of the wall. After a longer period the number of chromophil granules became more numerous, and a sort of reticular distribution of the same substance was brought out, giving the entire body a mottled appearance. In the case of brilliant cresyl blue the cross-bar arrangement of the chromophil substance was distinct but this was followed by complete plasmolysis of the organism, indicating that the chambers were the result of an unfavorable or toxic effect of the dye. Coiled specimens usually showed no deeply staining granules with Bismarck brown or neutral red.

Stained Preparations.

Several methods were used for the study of the morphological characteristics of the cristispiras, but the best differentiation of the various constituents of the organism was obtained by application of Giemsa's stain and Heidenhain's iron-hematoxylin.

With Giemsa's stain the organisms were variously fixed. In some instances the liquefied styles containing the cristispiras were thinly spread over a clean slide and the moist film surface was immediately exposed to osmic acid vapor (1 per cent) for 1 minute. 30 minutes fixation in absolute alcohol followed. In other instances the moist films were at once immersed in a jar containing Schaudinn's sublimate alcohol, which was maintained at a temperature of 75°C. for 5 minutes and then allowed to cool. Before applying Giemsa's solution the films were rinsed in water, treated first with Lugol's solution, then with 0.5 per cent sodium thiosulfate solution, then thoroughly washed in water. In this procedure the films were never allowed to dry until after being stained with Giemsa's solution. In still other instances the films were first dried in the air, then fixed in methyl alcohol for 30 minutes, then stained with Giemsa's solution.

The object of these various procedures was to study the influence of fixation on the morphology of the organism.

The morphological features revealed by Giemsa's stain were very similar, whether the films were fixed by the osmic vapor or by Schaudinn's hot sublimate alcohol method. In both preparations the body showed a cross-bar structure consisting of deeply stained blue bars alternating with light, almost unstained or faintly pinkish areas. The bars, which were correspondingly wider in larger specimens, appeared somewhat narrower toward the extremities. The relative width of the bluish bar and of the juxtaposed light spaces was very variable in different specimens. In some the width was nearly the same (Figs. 29, 30, and 40); in others the light spaces were much wider than the cross-bars, which then appeared like thin cross lines (Figs. 12, 13, and 31); in still others the reverse was true, in which case a deeply stained bluish body seemed to be segmented by narrow light cross spaces (Figs. 14, 15, 17, and 32 to 34). In the specimens fixed in sublimate alcohol there were specimens showing in some portion of the body several consecutive bars, so thinned out near the the middle as to be almost broken (Figs. 13, 17, and 35), the appearance being not unlike that of the organism in the fresh state. The bluish cross-bars were not of uniform width or sharply defined borders, but one end was usually broader than the other, and sometimes a few here and there were placed obliquely to the right angle formed by the wall and the hypothetical axis of the body. In contrast to fresh specimens, the stained organism generally had a tapering form, ending in sharply pointed extremities (Figs. 12 to 14, and 29 to 35). The crista took a brilliant red hue, showing one or more heavily stained marginal fibers, and it ended near the end of the body (Figs. 29 to 35). The connection between the body and the heavy marginal fibers of the crista is so thin that the finer fibrillar structure is seldom brought out. One may encounter in stained preparations specimens caught in full activity (Figs. 12, 14, 15, 17, 19, 29 to 33, and 35) or in the exhausted condition, yielding to the elastic crista, with beginning plasmolysis (Figs. 20 and 21) or with the crista skeleton (Figs. 23 and 36). Sometimes the crista alone may be found (Figs. 37 to 39).

The specimens fixed in methyl alcohol after being air-dried did not show so sharp a structure as those just described. The organism

stained more reddish in general, and the bluish bars were not clearly distinguished (Figs. 16, 18, and 19).

Heidenhain's iron-hematoxylin stain was applied to film preparations of the cristispira derived from clams and Mallory's to sections of oyster styles. In the latter the organisms were present in large numbers, usually lying parallel with the course of protein lamellæ, which run concentrically along the long axis of the style like a scallion. Some were coiled, but most assumed wavy forms (Fig. 22). The cross-bar structure was easily distinguished. The crista is occasionally revealed—it is perhaps held closely to the body. (In fresh specimens the crista can always be seen.) Occasionally there are found specimens cut across so that they are visible as a ring. The details of structure are more clearly brought out in the film preparations by adequate differentiation (Figs. 25, 27, and 44). The body is cross-barred by thin, dark grayish lines, with a more definite contour of the cell wall. The crista is fibrillar and retains a light bluish gray color which is deeper along the marginal fibers. The extremities of the body are not sharply drawn out but are blunt points.

In one of the clams collected in New York Bay in September, 1916, I encountered a rather long variety of *Cristispira*. It was similar in all other respects to the variety met with in the clams studied at Woods Hole. Whether this specimen of *Cristispira* is a new species or merely a variety due to temporary factors has not been determined (Figs. 24, 26, 28, 45, and 46).

Effects of Chemicals.

The microchemical reactions of various components of *Cristispira balbianii* were studied with a view to gaining an insight into the structure of the organism. Previous investigators have obtained varying results in a similar study of the European specimens. The writer's studies with the American variety were made with the following substances: sodium taurocholate, sodium glycocholate, sodium oleate, saponin, cobra lecithid, acetic acid, hydrochloric acid, sulfuric acid, potassium hydroxide, and ammonia.

Method of Study.—One part of the style emulsion containing numerous active specimens of *Cristispira balbianii* was mixed on a coverglass with one part of the substance, dissolved in sterile sea water;

TABLE III.

Effect of Chemicals on Cristispira.

Date.	Chemical.	Concentration.	Result.
1916		*per cent*	
Aug. 23	Sodium taurocholate.	10	Plasmolysis complete in 5 to 10 min. Skeleton, or crista, resistant. *Spirillum ostreæ* dissolved; bacilli still motile. After 20 hrs. at room temperature skeletons (cristas) showing their fine fibrillar composition are more completely denuded of the cell plasma.
		1	Practically the same as with 10 per cent.
		0.1	Nearly all have undergone plasmolysis; otherwise the same as preceding experiment.
	Sodium glycocholate.	10 1 0.1	Similar in effect to the taurocholate solution.
	Sodium oleate.	10 1 0.1	This salt produced such a viscid emulsion that no observation was possible.
	Cobra lecithid.		Almost insoluble in sea water. Observations indecisive.
	Saponin.	10	Complete plasmolysis in 5 to 10 min. Crista not affected but assumes more regularly waved spiral bundles of fibrils (Fig. 9), to which irregularly protruding masses of protoplasm are seen to be attached at some points. *Spirillum ostreæ* and bacilli still motile. After 20 hrs. at room temperature the cristas are still intact. They show their finer structure and their relation to the bodies, which are now mere masses of broken up protoplasm scattered along the regularly undulated spiral cristas.
		1	Same as with 10 per cent.
		0.1	Nearly all have undergone plasmolysis.
	Controls in sea water.		Actively motile for 2 hrs. after being sealed on slides; thereafter gradually became immotile; structure unchanged.
			After 20 hrs. no degeneration but all in "relaxed" state.

TABLE III—*Concluded.*

Date.	Chemical.	Concentration.	Result.
1916		*per cent*	
Aug. 24	Acetic acid.	50	After 2 hrs. body smooth, showing cross-bar structure and heavy cell wall; crista shrivelled and indistinct; *Spirillum ostreæ* swollen and indistinct.
		10	Same as with 50 per cent.
		1	" save for the presence of many round bodies suggesting extruded protoplasm of the body of *Cristispira balbianii.*
	Hydrochloric acid.	50	After 2 hrs. body shows more densely set cross-bar appearance than in acetic acid, but the crista, though perhaps thickened and less distinct than normally, is still attached to the body.
		10	Same as with 50 per cent.
		1	" except for some nodular swelling of the body in parts.
	Sulfuric acid.	50	Similar to results with hydrochloric acid except for more granular appearance of the body substance and less distinctness of the crista.
	Potassium hydroxide.	15	Within 2 hrs. the organisms have practically disappeared, except for a few fragments of the bodies in which no structure save the cell wall can be recognized; no crista found.
		3	Within 2 hrs. the body shows irregularly contoured thick wall with vestiges of thinned out, broken cross-bars; crista seems to be closely attached to the body.
		0.3	Within 2 hrs. there are apparently no changes; body shows distinct cross-bars and heavy cell wall; crista seems to be intact.
	Liquor ammoniæ fortis.	50	Within 2 hrs. no changes; immobilized.
		10	" 2 " " " "
		1	" 2 " " " "
	Controls in sea water.		After 2 hrs. almost all are still active.

the cover-glass was then sealed with vaseline on a slide and examination made under the dark-field microscope. The concentration of each substance and the effects of each upon the organism are briefly summarized in Table III. The observations were made at room temperature (23°C.).

Survival of Cristispira balbianii under Different Conditions.

An oyster emulsion containing actively motile cristispiras was used to study the effects of tonicity. The observations were made in hanging drop preparations after 2 hours at room temperature (22°C.). When mixed with distilled water the organisms became immotile, then the cell body assumed a regularly spiral course in conformity with the skeletal crista, presenting at the same time accumulations of a highly refractive substance in round or oval masses along the body at each turn of the spiral skeleton. The phenomenon may be interpreted as due to plasmolysis through hypotonicity. On the other hand, in 10 per cent sodium chloride solution the organisms remained intact, but many coiled forms (Fig. 40) were seen. The body appeared less refractive, but there was no plasmoptysis. In the control preparations made with sea water, most of the organisms became immotile after 2 hours at room temperature; in those kept at 6–8°C. all the cristispiras were still actively motile at the end of 2 hours, and one-fifth to one-tenth after 24 hours; after 40 hours only a few were found to be active in one of the three slides. In dark-field preparations (not hanging drop method), sealed with paraffin and kept at 6–8°C., all cristispiras were disintegrated at the end of 24 hours.

Attempts at Cultivation.

Several unsuccessful attempts were made to obtain a culture of *Cristispira balbianii*. The chief obstacles in the work were (1) the lack of bacteriological facilities, and (2) the impossibility of obtaining a bacteria-free suspension of *Cristispira balbianii*.

The culture media employed in the first attempts were made with a filtrate of various styles (oysters and scallops), obtained by passing the sea water solution (or emulsion) of styles (50 styles in 100 cc. of sea water) through a Berkefeld filter V. To 3 cc. of the filtrate were

added, in one set, 1 cc. of 2 per cent glucose agar; in another set 1 cc. of 2 per cent plain agar; in a third set 1 cc. of ascitic fluid; in the fourth 1 cc. of glucose broth; in the fifth 1 cc. of sterile sea water; and in the sixth (controls) 4 cc. of sterile sea water without the style filtrate. All tubes were covered with a thin layer of sterile paraffin oil to prevent evaporation of the culture media. The cultures were set up as follows: All tubes (1 cm. in diameter and 22 cm. high) first received 0.2 cc. of a rich cristispira emulsion from oysters. 3 cc. of the style filtrate were then added to all except the control (sixth) set. The first and second sets were next mixed with 1 cc. of 2 per cent melted glucose agar and plain agar, respectively, the temperature of the agar being about 42°C. at the time of mixing. The tubes were cooled quickly by immersion in the aquarium. The glucose broth and ascitic fluid were added to the other sets and the paraffin oil was finally added to all. Each set was made up in duplicate, one being kept at room temperature and the other at aquarium temperature (about 7°C.).

Examination of the culture tubes was made daily during 8 days, but no growth of cristispira was ascertained. They were brought to The Rockefeller Institute in an ice-packed container and followed for 3 weeks longer, but except in one instance, in which a peculiar large non-motile organism was seen to grow, the results were negative. This organism, which grew in the first set of media (semisolid), bore a close resemblance to *Cristispira*, except that there was no crista. It suggested Gross' *Saprospira*. Whether it was a cristispira modified through artificial cultivation (loss of crista) or an altogether different organism is still a question. Most of the tubes were more or less contaminated with members of the *mesentericus* group.

A spiral organism of oysters, *Spirillum ostreæ, n. sp.* (Fig. 43), likewise failed to grow. This spiral organism measures about 8 to 16 μ in length and 0.5 μ in the widest portion of the body. Both ends gradually taper off to fine filaments. The number of spirals varies from 4 to 8 and the curves are rather shallow but very regular. The body is elastic but not perceptibly flexible. They rotate rapidly but seldom proceed one way or the other.

In another experiment styles were ground in a mortar with Ringer's or Locke's solutions as well as with sea water and the whole was

sterilized in an autoclave. The opalescent fluids thus obtained were used in experiments similar to those preceding with unsatisfactory results.

The tissue culture technique was employed in order to determine the viability of cristispiras in the presence of various extraneous substances. By careful examination it was possible to collect a dozen styles rich in cristispiras and apparently free from ordinary bacteria. *Spirillum ostreæ* was the only variety present and occurred in rather large numbers in some styles. The styles were dissolved in 3 cc. of sterile Ringer's solution; the work was carried out as aseptically as circumstances permitted.

To 1 drop of the style emulsion was added 1 drop of the following liquids respectively: (1) mixture of oyster style-Locke's solution infusion filtrate; (2) oyster broth-Locke's solution-infusion-style filtrate; (3) oyster juice filtrate; (4) oyster broth; (5) Locke's solution; (6) sea water; (7) distilled water; (8) ascitic fluid; and (9) bouillon. Eight Ringer's solution preparations were made as controls.

At the end of 24 hours at 6–8°C. many *balbianii* remained active in three of the eight control (Ringer's solution) hanging drop slides, and in the slide containing ascitic fluid. In all other instances the organisms were inactive and diminished in numbers; there was an increasing mass of bacteria more marked in the slides containing albuminous solutions like oyster-filtrate, Locke's solution-style infusion, and bouillon. At the end of 48 hours only a few cristispiras were still sluggishly motile in the slides containing ascitic fluid and in one of the Ringer's solution controls.

This observation serves to emphasize the difficulty of obtaining a culture of this organism under the conditions of the experiments just described.

DISCUSSION AND SUMMARY.

Ten varieties of North American shellfish were examined for the occurrence of *Cristispira* in their styles. A cristispira was found in various numbers in *Ostrea virginiana*, *Venus mercenaria*, and *Modiola modiolus*, but none in *Ensis americana*, *Mya arenaria*, *Mactra solidissima*, *Pecten irradians*, *Mytilus edulis*, *Fulgur canaliculatus*, or *Nassa obsoleta*. Of 298 oysters, only 128 showed the crystalline styles, in

which cristispiras were present in 99. Active cristispiras were found in 59 styles only and degenerated forms in the remaining 40. In 110 clams (*Venus mercenaria*) 70 styles were found, and only 8 of these contained cristispiras; 5 yielded active and the other 3 degenerated cristispiras. In 97 modiolas there were 73 styles, only 4 of which contained cristispiras.

The physical properties of the crystalline styles of these shellfish varied considerably. The styles of the oysters were moderately soft, and when exposed to the air or mixed with sea water they underwent liquefaction, forming a clear, viscid material. The styles from clams and modiolas were opaque and were more firm, not easily crushed even in a mortar. The styles of the scallops were the most solid of all the styles examined. It happened that the softer the styles, the more frequent was the occurrence of the cristispira; in fact, no cristispira was detected in styles other than those of oysters, clams, and modiolas, of which oysters had the softest styles and the largest percentage of cristispira invasion.

The following observations were made regarding the structure of the cristispira found in oysters. The body is a long, flexible cylinder, with blunt extremities, towards which the diameter gradually diminishes. In motion the body rapidly stretches and contracts, forming in the contracted state several serpentine undulations. A membranous appendage (Gross' crista) winds about the body throughout its entire length. The inner margin is in connection with the body, the outer margin is free and is distinctly heavier. The latter is undulatory; that is, the width of the membrane, or crista, is narrower at some points than at others. The membrane is composed of numerous fine fibrils running in a roughly parallel or slightly oblique course, showing interwoven narrow meshes; at the outer margin there is a dense smooth ridge.

The contour of the body is highly refractive, as if possessing a cell membrane. The interior structure, as revealed by dark-field illumination, is an almost homogeneous, less refractive substance, but there are present minute highly refractive granules more or less symmetrically arranged. There is no definite cross-bar or chambered structure. On the other hand, when vital staining with brilliant cresyl blue is applied, there appear numerous paired masses of lavender hue at

fairly regular intervals, suggesting the cross-bar aspect of a stained specimen. In a few specimens there was seen a dim outline of cross-bar effect. Neutral red, Bismarck brown, and crystal violet all bring out deeply stained granules and reticular structure but no definite cross-bars.

While it is difficult to recognize any definite chambered structure in the fresh state, it is very easy to bring out the characteristic banded body by staining the organism with Giemsa's solution. The bands, or cross-bars, which take up a deep bluish color, are of variable width, ranging from a thin line to a square or even oblong block. In some specimens the body is almost blue with thin white cross lines, while in others the blank body is banded with thin bluish bands at irregular intervals. The crista, or undulatory membrane, is stained bright red, particularly along the outer free margin. The fibrillar structure of the crista is usually recognizable. In the specimens stained with Heidenhain's iron-hematoxylin the cross bands, which appear bluish gray, are more distinct and delicate. The bands are not, however, always thin and sharp, but in certain specimens are almost square. The irregularity in the width of the cross bands in various parts of one specimen and in different specimens suggests that the peculiar banded appearance may be a result of fixation and not the natural structure of the organism. At least, no such structure was recognized under the dark-field microscope, which would have revealed such definite septa if they existed. I believe that the cross bands are formed from a homogeneous mass through sudden contraction due to dehydration with absolute alcohol during fixation. In the preparations first dried in the air and then fixed with methyl alcohol this banded appearance is absent. That *Cristispira* has a chambered body, each chamber being equivalent to a single cell, is Gross' interpretation, which is not supported by the observations reported here. Moreover, the protoplasm of the organism readily escapes from the body in distilled water, saponin, or other chemical agents, forming many round masses of extruded protoplasm in different parts of the body. No septa are to be seen in these plasmolyzed or degenerated organisms. Acids, hydrochloric and acetic, failed to bring out any banded structure of the body. It may be deduced that the external surface of the body is a highly refractive cell membrane, and that

within the cell body are homogeneous protoplasm of reticular form and numerous highly refractive granules of varying sizes. Some of the contents take the dark bluish color when stained with Giemsa's solution. In a fixed specimen this particular substance becomes contracted into a series of masses of varying dimension, leaving between each mass a blank space of varying width, and giving a characteristic cross-barred or banded (so called chambered) structure. In some specimens the cross-bars are not seen, but instead a series of broken lines running parallel with the longitudinal axis of the body (Figs. 41 and 42).

The crista is a fibrillar structure, connected with the body at its inner edge. The outer margin is a thickened bundle of fibrils running an undulating course along the entire length of the crista. The crista is elastic and when detached from degenerated organisms assumes a rather regularly wound spiral, consisting of longitudinal bundles of fibrils (Figs. 36 to 38). A fragment of two or three waves may be encountered in a preparation containing many degenerated organisms (Fig. 39). The composition of the crista can best be studied in degenerated remains of the organism. During the life of *Cristispira* it is stretched or relaxed according to the contraction or extension of the body. The elasticity of the crista appears to furnish the organism with a propelling and rotating power upon its extension after being drawn tightly to the body by some contractile apparatus (myoneme) present somewhere within the cell body. The crista serves as a rudder and propeller for the swimming organism. It is interesting to compare here the elastic and regularly waved flagella of certain bacteria and spirochetes; it is possible that the crista of *Cristispira* is a highly modified form of flagella.

The nature of the substance which stains dark blue with Giemsa's stain is not known, but it does not give a chromatin reaction. By Heidenhain's iron-hematoxylin method it takes a dark grayish tint, similar to the cell wall or crista, which are also dark gray. This substance was regarded by Gross and Zuelzer as volutin, which is of nutritive origin. It is probable that there are also embedded within it minute chromidial elements. Multiplication is by transverse fission.

Cristispira balbianii is parasitic and does not survive more than a few days in ordinary sea water emulsion, even at its optimum

temperature. In its natural habitat, or the crystalline style, it is usually pure, but is sometimes found in association with a tiny spiral organism (*Spirillum ostreæ*). The cristispiras in the styles seem to diminish rapidly when oysters are collected from their beds and transferred elsewhere; oysters kept in tanks or cars for several days do not contain the cristispiras, and in opened oysters the styles disappear promptly at room temperature.

All efforts to cultivate this organism have failed.

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EXPLANATION OF PLATES.

PLATE 21.

Magnification uniformly × 1,000.

FIGS. 1 to 3. Dark-field view of living specimens of *Cristispira balbianii* from an oyster. Photographed while they were temporarily at rest. The smooth wavy body with sharp contour along the wall, and a somewhat hazy alveolar content are well shown. The undulating crista loosely attached to the body of the organism can be seen. What appear like pointed sticks attached to the organism in Figs. 1 and 3 are the spirillar organisms found in fresh oysters which were merely entangled in the crista.

FIGS. 4 and 5. Dark-field view of *Cristispira balbianii* in the styles of oysters. In the styles there are different forms undoubtedly representing various stages of the life of the organism. In Fig. 4, there are one degenerated and two well preserved specimens, while in Fig. 5 there are also two intact and one long skeleton of a degenerated organism.

FIGS. 6 to 9. Different phases of degeneration of *Cristispira balbianii* due to unfavorable conditions or to age. Fig. 9 shows the protoplasm bulb accumulated near the middle of the body under the influence of a concentrated solution of saponin. Figs. 6 and 8 show the elementary fibrils which constitute the crista.

FIG. 10. Dark-field view of a cristispira from the style of a clam. In every respect this organism is comparable to the *balbianii* found in oysters. It may be the same as that described by Dobell as *Cristispira veneris*.

FIG. 11. Dark-field view of a cristispira from the style of a modiola. Generally somewhat longer than the *balbianii;* otherwise appears identical.

PLATE 22.

Magnification uniformly × 1,000.

FIGS. 12 to 15 and 17. *Cristispira balbianii* from oysters, stained with Giemsa's solution after osmic acid or sublimate alcohol fixation. They are all well preserved.

FIGS. 16, 18, and 19. *Cristispira balbianii* stained with Giemsa's solution after methyl alcohol fixation. All except Fig. 16 appear in good condition, although no definite cross-bar structure can be distinguished.

FIGS. 20, 21, and 23. *Cristispira balbianii* in process of degeneration. Fig. 20 shows the body still solid, almost intact, with a regularly wavy crista. In Fig. 21 the body is still fairly well preserved, but it follows the regular waves of the crista, indicating the absence of any resistance to the form of the crista. Note also the protruding mass of protoplasm near the middle of the body. In Fig. 23 there is only a shadowy trace of the body and the spiral elastic skeleton, or crista, is distinct.

FIG. 22. A section of the oyster style fixed in sublimate alcohol and stained with Mallory's iron-hematoxylin. The organisms show the chambered structure, but no crista can be distinguished.

FIGS. 25 and 27. *Cristispira balbianii* in films from fixation sublimate alcohol and stained with Heidenhain's iron-hematoxylin.

FIGS. 24, 26, and 28. Cristispira from a clam caught in New York Bay. The film was fixed in sublimate alcohol and stained with Heidenhain's iron-hematoxylin.

PLATE 23.

Magnification × 1,250.

Drawings from film preparations fixed in osmic acid vapor and absolute alcohol and stained with Giemsa's solution.

FIGS. 29 to 42. Different specimens of *Cristispira balbianii* showing the general chambered or cross-barred appearance of the body and undulatory membranous appendage, called the crista by Gross. The blue-stained substance which constitutes the cross-bars, or partitions, is seen to be regularly or sometimes irregularly distributed in amount or distance. In some, the substance may not be attached to the wall at all, but forms longitudinal rods of varying lengths as seen in Figs. 41 and 42. In such instances there is no suggestion of a chambered structure.

FIGS. 36 to 39. The final steps of degeneration of *Cristispira balbianii*. Fig. 36 alone still shows the degenerated body while the others show nothing but the fibrous skeletons, or cristas, of the organism. Fig. 39 is a fragment of the crista.

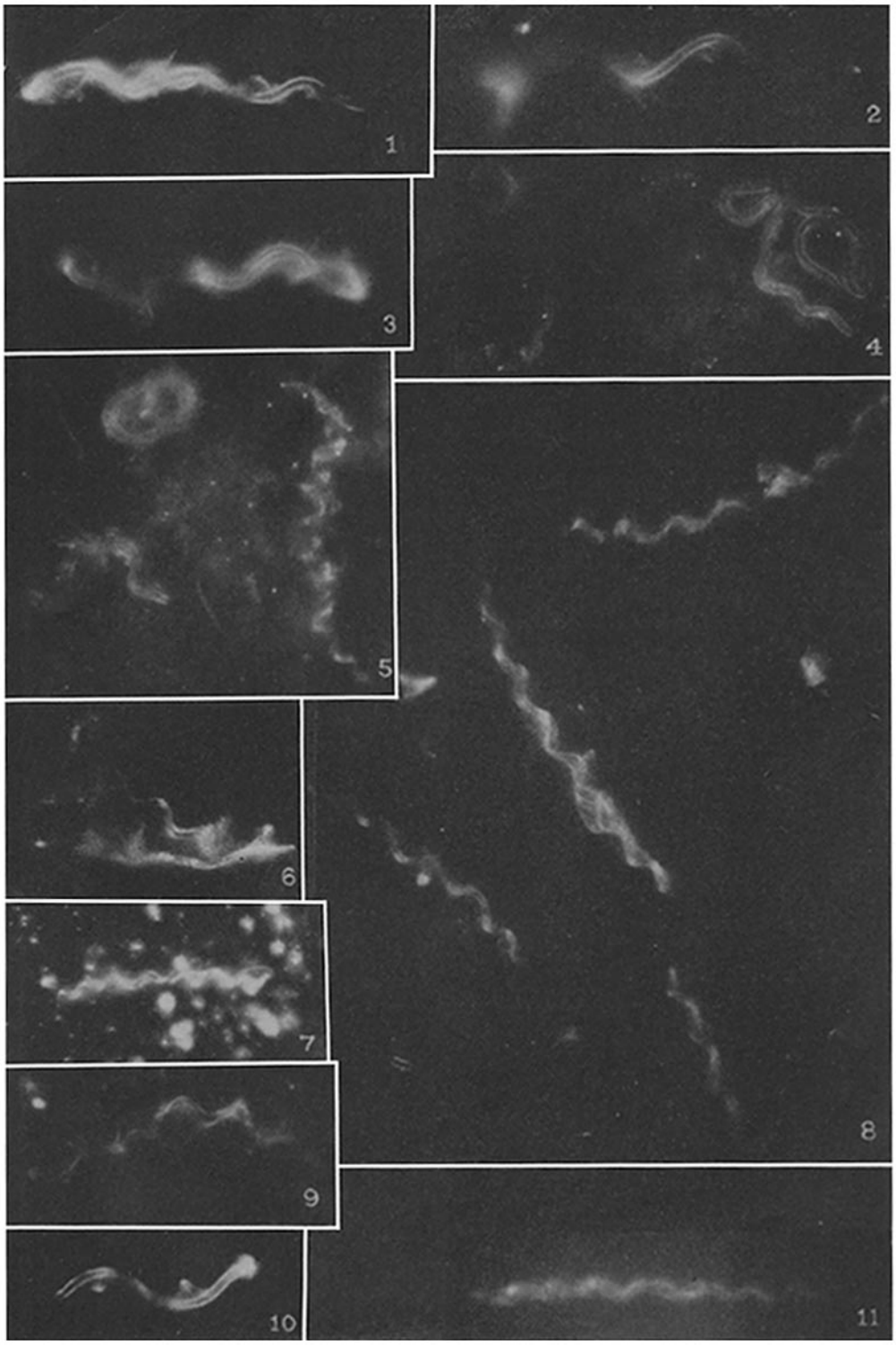
FIG. 43. The spiral organism found in the crystalline styles of fresh oysters caught in Woods Hole Bay.

PLATE 24.

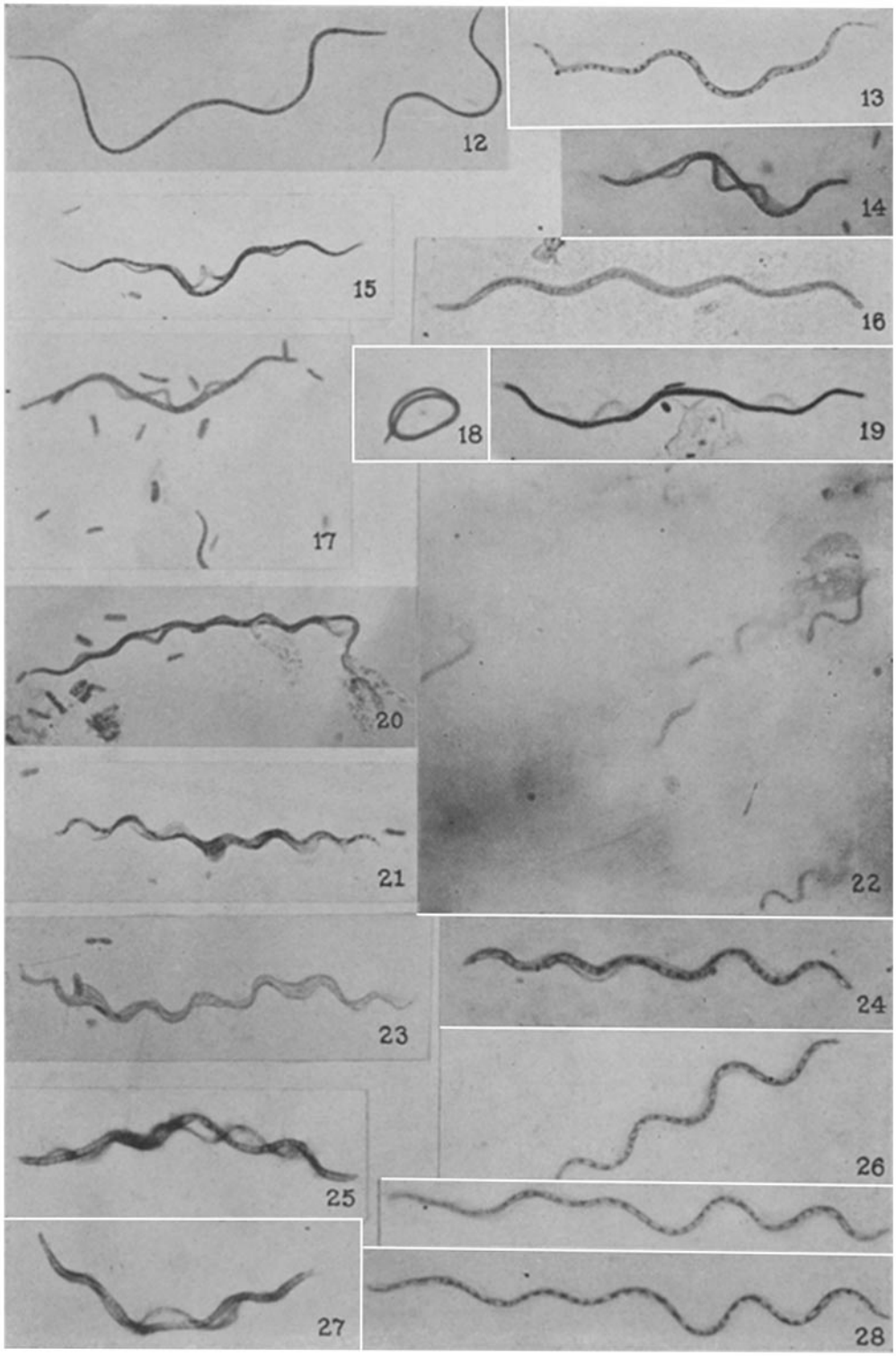
Magnification × 1,250.

FIG. 44. *Cristispira balbianii* from an oyster. Film preparation, fixed in sublimate alcohol and stained with Heidenhain's iron-hematoxylin.

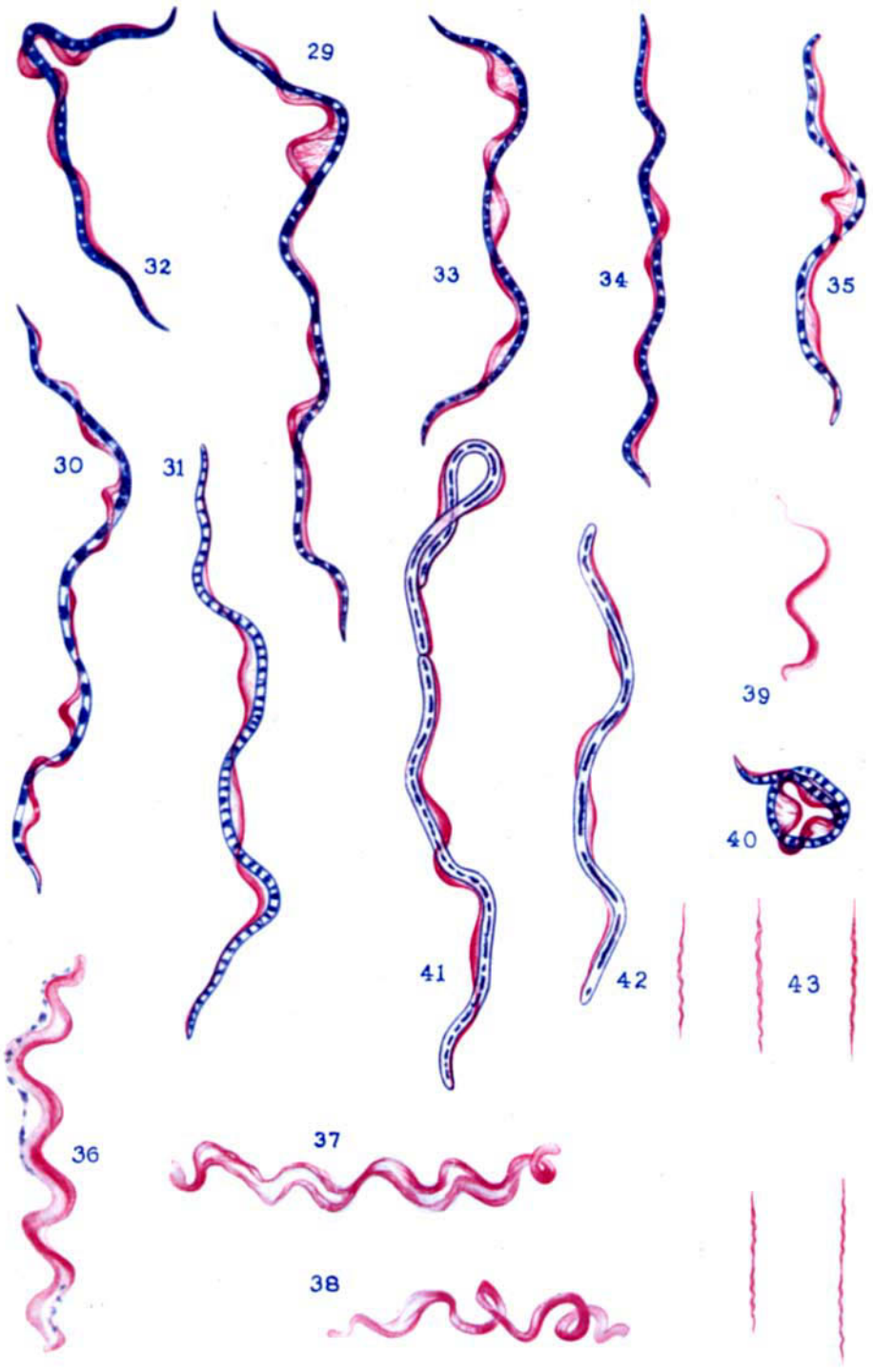
FIGS. 45 and 46. A cristispira (*Cristispira veneris?*) from a clam collected near New York Bay. Sublimate alcohol fixation and Heidenhain's iron-hematoxylin.



(Noguchi: *Cristispira* in North American shellfish.)



(Noguchi: *Cristispira* in North American shellfish.)



(Noguchi: *Cristispira* in North American shellfish.)

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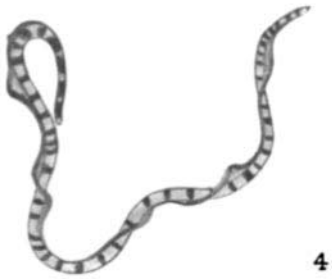

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(Noguchi: *Cristispira* in North American shellfish.)