

other mollusks, such as the oyster and clams, which have similar free-swimming larvae, for these survived in unusual numbers the same year.

The presence of even a few adult teredos in piling or in other submerged wooden structures may thus lead to a repetition of this unusual behavior whenever the environmental conditions are such as to favor the survival of their innumerable progeny, provided there is not sufficient wood in the areas to which the pelagic larvae are carried by the currents in the water.

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### THE EFFECTS OF MEDIA ON BACTERIAL FILTRABILITY

THE fact that Seastone and Lawrence<sup>1</sup> were unable to duplicate Kendall's filtration experiments on the Rawlins strain of *B. typhosus*, even with "K" medium made from "K" powder supplied by him, indicates the probable existence, in this work, of one or more neglected but variable factors. This is further evidenced by the statement of Seastone and Lawrence that their "K" medium showed no uniformity of pH from tube to tube, and also that it occasionally developed a spontaneous turbidity without inoculation.

I query whether bacteriologists, in carrying out filtration experiments, consider or give due weight to the following physico-chemical or colloid factors operative in their media, even when using identical filters; for variations in the filters themselves involve a series of additional factors, based on the specific attractions between filter constituents, bacteria and media constituents:

- (1) Colloids may favor the passage of fine particles through filters.
- (2) Variation in bacterial deformability may affect filtrability.
- (3) The flocculative or deflocculative power of the medium, at the time of filtration, must be considered.

At a symposium on filtration held by the American Society of Bacteriologists at Philadelphia in December, 1926, I heard no reference to factors (1) and (2), and felt constrained to draw attention to them in the open discussion and later on in *SCIENCE* for February 25, 1927.

Factor (1) was dealt with by R. Zsigmondy ("Colloids and the Ultramicroscope" [1909], Chapter 14 on Filtration Experiments), and the fact that moistening the gut with bile increases its permeability to some products of digestion is known to physiolo-

<sup>1</sup> *SCIENCE*, 77: 259, 1933.

gists. Factor (2) was pointed out by Bechhold and Neuschloss,<sup>2</sup> in connection with work on lecithin emulsions where the individual droplets, several  $\mu$  in diameter, under a pressure above 150 g./cm<sup>2</sup>, passed through an ultrafilter which completely retained hemoglobin, and whose pores were less than 30 m $\mu$  in diameter. Bechhold's explanation is that the lecithin passes the filter-pores in filiform fashion, and reforms droplets after its exit.<sup>3</sup> Apart from the stage of growth of the inoculum, and the relative growth-producing quality of the medium for the bacteria, both of which affect bacterial size, it is not impossible that changes in the medium (and pH is only one factor) may affect the *turgidity* of the bacteria present, and therefore their filtrability under constant pressure.

As to factor (3), the protective or coagulative action of any medium is the summation of various factors, including the specific colloids present. Filtration conditions are affected by pH, salts, temperature, cumulative protective relations, etc. There is an extensive literature on the wide variation in the protective action of colloids, especially of albumins, albumoses and their fractions, toward colloidal gold ("gold number"). Some fractions, instead of being protectors, are active coagulators.<sup>4</sup>

On first reading of Kendall's results,<sup>5</sup> it seemed possible that they might, in part, be accounted for by some or all of the factors above stated. Through the kindness of Dr. L. W. Famulener and his staff at the Pathological Laboratory of St. Luke's Hospital (New York), I was able to have made some preliminary tests on the relative protective behavior of three bacterial media, obtained through courtesy of the New York City Board of Health.

On March 24, 1932, samples of beef broth, veal broth and "K" medium were subjected to the "colloidal gold reaction," according to the technique described by Karl M. Vogel.<sup>6</sup>

The results were:

Beef broth .....	5	5	0	0	0	0	0	0	0	0
Veal broth .....	0	0	0	0	0	0	0	0	0	0
"K" medium .....	0	0	0	0	0	½	1	2	2	1

Here 0 represents satisfactory protection, 5 represents complete coagulation and precipitation of the gold, and the intermediate numbers represent varying degrees of aggregation of the gold ultramicros. The figures in the first column are for the original concentrations; those in subsequent columns are the results for progressively doubled dilutions.

<sup>2</sup> *Kolloid Zeitschrift*, 1921.

<sup>3</sup> H. Bechhold, "Colloids in Biology and Medicine," 1920.

<sup>4</sup> Zsigmondy, *lib. cit.*, pp. 79-89.

<sup>5</sup> *SCIENCE*, 75: 295-301, 1932.

<sup>6</sup> *Arch. Int. Med.*, 22: 496-516, 1918.

While these results are no criterion for the protective action of media wherein bacteria have grown, towards these same bacteria, they do indicate that media may exhibit wide differences in "gold number," which often parallels protective and deflocculative action, as well as the ability to aid the passage of particles through filters. Thus, Hans Zinsser<sup>7</sup> reported that Ward and Tang, in his laboratory, found that the agents of vaccine and herpes would pass through a filter more readily when suspended in certain types of broth than when suspended in isotonic salt solutions, irrespective of pH; further, that Grinnell found that *B. prodigiosus* passed through all their "V" Berkefeld and Mandler filters, and through most of grade "N," if taken from old cultures and suspended in hormone broth of about pH 7.8.

Speaking of filtrable viruses, T. M. Rivers stated:<sup>8</sup> "Methods of filtration are crude and inaccurate, and the most any one can say concerning viruses is that under given experimental conditions they either pass or do not pass through certain filters. The failure to pass through a filter, however, is certainly not determined in every instance by the size of the virus."<sup>8</sup>

There are, no doubt, many other factors to be considered, besides the three above suggested. Thus, discrete particles in the medium might adsorb otherwise filtrable particles; or oppositely charged particles might form with bacteria a non-filtrable coagulum or union. As pointed out by May Annetts,<sup>9</sup> changes in conductivity, pH and stability may be expected to accompany the filtration of suspensoid sols. Filters described as "certain" may prove to be very uncertain, under special conditions. There is a wide call for bacteriologists, suitably equipped, to give proper recognition and evaluation to all these physico-chemical factors, while attempting to establish exact technique for reproducible filtration experiments.

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#### A TEST FOR THE PRESENCE OF NOVOCaine IN NERVOUS TISSUE

DURING the course of some research work upon spinal anesthesia, it became desirable to determine the degree of penetration of novocaine solution into the substance of the spinal nerve roots, spinal cord and medulla, when injected into the subarachnoid space of live animals. At first we attempted to follow the course of the novocaine by adding a dye (methylene blue) to the novocaine solution and checking the distribution of the color post-mortem. The dye was

effective in coloring the surface of the nervous tissue and membranes but, on section, none was found within the substance of the nervous tissue. If the extension of the dye were an indication of the extension of the novocaine itself, the failure of the color to penetrate the nervous substance was not in accord with the production of anesthesia and, in some cases, the death of the animal. That the novocaine penetrated further into the nervous tissue than the dye with which it was in solution was the inevitable conclusion. To obtain direct evidence of this as well as to determine the exact distribution within the nervous tissue, a method of recognizing novocaine within the substance of the latter was sought. It occurred to one of us (Beber) that the novocaine might be diazotized, and a color reaction obtained with beta naphthol.

The method evolved is briefly as follows: The nervous tissue to be tested is placed in a beaker of cold 5 per cent. sodium nitrite solution. After a few minutes hydrochloric acid (1:10) is added in the proportion of one of the latter to five of the former solution. This liberates nitrous acid, which in turn diazotizes the novocaine. It is essential that the solution be kept cold for this reaction. After five minutes, the specimens are washed in distilled water and transferred to a 5 per cent. alcoholic solution of beta naphthol. The tissue containing novocaine takes on an orange red color which is greatly intensified by transferring to a weak solution (2 per cent.) of sodium hydroxide. The color fades if placed in water or carried through the usual solutions used in preparing paraffin sections. For section work it is found best after bringing out the color reaction to fix the tissue in 10 to 20 per cent. formalin for fifteen to twenty minutes, freeze and cut thick (fifty  $\mu$ ).

Control experiments, in which the nervous tissue was either treated with distilled water instead of novocaine or not treated at all, failed to produce a similar color reaction when subjected to the same process.

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#### SPONGE SPICULES FROM THE LOWER ORDOVICIAN OF WISCONSIN

WHILE examining insoluble residues from the Oneota dolomite (Lower Ordovician) of Wisconsin the writer came upon numerous detached sponge spicules from one locality. The location in question is on U. S. Highway 12, about three or four miles south of Springfield Corners, Wisconsin. Although the writer has examined several hundred samples of the Oneota from numerous localities in the Upper

<sup>7</sup> SCIENCE, 75: 257, 1932.

<sup>8</sup> Jordan and Falk, "The Newer Knowledge in Bacteriology and Immunology" (1927), p. 519; see also S. P. Kraemer, *ibid.*, 557.

<sup>9</sup> *Phys. Chem.*, 36: 2939, 1932.