THE PENETRATION OF BACTERIA THROUGH CAPILLARY SPACES

I. MOTILITY AND SIZE AS INFLUENCING FILTERABILITY THROUGH BERKEFELD CANDLES

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The contact surface of the pores of a Berkefeld filter and the water bathing them is the site of an electric potential difference, an ordinary Helmholtz electric double layer, the solid walls taking the negative, and the fluid the positive, charge (Mudd, 1923). If a solution of a "basic" dye, such as methylene blue, is drawn through the filter, electro-positive color ions are taken out by the electro-negative filter capillary walls, and the first portions of the solution may be partially or completely decolorized; more prolonged filtration results in saturation of the filter pores, a colored filtrate and colored wash water. Acid dyes, such as eosine, on the contrary may be filtered without demonstrable change in color; the electronegative color ion does not adhere to the negative capillary walls. The filter is "basophilic."

The filterable microörganism described in the preceding paper, *Vibrio percolans*, in ordinary culture media, was found to be negatively charged with reference to the suspending medium; migration in an electric field, studied with the aid of a microcataphoresis chamber, was toward the anode. The hope was entertained that by acidifying the medium a hydrogen ion concentration could be reached at which the vibrio would still maintain its activity but would carry the positive component of the electric double layer, its filterability thus being suppressed. However vibrios are known to be especially susceptible to acid, (Besson, 1914), and a hydrogen ion concentration of about

5×10^{-6} (pH = 5.3) or above was found to inhibit the motility of *V. percolans*, although insufficiently high to reverse the direction of the surface potential difference. Therefore the question whether or not a filterable microörganism will be found whose filterability can be suppressed by reversal of the interfacial potential with consequent adsorption on the negative filter walls remains an open one.

It was observed, however, that when cultures of *V. percolans* were filtered in media with hydrogen ion concentrations greater than 5×10^{-6} (pH = 5.3) no vibrios grew out in media inoculated with the filtrate: this raised the question whether the motility of a culture could be a determinative factor in its filtration. It has subsequently been found that the passage of *V. percolans* through Berkefeld V candles can be prevented by suppressing its motility with acid, with ether and chloroform, or with cold.[1]

SUPPRESSION OF FILTERABILITY OF V. PERCOLANS BY ACID

The reaction of the culture of *V. percolans* was at first adjusted by adding a sufficient amount of buffer solution of the desired hydrogen ion concentration. This method in the one experiment tried resulted in stoppage of motility and a negative filtrate; adjustment was made to H-ion concentration of 6×10^{-5} (pH = 4.2). Uncertainty as to possible toxic effect of the buffer ions led to abandonment of this method in favor of a safer one, namely, the washing out of the CO_2 from the culture with a stream of hydrogen and the addition of N/10 HCl until the desired reaction was reached. The hay infusion culture, usually at an initial H-ion concentration not far from 5×10^{-7} (pH = 6.3), by bubbling hydrogen through it for a half to three quarters of an hour could be brought to an approximately stable reaction around 1×10^{-8}, (pH = 8). Acid was then added as desired. The CO_2 could be bubbled from the culture somewhat more quickly if it was kept a little acid by addition of HCl during

[1] By "prevented" is meant prevented at least for intervals slightly more than sufficient to allow the motile control culture to inoculate a filtrate under similar conditions. It is possible, however, that a more prolonged filtration of the culture with motility inhibited might have resulted in inoculation of the filtrate.

the process (Henderson, 1920). The reaction was followed throughout with a Bovie direct-reading potentiometer (Bovie 1915–1916). The condition of the vibrios was followed by observing samples microscopically at frequent intervals with dark field illumination. No change was observed when the cultures were rendered slightly alkaline or acid, but when the H-ion concentration rose above 5×10^{-6} (pH = 5.3) motility became obviously impaired, and above 1×10^{-5} (pH = 5) was practically stopped, save in one culture which had been grown in a medium more acid than usual and in which the H-ion concentration had to be raised to about 4.5×10^{-5} (pH = 4.35) in order to stop locomotion. When motility was observed practically to have ceased the culture was filtered, and, in the seven experiments performed, proved uniformly negative for vibrios; the control filtrates from unacidified cultures were regularly positive, i.e., contained vibrios.

The complete experimental procedure was typically as follows:

The suspension filtered was always a mixed hay infusion culture of *V. percolans* and a weakly motile strain of *Erythrobacillus prodigiosus*. The vibrios were oridinarily the outgrowth in sterile hay infusion tubes of the filtrate from the previous control experiment. The culture was strained if necessary through coarse meshed cloth to remove flocculi and sediment. It was then put in a titration vessel of the Bovie potentiometer, the CO_2 washed out and the hydrogen ion concentration adjusted at from 1 to 5×10^{-6} (pH = 6 to 5.3). Motility of the vibrios at this reaction was still normal as seen by dark field observation. The culture was then divided into two equal parts, the first of which was rendered sufficiently acid to stop locomotion and then filtered, with negative pressure (suction), starting at zero and increasing the pressure difference on the two sides of the filter to several hundred millimeters of mercury; the filtrate was inoculated into sterile hay infusion tubes. Various amounts of the residual unfiltered acid culture were next inoculated into similar sterile hay infusion tubes as controls. The fact that both *V. percolans* and *E. prodigiosus* uniformly grew out in the latter tubes proved that the culture had not been

sterilized by the short acid exposure. The second half of the original culture, with motility slightly if at all impaired, was then passed through the same filter used for the acid filtration, and the filtrate inoculated into the sterile hay infusion tubes; *V. percolans* regularly grew out of these tubes. *E. prodigiosus* was regularly absent in all filtrate and filtrate-inoculated tubes.

The filters used in the experiments of this paper were Berkefeld "V" (No. 3, $2\frac{1}{2}$ inches long by $\frac{5}{8}$ inch in diameter) with wall thickness of approximately 4 mm. The filtration lasted only two to six minutes, as shown in the tables. Twenty-five to 60 cc. of filtrate were drawn through the candle for each experiment. The unfiltered culture was invariably turbid and the filtrate clear; several examinations of filtrate specimens under the dark field microscope immediately after filtration did not serve to detect vibrios, though these ordinarily grew out in twenty-four hours or less when the filtrate was positive.

From all analogy it seems certain that the acidification of the culture decreased the potential difference at the filter-fluid and bacteria-fluid interfaces. The fact that seven experiments in which motility was stopped with acid gave clearly negative filtrates, whereas when motility was inhibited by other agencies (see below) only five cultures showed complete suppression and five partial suppression of filterability, may possibly be of significance; decreasing the charges on bacteria and filter wall may have allowed the molecular adhesion, "stickiness," to act more effectively in holding the bacteria to the filter capillary walls. This suggestion is advanced at this time, however, purely as a speculation.

As to possible changes in the size of the organisms with acidification of the media, none was ever observed under the dark field, though a more sensitive measure than mere inspection might with profit be employed. However we would expect such a change if it occurred to have been in the direction of a shrinking rather than a swelling, as acid was added and the organisms were thereby brought toward their point of zero potential difference against the medium (Loeb, 1922) just as hydration of gelatin particles decreases as they are brought toward their isoelectric point.

SUPPRESSION OF FILTERABILITY OF V. PERCOLANS BY NARCOTICS

The coincidence of the H-ion concentration at which the motility of *V. percolans* ceased with that at which filterability was lost made it desirable to stop motility by other means. This was done with narcotics, and filterability was again found to be coincidently suppressed.

For each pair of experiments an actively motile culture was as before divided into two halves; one-half was shaken with a small amount of ether or chloroform and allowed to stand until dark field examination showed that locomotion had practically ceased. This was then filtered, the filtrate inoculated into sterile hay infusion tubes and other tubes inoculated with the unfiltered narcotised culture as a control of its viability. The untreated half of the original culture was then passed through the same filter and the filtrate inoculated into sterile tubes.

The residue of the narcotised unfiltered culture was allowed to stand in open cylindrical vessels until the anaesthetic had evaporated. Subsequent dark field examinaton showed the culture swarming with motile vibrios indicating, as did the inoculated control experiments mentioned above, that the chloroform and ether narcotised but did not kill the majority of the vibrios.

SUPPRESSION OF FILTERABILITY OF V. PERCOLANS BY CHILLING

Finally confirmation of the belief that suppression of the motility of the vibrio could inhibit its filtration through Berkefeld V candles was obtained by chilling the cultures. For each pair of experiments an actively motile culture was put in the ice box surrounded by ice water, and with melting ice in the culture vessels themselves. The filter was also chilled. One half of the culture was filtered at about 0°C.; the other half was allowed to warm for from twenty odd to seventy odd minutes and was then passed through the same filter. Vibrios regularly appeared in tubes inoculated with the latter filtrate, but were absent in some or all of the tubes inoculated with the filtrate from the filtration at 0°. Control inoculations of the culture at 0° into

TABLE 1

Effect of reaction of the medium on filtration of V. percolans

EXPERIMENT NUMBER	FILTER NUMBER	H-ION CONCENTRATION	MOTILITY	FITRATION TIME	FILTRATION PRESSURE	RESULT	REMARKS
				minutes	*mm. Hg.*		
V_1	1	6×10^{-5}	Almost stopped	6	0-286	−	Na-acetate acetic acid buffer used
V_2	1	1×10^{-6} 1×10^{-7}	Normal	5	0-295	+	*E. prodigiosus* control showed filter tight
V_3	1	2.3×10^{-5}	Practically stopped	4	0-155	−	CO_2 bubbled out in this and later experiments
V_4	1	Ca. 1×10^{-7}	Normal	4½	0-195	+	
V_5	2	7.4×10^{-6}	Little if at all impaired	3½	0-225	+	New filter used
V_6	2	7.9×10^{-8}	Little if at all impaired	3	0-245	+	Acid culture from V_5 rendered alkaline and filtered
V_7	2	Not acidified	Normal	3-4	0-245	+	
V_8	2	Not acidified	Normal	7	0-145	+	
V_9	2	Not acidified	Normal	3	0-235	+	*E. prodigiosus* control showed filter tight
*V_{10}	3	7.2×10^{-6}	Impaired	3½	0-255	−	New filter used
*V_{11}	3	9.5×10^{-7}	Normal	4	0-225	+	*E. prodigiosus* control showed filter tight
V_{12}	Vibrios apparently killed by twenty odd minutes exposure to H-ion concentration about 1.5×10^{-5}. No growth in control tubes						
V_{13}	3	3×10^{-8}	Normal	2½	0-195	+	*E. prodigiosus* control showed filter tight
V_{14}	3	$1.2\text{-}1.3 \times 10^{-5}$	Much impaired	2½	0-245	−	
V_{15}	3	2×10^{-6}	Little if at all impaired	5½	0-245	+	*E. prodigiosus* control showed filter tight

V_{16}	4	1.6×10^{-5}	Much impaired	$6\frac{1}{2}$	0–109	−	New filter used
V_{17}	4	3.6×10^{-6}	Little if at all impaired	5	0–109	+	*E. prodigiosus* control showed filter tight
V_{18}	4	1.7×10^{-5}	Practically stopped	$3\frac{1}{4}$	0–225	−	
V_{19}	4	3.2×10^{-6}	Slightly impaired	$3\frac{1}{2}$	0–225	+	
V_{20}	Results inconclusive because of bad culture medium						
V_{21}	Results inconclusive because of bad culture medium						
V_{22}	5	4.5×10^{-5}	Practically stopped	$3\frac{1}{4}$	0–145	−	
V_{23}	5	3.2×10^{-6}	Little if at all impaired	2	0–145	+	*E. prodigiosus* control showed filter tight

* Experiments bracketed performed with two halves of same culture as above described.

TABLE 2

Effect of narcotics on filtration of V. percolans

EXPERIMENT NUMBER	FILTER NUMBER	APPROXIMATE PER CENT NARCOTIC ADDED*	MOTILITY	FILTRATION TIME	FILTRATION PRESSURE	RESULT	REMARKS
				minutes	*mm.Hg.*		
V_{26}	3	Chloroform 0.28 per cent added; not all dissolved	Much reduced	2¼	0–200	Negative all tubes	Filter had been cleaned with Na_2CO_3 and water since using for V_{15}
V_{27}	3	Untreated	Active	2½	0–180	Positive 4 tubes, negative 1 tube	
V_{28}	3	Ether 2.7 per cent	Practically stopped	5½	0–100	Negative all tubes	
V_{29}	3	Untreated	Active		0–105	Positive all tubes	*E. prodigiosus* control showed filter tight
V_{30}	2	Ether 6.5 per cent added	Practically stopped	3¼	0–220	Negative all tubes	
V_{31}	2	Untreated	Active	3	0–220	Positive all tubes	*E. prodigiosus* control showed filter tight
V_{32}	5	Ether 3.9 per cent added	Practically stopped	2	0–270	Negative 5 tubes, positive 1 tube	
V_{33}	5	Untreated	Active	2	0–270	Positive all tubes	*E. prodigiosus* control showed filter tight
V_{34}	3	Ether 3.2 per cent added	Greatly reduced	3½	0–335	Negative 3 tubes, positive 2 tubes	
V_{35}	3	Untreated	Active	3½	0–335	Positive all tubes	

* The percentage of narcotic tabulated is probably somewhat greater than that actually present in the cultures because of partial evaporation during additions of the ether and examinations of the cultures.

sterile hay infusion tubes at room temperature gave uniformly positive results.

The organisms regained active motility with surprising quickness on rewarming after chilling. Preparations could scarcely be got on the dark field microscope without showing motility, and within a few seconds locomotion was active.

ATTEMPTS TO FILTER VIBRIO PERCOLANS THROUGH N AND VIBRIO COMMA THROUGH V CANDLES

Inspection under the dark field microscope of living cultures of *V. percolans* and of *V. comma* impresses the observer with the general similarity of the two organisms but also with two peculiarities of *V. percolans* which are doubtless significant in determining filterability. These are, first, the frequency of highly motile, small, almost coccoid forms in *V. percolans* cultures, and, second the greater slenderness of *V. percolans*. Filar micrometer measurement has given as the average diameter of one hundred each of the organisms, *V. comma*, 0.46μ; *V. percolans*, unselected, 0.35μ; *V. percolans* (smaller individuals) 0.31μ, and *E. prodigiosus*, 0.31μ. The range of diameters in the *V. percolans* vibrios measured was 0.28μ to 0.51μ.

Doubtless in correlation with these characteristics is the fact that *V. percolans* under normal cultural conditions regularly gives a positive filtrate with Berkefeld V candles (though uniformly negative in six attempts at passage under similar conditions through three N candles) whereas *V. cholerae* is not a filter passer. Four unsuccessful attempts were made to pass *V. comma* through three Berkefeld Vs which had been shown before and after the attempted *V. comma* passage to be pervious to *V. percolans*.

DETECTION OF V PERCOLANS IN GROUND DOWN FILTER SECTIONS

Berkefeld filters, as is well known, are made of baked kieselguhr (diatomaceous earth). By microscopic examination of scrapings from a dry filter the fine irregular silicious granules

TABLE 3

Effect of chilling on filtration of V. percolans

EXPERIMENT NUMBER	FILTER NUMBER	PREPARATION	MOTILITY	FILTRATION TIME	FILTRATION PRESSURE	RESULT	REMARKS
				minutes	*mm.Hg.*		
V_{36}	6	Chilled	Greatly reduced	2¼	0–312	Negative all tubes	
V_{37}	6	Chilled and rewarmed	Active	2½	0–310	Positive all tubes	
V_{38}	6	Chilled	Much reduced	3¾	0–240	Negative 4 tubes, positive 1 tube	
V_{39}	6	Chilled and rewarmed	Active	3¼	0–240	Positive all tubes	*E. prodigiosus* control showed filter tight
V_{40}	7	Chilled	Much reduced	3¼	0–300	Negative all tubes	
V_{41}	7	Chilled and rewarmed	Active	2¾	0–250	Positive all tubes	*E. prodigiosus* control showed filter tight
V_{42}	8	Chilled	Reduced	4½	0–355	Negative 2 tubes, positive 3 tubes	
V_{43}	8	Chilled and rewarmed	Active	1¾	0–342	Positive all tubes	
V_{44}	7	Chilled	Reduced	6	0–400	Negative 5 tubes, positive 1 tube	
V_{45}	7	Chilled and rewarmed	Active	3½	0–395	Positive 4 tubes, negative 1 tube	

may be seen. Study of filter sections ground down to microscopic thinness shows a matrix of the fine granules containing at intervals spaces or lacunae of much greater size, i.e., a few to more than a hundred micra. For photomicrographs, see von Esmarch (1902) and Hofstädter (1905).

It has been the judgment of most authors who have considered the point that these lacunae could not afford a continuous passage through the filter wall and that a part, and of course a critical part, of the path of particles traversing the filter must be through the matrix or actual granular filter substance itself. This view has not been universally accepted, however, so that it seemed necessary to study the distribution of the vibrios in the filter.

A used V filter, no. 8, was again immersed in a vibrio culture and a filtration pressure maintained for a few minutes. It is to be emphasized that the bacteria were carried into the filter by filtration of only a few minutes duration and with filtration pressure of a few hundred millimeters of mercury. They were not allowed to "grow" into the filter. Absolute alcohol was drawn through to fix the freshly deposited vibrios. Fuchsin (acid) was drawn through for several hours in an attempt to stain the organisms satisfactorily; this later proved to have been unsuccessful. The filter was dehydrated and balsam in xylol was drawn through by suction and then allowed to seep through for a day or more. The balsam-impregnated filter was allowed to harden for a couple of weeks in the incubator. Rings were then cut with a knife and ground down with a coarse stone. A ring was mounted in balsam on a glass slide and final reduction to microscopic thinness carried out by prolonged grindings on fine stones. The balsam was removed by soaking in xylol, the xylol allowed to evaporate, and the organisms stained *in situ* with carbol fuchsin (acid) or methylene blue. The filter fragment was finally washed, dried and mounted in xylol balsam and studied with oil immersion.

The result is quite conclusive (see plate 2). The organisms may be seen in a stained coagulum on the surface and extending down into the superficial lacunae, and in among the granules of the matrix. By focussing with the fine adjustment the trans-

parent granules may be brought into focus above and below the vibrios, proving the latter to be in the capillary interstices between the silicious grains of the actual filter substance.

In plate 2 the organisms are readily discernible, a few in focus, others below and above the focal plane. No lacunae are shown in the photomicrograph, though they are present in other fields of the section. Intense illumination was used for the photomicrograph in order to give definition to the vibrios and the detail of the filter structure was thereby sacrificed. A few silica granules can, however, be made out.

THE INTERGRANULAR DIAMETERS AND RELATIVE POROSITY OF DIFFERENT BERKEFELD FILTER TYPES

The pore size of a number of Berkefeld filters has been estimated by the methods of Bechhold (1908). Compressed air was forced through the filters and the pressure in millimeters of mercury at which streams of bubbles began to issue from six or more pores was substituted as p in the formula:

$$D = \frac{4\beta \times 760}{p \times 1.933 \times 10^5}$$

β is the capillary constant of water at the temperature of the determination. D is the diameter in millimeters of the narrowest portion of the pores from which the bubbles emerge, i.e., it is an estimate of the average value of the spaces between the granules of the actual filter substance.

By using a small escape valve in the air line and a mercury manometer air pressure could be maintained within a few millimeters of any desired level. The values given in table 4 are in each case the average of several observations; they agree within a few mm. for any clean filter, but in used filters may vary by as much as 40 mm. Hg.

The variations in the mean values for the three types of filters, V. N. and W, is seen to be small; moreover it is in the wrong direction, i.e., the V's or most porous, appear to have the smallest intergranular spaces. These differences are thus probably fortuitous.

It may be pointed out that some of the used filters whose pores had been contaminated with dyes and proteins emitted bubbles at much lower pressures than the new ones, apparently indicating larger intergranular diameters. A similar effect was produced by mixing alcohol with the distilled water wetting the filters. It is thus evident that Bechhold's formula gives too high values for pore size in the presence of substances lowering the surface tension of the water in the pores.

TABLE 4

FILTER	SIZE	CONDITION	PRESSURE	CALCULATED DIAMETER	AVERAGE DIAMETER FOR FILTER TYPE
	inches		*mm. Hg*	μ	μ
V (10)	$2\frac{5}{8} \times \frac{5}{8}$	New	520	0.414	
V (11)	$2\frac{5}{8} \times \frac{5}{8}$	New	567	0.380	
V (16)	$2\frac{5}{8} \times \frac{5}{8}$	New	585	0.373	0.38
V (17)	$2\frac{5}{8} \times \frac{5}{8}$	New	581	0.376	
V (18)	$2\frac{5}{8} \times \frac{5}{8}$	New	588	0.371	
N (X)	$2\frac{5}{8} \times \frac{5}{8}$	New	466.5	0.461	0.45
N (XI)	$2\frac{5}{8} \times \frac{5}{8}$	New	493	0.437	
W (1)	$2\frac{5}{8} \times \frac{5}{8}$	New	410.5	0.524	
W (2)	$2\frac{5}{8} \times \frac{5}{8}$	New	450	0.478	0.43
W (X)	$2\frac{1}{2} \times 1$	New	592	0.369	
W (XI)	$2\frac{1}{2} \times 1$	New	636	0.343	
V (6)	$2\frac{5}{8} \times \frac{5}{8}$	Used	588	0.366	
N (1)	$2\frac{5}{8} \times \frac{5}{8}$	Used	617.5	0.349	
N (2)	$2\frac{5}{8} \times \frac{5}{8}$	Used	468	0.460	
N (VIII)	$2\frac{1}{2} \times 1$	Used	287	0.761	
W (IX)	$2\frac{1}{2} \times 1$	Used	307.5	0.710	

Tested by the second method, namely that of determining the rate of flow of distilled water through the filters as a function of the pressure head, the filters of course showed themselves more porous in the order V > N > W. Plotting pressure against rate of flow, the points fall along straight lines passing very nearly through the origin. The slopes indicated the following rates of flow for three new $2\frac{5}{8} \times \frac{5}{8}$ inches filters:

V (10), 0.45 cc. per second per 100 mm. Hg pressure head
N (X), 0.21 cc. per second per 100 mm. Hg pressure head
W (1), 0.125 cc. per second per 100 mm. Hg pressure head

The filters used thus evidently differed in the number and size of the gross pores or lacunae rather than in the size of the intergranular spaces.

With the foregoing conclusion in mind further attempts were made to filter *V. percolans* through N filters, as follows:

Approximate volume filtered, 150 cc.; filtration pressure, 0–400 mm. Hg; result negative
Approximate volume filtered, 125 cc.; filtration pressure, 0–500 mm. Hg; result negative
Approximate volume filtered, 575 cc.; filtration pressure, 0–700 mm. Hg; result negative
Approximate volume filtered, 480 cc.; filtration pressure, 0–744 mm. Hg; result negative

The passage of filterable organisms through the filter wall is evidently then greatly facilitated by the lacunae, even though one critical part of the passage has to be through the fine interstices of the granular filter substance.

DISCUSSION

Porous filters of the Berkefeld type, as of course is well known, have shown themselves of much practical utility in freeing solutions of thermolabile substances from bacteria, in separating the ordinary bacteria from the "filterable viruses" (Wolbach, 1912), in purifying water, and in other ways. The majority of these filters under the usual conditions of short duration of filtration and moderate pressure give filtrates sterile for most known microörganisms. However, the familiar fact that the microörganisms penetrate the filter if contact of the culture with it is sufficiently long-continued, especially if suitable nutrient material is at hand in the filter pores (Hofstädter, 1905), would seem to indicate that the filter owes its tightness to the tortuosity and length of the passage to be traversed as well as to its narrowness, rather than to an absolute excess in all cases of the smallest dimensions of the microörganisms over the smallest diameters of the pores they must pass through.

Adhesion also doubtless plays a part in holding back the bacteria (Bechhold, 1918). In dark field examination of cultures of *V. percolans* in films between cover-glass and slide the vibrios are found adherent to the upper or lower glass surfaces in increasing numbers as time goes on. Often a vibrio can be seen to swim against the cover glass and stick there; occasionally an organism will stick to the cover glass only at one point and thresh about in the medium with this contact as a fixed point. With *E. prodigiosus* this stickiness doubtless plays even a more considerable part, for colonies on solid agar are extremely adherent and when scraped with a platinum loop come off in long strings. A fluid culture of *E. prodigiosus* can be distinguished from one of *V. percolans* also by the stringing out of the former when it is filtered through cheese-cloth or even when a platinum loop is withdrawn from the prodigiosus culture.

The "effective pore size" of a bacteria-tight filter has been defined by Rosenthal (1908) as "the narrowest diameter which is present in each of the extraordinarily many porous passages, at least in one place; it becomes, if we consider layers of one and the same mass increasing regularly in thickness, smaller, at first rapidly, then more slowly." He estimates the effective pore size as between 0.5 and 2μ.

Schmidt (1910) from the fact that he was able to filter *Ps. fluorescens-liquefaciens* but not staphylococci and Gram-negative diplococci, whose diameter was about 0.8μ, gives 0.2μ and 0.8μ as upper and lower limits of the effective pore size of Berkefeld filters. "One will certainly not be far from the true value, if he assumes a mean value of about 0.5μ." Assuming that the smallest quartz grains visible in ground-down sections of Berkefeld filters approximate close-packed spheres, he calculates the actual diameter of the finest pores between them as about 0.3 to 0.4μ.

Bechhold (1908) has calculated the diameter of pores of filters by the minimum pressure able to force air through. He gives the mean pore diameters of the larger pores of a new chamberland F filter as 0.23 to 0.41μ.

Our own estimates on Berkefeld filters are given above.

In view of these estimates it is of interest that under the circumstances of our filtrations motile *V. percolans* passes through Berkefeld V but not Berkefeld N candles and *V. comma* through neither. The measured diameters of one hundred unselected individuals of *V. percolans* vary from 0.28 to 0.51μ, with an average value of 0.35μ. Selecting the smaller individuals, the mean diameter found was 0.31μ. The average diameter of one hundred unselected cholera vibrios was 0.46μ.

The mechanics of the situation determining sterility or nonsterility of the filtrate from any given bacterial culture are evidently then such that under ordinary circumstances we may expect bacteria-free filtrates, but in the presence of any circumstances exceptionally favorable to passage through the filter, we anticipate a seeding of the filtrate. This expectation the literature shows indeed to be fulfilled.

The favoring circumstances may be small size alone e.g., the globoid bodies of poliomyelitis (Flexner and Noguchi, 1913), of 0.2μ average diameter, the coccoid bodies described by Foster as a cause of coryza (1917), typically 0.2 to 0.3μ in diameter, *Bacterium pneumosintes* (Olitsky and Gates, 1921, 1922), 0.15 to 0.3μ in length, a half to a third as large in diameter and the minute Gram-negative anaerobes isolated from human throats by Olitsky and Gates (1922), and probably the coccoid bodies described by MacCallum and Oppenheimer in vaccine lymph as about one-tenth the size of ordinary streptococci (1922).

Or small size and motility may combine to produce filterability, e.g., *Spirillum parvum* of v. Esmarch (1902), diameter 0.1 to 0.3μ, and the filterable spiral organisms and the protozoan of tap water discovered by Borrel (1903):

> Ceux qui passent le plus ordinairement sont des vibrions très polymorphs dont certains sont à la limite de la visibilité, reconnaissable à leur cil unique. Ces formes sont celles qui passent à tràvers les pores de la bougie, puis, dans la culture, il se développe des vibrions de dimensions variable, quoique cette culture paraisse tout a faire pure.
>
> Dans d'autre cas, ce sont des formes spirillaires qui passent et la culture montre des très longs filamentes grêles, invisible à l'état frais, assez semblables aux spirilles de la fièvre recurrente, mais plus courts. . . . .

Dans ce même milieu, j'ai obtenu, par filtration, la culture pure d'éléments très particuliers, que je considère comme appartenant au groupe des protozoaires, et que j'ai désigné sous le nom de *Micromonas mesnili.* Ce sont très ordinairement des éléments ovoïdes allongés de $\frac{1}{4}\mu$ de largeur sur 3 à 4μ de longeur, munis de deux cils trepus, plus gros que des cils de bacteries, plus rigide . . . . etc.

A clear case of the determinative value of motility is the passage of motile *V. percolans* through Berkefeld Vs and the failure to pass of non-motile percolans.

Slenderness, motility and flexibility may all be complementary factors in enabling organisms to pass the filter, e.g., the filterable spiral organisms of Wolbach and Binger, *Sp. elusa* (1914) and *Sp. biflexa* (1915), and *Leptospira icterohaemorrhagiae* (Noguchi 1919), diameter 0.25μ, and Noguchi's *Leptospira icteroides* (Noguchi, 1919), 0.2μ in diameter.

Again slenderness, motility and flexibility may not suffice and specially favorable pressure conditions may be require in addition, e.g. the experience of Todd and Wolbach (1914), who conclude that:

Sp. duttoni in an infective form can be forced through a Berkefeld filter by pressures of over 50 pounds to the square inch.

Sp. duttoni cannot be filtered through a Berkefeld filter in an infective form by atmospheric pressure.

Similarly Hofstädter, in an admirable work on the penetration of bacteria through the finest capillaries (1905), was able to force bacteria through capillaries under a filtration pressure of 50 to 100 atmospheres which would not permit their passage under pressures of two to three atmospheres or without a pressure head.

It is a pleasure to acknowledge the many courtesies of the late Prof. H. C. Ernst, of Profs. S. B. Wolbach and W. T. Bovie and assistance on a number of points of Mr. Shields Warren and Emily B. H. Mudd.

SUMMARY

Cultures of *Vibrio percolans*, grown in hay infusion whose reaction was approximately neutral, when brought to a hydrogen-

ion concentration of 5×10^{-6} (pH = 5.3) or above rapidly lose motility. Such non-motile cultures gave negative filtrates with Berkefeld V candles through which motile vibrios regularly passed.

Similarly the filterability of *V. percolans* under the conditions of our experiments was suppressed completely five times and partially five times by inhibiting motility with ether, with chloroform or by chilling.

In all cases the viability of the non-motile culture, at least for the duration of the experiment, was proved by positive subcultures. Growing with *V. percolans* in the cultures which were filtered was a feebly motile strain of *E. prodigiosus*. This organism did not appear in the filtrates whether positive or negative for *V. percolans*.

It is thus shown that when a culture is drawn through an irregular capillary bed under circumstances in which a few organisms may or may not pass through, motility may be a critical factor in determining passage. Motility doubless aids passage both in purely mechanical ways and by combating the tendency of the organisms to adhere to the pore walls.

V. percolans did not in ten attempts made pass through Berkefeld N candles. Attempts to pass *V. comma* through three Berkefeld V's shown before and after to be pervious to *V. percolans* were likewise unsuccessful. *V. comma* repeatedly showed itself able to pass by its own powers of growth and locomotion through a layer of ten centimeters of quartz sand in shorter time than *V. percolans*. Its inability to traverse Berkefeld filters is therefore not referable to inferior motility but to slightly larger size than *V. percolans*. The average diameters found for cholera vibrios (unselected) was 0.46μ, for *percolans* (unselected), 0.35μ, for *V. percolans* (smaller individuals), 0.31μ, for *E. prodigiosus* (unselected), 0.31μ.

Estimation of the diameters of the intergranular spaces of Berkefeld filters by Bechhold's method of forcing air through gave the following average values:

V type, 0.38μ; N type, 0.45μ; W type, 0.43μ. The differences are interpreted as fortuitous.

Estimation of porosity by determining the rate of filtration of water at various pressures shows the V type the most and the W the least porous. The following filtration rates with distilled water for $2\frac{5}{8} \times \frac{5}{8}$ inch candles were obtained:

V, 0.45 cc. per sec. per 100 mm. Hg pressure head
N, 0.21 cc. per sec. per 100 mm. Hg pressure head
W, 0.125 cc. per sec. per 100 mm. Hg pressure head

The differences in porosity are then evidently a matter of the relative size and numbers of the gross pores rather than of the intergranular spaces.

Sections of a Berkefeld V candle used for filtration of *V. percolans* have been ground down to microscopic thinness and the vibrios stained and demonstrated in the interstices between the silicious granules of the filter substance. The intergranular diameters are therefore of critical importance in determining the penetrability of the filters by bacteria.

That the relative numbers and sizes of the gross pores or lacunae may also be of critical significance is shown by the successful passage of motile vibrios through V filters and their failure to pass N's.

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PLATE 1

FIG. 1. E. PRODIGIOSUS, TWENTY-FOUR HOUR AGAR CULTURE IN 0.5 PER CENT AGAR MOUNT

Five inches, 1.9 mm., 7 oc. × 1120 diameters. Ultraviolet light, $\lambda = 2800$ Å, Mg electrodes.

FIG. 2. V. CHOLERAE, TWENTY-FOUR HOUR CULTURE

Conditions same as above

FIG. 3. V. PERCOLANS, TWENTY-FOUR HOUR CULTURE

Conditions same as above

Photomicrographs by Mr. Shields Warren

PLATE 2

GROUND DOWN SECTION OF BERKEFELD V FILTER WITH VIBRIO PERCOLANS STAINED IN SITU WITH METHYLENE BLUE

A few vibrios in focus, others below and above the focal plane. Darker zone along left margin of field deeply stained coagulum at surface of filter. Oil immersion. × 1000 diameters. Photograph by Prof. S. B. Wolbach.

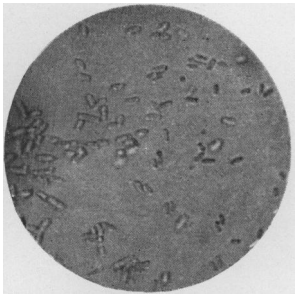


FIG. 1

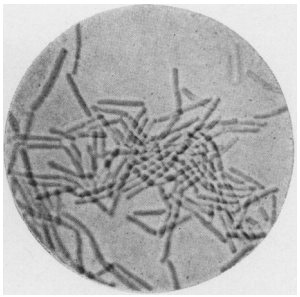


FIG. 2

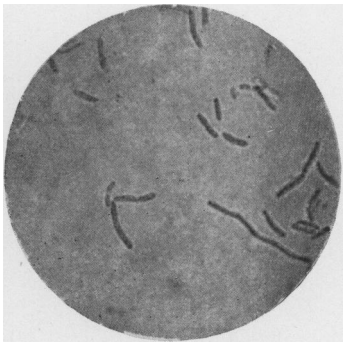
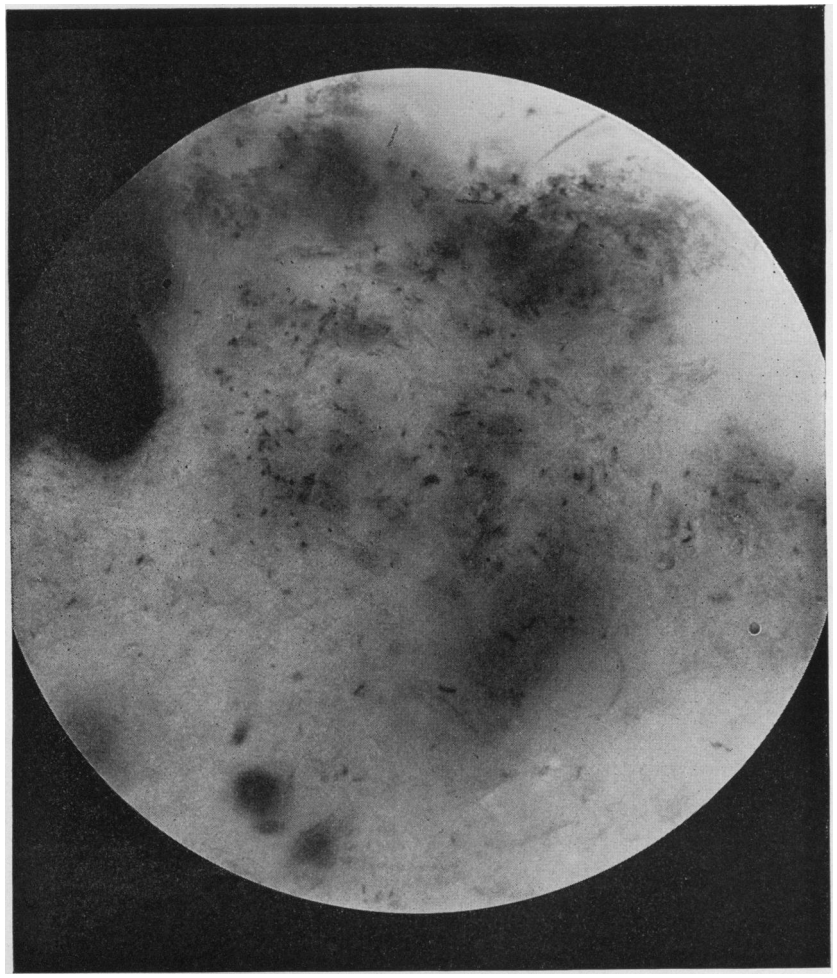


FIG. 3

(Mudd: Penetration of bacteria.)



(Mudd: Penetration of bacteria.)