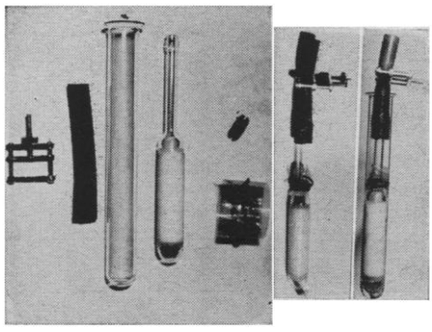

Fig. 1 (left). Materials for the ultrafiltration apparatus. (Left to right) Clamp, vacuum tubing, test tube, fritted immersion filter, grommet, dialysis tubing, and rubber band. Fig. 2 (right). Ultrafiltration apparatus (left) assembled, showing how the rubber band is used to seal the dialysis bag to the grommet, and (right) complete with close-fitting test tube; 2 ml fills the tube to the level of the grommet.

positioned as close as possible to the fritted portion of the immersion filter (Thomas, No. 5151-C; fritted length, 2 in.). The moistened dialysis tubing (diameter when inflated, 5/8 in.) is formed into a bag by tying a knot in one end and is gently pulled over the moistened filter and grommet. A rubber band is wrapped around the bag two or three times, forcing the dialysis tubing into the groove of the grommet to provide a vacuum-tight seal (see Fig. 2, left).

The assembly is then evacuated with a water aspirator and sealed with the clamp. Wetting the entire bag during evacuation helps to obtain maximum vacuum. The material to be concentrated or dialyzed is placed in a test tube of a size determined by the volume of liquid. A thin-wall test tube (Thomas, No. 9444; 150 × 18 mm) of 18-mm outside diameter is suitable for 1 or 2 ml of liquid. If proteins are to be concentrated, the completed assembly (Fig. 2, right) is placed in the refrigerator. In about 2 hours 75 percent of the liquid will be inside the bag. It can be removed by inserting an 8-inch length of thin plastic tubing (Adams, intramedic No. PE 205) and withdrawing the liquid into a syringe (fitted with a 16-gauge needle for use with the designated tubing). The assembly can be re-used if it is stored in water to prevent drying out of the bags.

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8 March 1962

Defect in Small Millipore Filters Disclosed by New Technique for Isolating Oral Treponemes

Abstract. Three Millipore filters (100, 50, and 10 mμ) can effectively and easily be used to isolate oral spirochetes from a mixed oral inoculum. The spirochetes appear pure in the underlying agar, owing to their ability to grow through the filter. Their passage through the 10-mμ filters may be attributed to the instability of this pore size on storage.

Strains of spirochetes will filter or grow through porcelain, paper, and collodion filters into underlying agar or broth media (*1*). The pore size of these filters was sufficiently large to permit passage of the slender, motile spirochetes through the filter (*2*). Membrane filters with more uniform pore sizes (Millipore Filter Corporation, Bedford, Mass.) have been used by us in experiments intended to separate oral treponemes from a mixed oral inoculum, and to determine the smallest pore size that would permit passage of the spirochetes (*3*).

The 0.45- and 0.3-μ filters were sterilized by autoclaving at 15 pounds for 15 minutes; the 100-, 50- and 10-mμ filters were sterilized by high voltage irradiation (MF types HA, PH, VC, VM, VF-white-plain-47 mm) before purchase. The filters were placed on the surface of PPLO medium (Baltimore Biological Laboratories) containing 1.2 percent agar, with sterile forceps, touching only one peripheral area of the filter. The inoculated filter plates were incubated in the upright position, anaerobically, in Brewer jars, in an atmosphere of 95 percent H_2 and 5 percent CO_2 at 37°C for a 5- to 8-day period. A drying agent, $CaCl_2$, was placed in each jar to remove excess water.

For primary isolation of spirochetes, freshly obtained debris from the gingival crevice of man was used. This mixed inoculum was collected in tubes containing 2 ml of PPLO broth or phosphate buffer (*p*H 7.0) and dispersed on a Vortex mixer. It was inoculated with a Pasteur pipette as approximately 0.05-ml drops on the filter surface within 30 minutes after collection. Oral bacteria, as well as spirochetes, were found beneath 0.3- and 0.45-μ filters, demonstrating the inadequacy of these pore sizes in the mechanical separation of treponemes from a mixed oral inoculum. When filters measuring 100, 50 and 10 mμ were used, spirochetes appeared as multiple foci of dense growth beneath most inoculation sites (Table 1). Occasionally, a Gram-positive coccus or rod was recovered from beneath the 100-mμ filter. These contaminants on subculture were capable of growing into the agar medium. When the oral inoculum contained swarming organisms, they would cover the entire surface of the filter and adjacent agar but would not migrate under the filter from the periphery.

A laboratory strain of *Treponema microdentium* with a diameter consistently greater than 100 mμ as determined by electron photomicrographs was employed to study this grow-through phenomenon in greater depth. This particular strain grows on most commercially available media when in association with an anaerobic diphtheroid (strain JB3B) and a fusiform (strain JF_5) (*4*).

This combination was prepared for inoculation by removing agar, containing spirochetes and supporting organisms, from 5-to-10-day-old well plates with a sterile scalpel. This material was introduced into a glass tissue grinder and homogenized with 3 ml of PPLO broth. The resulting mixture was placed as single drops from a Pasteur pipette at five or more sites on each filter surface. The spirochetes appeared beneath the 100-, 50-, and 10-mμ filters at most inoculation sites, with results varying with shipments of filters (Table 1).

Experiments were designed to determine whether the spirochetes were growing through the filter or migrating laterally to by-pass the filter. No migration of bacteria or spirochetes beyond the edge of the filter and back under it to the site of spirochetal proliferation was ever observed. The hazes continued to appear when the filter borders were sealed with sterile paraffin, or when the filter edge extended beyond the support of the underlying agar. The pattern of the haze below the filter corresponded to the outline of the inoculum on the filter surface. When spirochetes and sup-

Table 1. Passage of spirochetes through millipore filters. Data are given in terms of haze/inoculation site.

100-mμ filter (VC)	50-mμ filter (VM)	10-mμ filter (VF)
	Oral debris	
12/34*	2/10	8/12
15/15*		
	Treponema microdentium†	
4/21*	55/55*	106/112*
117/117*	1/45*	17/140*
90/90*	0/20*	

* Results obtained with different shipments of filters. † In conjunction with supporting organisms, JB3B and JF5.

porters were inoculated in the center of the filter, and when supporters alone were inoculated along the filter periphery, spirochetal growth occurred only under the center inoculation site.

It was conceivable that the vacuum drawn in the anaerobic technique might disrupt the filters. When anaerobiosis was established by flushing with N_2 and CO_2 without the use of a vacuum, typical hazes were found beneath the filter. Nonirradiated, nonsterile filters could be substituted for the sterile filters without affecting the ability of the spirochetes to pass through them.

Ten-millimicron filters which had permitted the passage of *Treponema microdentium* were returned to the Millipore Filter Corporation for evaluation. They found these filters to be defective (*5*). They reported retesting their own stock supplies of 10-mμ filters and detecting an alteration in their pore size. They stated that the pores in this filter measure 10 mμ $\pm$ 20 percent immediately after manufacture, but the pore size enlarges during storage. They found no demonstrable alteration of stock supplies of 100- and 50-mμ filters. They advised us that steps are being taken to assure the manufacture of a stable 10-mμ filter.

We believe this defect was primarily responsible for spirochetal passage through the 10-mμ filter. However, with the 100- and 50-mμ filters, the possibility exists that the absence of a rigid cell wall in the ordinary bacterial sense could permit the spirochetes to penetrate through pores significantly smaller than the organism's normal diameter, or an L-type form might traverse the narrow pores.

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21 May 1962

Jamin and "True" Meniscal Resistances: A Single Meniscus Apparatus

Abstract. Meniscal resistance to movement of aqueous liquids in glass capillary tubes is due either to soiled walls or to dissolved surface-active agents. With the latter there are sharp maxima at concentrations of ¼ to ½ gram per liter. Blood serum and plasma exhibit significant "true" meniscal resistance.

Jamin reported in 1860 that a chain of droplets in a fine tube could withstand a finite force (*1*). Smith and Crane showed that this resistance is absent in clean tubes filled with pure water (*2*). But Calderwood *et al.* found it present under most ordinary circumstances (*3*). The resistance in moving menisci has been attributed to surface tension by Barr (*4*) and others. It appears that all these authors were occupied with resistance due to soiled walls.

The instrument shown in Fig. 1 was devised to observe single menisci. It avoids (i) the combination of advancing and receding menisci and (ii) the effects of varying bubble length that complicate work with bubbles in a capillary. The device consists of a horizontally placed precision bore (0.25 mm) glass capillary tube *A* about 180 mm long, bent and flared to join larger tubes as shown. The vertical reservoir *B* is about 25 mm wide and 150 mm high. Tube *F* and diaphragm stopper *C* allow liquid to be added or removed by hypodermic needle and syringe.

During operation the liquid level in the reservoir *B* is adjusted to put the capillary meniscus in tube *A* at rest in the middle of the capillary. Then the capillary attraction of the meniscus in *A* is precisely balanced by a hydrostatic force and the meniscus is free to move in either direction in response to small forces.

Two or more controlled air pressures are applied alternately to *D* and *E*, and the movements of the meniscus back and forth over a 10-cm course are timed. To assure constant speed over the timed course, a run of 3 cm is always made before the meniscus reaches the starting mark.

When there is little or no meniscal resistance the rate-pressure plots are linear and cross the origin. The advancing and receding menisci move at identical rates at a given pressure.

When meniscal resistance is present the meniscus no longer responds to

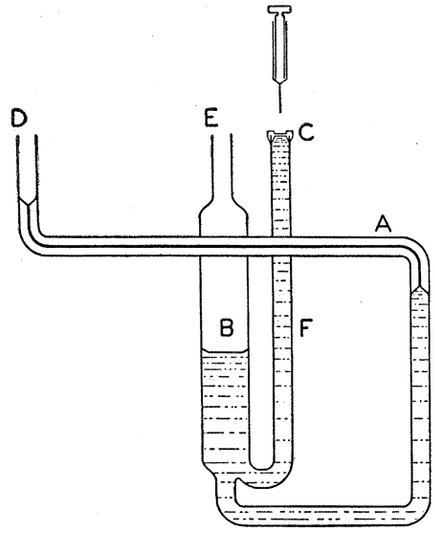

Fig. 1. Apparatus for single menisci.

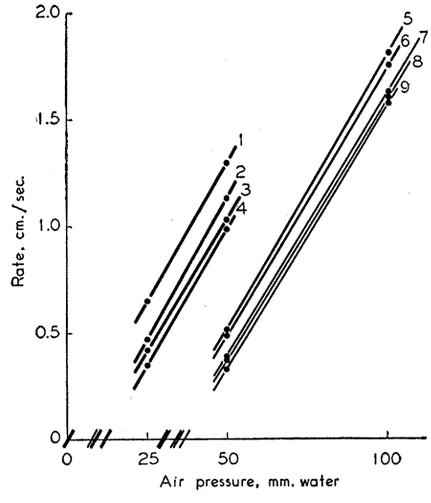

Fig. 2. Flow lines for water and eight aqueous solutions of surface active agents, all at 1 g/1: *1*, water; *2*, hexyl resorcinol; *3*, nonyl phenol 20 EO; *4*, Aerosol OT; *5*, dinonyl phenol 20 EO; *6*, Tween 80; *7*, sodium oleate; *8*, ammonium oleate; *9*, Triton A-20.

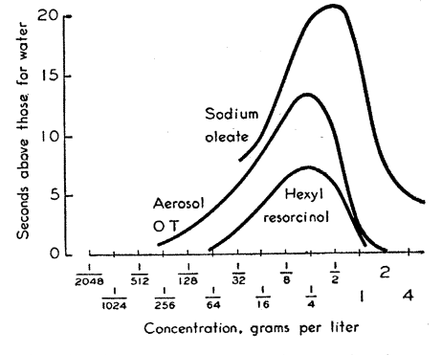

Fig. 3. Maxima in "true" meniscal resistance. Concentrations in grams per liter at which maxima occur. Time in seconds above those required for water alone (7.8 sec). Temperature, 37°C; capillary, 0.25 mm; pressure, 50 mm of water.