A READILY CULTIVABLE VIBRIO, FILTERABLE THROUGH BERKEFELD "V" CANDLES, VIBRIO PERCOLANS (NEW SPECIES)

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During the investigation of certain electrocapillary effects of importance in filtration through laboratory filters, (Mudd 1923), it became desirable to have a filterable living organism whose surface electric potential could be studied in connection with its filterability. Search was accordingly begun, at the suggestion of Prof. S. B. Wolbach, and in October, 1921, an undescribed and easily cultivable vibrio was isolated in the filtrate from hay infusion made from fresh water from near Boston. This vibrio passes readily through Berkefeld "V" candles impervious to *Erythrobacillus prodigiosus* and *V. comma.*

The point of zero potential difference between this vibrio and its medium unfortunately corresponds to a concentration of hydrogen ions so high as to be incompatible with the organism's normal activity, so that it has proved unsuitable for determining the effect on filterability of reversing this interfacial potential. On the other hand, the new vibrio has proved so useful in a variety of ways for testing other factors in filtration that its description seems merited.

This organism is a short, actively motile, comma-shaped to straight, rod with rounded ends, occurring singly or in short chains. There is typically one polar flagellum, though frequently two or three are present. The flagella are rarely bipolar; in length they range from 3 to 7μ. With the fuchsin encre stain of Nicolle and Morax they are readily demonstrated.

In length this vibrio ranges from 0.5 to 2.5 μ, the large majority being 1.5 to 1.8 μ long, as determined by measurement of photomicrographs and by the use of a filar micrometer. The thickness is about 0.3 to 0.4 μ.

In young cultures (eighteen to twenty hours) filamentous forms are frequently found, some of which are chains of organisms, but in which, in other instances, subdivision is with difficulty, or not at all, detectable. The filaments are typically long slender spirals; in smears, however, they often appear straight or with only slight curvature.

The organism is Gram-negative. It stains with the ordinary laboratory dyes.

There are no spores, and usually the protoplasm, as seen by dark-field illumination and in stained specimens, appears homogeneous, except for one or more fine granules in an occasional organism. However, when a culture some days old, containing many individuals, which have lost motility and presumably vitality, is examined with dark-field illumination many of the non-motile organisms appear to consist of brightly shimmering, coarse granulations. This same coarse granulation has been occasionally noted, in hay infusion too acid for optimum growth, even in a majority of the motile individuals of the culture. Subcultures on media with proper reaction return to the normal appearance.

Very occasionally, in old (ten to twenty days) acid hay infusion cultures, "involution" forms similar to those of *V. comma* are found; large globoid forms which stain faintly, spoon-shaped and clubbed organisms, and forms which show a banded appearance with carbol-fuchsin. However, the appearance of these markedly atypical "involution" forms is not very common—not nearly so common as in the case of *V. comma*.

Our most satisfactory cultures of this organism have been grown on hay infusion of reaction near neutrality. Under optimum conditions, the medium is diffusely clouded in six to ten hours, and in twenty-four hours a pellicle forms, sometimes tough and whitish, sometimes merely a scum which is faintly iridescent. After two to three days a slimy deposit settles out

at the bottom of the tube. When the tube is swirled, this deposit comes up in the form of a coarsely twisted rope, looking much like an inverted water-spout.

In bouillon and in Witte's pepton growth is similar to that in hay infusion, but rather less abundant.

Litmus milk is unchanged, and no pellicle forms, though growth occurs.

At first the vibrio grew with difficulty on nutrient agar; after one or two generations, however, growth became profuse. The typical twenty-four hour colony is round, convex and slightly flattened on top, 1 to 2 mm. in diameter, with entire margin. The consistency of the colonies is mucoid, and they tend to become confluent. By reflected light they appear bluish-white and glistening; viewed by transmitted light under low magnification, they are yellowish and finely granular. Colonies five to six days old, where there is sufficient room for growth, reach 6 to 7 mm. diameter and show a distinct central boss (pl. 1). The deeper agar colonies are lenticular.

There is no liquefaction of gelatin. The character of the colonies is the same as on agar. In a gelatin stab, while growth is good on and near the surface, it is very faint and scattered along the line of the stab. Growth is not so abundant on Loeffler's blood serum as on either agar or gelatin, and there is no digestion of the medium. On potato the colonies are small, white, and slimy. There is no diastatic action on starch. Nitrates are not reduced, nor is gas formed. No indol is produced.

There is no fermentation of the following carbohydrates: glucose, lactose, sucrose, maltose, mannitol, adonitol, rhamnose and xylose. The most favorable temperature for growth is about 30°C.

The vibrio is actively motile, both in fluid media and in suspensions of young cultures from solid media. Examination of eighteen to twenty-four hour hay infusion cultures shows most of the individuals free-swimming, relatively large and slow-moving; a few small groups of agglutinated organisms are to be seen. In older cultures (several days old) agglutinated masses are much more in evidence and the free-swimming organisms are smaller,

a few even coccoid, and motility appears much swifter and more vigorous. In hay infusion cultures, weeks or months old, motility may be almost absent, although subculture proves viability still present.

Progression with rotation on the long axis is the characteristic mode of motion with the vibrio. A greater or less degree of precession, or "wobbling" of the long axis, is also to be seen in a large number of the organisms. The combination of these movements in the small, swiftly moving forms produces a strong impression of a progressive undulation passing down the organisms, which, however, is apparently illusory, since the long, slow-moving, filamentous forms present in young hay infusion cultures can be seen to retain their shape during motion, and to be without "notable" flexibility. During division, when the daughter individuals are separated by an obvious constriction, their axes may be quite movable with reference to each other.

The direction of motion is reversible. An individual may often be seen to dart forward, then backward, then forward again in a slightly different direction in a way suggestive of the avoiding reaction of paramecium.

A filterable spiral form, *Spirillum parvum*, has also been described by von Esmarch (1902). To this, our organism in some ways appears similar. However, *Sp. parvum* does not grow on either potato or milk. Old agar colonies have a violet tinge. Moreover, it grows very slowly, rendering fluid media turbid only after eight or ten days. Among the larger forms of filterable organisms should be mentioned the spirochaetes, both parasitic and free-living, whose passage through the Berkefeld filter Wolbach (1915) has demonstrated, and the closely allied Leptospirae (Noguchi, 1918, 1919). In view of the distinctive morphological and cultural characteristics of the new organism and its filterability, we propose the name *Vibrio percolans*.

An agglutinating serum for *V. percolans* can be produced in guinea pigs by intraperitoneal injections of 2 cc. of a twenty-four bouillon culture of the organism every other day for fourteen days. This serum, if allowed to act for twenty-four hours at room temperature, agglutinates *V. percolans* in dilutions up to

1:750. Normal guinea-pig serum has no agglutinating action on the organism. *V. percolans* agglutinating serum has no effect on *V. comma*, *Sp. Finkler-Prior*, or *Sp. metchnikoffi.*

Table 1 summarizes the results obtained in determining the thermal death point of *V. percolans*. The cultures used were twenty-four hours old in the bouillon of $(H^+) = 1.3 \times 10^{-7}$ (pH = 6.9). Approximately one and a half minutes was required to raise the culture to the given temperature. The results were read after forty-eight hours incubation of the subcultures at 30°C.

TABLE 1

DEGREES CENTIGRADE	TIME														
	½ minute	1 minute	1½ minutes	2 minutes	2½ minutes	3 minutes	4 minutes	5 minutes	6 minutes	8 minutes	9 minutes	12 minutes	15 minutes	16 minutes	20 minutes
56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
55	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0
54	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0
53	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0
52	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0
51	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0
50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ indicates that the subcultures made at the time specified gave a growth of the vibrio; 0 indicates negative subculture.

The organism is fairly resistant under various conditions. It survived after at least eight days at 0°C. in bouillon. At laboratory temperature one glucose broth culture, plugged merely with cotton, was viable after two months. One of the original subcultures, kept at room temperature on a paraffin plugged agar slant, is alive after seven months. Subcultures made from hay infusion cultures kept only with cotton plugs at laboratory temperature for more than six months in several instances have been positive, as is illustrated by the following protocols.

Large test tube (3.5 cm. diameter) of hay infusion inoculated December 11, 1921 with *V. percolans*. Kept in laboratory with cotton plug until June 29, 1922. Then had evaporated to a little less than

half original volume. Short, medium and filamentous forms of *V. percolans* to be seen under dark field, a very occasional individual motile. Subculture in twenty-four hours swarming with vibrios of high motility.

Test tube (2.5 cm. diameter) of hay infusion inoculated November 26, 1921. By June 29, 1922, had evaporated to a little less than a quarter original volume; opaque, chocolate color; under dark field, heavy suspension of vibrios, an occasional one still motile. Subculture positive.

Test tube (2 cm. diameter) inoculated October 11, 1921. By June 29, 1922, evaporated to a little less than quarter original volume occasional motile vibrio present. Subculture positive.

This vibrio is not pathogenic for white mice, white rats, wild rats (*Rattus norwegicus*), guinea-pigs, and rabbits, either by subcutaneous or intraperitoneal injection or by ingestion. One of us (S. W.) swallowed 5 cc. of a twenty-hour broth culture without detecting any effect.

We would call attention to the photomicrographs illustrating this paper and the following paper taken by means of the apparatus for ultra-violet photomicrography kindly placed at our disposal by the late Prof. H. C. Ernst.

The light source is the 2800 Å. line of a magnesium arc. By means of an apparatus for mechanical focussing, devised by Dr. W. T. Bovie, it is possible to focus accurately on the bacteria, using for illumination the harmless green portion of the magnesium spectrum, and then to change directly to the proper focus for ultra-violet light. In this way, the organisms are subjected to ultra-violet light during only the actual exposure of the plate (three to five seconds).

In order to be sure the organisms were not killed by this exposure, the photographed preparations, which were made in 0.5 per cent agar in saline, were rubbed up in sterile broth and plated out. In every case a heavy growth was obtained.

Both from the point of view of theory (V. Sabine (1906) and Ernst and Wolbach (1906)) and of practical results, it is believed that the ultra-violet microscope gives more nearly the actual structure of the living bacterial cell than does any other method of direct observation.

POWER OF LOCOMOTION OF V. PERCOLANS AS COMPARED TO THAT OF V. COMMA AND E. PRODIGIOSUS

In correlation with the study of motility as affecting filterability described in the paper subsequent, investigation was made of the passage of *V. percolans*, *V. comma* and *E. prodigiosus* through a coarse-pored filter without aid by any pressure head and dependent solely upon their own powers of locomotion and growth. The following modification of the method of Carnot and Garnier (Besson, 1914) has been used:

A U-tube, 14 mm. inside diameter, with arms 20 cm. long, has a plug of glass wool placed in the bottom of one arm. Above this is placed a layer of 60/120 quartz sand (average diameter of grains 0.23 mm.), acid-washed, about 10 cm. in depth. Hay infusion or nutrient bouillon is poured in, filling both arms to a level one centimeter above the sand, the arms plugged with cotton, and the whole autoclaved. When ready to inoculate, 2 to 3 cc. of the medium is removed from above the sand with a sterile pipette, and replaced with an equal amount of a suspension of the organism to be tested. The tubes are then incubated. Appearance of a faint clouding in the uninoculated arm of the tube indicates the end point. By dividing the time in hours from inoculation to clouding of the sand-free arm by the depth in centimeters of the sand layer, the approximate average time required by the organism to pass 1 cm. of the sand is obtained.

In the experiments each organism was placed under its optimum conditions of growth. *E. prodigiosus* and *V. comma* were grown at 37°C., the former in bouillon and the latter in pepton bouillon. *V. percolans* was grown at 30°C. in faintly alkaline hay infusion. The slightly lower temperature of cultivation may have been a serious handicap to *V. percolans* in comparing its rate of passage with that of *V. comma;* if so *V. percolans* was also handicapped in the filtration experiments in which its temperature was again somewhat lower.

V. comma showed consistently greater speed in effecting passage than did *V. percolans*. The optimum rates obtained were as follows:

ORGANISM	TIME FOR PASSAGE 1 CM. OF SAND
V. comma (Rosebank strain)	1 hour 48 minutes
V. percolans:	
Strain V_{15}	2 hours 24 minutes
Strain V_{19}	2 hours 29 minutes
Strain V_{29}	2 hours 30 minutes

E. prodigiosus[1] did not pass the sand in the six experiments performed: three experiments were of one week and three of two weeks' duration.

A culture of *V. percolans* has been deposited in the Army Medical Museum, Washington, D. C., whence any worker interested may obtain a culture.

REFERENCES

MUDD, S. 1923 Certain factors affecting filtration through Berkefeld candles. Amer. Jour. Physiol., **63**, 429.

BESSON, A. 1914 Technique Microbiologique et Sérothérapique, Paris, 149.

ERNST, H. C., AND WOLBACH, S. B. 1906 Ultra-Violet Photomicrography. Jour. Med. Res., **14**, 463.

VON ESMARCH, ERWIN 1902 Ueber kleinste Bakterien und das Durchwachsen von Filtern. Centralbl. f. Bakt. u. Parasit., 1 Abt., Origin., **32**, 561.

NOGUCHI, H. 1918 Morphological Characteristics and Nomenclature of *Leptospira* (*Spirochaeta*) *Icterohaemorrhagiae* (Inada and Ido). Jour. Exp. Med., **27**, 575.

NOGUCHI, H. 1919 Etiology of yellow fever. VI. Cultivation, morphology, virulence and biological properites of *Leptospira icteroides*. Jour. Exp. Med., **30**, 13.

SABINE, W. C. 1906 The optical advantages of the ultra-violet microscope. Jour. Med. Res., **14**, 155.

WOLBACH, S. B. 1915 On filterability and biology of spirochaetes. Amer. Jour. Trop Dis. and Prev. Med., **2**, 494.

[1] The *E. prodigiosus* strain used was from the Medical School stock. Transplants on certain media showed limited motility. In bouillon or suspension from plain agar for instance a few organisms were motile. A culture on blood agar was somewhat more motile. A culture on wet potato showed greatest motility. Of the organism in the fluid at the bottom of the potato tube a considerable number, possibly five per cent, were motile. Motility in hay infusion cultures was not with certainty observed. Such individuals as were motile on any media, with the possible exception of the potato fluid, were quite obviously less vigorous and rapid in their movements than the cholera or percolans vibrios.

EXPLANATION OF PLATE

PLATE 1

FIG. 1. LIVING TWENTY-FOUR HOUR HAY INFUSION CULTURE OF V. PERCOLANS.

Ultra-violet light, magnesium line of λ2800 Ångstrom units. Five seconds exposure. ×1120.

FIG. 2. PLAIN AGAR COLONIES FIVE DAYS OLD

Natural size. Drawn as seen by reflected light

FIG. 3. PLAIN AGAR COLONIES

Transmitted light; low magnification

FIG. 1

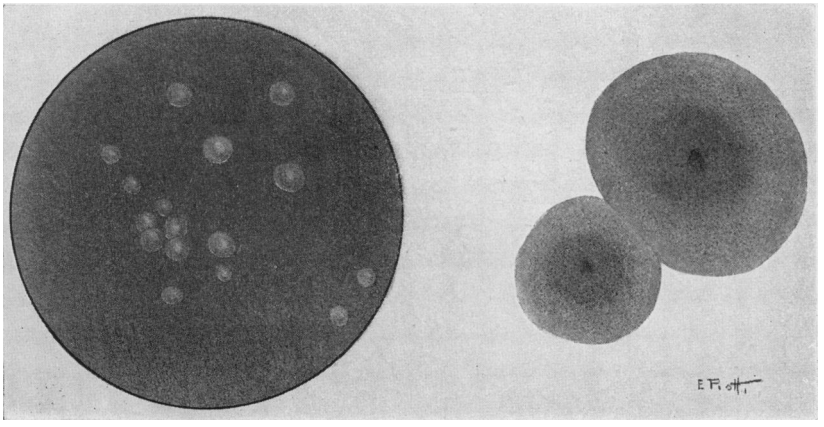

FIG. 2 FIG. 3

(Mudd and Warren: A readily cultivable vibrio.)