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# The Removal of Entamoeba Histolytica Cysts from Water by Porous Filter Septums Either with or without Filter Aid

by

E. Robert Baumann

Harold E. Babbitt

UNIVERSITY OF ILLINOIS BULLETIN

### A REPORT OF AN INVESTIGATION

Conducted by THE ENGINEERING EXPERIMENT STATION UNIVERSITY OF ILLINOIS

In Cooperation with

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# The Removal of Entamoeba Histolytica Cysts from Water by Porous Filter Septums Either with or without Filter Aid

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## I. INTRODUCTION

## 1. Purpose of the Investigation

The purpose of this investigation has been primarily the discovery or development of a filter septum that will remove the cysts of Entamoeba histolytica from water. Sand filters described by Black and Spaulding<sup>(1)\*</sup> were used by the United States Army during the early years of World War II. In an investigation<sup>(2)</sup> of these filters made at the Engineer Research and Development Laboratories at Ft. Belvoir, Virginia, sand filters were shown to be unsatisfactory in removing amoebic cysts at the rates of filtration normally employed in the field. That investigation led to the development by the Army of filters using a porous septum with a precoat of diatomite. It has been shown that such filters will remove amoebic cysts from water when operated under carefully controlled conditions. However, if these filters are operated under conditions in which the precoat is not formed completely or is absent, the filters will not remove amoebic cysts and the filtered water supply may be contaminated. Cyst removal is effected by these filters only by the successful formation of a precoat on the filter septum. Studies were started at the University of Illinois in June 1947 under a cooperative agreement with the Engineer Research and Development Laboratories at Ft. Belvoir, Virginia, one purpose of which was to offset this objectionable feature of diatomite filters.

At present, in the use of diatomite filters for water, filter aids are intended to remove turbidity and cysts, and chlorine sterilization to destroy pathogenic bacteria. Since the cysts of *Entamoeba histolytica* are not destroyed by the dosages of chemicals in normal use for potable water supplies,<sup>(3)</sup> they must therefore be removed by the filtration process. Filter aids normally used in precoat formation on porous septums remove cysts, ranging in size from 5 to 25 microns, together with coagulated material and bacteria of much smaller sizes. It is generally believed that amoebic cysts smaller than about 9 microns are not pathogenic.<sup>(4, 5)</sup>

\* Parenthesized superscripts refer to correspondingly numbered entries in the Bibliography.

A decided improvement in filtration apparatus would result if a filter septum were developed which, without a filter aid, would remove amoebic cysts and would allow long filter runs. A septum providing permanent protection against the passage of amoebic cysts would permit the use of the precoat material in its proper role as a filter aid for the lengthening of filter runs by the entrapment of cysts and particles of turbidity. In a normal filter run the filter aid, used effectively, will remove all of the cysts and turbidity and a very large proportion of the bacteria and other smaller particles. The harmless particles of small size which pass through the filter aid will also pass through the filter septum with no decrease in the length of filter run. If the improved septum were developed, the occasional cyst which might reach the septum due to a failure of the filter-aid precoat would be retained by the septum. In this manner, the septum would serve as a filter for the removal of cysts only in the event of the failure or absence of the precoat. The reduced length of filter run resulting would serve as a warning that a breakdown of the precoat had occurred, yet the filtrate would be protected against cyst contamination.

Objections to the use of septums with pore sizes small enough to remove small organisms such as pathogenic amoebic cysts have been based on the large head losses involved with corresponding decrease in filter capacity, on the structural weakness of the septum materials, and on the relatively great weight of the septum. Some of these objections result from attempts to utilize septums with pores small enough to remove all bacteria from water. The objective of the tests made in this investigation was to find a porous medium that would remove all pathogenic amoebic cysts, the problem being simplified to some extent by the size differential between bacteria and pathogenic amoebic cysts. The largest dimension of a bacterial cell usually will not exceed 3 microns, whereas cysts which are pathogenic to man range in size from 9 to 10 microns upwards. Kudo<sup>(4)</sup> indicates that there are various races in E. histolytica, some of which are pathogenic and some of which are not. Since 1918, various investigators have concluded that there is one small race of the organism which has only a weak capacity for invading the intestinal wall and thus is not pathogenic to man. In 1942, Sapero, Hakansson, and Louttit<sup>(5)</sup> reported two races which can be distinguished by the diameter of the cysts. The dividing line was set between 10 microns and 9 microns in living and balsam-mounted specimens respectively. The race with large cysts gives rise to trophozoites which are more actively motile, ingest erythrocytes, culture easily, and are pathogenic to man. The race with cysts below 10 microns in diameter develops into less actively motile amoeba which do not ingest erythrocytes, are difficult to cultivate, and are not pathogenic to hosts. This discovery alone is important in determining the type of material that may be used in a filter septum.

#### 2. Sponsors

On June 1, 1947, a contract was completed between the War Department, Water Supply Branch of the Engineer Research and Development Laboratories (hereinafter referred to as E. R. D. L.), the Engineer Center, Ft. Belvoir, Va., and the University of Illinois, under the terms of which the University was to conduct studies on the filtration of water through permeable septum media. One portion of the studies pertained to the determination of the "type and grade of filter media required for the removal of amoebic cysts from water and the corresponding hydraulic and physical properties of such materials in the form of filter septum elements." The contract and the work were completed on July 31, 1950.

#### 3. Work of Other Investigators

A review of the literature relative to apparatus and procedures used in the experimental determination of the removal of amoebic cysts by filtration indicated that the most recent work was that reported by the E. R. D. L.<sup>(1)</sup> The Water Supply Branch of the E. R. D. L., which is responsible for the development of water supply equipment for the Army, was authorized by the War Department in March 1943 to study the effectiveness of Army purification equipment in removing cysts of *E. histolytica* from water.

The tests were conducted at the E. R. D. L. by introducing a predetermined number of cysts, cultivated in a laboratory, into the raw water, passing this water through the filter, and collecting samples of the effluent at regular intervals for examination under a microscope. When preliminary treatment consisting of coagulation and sedimentation was employed, a uniform suspension of cysts was obtained in the water to be treated by introducing the organism into the water at a constant rate during the period that the settling tank was being filled.

In the E. R. D. L. tests, cultures of the NRS strain of E. histolytica obtained originally from monkeys were prepared and maintained. They averaged about 15.2 microns in size, the size that covers the pathogenic strains of E. histolytica. "To procure the cvst, the amoebae, maintained on egg slant cultures with an overlay of Stone's modification of Locke's solution, were transferred to a similar medium containing rice starch. After 72 hours of incubation with this medium, the cysts were harvested, washed with distilled water, and stored in the refrigerator at least 24 hours before use. Estimates of the numbers of cysts were made with the use of a Fuchs-Rosenthal counting chamber, while the identification and counting of the cysts present in the effluent samples were made in a Sedgwick-Rafter counting cell."<sup>(2)</sup>

In general, the methods used at the E. R. D. L. for the examination of water samples for cysts were as follows: Samples of the effluent waters were allowed to settle in a cool place for 18 to 24 hr. The supernatant was then gently siphoned off and the residue was placed in a smaller bottle. Ammonium alum and soda ash were added to these bottles which were left in a cool room overnight. The supernatant was again siphoned off and the residue placed in 50 cc centrifuge tubes and centrifuged. The sediment from each tube was again concentrated in a centrifuge. One or two ml of M/10 oxalic acid were added to redissolve the coagulum and the volume was made up to 5 or 10 cc with distilled water. One-cc samples from these tubes were mixed with one drop of 1 percent iodine in a Sedgwick-Rafter counting cell, and the number of cysts in the sample was determined using a microscope.

The E. R. D. L. personnel concluded that a combination pretreatment, sedimentation, and filtration gave considerably better cyst removal than filtration alone. However, when the U. S. Army Portable Water Purification Unit (sand filter) was operated at flow rates practical for field use, the complete removal of *E. histolytica* cysts was not accomplished. One series of tests was made with a Portable Army Water Filtering Unit that had the sand replaced by a diatomite filtering medium. The tests demonstrated that this equipment, properly operated, could be used to effect complete removal of amoebic cysts. By 1944 the Engineer Board had developed a practical portable water filter using diatomite on the basis of the earlier experiments.<sup>(1)</sup> This was a pressure-type precoat filter which, when operated with pretreated water, gave an effluent with less than 0.1 ppm turbidity, better than 90 percent bacterial removal, and complete amoebic cyst removal.

Research by other investigators on the problem of cyst removal by filtration is limited. Brady, Jones, and Newton<sup>(3)</sup> showed that dosages of chlorine used in water works practice do not necessarily destroy the cysts of E. hystolytica. Baylis, Gullan, and Spector<sup>(6)</sup> demonstrated that rapid sand filters operated at flow rates of 2 gpm per sq ft would effectively remove 99.99 percent of applied cysts. The cysts used in their tests were obtained by making up water suspensions of cysts from feces of persons infected with the disease. All cyst determinations were made by counting iodinestained organisms in a Sedgwick-Rafter counting cell. Sedimentation and centrifuge methods were used to concentrate the organisms from the large effluent volumes.

#### 4. Acknowledgments

The authors wish to express their appreciation to the Water Supply Branch of the Engineer Research and Development Laboratories and to the Engineering Experiment Station of the University of Illinois, who made it possible to conduct the investigation. The authors are indebted to Harry N. Lowe, Jr. and Charles H. Spaulding of the E. R. D. L. for their suggestions and encouragement during the progress of these tests.

The test program was planned and executed under the direction of H. E. Babbitt, Professor of Sanitary Engineering, and was conducted under the supervision of E. R. Baumann, Research Associate in Civil Engineering. The cyst filtration tests and pore size analyses were made by Baumann and H. F. Zobel, Research Assistant in Civil Engineering.

The cultivation of the trophozoites of Entamoeba histolytica and the production of cysts was originally undertaken in 1948 by Murray Wittner, graduate student in protozoology. With the advice and encouragement of Dr. R. R. Kudo, Professor of Zoology at the University, and of Dr. William S. Balamuth, Professor of Zoology at Northwestern University, Wittner was able to set up laboratory procedures and techniques for the successful production of cysts and for the concentration, identification, and counting of cysts in water samples. Dr. Balamuth was particularly helpful in providing our laboratory with unpublished details of his methods of cyst production. Wittner was succeeded by Sidney Kantor, also a graduate student in protozoology. In addition to the determination of the effectiveness of filter septums in removing cysts, the graduate protozoologists conducted other studies pertaining to the causes of encystment and the viability of filtered cysts.

### **II. PRECOAT FILTERS**

#### 5. Description

Figure 1 shows a diagram of a typical constantrate, diatomite filter equipped for air-bump backwash, precoat and continuous filter-aid body feed. The apparatus consists essentially of a filter housing containing a filter septum or filter septums upon which the filter aid is deposited, a centrifugal pump, an unfiltered water tank, a slurry-mixing tank, and a filtered-water storage reservoir.

The filter septum, which is one of the chief components of a precoat filter, is of importance because its design can affect its ability to be precoated and backwashed. Filter septums are usually designed to serve only as a supporting medium for the filter-aid precoat which serves as the filter. This wrapped with wire, or of porous septums of metal, carbon, or Aloxite.

Two different terms, precoat and body coat, are used to refer to the filter-aid material used in filtration. Precoat filter aid refers to the filter aid applied initially to the septum before the start of filtration. It serves as the filtering medium during the period of filtration. Body coat refers to the filter aid which is added continuously to the raw water during filtration. It is caught by the precoat and serves to maintain a porous layer of filter aid and removed-solids around the precoat, thus prolonging the filtering cycle.

Two methods are used in the operation of precoat filters. They are, respectively, constant-rate



Fig. 1. Schematic Diagram of Typical Diatomite Filter Apparatus

is the case with such septums as the Aloxite cylinder, the Stellar, wire-wrapped septum, and the Johnson, well-screen septum. Other filter septums such as the diatomite filter candle and cellulose filters are disposable septums designed to serve as the filter for the removal of all solids which are to be removed. Disposable septums are thrown away after they no longer filter water at the desired rate of filtration. Filter aids, however, may also be used in conjunction with disposable septums to provide longer filter life. Filter septums which serve only as a supporting medium are better suited for water filtration. They usually consist of a plastic or metal core wrapped with metal screen, or helically and constant-pressure. In a constant-rate cycle, a predetermined minimum rate of filtration is set, usually from 1 to 6 gpm per sq ft of filter surface area. The filtration is continued at a constant rate until the filtration rate begins to decrease when the head loss through the filter cake reaches the maximum head available. The filter is then backwashed, reprecoated, and filtration is resumed.

In a constant-pressure cycle, the pressure-drop across the filter is maintained approximately constant, and the rate of filtration on the filter is allowed to drop off as the filter becomes clogged. Whenever the flow drops to a predetermined minimum rate, a new cycle is started. Precoat filter aid may be applied to the filter either with or without circulation of the effluent. With circulation preceding the start of a run, the required amount of filter aid, usually between 0.10 and 0.15 psf of filter septum surface, is mixed with filtered water in the slurry-mixing tank and is pumped through the filter and back to the slurry tank. The filter aid is retained on the septum. The circulation is continued until the precoat is formed and the effluent runs clear. Then, the raw water is drawn from the raw-water storage tank, and filter effluent is diverted to the filtered-water storage.

Precoat application without circulation is accomplished by placing the required amount of filter aid in the bottom of the filter housing and starting the filtration cycle. The filtrate is wasted for 2 or 3 minutes until the precoat is completely in place.

Body-coat filter aid is added to the raw water with special positive-displacement slurry feeders or with other apparatus. In the apparatus in Fig. 1, the body-coat filter aid may be mixed with filtered water in the slurry-mixing tank and added at the suction of the main pump.

At the end of a filtration cycle, the used filter cake consisting of precoat, body-coat, and impurities removed from the water must be washed from the septum in order to start a new cycle. The septum backwashing, as the operation is termed, is usually accomplished by the back-flow of water through the septum in a direction reverse to that indicated in Fig. 1. This operation may be accomplished in many ways, for example as by the use of pumps or compressed air.

#### 6. Commercial Septums

Filter septums used in industrial processes for precoat filtration differ in size, shape, area, and filtering capacity. Generally, the filter septum is used as a supporting frame for the filter aid, which is the filtering medium. In some cases, however, the septum acts as the filtering medium and no filter aid is used. In either case, the size of particle retained by a filter septum, whether the particle be filter aid, suspended matter, bacteria, or cyst, is determined largely by the pore diameter of the septum. Types of filter septums used in this study are listed below.

#### SEPTUMS AVAILABLE FOR STUDY

Septum 1. Mandler filtering cylinder #45061D, Mandler diatomaceous regular, a porous cylinder made from the purest diatomaceous earth of United States production and fitted with a metallic headpiece with lock nut, two rubber washers, one fiber washer, and nipple. Tested for 6 to 9 lb air pressure, height 8 in., diameter 1 in., length of nipple 2 in., diameter of nipple  $\frac{5}{16}$  in.

Septum 2. Mandler filtering cylinder #45062D, Mandler diatomaceous fine. Same as Septum 1, except for porosity. Tested for 10 to 16 lb air pressure.

Septum 3. Mandler filtering cylinder #45063D, Mandler diatomaceous, preliminary. Same as Septum 1, except for porosity. To be used in preliminary filtrations.

Septum 4. "Aloxite" porous tube, O. D. 3 in., I. D. 2 in., length 24 in., open ends, Grade 5 (Carborundum Company), (old style material).

The permeability or "Grade" of a 1-in. thick Aloxite septum represents "that quantity of free air, in cubic feet per minute, which will pass through 1 sq ft of area of tube, when tested dry at  $70^{\circ}$  F, under a pressure equivalent to 2 inches of water."

Septum 5. Same as 4 except Grade 25. (Old style material.)

Septum 6. "Aloxite" porous tube, O. D. 3 in., I. D. 2 in., length 13 in., open ends, Grade 40 (Carborundum Company), (old style material). See Fig. 2.

Septum 7. Selwyn leaf filter,  $24 \ge 110$  mesh, plain Dutch weave monel,  $0.25 \le q$  ft.

Septum 8. Bowser #611, cellulose septum, filters from both sides, length 30½ in., O. D. 5 in., I. D. 2¾ in., filtering area 6.21 sq ft.



Fig. 2. Typical Filter Septums

Septum 9. Bowser #610, monel metal, filters from both sides, length 35 in., I. D.  $3\frac{1}{2}$  in., O. D.  $4\frac{5}{8}$  in., filtering area 5.15 sq ft.

Septum 10. Nylon filter cloth, nylon 1000, Filter Media Corp., Hamden 14, Conn.

Septum 11. Saran filter cloth, saran 105, Filter Media Corp., Hamden 14, Conn.

Septum 12. Vinyon filter cloth, vinyon 319, Filter Media Corp., Hamden 14, Conn.

Septum 13. Wire mesh, 50 x 700, chromel, Michigan Wire Cloth Co., Detroit 16, Michigan.

Septum 14. Wire mesh, 24 to 110, brass, Michigan Wire Cloth Co., Detroit 16, Michigan.

Septum 15. Rubber filter media, style 601, pores per sq in. 3200, average diameter of pores .005 in. to .006 in., approximate percent of voids 5 to 7, thickness of material 0.07 in., Filter Media Corp., Hamden 14, Conn.

Septum 16. Allen #2 unglazed porcelain, fine, length 8 in., diameter 1 in., length of nipple 2 in., diameter of nipple  $\frac{5}{16}$  in., tested for 28 psi air pressure.

Septum 17. A-23400, Stellar element, #.65-2.5, ½-in. lucite core with ½-in. holes spaced on ¼-in. centers over entire area, length 12 in., 0.028-in. wire. See Fig. 2.

Septum 18. A-22886, Stellar element, #.78-3,  $\frac{1}{2}$ -in. lucite core with 2 rows of  $\frac{1}{2}$ -in. holes at top, 2 rows at the center and 6 rows at the bottom of the septum, holes spaced  $\frac{1}{4}$ -in. apart, length 12 in., 0.020-in. wire. See Fig. 2.

Septum 19. A-22221, Stellar element, #.52-2.

Septum 20. Wire mesh, 24 x 110, 0.015 in. x 0.010 in., plain Dutch weave monel, Michigan Wire Cloth Company.

Septum 21. Wire mesh, 30 x 150, 0.009 in. x 0.007 in., plain Dutch weave brass, Michigan Wire Cloth Company.

Septum 22. Wire mesh, 40 x 200, 0.007 in. x 0.005 in., plain Dutch weave brass, Michigan Wire Cloth Company.

Septum 23. Wire mesh, 30 x 250, 0.010 in. x 0.008 in., twilled Dutch weave monel, Michigan Wire Cloth Company.

Septum 24. Wire mesh, 20 x 325, 0.007 in. x 0.0065 in., twilled Dutch weave nickel, Michigan Wire Cloth Company.

Septum 25. Wire mesh, 28 x 500, 0.007 in. x 0.0042 in., twilled Dutch weave nickel, Michigan Wire Cloth Company.

Septum 26. Wire mesh, 200 x 200 (#42476), A. S. LaPine Company.

Septum 27. Berkefeld "W" diatomaceous earth filter candle, German make, 8 in. x 1 in. cylindrical, area 0.169 sq ft.

Septum 28. Everpure Water Purifier, model 30T, 150 refill, area 1.0 sq ft, length over-all  $9\%_{16}$  in., diameter 5 in., working pressure 127 psi.

Septum 29. Carbon tube, 1¾ in. O. D. x 5% in. I. D. x ¼ in. long, National porous carbon tubes, blind ends, Grade 60.

Septum 30. Porous stainless steel, 2 in. x 2 in. x ½ in., Grade D, Micro Metallic Corporation, 193 Bradford Street, Brooklyn 7, N. Y.

Septum 31. Same as 30 except Grade E.

Septum 32. Porous stainless steel, cylindrical filters 1½ in. O. D., 1¼ in. I. D., length 85% in., Grade F, Area 0.27 sq ft, Micro Metallic Corporation, 193 Bradford Street, Brooklyn 7, N. Y. Top end connected to filter housing using  $\frac{1}{-1}$  using  $\frac{1}{-1}$  using stainless steel plate. See Fig. 2.

Septum 33. Same as 30 except Grade G.

Septum 34. Same as 30 except Grade H.

Septum 35. Battery plate separator. (Made available to project by Mr. C. H. Spaulding.)

Septum 36. Foam rubber, medium, U. S. Kaylon.

Septum 37. Sparkler filter, model W-5, filter pads "W,"

"Wa," and "Wb," Sparkler Manufacturing Co., Mundelein, Illinois.

Septum 38. Johnson well screen septum, brass, length 12 in., diameter  $2^{11}/_{16}$  in., area 0.705 sq ft.

Septum 39. Filter cloth #929, Filter Media Corporation.

Septum 40. Filter cloth  $\#\,998,$  Filter Media Corporation.

Septum 41. Filter cloth, vinyon 302, National filter cloth.

Septum 42. Filter cloth #204, National glass.

Septum 43. Filter papers, Visking Product Co.

R	a.	R-35-D
	b.	R-45-D
	c.	R-45-S
	d.	R-55-D
	e.	R-55-S
	f.	C-35-D
	g.	C-45-D
	h.	C-55-D
	i.	C-65-D

Viskon

Septum 44. Johnson well screen septum,  $2\frac{1}{2}$  in. O. D. x 12 in. long, 14 interior vertical supports, opening 0.002 in. x about  $\frac{1}{16}$  in., area 0.65 sq ft. See Fig. 2.

Septum 45. Wallace and Tiernan wire wound element, 3 in. O. D. x 13 in. long, lining of crisscross metal mesh with about <sup>1</sup>/<sub>4</sub>-in. openings, area 0.85 sq ft. See Fig. 2.

Septum 46. Purolator element, 3 in. O. D. x 5% in. long, area 0.369 sq ft, fine brass wire mesh for filter aid support, brass cylinder core with perforations, closed lower end, upper end has threaded 1-in. pipe connections. See Fig. 2.

Septum 47. Porous bronze filter tubes; 1½ in. O. D. x 6 in. long, variable areas due to brazing, porosity regular, The Permutit Co., 330 West 42nd Street, New York 18, N. Y. These septums are made of spherical bronze material molded into shape with ½-in. wall thickness. They have a bronze plate on the lower end and ¼-in. pipe connection on the upper ends. See Fig. 2.

Septum 48. Fisher Microfilter, porous bronze tube, 1.27 in. O. D. x 4 in. long, total area 0.111 sq ft, porosity medium, open ends, Oscar Fisher Co., Inc., N. Y. 13, N. Y.

Septum 49. "Aloxite" porous tube,  $2\frac{1}{2}$  in. O. D.,  $1\frac{11}{16}$  in. I. D., variable length, open ends, permeability 5 (new manufacturing process). See Fig. 2.

Septum 50. Same as 49 except permeability 10. See Fig. 2.

Septum 51. Same as 49 except permeability 20. See Fig. 2.

Septum 52. Same as 49 except permeability 60.

Filter septums used in clarifying beer, sugar, vinegar, and glue usually consist of plates or leaftype filters that have a woven cloth for a septum

upon which the diatomite layer is formed. In the filtration of sulphuric acid, sulfite cooking acid, and may other similar substances, filter septums consisting of tubular elements are usually employed. These elements are made of one of the following: hollow plastic cores, helically wound with stainless steel or monel metal wire; carborundum stone; carbon tubes; hollow copper or stainless steel cores wound with copper or monel metal wire; or cylindrical fluted bronze or copper cores around which porous filter pads, cloths, or wires are wrapped. The materials differ with respect to durability, strength, response to backwash and liability to clogging. Septums 20 through 26 in the preceding list are examples of wire cloths used in filters of the leaf type. Septums 10 through 12 are examples of the type of filter pads or cloths used in both the leaf-type and cylindrical-type septums. Septums 4, 5, 6, 8, 9, and 17 are typical cylindrical septums; some of these are illustrated in Fig. 2.

The characteristics of the liquid to be filtered will usually dictate the style of septum to be used. If the liquid is viscous and sticky, a filter press with a leaf-filter septum may be needed because a higher pressure can be obtained economically for a given rate of filtration. On the other hand, if the liquid does not have high viscosity and flows readily, such as water, a cylindrical septum may be more desirable since it offers a larger area in a more compact unit, is structurally stable, relatively easy to manufacture and replace, and is stiffer under variations in pressure. The clothenvelope type of septum, septum 28, attempts to secure large filtering area at relatively low cost, but at a corresponding decrease in durability and ability to withstand large differences in pressure. Bayonet-type filter elements consist of two sheets of filter material welded together along the edges and at one end, and connected to a standard pipe at the other. They are reported by manufacturers to be less expensive than cylinders of equal area, and they can be reinforced to withstand any required external pressure up to 10,000 psi. Starshaped septums, whose multiple surface convolutions present an extremely large area in a compact element, give larger areas more compactly than cylindrical septums. Star-shaped septums reportedly may be reinforced to withstand pressure differentials of 1000 psi more cheaply than can cylindrical elements. One septum manufacturer has developed a cylindrical septum which filters through the inside and outside walls of a double walled cylinder, as in septum 8.

The size or shape of a septum, for all practical purposes, can be controlled in its manufacture. Therefore, if a septum material proves to be successful in accomplishing the desired degree of filtration, it can be formed into any one of the above types. Hence, test runs in this study were conducted to determine efficiencies of materials, not of shapes.

Septums used only as supporting mediums for filter aid have, in general, rather large pore diameters. This is true of cloths, wire mesh, and helically wound elements. Septums used to accomplish filtration without filter aid were made of materials with smaller pore diameters such as porcelain, bonded diatomite, plastic cellulose, porous metal, rubber, carbon, and asbestos. Some of these septums are however seldom, if ever, used in potable water filtration but are confined to specialized industrial processes.

The most interesting and most promising of the septums which may be developed for use without filter aid are the type whose pore diameter is controlled in the manufacturing process. Among these septums are bonded diatomite (septums 1 through 3), carborundum (septums 4 through 6), carbon (septums 29), and porous metal (septums 30 through 34). Aluminum oxide or Aloxite septums are made so that the porosity (ratio of pore volume to total volume of septum) is kept nearly constant regardless of the tube permeability. Coarse-grained tubes have a relatively small number of large pores and fine-grained tubes have a greater number of fine pores.

Porous metal septums are made by cementing together small particles of stainless steel or bronze. The process has been perfected so that average mean pore diameters of from 5 to 60 microns are available. Bonded diatomite septums are also manufactured with various pore diameters.

#### 7. Sand Filters vs. Precoat Filters

Comparative data on sand and diatomite filters are shown in Table 1. The information in the table emphasizes the advantages of the diatomite precoat filter in that its size and weight, for equal capacity, are less than in pressure or gravity sand filters. The advantages of diatomite filters over conventional sand filters are reported, in general, to be

		Compariso	on of Diatomite	and Other Filter	s		
Characteristic	U.S. Army Pack Diatomite Filter*	U.S. Army Pressure Sand Filter*	U.S. Army Mobile Diatomite Filter*	Pressure Filters U.S. Army Pressure Sand Filter*	General Filter Co. Sand Filter† (Vertical)	Proportioneers FAB-28½ Diatomite‡ Filter	Gravity Sand Filter§
Filter Area, ft <sup>2</sup> Capacity, gpm Weight, lb (filter only) Diameter, in. Over-all Height, in. Floor Space, in. or ft	3.6 15.0 50.0 8 22 10" x 10"	7.1 15.0 2860 36 73 37" x 49"	$10.0 \\ 50.0 \\ 350.0 \\ 18 \\ 30 \\ 24'' \ge 24''$	19.6 50 8050 60 82 $61'' \ge 78''$	38.5 77.0 17,800 84 60 8' x 10'	40.0 80.0 475 21 <sup>1</sup> ⁄ <sub>2</sub> 81 30″ x 30″	40 80 24,000 118 90 10' x 10'
Industrial and Engineerin	g Chemistry, Vol.	39, No. 11, p. 141	3, 1947.				

-3-50-G.

General Filter Co., *Filters*, p. 48-F-5, Bulletin 48-F-15 Proportioneers, Pur-O-Cel Filters, p. 7, Bulletin 1800. Estimated.

that they occupy 1/10 of the space and cost considerably less than sand filters.<sup>(7)</sup> Kominek<sup>(8)</sup> reports that the estimated cost of diatomite, power, and wash water for operating diatomite filters would range from a minimum of 1.3 to a maximum of 3.6 cents per 1,000 gal of filtrate; a similar estimate of the cost of operating comparable sand filters gave a minimum cost of 0.20 and a maximum cost of 1.07 cents per 1000 gal. Although the operating costs of diatomite filters would thus be greater than the operating costs of sand filters, the original cost of the diatomite filters installed is usually from 30 to 50 percent less than for the sand filters.(8)

In addition to the savings in space and weight, diatomite filters have the added advantage that they are more effective in removing amoebic cysts and can be operated without preliminary coagulation of the raw water. Although coagulation of the raw water will result in longer filter runs with diatomite filters and is to be recommended where feasible, preliminary coagulation is not required to secure a clear, sparkling, safe filtrate. In most cases, rapid sand filters operated without coagulation would be unsatisfactory. It may be concluded that, in general, in spite of their higher operating costs, evaluation of all of the factors involved may favor the use of a diatomite filtration plant.

## **III. LABORATORY INVESTIGATIONS**

#### 8. Introduction to the Experimental Approach

In order to accomplish the purpose of this investigation, nearly all septums and septum materials available commercially during the period of the research were evaluated and tested. Letters were written to manufacturers and engineers asking for information and assistance in securing a wide range of septums for test. As a result of such correspondence, 52 septums were received and numbered as in Section 6. The septums are listed in the order of their availability to the project.

The study of the types of filter septums was organized by setting up a tabulation of the various qualitative relations probably existing among septums of various degrees of permeability. For convenience, the range of permeability of filter septums was arbitrarily divided, as suggested by Charles H. Spaulding, into five grades progressing from finest to coarsest. See Table 2. Classification of

 Table 2

 Assumed Qualitative Relations of Septums

Reten- tion Grade	Retention	Capacity	$Head \\ Loss$	Function of Filter Aid
1	Perfect, will remove all bacteria	Minimum	Excessive	Retards plugging
2	Sufficient for cyst removal	Low	Moderate	Retards plugging
3	Important but im- perfect	Increased	Reduced	Supplements re- tention and re- tards plugging
4	Inadequate except for coagulated waters	Moderate	Low	Supplements re- tention for raw water, retards plugging for either raw or coagulated
5	Inadequate for any water	Maximum	Insignificant	Sole filter medium

available septums according to this schedule necessitated the development of various test filters and procedures. A study of head losses was made to give the quantitative head-loss relations measured with various septums in grades 1, 2, and 3. This report is concerned, however, chiefly with the tests to classify the various septums in accordance with their retention of bacteria and amoebic cysts.

Almost all of the septums received were first tested to determine their efficiency in removing bacteria from water without the use of filter aids. Cultures of *Serratia marcescens* were used as the water-polluting organisms. Three main objectives were sought in these tests: first, the suitability of the material as a septum; second, techniques to be applied later in the cyst filtration tests; and third, an indication as to whether or not a septum might remove cysts.

Those septums which were found to offer some but imperfect bacterial removal were tested also to determine their efficiency in removing cysts. The results of these tests served to provide data for the classification of septums in retention grade 2 or in higher grades. In view of the effectiveness of some of the porous septums in removing cysts, the study was extended to determine the feasibility of methods of specifying and testing the pore size of septums required to remove cysts.

#### 9. Preliminary Screening Tests

In order to make preliminary laboratory tests of the bacterial filtering efficiencies of various septums, test filter apparatuses, as shown in Figs. 3 and 4, were developed. The apparatus shown in Fig. 3 included a storage tank containing contaminated water connected to an air-pressure line; one or more filter housings; an orifice-piezometer gage; a pressure gage; and a filtrate collection system. A similar setup was used in subsequent cyst filtration tests described in Section 12. The septum to be tested was usually installed in a housing similar to that shown in Fig. 3, but in some cases it was equipped with its own housing, which was fitted into the setup.

Before conducting a test with the preliminary screening apparatus, the water storage tank was filled with distilled water containing 50 ppm of chlorine. This water was allowed to flow through the entire system and was allowed to stand, usually overnight, until the apparatus was sterilized. Immediately before use in a filtration test, the system was then thoroughly flushed with boiled water to remove all traces of chlorine. The septum was sterilized by boiling it under pressure of 15 psi for 15 min and was then carefully installed in the filter housing.



Fig. 3. Bacteriological and Cyst Filtration Apparatus

At the start of a run, the water storage tank was filled with boiled distilled water, and about 300-500 ml of a lactose-broth culture of *Serratia marcescens* was added. This tank was closed and the air pressure regulator was adjusted to provide the desired pressure in it. The contents of the tank were thoroughly mixed by the air bubbling up through the liquid. The needle valve on the filtered-water line was opened, causing raw water to flow from the water storage tank to fill the filter housing and thence to flow through the filter septum.

As soon as filtration commenced, the time and pressure were recorded and a sample of the raw water was taken. In the early tests, the filtrate was collected in a 1000-ml graduate and the time to filter 700 to 800 ml was recorded. The rate of filtration was calculated from this determination. Several filtered samples were collected during the course of a test. Immediately before the test run was concluded, the pressure was again recorded, another raw sample was obtained, the time was recorded, and the total volume of filtrate was noted. As soon as a filtration run had been completed, the entire apparatus was sterilized by running through it water containing 50 ppm of chlorine. This water was allowed to remain in the apparatus until it was used again.

The raw and filtered samples collected were removed to the bacteriological laboratory, and the number of bacteria in each was determined. This was accomplished by making duplicate nutrientagar plate counts of each sample for dilutions ranging from 1:1 to 1:1,000,000. The plates were incubated for 48 hr at a temperature of about 27.5 deg C. The final count was determined by taking the average of the plates showing between 30 and 300 colonies per plate. Only colonies of Serratia marcescens were counted.



The bacterial cultures used in the filtration tests were obtained by inoculating lactose broth with a colony of *Serratia marcescens*. Several cultures were maintained at all times, so that one or more would always be available. No difficulty was experienced in culturing, identifying or counting. *Serratia marcescens* (*Bacillus Prodigiosus*) was used as the measure of filtering efficiencies in all of the bacteriological filtration tests. The organism is a red coccobacterium occurring singly and occasionally



Fig. 4. Cyst Filtration Apparatus

in chains of 5 to 6 elements. It is motile, with four peritrichous flagella. The organisms measure about 0.5 by 1.2 microns and flourish at an optimum temperature of 25-30 deg C. They show no growth at 37 deg C. The size, color, and ease of cultivation and of identification made this organism ideal as a bacterial measure of filtering efficiencies.

#### 10. Life Cycle of Entamoeba Histolytica

The life cycle of E. histolytica is comparatively simple. The formation of infective cysts in the large intestine of man is followed by excretion of the cysts in the feces, their extra-corporeal existence, and their ingestion by a new host.<sup>(9)</sup> E. histolytica is found in feces in three morphological phases: the trophozoic, the precystic, and the cystic.

The trophozoite form ranges in size from 15 to 40 microns in diam and multiplies by binary fission. The variation in size of the amoebae may be due to the fission or to the existence of individual races.<sup>(5)</sup> The amoeba lives normally in the tissues of the intestinal wall of man and brings about characteristic ulceration of the colon which is often accompanied by symptoms of amoebic dysentery. The amoeba may enter other organs through the portal vein and cause an infection referred to as amoebiasis. The organisms have been reported in monkeys, dogs, cats, rats, and hogs, and experimental infections have been established in many other animals. In numerous surveys which have been made to determine the prevalence of E. histolytica in man in various parts of the world, it has been found to be most prevalent in the tropics, sub-tropics, and in countries where sanitary conditions are poor. In the United States, the average incidence is about 10 percent.<sup>(10)</sup>

In some circumstances, the amoebae in the intestines remain small after division. Conditions resulting in small-size cysts are not understood, but they are being studied by many investigators. Such small amoebae are sluggish and are known as the precystic forms. These forms ultimately secrete a resistant wall and become encysted. The cyst form is spherical and measures from 5 to 25 microns in diam. All forms of *E. histolytica* may be found in dysenteric feees, but only cysts are usually found in normal feeal material from an infected person.

The trophozoites voided in feces will perish in a comparatively short time. Even if they are encountered in food or water supplies, the trophozoite will be killed by the normal body juices found in the stomach. The cyst form, however, will survive for a considerable period outside of the body and will pass through the body into the intestines unharmed. There excystment occurs, and a new cycle is started. Thus the dissemination of the infection is carried on exclusively by the cyst.

Viable cysts may be transmitted in many ways, principally by the contamination of food through contact with cyst carriers or with contaminated water, or by the direct ingestion of contaminated water. Normally, the only public water supplies subject to infections are surface water supplies.

#### 11. Cyst Cultivation

The strain of E. histolytica used in this series of experiments had been under cultivation for more than 20 years. It was first isolated<sup>(11)</sup> from a Macaques nemistrinus, a Macaques monkey, on August 3, 1927, by Clifford Dobell at the National Institute for Medical Research in London. It was subsequently cultured and passed through two other species of Macaques. M. rhesus and M. sinicus. The strain finally isolated from the third monkey on April 26, 1929, was designated as the NRS strain, showing that it had passed through the three species of monkey. The NRS strain was found to grow well in test tubes, or in vitro, and to produce cysts fairly readily. It was studied intensively by Dobell<sup>(11)</sup> and was brought to this country where the strain has been maintained by a number of investigators. The original cultures used in these tests were obtained from Dr. William Balamuth of Northwestern University. The organisms were cultured in our laboratory continuously between the spring of 1948 and the summer of 1950. They grew well and could be made to encyst by following the Balamuth method. The cysts used in these tests ranged in size from about 10 to 20 microns with an average diameter of about 14 microns. These cysts are of the larger pathogenic race of E. histolytica.

Stock cultures of trophozoites of *E. histolytica* were maintained in this laboratory in a medium developed especially for the organism by Balamuth.<sup>(12)</sup> The medium is an egg-yolk infusion in physiological saline buffered to a pH of about 7.3 with phosphate. The medium is pale yellow and almost clear when correctly prepared. For cultivation it was found to have the advantages over other media of being easily prepared and of requiring less frequent subculture. The medium also suppresses *Blastocystic*, an intestinal parasite almost invariably found in cultures of intestinal protozoa.

Many investigators have experienced a considerable degree of success in maintaining cultures of trophozoites of *E. histolytica*.<sup>(11-16)</sup> Up to the present, however, little is known of the factors which control encystation *in vitro*. Most investigators have experienced periods in which previously successful methods have failed to produce cysts. Chang,<sup>(17, 18)</sup> for example, even found that the best encystment occurred in different pH ranges of the media at different times. In view of the uncertainties involved in producing successful encystment of *E. histolytica*, special care and techniques were employed at all times to eliminate as many sources of interference as possible. The factors at present believed to be involved in encystation *in vitro* may be grouped under three general headings:

(1) Carbohydrate or carbohydrate derivatives. Carbohydrate is necessary in the medium in some form.<sup>(11, 16, 19 to 21)</sup> Just what immediate role the carbohydrate, usually used in the form of rice starch, plays in encystation is not known. It may be that it is responsible only for enriching the medium so that rapid growth and multiplication takes place. However, the carbohydrate may serve merely as a raw material for some ultimate break-down project produced by the activity of bacteria or of the amoebae themselves which influence the encystment process.

(2) Bacteria. E. histolytica cannot be cultured and caused to encyst without the presence of an associated bacterial flora.<sup>(11, 19, 21, 22, 23)</sup> On the other hand, if a contaminating flora is present, encystation will be inhibited. The bacteria, at the very least, serve as an important source of food for the amoebae in culture. They also, probably, serve to maintain the necessary conditions of oxidation-reduction and pH.<sup>(17, 18, 24)</sup> However, in view of their importance in cultures, it is more than likely that they provide other important factors, possibly nutriment in the form of protein, fat, or carbohydrate break-down products which are the actual encystment factors.

(3) Rapid growth. Most authors agree that the rapid growth of the cultures is a necessary preliminary to encystment.<sup>(17, 18, 19, 24)</sup> It may be that rapid growth is only a concomitant result of the factors leading up to encystment. The use of serums, liver extracts, and infusions possibly serves only to enrich the medium and thus aid in the stimulation of rapid growth and multiplication. Protozoologists are investigating these and other factors to determine their qualitative and quantitative effects on encystment.

Since Entamoeba histolytica is cultured in the presence of certain bacteria, careful bacteriological technique is necessary in handling the cultures to keep extraneous bacteria from entering the culture. A changed bacterial flora in the culture tube usually resulted in a decrease in the amoebae population in that tube. If the amoebae survived at all, the population density would gradually rise in ensuing cultures as the amoebae adjusted to the new flora. Unfortunately, such an occurrence would result in considerable delay before the cultures could be used in encystment media.

The procedures used in this investigation for maintaining a culture stock of trophozoites of E. histolytica were followed in a standard routine. Each month, a 30-day supply of culture tubes containing Balamuth's (12) medium was made up and sterilized. Immediately preceeding the inoculation of a sterile culture tube, a small amount of sterile rice powder was added to it. A culture was allowed to grow for 72 hr in a tube before inoculation of a fresh sterile tube. At that time, a small portion of the sediment in the tube containing the growing culture was removed and examined microscopically. The relative population density, size, activity, and cellular inclusions of the amoebae were noted and recorded. If their appearance was normal, some of the amoebae were transferred with their bacterial population to a sterile culture tube containing rice powder. That tube, in turn, was subcultured after about 72 hr. Usually, two to four new tubes were inoculated at each subculture. The tubes from which the inoculations were made were retained in the incubator for another 72 hr until the next subculture was completed. The double precaution of inoculating several new tubes and retaining the old tubes served as a guard against the loss of the culture and the delay in obtaining a new one which might, even then, produce cysts less readily.

The methods used most successfully in this series of investigations to cause the dysentery amoeba to encyst were developed by Balamuth (unpublished personal communication). In brief, the method consists of subculturing the amoebae into a pre-encystment medium for a period of about two weeks during which time the cultures are depleted of rice powder. At the end of this time, the few amoebae growing in the tubes are transferred to an encystment medium containing an extra amount of all nutrients required by the amoebae. In this rich, ideal environment they grow and divide rapidly for two or three days and then encyst. The simplified process is diagrammed in detail in Fig. 5. The cysts were usually removed from the culture medium, washed in sterile distilled water, and stored at 4 deg C in the refrigerator until the filtration test. Cysts were found to remain viable for as long as four weeks, but all filtration tests and cyst counts were completed within two weeks from the date of harvest.

In the course of this investigation, Dobell's<sup>(11)</sup> techniques for cyst production were found to work, though they did not produce so many cysts as Balamuth's unpublished techniques under the conditions of this experiment. A vigorously growing culture was introduced into Balamuth-and-Sand-za's<sup>(12)</sup> medium containing no rice starch. During the two subsequent subcultures 3 days apart, the



number of trophozoites dropped from approximately 100 per field (20x objective, 10x ocular, draw tube 170 mm) to 1 or 2 in 2 or 3 fields. The third subculture was made in a tube containing the same medium, to which was added the amount of starch which could be picked up, level, in a scoop 4 mm in diam and 1 mm deep. On the third day, the growth was tremendous, so much so that the trophozoites covered the complete field and were in contact with one another. On the fifth day, fewer trophozoites were present and cysts appeared. On pooling and concentrating two such tubes, 4500 cysts were counted. Thus, in this medium at least, carbohydrate (rice starch) was necessary for growth and possibly for encystment.

In order to study what effect subculturing had on the protozoa, cultures were allowed to grow in Balamuth's medium<sup>(12)</sup> without subculturing. The organisms lived for 8 to 14 days and then died without the formation of cysts. Cooling apparently had no effect on encystment, for after cultures were placed in the refrigerator (with rice starch and subcultured three days previously), the organisms were dead at the end of three days and no cysts were found.

The accurate counting of the number of cysts present in a culture at any time presented a problem. The determination had to be quantitative and fairly simple. The criterion of simplicity was important because the counting of cysts after a filtration test had to be completed before any of the cysts had deteriorated. If the method were long or tedious, delay or inaccurate counting would result. The method of counting was a crucial link in the process; results had to be correct and reproducible.

The original method of counting cysts made use of a Spencer-Neubauer Haemacytometer.\* Several determinations were made with this instrument and its use appeared to be justified. However, it was found that a disproportionate number of cysts remained in the moat surrounding the calibrated chamber of the haemacytometer. Results of filtration tests were by no means invalidated, but the numbers of cysts both in the influent and effluent would be lower than a true random sample. Actually this error should have been expected with the use of a haemacytometer, since it is constructed to give a layer of erythrocytes only one cell in depth. The human erythrocyte measures 7 to 8 microns in diam while the E. histolytica cysts measure 10 to 20 microns with an average of about 14 microns. The cysts, therefore, could not all be drawn into the counting chamber.

As a result of this inaccuracy the method of counting was altered. The method used for a majority of the tests consisted of pipetting 0.02 ml from a 0.5-ml concentrated sample to a microscope slide and covering it with a 7/(s-in). coverslip. Then, all of the cysts in the 0.02-ml sample were counted and appropriate calculations made to determine the number of cysts contained in the 0.5-ml concentrated sample, and finally in the sample from which the concentrate was made. Duplicate or triplicate 0.02-ml volumes were counted from each sample. The count was made by means of the mechanical stage on the microscope. Approximately one half hour was required to scan the whole coverslip, ex-

\* American Optical Co. No. 1492, Spencer Bright Line Improved Neubauer Haemacytometer. amine cysts, record their sizes and reject bodies similar in appearance to cysts. Occasionally, one drop of 1 percent iodine was added to the 0.02-ml sample to stain the cysts before counting.

Counts were made of the number of cysts in the concentrated harvest culture, in the filter influent, and the filter effluent. All counts were made in exactly the same manner and were reproducible; that is, two successive counts on one sample agreed closely. The sizes of all the cysts in the filter effluent were recorded so that an average cyst size could be obtained. By measuring the size of the cysts it was possible to determine whether only small cysts would pass through a particular septum.

#### 12. Cyst Filtration

The filter apparatus shown in Figs. 3 and 4, was constructed for making filtration tests using water containing cysts of E. histolytica. This unit was designed to be as compact and simple as possible in order to permit ease of cleaning and sterilization.

The apparatus included a source of filtered air under pressure and regulated by means of an airpressure regulating-valve. The air under pressure supplied the head required to filter the water. The water containing the cysts was stored in a storage reservoir consisting of an 11- to 20-in. length of 6-in. lucite tube fitted tightly into grooves cut into circular top and bottom plates. An air-pressure line entered the storage reservoir at the top in such a way that air flowed through a copper tube extended to about  $1\frac{1}{2}$  in. from the bottom. The contaminated water was poured into the reservoir at the same location. The air, bubbling up through the liquid after leaving the copper tube, served to keep the cysts in suspension in the liquid during the test.

The bottom of the reservoir was connected by means of a  $\frac{1}{4}$ -in. pipe to two filter housings placed in parallel. Various septums of small area were installed in these housings for test purposes. The effluent from each filter passed through a needle-valve control to a calibrated orifice.

In order to make a filtration test using water contaminated with cysts, the apparatus, assembled as shown in the figure, was carefully checked for leaks after the test septums were installed. A known quantity of cysts of E. histolytica was added to water in a mixing bottle and shaken to distribute the cysts throughout the water. The top cap of the storage reservoir was removed and the effluent tube from the mixing bottle was inserted well down into the storage reservoir. The valves on the effluent

from the filter housings and the valve controlling the flow to the housings were opened. Air pressure was applied to the mixing bottle, forcing the water and cysts over into the storage reservoir. The water and cysts flowed into the filter housings, forcing out the air. As soon as water flowed from the orifices, the valves were closed allowing the storage reservoir to fill. Figure 4 shows a photograph of the apparatus during the filling operation. The air valve on the compressed air line leading to the reservoir shown in Fig. 3 was opened slightly to allow air to bubble up through the liquid to keep the cysts in suspension. As soon as the reservoir was filled to within  $\frac{1}{2}$ -in. of the top, the influent tube was clamped, and the tube was removed from the reservoir. The cap was replaced on the storage tank, and the air pressure brought to the desired level by adjusting the pressure-regulating valve. Some air was allowed to escape continuously through the cap, so that the bubbles of air rising through the liquid would keep the cysts well distributed in the water. The bottle used for mixing the cysts was immediately sterilized by the addition of 200-500 ppm of chlorine to the remaining liquid. A large pan, shown under the apparatus in Fig. 4, was partly filled with water containing 500 ppm of chlorine to serve as a protection against the spilling of any of the cyst-contaminated water.

The effluent valve to one of the filter housings was adjusted to provide a desired constant-flow rate as determined using the calibrated orificepiezometer equipment. The filtration was continued at that rate, and 10-ml samples of effluent were collected in sterile test tubes at various periods of time. As soon as 3 or 4 samples were collected, or when the filtration rate could not be maintained, the filtration was discontinued. The other filter was then operated in an identical manner. The installation of the two filters in parallel made it possible to test two septums using the same culture of cysts.

As soon as all of the samples had been collected and labeled, the apparatus was filled with chlorine solution and allowed to stand overnight. All external surfaces and equipment were washed with phenol and 50 percent alcohol to sterilize any part of the equipment which might have become contaminated during the test. Twenty-four hr after the test, the chlorinated filter was removed and placed in an autoclave at 15 psi pressure for 20 min to complete the sterilization process. The apparatus was then completely disassembled and cleaned. In making tests on filter-candle septums, a suction apparatus was used. The apparatus consisted of a glass mantle-type holder, in which the filter candle was fastened. The outlet from the candle was then connected to a graduated suction bottle, which in turn was connected to an aspirator. The filter candle was covered with the contaminated water, the suction was applied, and the filtrate was collected in the suction bottle. The flow rate was determined approximately by means of the calibrated suction bottle and a stopwatch. The suction pressure was determined by means of a mercury manometer.

Biological Filtering Test No. 1 was made using the cyst filtration apparatus described in Section 12. Determinations of the number of cysts in the influent and in the effluent were made by collecting and evaporating all of the filtrate to a volume of 20 to 30 ml. The resulting volume of sample was centrifuged and the cysts were counted under a microscope using a Neubauer haemacytometer counting chamber. The approximate number of cysts in the culture had been determined using a microscope and the counting chamber before the culture was added to the influent. In this test, a total of 664 dead cysts were added per cubic centimeter of influent. The evaporation method of determination showed only 334 cysts per cc in the influent for a cyst recovery of about 50.4 percent. No cysts were found in the effluent.

In order to improve the method of determining the number of cysts present in the water, three suction filtration tests were made using septums 1, 2, and 3. The influent water for all three tests was contaminated by adding 1 cc of cyst culture containing about 1,526,000 cysts to 3500 cc of distilled water. Two samples of the influent were tested using two different methods of determining the number of cysts present: (1) by evaporating to reduce the volume, centrifuging, and examining the concentrated sample microscopically; (2) by centrifuging and examining three 10-ml portions of the sample microscopically. The evaporation method of determination showed a total of only 105 cysts per cc in the influent for a recovery of only 24.1 percent. The average of the counts from the three 10-ml portions, however, showed about 400 cysts per cc or a recovery of 92 percent.

In view of the fact that the use of three 10-ml portions of the sample appeared to give better results, cyst filtration tests using porous stainless steel septums were undertaken with living cysts. Test No. 2 was made on the same septum used in Test No. 1. One cc of a culture containing 2,083,000 cysts was added to 4000 cc of distilled water. The water, therefore, contained about 521 cysts per cc of influent. The determination of the number of cysts in the influent was checked by the two methods described above. The evaporation method showed a total of only 95 cysts per cc to be accounted for, giving a recovery of only 18.2 percent. The aliquot-portion method accounted for 469 cysts per cc for a recovery of 90 percent. It is apparent from these tests that the use of three 10-ml samples of the water provided a more accurate count than did the evaporation method. For that reason, the evaporation method was not used in later tests. The use of the haemacytometer for counting was discontinued after Test No. 6.

#### 13. Septum Pore-Size Measurements

Even though the cyst filtration tests might indicate that certain porous materials will effectively remove amoebic cysts from water, their use in the field would be impractical unless a fast, simple, and accurate method of evaluating septum effectiveness were available. Each septum, as manufactured, must invariably contain a variation in the diameter of the pores. Usually, such variations are accounted for by expressing the septum porosity in terms of its average pore diameter. Porous stainless steel materials are graded, for example, as 60-, 35-, 20-, 10-, and 5-micron diam materials according to their mean pore size. Actually, the maximum diameter of pore may be up to 5 to 10 times the diameter of the mean pore.

If only porous septums of correct pore size to remove cysts were utilized in water filters, many septums would be required and the cost of evaluating each commercial filter septum individually as to effectiveness in cyst removal by conducting cyst tests would be prohibitive. Therefore, two methods have been suggested by means of which satisfactory cyst performance could be determined by making faster, less expensive determinations. The two methods were dependent on: (1) a correlation between the removal of a particular bacterium and amoebic cysts by numerous porous septums, and (2) a correlation between cyst removal and the pore size of various septums.

The latter method of correlation is the most economical and is probably the most accurate. Two problems, however, were faced immediately in making such a correlation: (1) an easy and accurate method for the determination of pore size; and (2) the selection of the control pore size. The first problem was not difficult to solve as is evidenced by the references to such methods in the literature.<sup>(25-44)</sup> The second question, however, involves much conjecture not easily subject to experimental verification.

In the event that the head loss is considered to be the prime source of interest relative to a septum, it is reasonable to use the mean pore size as the control size, as that value would have a definite relationship to septum head-loss characteristics. If, however, the smallest size of particles which can be removed by the septum is of prime importance, it is reasonable that the pore size should be expressed as the size of the maximum pore, for that is the pore which would govern whether a given filter would or would not pass cysts. Any specification for a septum to be used as filtration protection against cyst passage should probably include, therefore, a specification of both the mean pore size and the maximum pore size.

Maximum Pore Size. Even though the size of the maximum pore would control the size of particle removed, there is some doubt as to the significance of such a determination in these tests. If we consider an area of 0.2 sq ft of a Grade H porous stainless steel septum whose mean pore diameter is 5 microns, the order of magnitude of the total number of pores in the area<sup>(43)</sup> is  $5 \ge 10^7$ . If it is assumed that the largest pore carries 50 times as much of the flow volume during filtration as a pore of "average" size, the proportion of the total flow carried by the largest pore is still only one in one million. If this is translated in terms of cyst count in a given volume of water flowing through the filter, the order of magnitude of probability of a cyst actually passing through the largest pore is seen to be small. On the basis of such reasoning, it was concluded that a 5-pore average would be more significant as a measure of the maximum pore size. The possibility of the maximum pore not allowing the passage of cysts in any one test must be considered for the above reason. P. C. Carman<sup>(42)</sup> is of the opinion that "the measurement (of maximum pore size) is best referred to the pressure required to produce an arbitrary rate of bubbling rather than to the first appearance of bubbling, to prevent the largest pore carrying too much weight."

The basic research verifying the accuracy of the pore size determination method used in this study was found in a report by H. Knöll.<sup>(36, 37)</sup> Knöll was interested in applying a method introduced earlier for the determination of the pore sizes of sintered glass filters manufactured by the Jena Glass Works. The determination depends upon "that specific air pressure which is necessary to force water from a capillary, forming an air bubble tangent to the filter surface." Knöll discussed the accuracy of two methods of determining that pressure and converting it to a pore diameter.

In the first method, known as the "Erste" pressure method, the filter was submerged in a liquid such as water or carbon tetrachloride whose capillary constant was known at various temperatures. Air was forced through the filter, and the "Erste" pressure was noted on a differential manometer at the instant that the first bubble of air broke the surface of the liquid. In another method, the pressure was applied until the filter was bubbling freely and then the pressure line was shut off from the filter. At the instant that the last bubble broke loose and rose to the surface due to the decreasing pressure, the "Letzte" pressure was recorded. The nominal diameter of the maximum pore, assuming it to be of uniform size for its entire length, may be computed according to the formula:

$$D = \frac{4B \times 760 \times 10^4}{p \times 1.033 \times 10^6}$$
(1)

Where D = diameter in microns

B = capillary constant at the temperature of the test fluid, dynes per cm p = applied pressure in mm of mercury

Knöll collected considerable preliminary data before attempting to determine the pore sizes of filters. He first worked with small capillaries of known diameter and of varying length. As a result of those tests, Knöll concluded that the "Letzte pressure" method gave excellent results which were independent of the length of the capillary (or thickness of the material) or the rate of pressure application. The "Erste pressure" method was preferred for bacterial filters, however, because of the longer time required in the "Letzte pressure" method while waiting for the last bubble to form. Knöll, therefore, conducted experiments so that improved apparatus would permit the use of the "Erste pressure" method with glass filters.

In view of the accuracy and lower costs of making "Letzte pressure" determinations, such a method was used in this study for the determination of the 1-bubble and 5-bubble average pore diameters of filter septums. The 1-bubble pore refers to the determination of the theoretical diameter of the largest pore. The 5-bubble average pore refers to the theoretical diameter computed from the pressure when 5 pores are still passing air.<sup>(42)</sup>

A porous filter material or septum is usually assumed to be composed of a large number of capillaries. In order to develop the formula for the pore diameter of such capillaries, it is necessary to assume that these filter capillaries are straight and round. Since the actual opening through the filter follows a tortuous path, the actual size of particle which will pass a given filter must be smaller than the corresponding equivalent capillary diameter based upon an assumption that the capillary is straight and round. This is because a passageway of a certain equivalent capillary diameter must, obviously, be larger in some spots and smaller in others; furthermore, its shape will change from one part of the passageway to the other. In one spot it may be approximately circular in cross section, while in another it will be approximately rectangular in cross section; thus a spherical particle will not be able to pass, even though it is substantially smaller than the equivalent capillary diameter. The Micro Metallic Corporation has indicated<sup>(43)</sup> that "as a result of years of field observation, we have come to the tentative conclusion that the maximum particle which will pass a filter of given mean pore opening is about one-third of the mean equivalent capillary diameter."

"The radius of a capillary pore can be determined by making use of a modification of the capillary ascension method for the determination of surface tension. In a capillary tube of radius, r, a given liquid will advance to a height, h. In order to force the liquid down to the level of the plane surface of the liquid, or, in other words, to prevent the advance of a liquid into a capillary tube due to capillary action, a pressure p, equal to the hydrostatic head, hd, will be required."<sup>(32)</sup> If S is the surface tension in dynes per cm<sup>(41)</sup>

$$S = \frac{rhdg}{2} \tag{2}$$

where r = radius of the capillary, in cm

h = height of capillary rise of liquid, in cm d = density of the liquid, grams per cm<sup>3</sup>

g = 980.665 cm per sec<sup>2</sup>

(3)

The pressure, p, which should be expressed in grams per cm<sup>2</sup> to maintain consistent units, may then be substituted in place of hd.

 $S = \frac{rpg}{rpg}$ 

or

$$r = \frac{2S}{nq} \tag{4}$$

Knöll's equation, Eq. 1, is set up so that the diameter of the pore, D, is measured in microns and the pressure, p, is measured in mm of mercury. Knöll's pressure in mm of mercury may be converted to pressure in grams per cm<sup>2</sup> by dividing the mm of mercury by 760 to convert to atmospheres of pressure. One atmosphere of pressure is equivalent to 1033.2 grams per cm<sup>2</sup>. In order to change the diameter, D, from centimeters to microns, it is necessary to multiply D by 10<sup>4</sup>. Equation 4 may therefore be written:

$$D = 2r = \frac{4S \times 10^4}{\frac{p}{760} \times 1033.2 \times 980.665}$$
$$= \frac{4 \times S \times 760 \times 10^4}{p \times 1.01325 \times 10^6} = \frac{3S}{p}$$
(5)

where D = diameter in microns

S = surface tension in dynes per cm

p = pressure in mm of mercury

Equation 5 differs from Knöll's equation (Eq. 1) in that Knöll has preferred to use a "capillary constant," B, in preference to "surface tension," S. In the foregoing discussion, the angle of contact between the surface of the fluid and the wall of the capillary has been assumed as zero. If the angle of contact is not zero, the radius of the capillary then becomes:<sup>(32)</sup>

$$r = \frac{2S\cos\theta}{pg} \tag{4a}$$

where  $\theta$  = angle of contact. Knöll has preferred to use the constant, *B*, because the angle of contact between the glass capillaries utilized in his experiments and water was not zero. Therefore, his capillary constant, *B*, may be described as

$$B = \frac{S \cos \theta}{0.980665} \tag{6}$$

Various experimenters have shown Knöll's B to vary between 70.60 and about 76.80, but Knöll consistently used a value of 72.53. If we substitute Knöll's B in Eq. 5, we obtain

$$D = \frac{4B \times 760 \times 10^4}{p \times 1.033 \times 10^6} \tag{1}$$

Figure 6a shows the type of laboratory apparatus designed and constructed for the determination of septum maximum pore size. The pore-size determination apparatus consisted of a pan filled with water or other fluid under which the septum undergoing test was submerged, a controlled source of filtered compressed air connected to the septum interior, two manometers for observing pressures applied, and a thermometer for the determination of the water temperature. Both a mercury manometer and a manometer containing oil whose specific gravity was unity were provided to cover pressure ranges up to about 800 mm of mercury. A septum preparation apparatus (Fig. 6b) was added after several preliminary tests had indicated its desirability. It consisted of a container B large enough to hold the largest septum tested, a source of vacuum, a mercury manometer for measuring the applied vacuum, and a storage container, A, holding distilled water or other fluid.

After setting up the test apparatus, several preliminary tests were made to check the reproducibility of the test results. After numerous attempts using the "Letze pressure" method for the determination of the pressure utilized for pore-size computations, it was found that the determination could not be repeated with any reasonable degree of accuracy. The trouble was believed to be due to the inconsistent wetting of the pore surfaces. Washburn,<sup>(38, 39)</sup> while studying the porosity of ceramic materials, had similar difficulties. He was able to show that a prior treatment was necessary to insure complete wetting of the inner surfaces. Washburn found that the most "trustworthy" procedure was one in which the material was first subjected to a vacuum, covered with liquid, and finally soaked in that liquid. The soaking period is necessary because time is required for the water to penetrate the smallest pores of the septum. The smaller the pores and the more numerous the small pores, the greater the time element required. Atmospheric pressure plus the surface tension pressure were used to force the water into the pores.

Washburn's theory<sup>(40)</sup> on the rate of penetration of porous bodies by liquids shows that the amount of liquid which penetrates a porous body is proportional to the square root of the time of soaking and to the square root of the ratio  $S/\mu$ . This is the ratio of the surface tension of the liquid to its viscosity. This holds true for all cylindrical pores except those of molecular dimensions.



Fig. 6. Maximum Pore-Size Determination and Septum Preparation Apparatus

A procedure similar to Washburn's pore wetting procedure was incorporated into the test operations. The test septum was placed in a container which could be sealed. This is indicated as B in Fig. 6. Water was placed in a large separatory funnel which is shown as A in Fig. 6. A vacuum was drawn by a vacuum pump through the vacuum line trap. The septum was allowed to remain under such a vacuum for 20 min. At the end of that time, water was allowed to flow suddenly into chamber B from chamber A. The septum was then allowed to stand at atmospheric pressure for 30 min. These periods were used to insure complete saturation of the septum pores with water.

After 30 min. of soaking in the fluid, the septum was removed from chamber B, and all of the excess fluid inside the septum was shaken out. The septum was immediately placed in the apparatus for poresize determination by connecting it to the air pressure line. In each case, the air was allowed to come from the interior to the exterior of the septum. The septum, located in the pan shown in the figure, was covered to a depth of about 1-in. with the liquid used.

After the system was checked for air leaks, the needle valve controlling the flow in the air pressure line was opened, and air passed through the septum. As soon as the air bubbles were rising freely and in sufficient numbers, the air pressure control valve was closed. The air remaining inside the septum was allowed to seep out through the pore openings. thus decreasing the pressure on the manometer. At the moment that the number of rising bubbles was reduced from 6 bubbles to 5 bubbles, a pressure reading was taken on the manometer. At the moment that the last bubble slipped off the septum and up through the fluid, the pressure was again observed and recorded. The manometer readings were converted to equivalent pressures in mm of mercury. All of the pressure determinations were made using water or xylene at a temperature of 20 deg C.

The depth of the last pore below the surface of water was measured and converted to equivalent depth in mm of mercury. That depth was then subtracted from the manometer pressure, so that the total effective air pressure was determined. After each test, the data were inserted in Eq. 1, and the 1-bubble and 5-bubble pore sizes were computed.

*Mean Pore Size.* The Micro Metallic Corporation, manufacturer of porous stainless steel septums, used the following procedure for routine pore-size analysis:

"The largest pore opening was taken as corresponding to the pressure at which the first bubble appeared in a number of tests. The mean pore opening was determined by first plotting a curve of flow versus pressure drop for air with a dry filter, and second, by plotting the same curve with the filter wetted with xylene. The point at which the flow on the second curve is exactly one-half of the flow on the first curve determines a pressure which corresponds to the mean pore opening. This is the *mean* in the sense that the amount of gas carried by all the pores above this size is equal to the amount of gas carried by all the pores below this size."

The method adopted for use in this laboratory for the determination of mean pore size consisted of an adaptation of work by Dr. F. E. Bartell<sup>(32)</sup> at the University of Michigan. Bartell's methods and treatment of data offered a more simple and accurate determination of mean pore diameter than those of the Micro Metallic Corporation and other researchers.

Bartell modified Posieuilles' original equation for the flow of liquids through capillaries to apply to the flow of liquids through a porous filter. The pressure P required to force a given volume of water Q, of viscosity  $\mu$ , in time t through a given thickness of filter L of measured pore volume  $V_o$ , is related to the radius of the pores by the equation:

$$R = \frac{\pi}{2} \cdot L \sqrt{\frac{8\mu Q}{P V_o g t}} \tag{7}$$

Where: R = radius of the mean pore, cm

- $\frac{\pi}{2}$  = correction factor, assuming closepacked spheres
- L = thickness of the filter material, cm
- $\mu$  = viscosity of medium at test temperature, poises
- Q = volume of water, ml

- $P = \text{pressure, gm cm}^{-2}$
- $V_o =$  volume of pores, cm<sup>3</sup>
- $g = 980 \text{ cm sec}^{-2}$
- t = time for volume Q to pass filter, sec

The septum pore volume  $(V_o)$  required in the above formulation of pore-size data may be determined by the use of a water saturation procedure in an apparatus such as is shown in Fig. 6. The dry weight of the septum is determined before the pores of the septum are filled with water. After saturation with a water of known temperature, the weight of the septum plus the water in the pores is determined. The calculation of pore volume may then be made as follows:

#### $V_o =$

### weight of septum saturated – weight of dry septum density of water at test temperature

Figure 7, which shows an apparatus for the determination of the mean pore size of porous filter septums, includes details of two types of laboratory test filters used in the pore-size and cyst-filtration tests. Detail A shows the type of porous bronze filter used, and detail B shows the type of construction used in testing porous stainless steel. A filter septum which can be disassembled more completely than those pictured would facilitate the determination of pore volumes as outlined above. The details illustrate the non-filter components of the complete septum which are necessary to seal and mount the filter successfully. During the saturation procedure the entire septum was wetted, making it necessary to dry those parts which are not porous in order to obtain an accurate measurement of the pore volume. Care had to be taken to insure the removal of water in excess of that which was required to fill the pores. For this reason, the mean pore volume was determined by taking an average of several determinations.

In the determination of the mean-pore size of a full-scale filter septum, the pore volume determination would be simplified. The porous material would probably be made in cylindrical tubes with open ends, which would facilitate both supersaturating, weighing, and the measurement of filter thickness.

Figure 7 shows a schematic diagram of the apparatus designed and constructed for the determination of the average or mean pore size of porous filters. The apparatus was designed to utilize the same filter septums which were used in the cyst



Fig. 7. Mean Pore-Size Determination Apparatus

filtration tests. The apparatus consisted essentially of a source of air pressure, a water storage reservoir, a filter housing in which the septum to be tested was installed, a series of manometers for measuring pressure differentials, and a means of controlling and measuring the flow of water through the septum. Compressed air at a pressure of about 75 to 85 psi was passed through a glass wool filter to remove particles of dust. The air line entered the high pressure side of an air pressure regulator which could be adjusted to provide any desired constant air pressure on the outlet side of the regulator. A mercury manometer,  $M_1$ , was connected to the air line at the outlet of the regulator in order to permit close regulation of the input air pressure. A small air reservoir was connected to the air line to provide additional air volume between the air pressure regulator and the water storage reservoir. The additional air volume served to reduce the pressure fluctuations when the apparatus was functioning. The air pressure line supplied, therefore, a nearly constant pressure of compressed air to a 5-gal pressure water storage reservoir. The compressed air served to force the water in the storage reservoir through the rest of the apparatus.

A water supply line led from the bottom of the storage reservoir to the bottom of a brass-lucite filter housing in which the test septum was installed. The filter housing is shown in Fig. 7. The effluent line from the filter housing led to flow control value 6, which is shown in detail C.

The pressure drop across the filter septum and housing at any influent pressure and filtration rate was measured by either of the two differential manometers,  $M_2$  or  $M_3$ , connected to the influent line and effluent line from the filter housing. The details of the manometer system incorporating manometers  $M_2$  and  $M_3$  are not shown in the diagram. The two manometers were interconnected with glass tees, rubber tubing, and glass valves so that either manometer could be quickly connected or disconnected from the system during pressure-drop determinations. Manometer M<sub>2</sub> contained a liquid with a density of 2.95 and was used for determinations in which the pressure drop was low. Manometer  $M_3$  was filled with mercury and was used for determinations in which the pressure drop was considerable.

A calibration curve was constructed for the apparatus to permit correction of the observed head loss on manometers  $M_2$  and  $M_3$  for the head loss through the filter housing and piping. In the calibration, the proper setting was made on value 6 and the influent air pressure was varied. The pressure difference on manometers  $M_2$  and  $M_3$  was then recorded at each influent pressure with no septum installed in the filter housing. In calculating the correct pressure drop across a septum only, the value of the pressure correction corresponding to the influent pressure used in this test could be read from the calibration. This correction was then subtracted from the pressure recorded on manometer  $M_2$  or  $M_3$ . The correction was insignificant in those tests in which the required air pressure was high.

The test procedure followed in the determination of the mean pore size of porous filter septums is summarized as follows:

1. The thickness of the filter septum was measured by taking an average of 10 to 15 measurements secured at random on the septum. This average thickness was recorded.

2. The septum was supersaturated and its pore volume was determined using the methods described on the preceding pages.

3. The septum was again supersaturated and installed in the brass-lucite filter housing. The housing and connecting piping were filled with water. (Wetting of the pores tends to give more reproducible results in both the maximum and mean pore size determination.)

4. Water was forced through the septum at a reasonable rate (about 2 gpm per sq ft of septum) by increasing the air pressure in the water reservoir. The pressure was checked, and the pressure drop across the septum was determined by the use of a suitable manometer.

5. The effluent water was collected in a 1000-ml volumetric flask as it left valve 6. The time required for filling the flask was recorded. Small volumes of water were added to the flask by means of a burette when necessary to bring the final volume of water to 1000 ml at the end of the test. The amount added was subtracted from 1000 ml to obtain the volume of filtrate collected in the measured time.

6. The influent and head-loss pressures shown respectively on manometer  $M_1$  and on  $M_2$  or  $M_3$  were recorded.

The data were then used to calculate the diameter of the mean pore as shown by the following example. SAMPLE CALCULATION OF MEAN PORE SIZE Wet weight 148.940 grams

1.665 = gms. water at  $25^{\circ}\text{C}$ 

$$\frac{1.665}{0.99707} = 1.67 = Vo$$
  
 $\mu$  at 25°C = 0.0089  
 $Q = 997.5$  ml  
 $t = 199$  sec  
 $g = 980$   
 $L = 0.278$  cm  
 $\frac{\pi}{2} = 1.57$   
 $P = \text{cm of manometer differential } (\rho - 1)$   
(The density of the manometer liquid is 2.95.)

$$P = (12.4 \text{ cm} - 5.2 \text{ cm})(2.95 - 1) = 7.2 (1.95) = 14.04 \text{ cm}$$

$$\therefore R = \frac{\pi}{2} \cdot L \sqrt{\frac{8\mu Q}{PV_o gt}}$$
  
= (1.57)(0.278)  $\sqrt{\frac{(8) (.0089) 997.5}{(14.04)(1.67)(980)}}$ (199)  
= (.436)  $\sqrt{15.5 \times 10^{-6}}$   
= (.436)(3.94×10^{-3}) = 1.72×10^{-3} = cm radius  
1.72×10^{-3}×2 = 3.44×10^{-3} = cm diam  
or 0.00344 cm  
1 cm = 10<sup>4</sup> microns

 $\therefore$  Average pore diameter = 34.4 microns

#### 14. Septum Hydraulic Characteristics

The total head loss through a filter septum and filter cake after a period of filtration with body feed is equal to the sum of the head losses through four separate layers possessing different hydraulic characteristics. These layers are (1) the septum, (2) the precoat not penetrated by the solids, (3) the precoat penetrated by the solids, and (4) the body feed plus solids layers. Figure 8 shows a schematic cross section of a septum, precoat, and filter cake after a period of filtration with and without body feed. The portion of the head loss due to the flow through the septum generally remains constant throughout the entire period of filtration. Similarly, the head loss through the precoat not penetrated by the solids also remains relatively constant, provided the effects of the compaction of the filter aid on the septum are small. The head loss through the portion of the precoat which is penetrated by the solids and the head loss through the body-feed cake increase throughout the run. The rate of increase depends on the rate of flow, the amount of body feed added, and the amount and character of the suspended material in the water.







Fig. 8. Cross Section of a Septum, Precoat, and Body Coat

In general, the terminal pressure drop across a filter septum at the end of a filter run will be between 40 to 60 psi (92.4 to 138.6 ft of water). The effective filtration pressure-drop is equal to the total terminal pressure-drop minus the initial pressure-drop across the septum plus its precoat. In general, the filter septums now used in water filtration have been designed to keep the initial pressuredrop at a minimum. As the mean pore size of a septum decreases, the initial head loss through the septum will increase. Porous septums made with pore size sufficiently small to remove cysts have not been used previously, because the high initial head losses through the septums reduced the filter capacity below economical levels. This has resulted from the utilization of septums with pore sizes much too small.

In order to observe the effect of using fine-pore septums in water filters, a study was made of the hydraulic characteristics of several typical filter septums. The apparatus used in the study of the head loss through filter septums was similar to that shown schematically in Fig. 1. A centrifugal pump was used to pump filtered water from a storage tank through the septums installed in the filter housing and back to the storage tank. During precoating, the discharge from the filter was fed back into the slurry tank and recirculated so that all of the filter aid in the slurry could be precoated on the septums. The effluent line from the filters discharged through a calibrated orifice-piezometer gage, which was used to control the rate of flow through the filters. Two manometers were installed for use in the head-loss tests. The manometers were installed so that one leg was connected to the housing on the raw-water side of the septums and the other leg was connected to the filtered-water side of the septums. One manometer contained mercury and was used for measuring high head losses. The other manometer contained Meriam Oil No. 3, a manometer fluid with a specific gravity of 2.95. The manometer readings showed the difference in pressure of the water just before and just after it passed through the septum. Thus, the pressure difference showed the head loss or pressure drop across the septum.

The procedure used to determine the head loss through a filter septum alone and through a filter septum plus precoat was as follows. The septum was installed in the filter housing, and the flow cycle was adjusted so that water was pumped from the slurry tank through the filter and back to the slurry tank. The slurry tank and filter housing were then filled with filtered tap water. The pump was started and the filtered water was circulated through the septum at a known constant rate of filtration. A thermometer was inserted in the slurry tank, and the water temperature was recorded at the same time that the head loss was measured on one of the manometers. Subsequently, all head-loss readings were converted to a standard temperature of 50 deg F. Periodically the rate of flow through the filter septum was varied until the relationship between head loss and rate of flow had been determined throughout the desired flow range.

In order to determine head losses through the septum plus a precoat, the slurry tank and housing were filled with filtered tap water until the system contained a predetermined quantity of water. A measured amount of filter aid corresponding to the desired weight of precoat was added to the slurry tank, mixed with the water, and circulated through the system at a rate of flow of 7.88 gpm per sq ft of filter area. As soon as the precoat was completely formed on the septum, the rate of flow through the septum was adjusted to different values and the head loss and temperature were recorded. The orifice-piezometer apparatus was used to measure and observe the rate of flow. The head-loss measurements were made as quickly as possible to reduce electrokinetic and compaction errors in head losses. As soon as sufficient data were obtained, the filter was backwashed and all of the water was drained from the filter system. The procedure was then repeated using other weights of filter aid.

The data for the tests were customarily plotted to show the head loss in feet of water at 50 deg F against the rate of flow through the septum in gpm per sq ft. Hydraulic characteristic curves of this type were prepared for all of the septums listed in Table 3.

	Table 3			
Effect of Grade of	Retention of	Filter	Septums	on
the Initial Head	d Loss Throug	h the	Septum	

Septum No.	Reten- tion Grade	Head Loss Septum O Water at	s Through nly (Ft of 50 deg F)	Head Loss Through Septum Plus 0.15#/ft of C-535 (Ft of Water at 50 deg F)			
		2 gpm/ft2	$4 gpm/ft^2$	2 gpm/ft2	4 gpm/ft <sup>2</sup>		
1	1*	56.7					
2	1*	135.0					
29	2†	1.48	2.96				
17	5	0.05	0.08	0.6	1.12		
47. Grade 2	3	0.05	0.10	0.5	0.92		
47. Grade 3	3	0.15	0.32	0.52	1.10		
47. Grade 4	2†	0.62	1.30	1.02	2.10		
32, Grade F	2†	0.30	0.80	0.80	1.70		
* Will rome	wo all avet	e and boataria					

+ Will remove all cysts and ba

Hydraulic characteristic curves for three grades of septums are shown in Figs. 9 through 12. Figure 9 shows the relationship between head loss and rate of flow for filter septums 1 and 2. Both of these septums are of the filter-candle type and will remove 100 percent of the bacteria and amoebic cysts in the water. Neither of the filter septums would permit filtration to be conducted at the normal desired constant-rate of 2 gpm per sq ft. Both filter septums are composed of material which results in a very uniform pore size and pore-size distribution. As a result, the head loss was found to vary directly with the rate of flow up to the maximum flow rates obtained in the tests.

Figure 10 shows the hydraulic characteristic curve obtained with a porous carbon septum whose mean and maximum pore sizes were not determined. The material removed 100 percent of the cysts from water, but the material appears to be too fragile for widespread use in filter septums. The filter septum, however, shows a greatly reduced pressure-drop through the septum when compared with the filtercandle septums.

Figure 11 shows the hydraulic characteristics of a typical filter septum such as those available commercially for use in diatomite filters. The figures show the head loss at various rates of flow through each filter septum alone and through the septum precoated with various amounts of filter aid. With these septums, the initial head loss should be based on the head loss through the septum plus its minimum weight of precoat, rather than on the head loss through the septum alone. This type of filter should *never* be operated without precoat in order to guard against unnecessarily clogging the filter septum. Since a minimum precoat of 0.15 lb of filter aid per sq ft of filter is recommended, all head-loss comparisons will be made on the filter precoated with this amount of filter aid. Figure 11 shows the results for a Stellar filter element similar to those which have been used in the U.S. Army 15-gpm pack filter. The results with the regular porosity, standard porous bronze filter element used in the Permutit Company's diatomite filters were also plotted graphically (Fig. 12). Both of these



Fig. 9. Hydraulic Characteristics of Grade One Filter Septums

filters are being used successfully in diatomite precoat filters. The Stellar septum is a retention grade 5 filter septum since it will remove no bacteria or amoebic cysts from water. The porous bronze septum may be classed as a retention grade 3 filter septum since it will remove a considerable portion but not all of the cysts in a water.

The hydraulic characteristic curves for porous bronze filter septums whose mean pore size is smaller than the regular porosity bronze septum currently being used commercially were plotted



Fig. 10. Hydraulic Characteristics of a Carbon Septum

graphically (Figs. 13 and 14). It was observed that the Grade 3 septum, which is a retention grade 3 filter septum, will remove some but not all of the cysts in water. The Grade 4 septum, which is a retention grade 2 septum, will remove all of the cysts from the water without the use of a filter aid precoat. This septum would provide positive protection against the passage of cysts at all times during a filter run. Figure 15 shows the hydraulic characteristics of a series of five porous stainless steel filter septums tested as one septum. These septums were Grade F septums whose average mean pore size of about 18.1 microns was sufficient to remove all cysts from the water. The maximum pore size of these septums was not determined. These septums are also Grade 2 septums and are available commercially. Figure 16 shows the hydraulic characteristics of four different grades of porous stainless steel when tested without a precoat.

Table 3 shows a comparison of the head-loss test results for all of the septums operated without body feed and with 0.15 lb of precoat per sq ft at flow rates of 2 and 4 gpm per sq ft. The two



septums which removed 100 percent of all cysts and Serratia marcescens in the water show initial head losses of 56.7 and 135.0 ft at the 2-gpm rate. Neither would be suitable for use in a precoat diatomite filter, as might be expected, since over half of the available pressure drop in the case of septum 1 and all of the available head loss in the case of septum 2 is used to satisfy the initial pressure drop through the septum.

The explanation for the greatly increased head loss through septum 2 when compared with septum 1 may be traced to the porosity of the two septums. Septum 2 is a very fine-pore material suitable for the removal of all bacteria from a fluid which it filters. Septum 1 is a coarse material and is suitable for preliminary filtrations in which only the larger bacteria, such as Serratia marcescens, are completely removed from the filtrate. Septums 32 and 47, retention grade 2, are septums which will remove all of the cysts from the filtrate without the use of filter-aid precoat. Assuming a normal total pressure drop of 100 ft of water, the initial head losses through septums 32 and 47, retention grade 2, without precoat are 0.30 ft and 0.62 ft respectively at a 2-gpm rate per sq ft and 0.80 and 1.30 ft at a 4-gpm per sq ft rate. In other words, the initial head loss through the uncoated septum would be less than 1.3 percent of the total available head loss.









Fig. 16. Hydraulic Characteristics of Porous Stainless Steel Septums

Similarly, the initial head loss at a 4 gpm per sq ft rate through either septum precoated with 0.15 lb of filter aid per sq ft of filter would be less than 2.1 percent of the total available head. Septums 17 and 47, Grade 2, are septums which are in retention grades 5 and 3, respectively. Neither will protect the filtrate against the passage of cysts. Both have been used commercially in water filters. At a 4 gpm per sq ft filtration rate through these septums, the initial head loss through a precoat of 0.15 lb of filter aid per sq ft of filter is, respectively, 1.12 and 0.92 ft of water. In other words, the initial head loss varies from 0.92 to 1.12 percent of the total available head for these filters which will not remove cysts as compared to 1.7 to 2.1 percent for those that will remove cysts. The filter capacity would be reduced by less than 1 percent by the use of these septums at a 4 gpm per sq ft rate and by less than 0.5 percent at a 2 gpm per sq ft rate. Such a reduction in capacity is necessary in order to obtain protection against the accidental passage of cysts in the event of the failure of the filter-aid precoat.

#### 15. Test Results

A few results of the bacteriological filtering efficiency tests are listed in Table 4. These tests were conducted as a means of making a preliminary screening of the septums into their proper grade. The tests have been assembled into groups, and the test results will be discussed for each group as follows:

- Group 1 Wire cloth, cloth, and rubber septums of grades 4 and 5 retention
- Group 2 Aloxite septums, probably of grades 3 or 4 retention
- Group 3 A porous rubber septum
- Group 4 A porous battery plate septum
- Group 5 A porous carbon septum
- Group 6 Porous stainless steel septums in retention grades 2 and 3
- Group 7—Porous ceramic and diatomite filter candles in retention grade 1.

Group 1. This series of tests indicated that cloth and wire cloth septums, designed for use as supporting media for filter aids, will not remove bacteria without the use of filter aid. The wire mesh tested ranged in size from  $24 \times 110$  mesh to  $50 \times 700$ mesh. The filtration rates varied between 1.55 and 18.55 gpm per sq ft.

Group 2. This series of filtration tests using porous aloxite septums 4, 5, and 6 disclosed that these septums are not suitable for the removal of bacteria without the use of filter aids.

					some b	acteriologic	a rittering	g Emicien	cy lesis				
Group No.	Test Run	Date	Septum No.	Rat Filtr	te of ation	Pressure	e Drop	Filte	ering me	Flow	S. Marcescens in	S. Marcescens	% Removal
				Max. gpn	Min. $n/ft^2$	Max. in. of	Min. hg.*	Min.	Sec.	ml	Influent/cc	Effluent/cc	
1	36	9/23/48	10	2.94	2.81	55.7	51.4	27	00	2,900	16,300	17,000	0
1	25	8/18/48	13	12.3	11.6	57.0	56.3	22	00	9,000	3,230,000	2,860,000	11
1	28	8/25/48	23	2.32	1.55	56.5	54.8	17	00	1,500	2,860,000	1,960,000	31.0
2	60	7/2/49	4	2.0	2.0	15  psi	15  psi	18	00	5,000	7,200	12.150	70.1
							1.1			(2)	22	21.520	78.8
												3 3,400	52.8
2	21B	8/12/48	5	0.78	0.76	53.5	46.9	21	00	7,500	2,110,000	2,400,000	0
3	38	10/2/48	36	4.0	2.9	54.9	54.7	29	00	2,800	13,900	3,500	75.0
4	35	9/14/48	35	12.3	6.61	56.7	44.3	24	00	10,000	23,400	13,000	45.5
4	57	6/21/49	35	2.0	2.0	23 psi	23 psi	60	00	3,850	950	1 730	23.2
100	10.0					000000000000						2 160	83.0
												3 118	87.6
5	17	6/24/48	29	14.6	3.25	42.8	39.1	30	00	18,000	480,000	129,000	73.0
1990	50	-,,	22.2								630,000	46,000	91.0
												65,000	
5	56	6/9/49	29	2.0	2.0	23 psi	7 psi	20	00	5.800	45,000	11,000	75.5
6	50	2/3/49	30.	2.09	1.81	14  psi	14  psi	47	00	2,500	36,000	110,400	71
		-/ -/	PSS-D			1000	0.000				100 M 100 10	215.100	58
			I.D9									3, -16, 100	55
6	61	8/24/49	30.	0.5	0.5	15  psi	10  psi	15	00		13,600	720	94.6
1		0/ = 1/ 10	PSS-D	1.0	1.0			16	00			520	96.2
			I.D8	2.0	2.0			15	00			270	98.2
				4.0	4.0			12	00			1,100	92.0
				6.0	6.0			15	00			2,450	82.0
				10.0	10.0			8	00			3,880	71.5
6	62	9/7/49	31.	0.5	0.5	14	14	20	00		1,070	155	85.5
		0/ 0/ 00	PSS-E	1.0	1.0			15	00		0.00000	265	75.2
			I.D6	2.0	2.0			15	00			425	60.3
				4.0	4.0			15	00			285	73.3
				6.0	6.0			15	00			325	69.6
				10.0	10.0			15	00			485	54.6
6	53	2/8/49	33.	2.72	1.81	14 psi	14 psi	48	00	2.750	10,450	1 50	99.5
	00	-, 0, 10	PSS-G									2 30	99.8
			I.D3									3 10	99.9
7	3	3/26/48	1.	0.74	0.66	47.3	46.5	7	54	3,500	1,300,000	0	100
	×.	0/ 00/ 10	Mandler										
			Reg.										
7	1	3/25/48	27.	0.55	0.48	48.7	47.3	30	30	10,000	1,000,000	0	100
	÷.	5, 20, 10	Berke-		1000		2010/22/2010	COMP.			-11000	100	77.7275
			feld F										

Table 4 Some Bacteriological Filtering Efficiency Test

\* Unless otherwise designated.

Group 3. Test #38 made with septum 36 indicated that the porous rubber media effected the removal of about 75 percent of the bacteria. The material, which is sponge rubber, is unsatisfactory as a filter septum.

Group 4. The porous battery plate (septum 35), tested in Runs #35 and #57, removed 45.5 percent of the applied bacteria at a flow rate between 6.6 and 12.4 gpm per sq ft. At a rate of flow of 2.0 gpm per sq ft, the percentage removal increased to a maximum of 87.6 percent. The effectiveness of this filter material in removing bacteria would undoubtedly be improved by increasing its thickness. It is doubtful, however, whether the material has merit for septums in water filters.

Group 5. Runs #17 and #56 were made using a porous carbon filter, septum 29. At a maximum flow rate of 14.6 gpm per sq ft, over 72 percent of the bacteria were removed. As the filtration rate dropped to 3.25 gpm per sq ft, the percentage removal increased to over 90 percent. At a constant rate of flow of 2.0 gpm per sq ft, the filter removed an average of 75.5 percent of applied bacteria. It is apparent that the rate of flow has a decided effect on the filtering efficiency of this septum. The good bacteria removals indicated the possibility that this septum might also remove cysts.

Group 6. This group of tests was made using 5 grades of porous stainless steel septums (septums 30, 31, 32, 33, and 34). The bacteriological filtering efficiency tests using these porous stainless steel septums present a means of comparing the percent removal of *Serratia marcescens* against the pore diameter of the material. The septums used in these tests were all of about the same filtering area and were about  $\frac{1}{s}$ -in. thick.

The data from Runs #50 through #55 were used to plot the graph in Fig. 17, which shows, roughly, the percentage removal of *Serratia marcescens* vs. the mean pore opening. The mean pore size listed is that determined in this laboratory using the procedures outlined previously. At a filtration rate of approximately 2 gpm per sq ft, the Grade D porous stainless steel septum (34.4 microns mean pore size) removed roughly 60 percent of the applied *Serratia marcescens*. The Grade G septum (8.9 micron mean pore size) removed well over 99 percent of the bacteria at the same flow rate. Runs #61, #62 and #63 were made using the biological filtering apparatus to test Grades D, E, and F porous stainless steel filter septums. In Run #61, the filtration was started at the lowest flow rate through a Grade D septum and periodically increased to 10 gpm per sq ft at the end of the test. The percentage removal of *Serratia marcescens* dropped off until only 71.5 percent were removed at the 10 gpm per sq ft flow rate. See Fig. 18. Run #62 was made in a similar manner with a Grade E



porous stainless steel septum. The percentage removal of *Serratia marcescens* for each rate of flow is shown in Fig. 18. This figure shows also a similar curve as determined for an F porous stainless steel septum in Run #63.

Group 7. All of the tests in this group were made with septums which removed 100 percent of the applied bacteria and consequently may be classed as retention grade 1 septums. These septums include the diatomite filter candles, septums 1, 2, 3, and 27, and a porcelain filter candle, septum 16. In each case, the maximum flow rate which could be obtained from these filters was less than 1 gpm per sq ft. The flow rate fell off very rapidly as the filtration continued.

A review of the results of the bacteriological tests of the filter septums indicates that the septums in groups 5, 6, and 7 were the septums which might be used to remove amoebic cysts without the use of filter-aid precoat. For this reason, a number of porous metal septums were fabricated for use in testing the ability of these septums to remove amoebic cysts. In order to correlate the effectiveness of the removal of the cysts with the pore size of the septum, the maximum and mean pore sizes of the porous metal septums used in the bacteriological and biological filtration tests were determined using the procedures described in Section 13.

*Pore-size determination.* A summary of the maximum pore sizes of the available porous metal septums used in the cyst-filtration tests is given in Table 5. The first and second columns in the table include a description of the septum and its identifying number. The fourth and sixth columns show the results of the average 1-bubble and 5-bubble maximum pore sizes, respectively.

In determining maximum pore size of porous metal filters using the 1-bubble determination, variable results were usually obtained. For example, a test made with a Grade D porous stainless steel



Fig. 18. Effect of Rate of Flow on the Percentage Removal of Serratia Marcescens by Porous Stainless Steel Septums

septum, I.D.-8, indicated that the maximum pore size was 316 microns on one day and 175 microns on another. The average pore size would be about 246 microns. The average 1-bubble pore size determined from 8 to 10 tests, however, was 218 microns, using water as the fluid in the test. The likelihood of obtaining discrepancies with the 1bubble maximum pore size was found to be greater using septums with a larger mean pore size.

The results of the 5-bubble average for the determination of the average pore size of the 5 largest pores indicate a more reliable determination. In most cases, the 5-bubble size determination produced a pore size considerably smaller than the

Table 5 Maximum Pore Sizes of Filter Septums Description of One-Bubble Five-Bubble

tity	Septum	Maximi	ize	Maximu Si	im Pore ze
ber		Xylene	Water	Xylene	Water
I.D 1	Porous Stainless Steel Grade H		23.7		17.5
I.D 2	Porous Stainless Steel Grade H		25.3		19.9
I.D 3	Porous Stainless Steel Grade G		41.2		33.9
I.D 4	Porous Stainless Steel Grade F	35.8	36.9	26.6	30.8
I.D 5	Porous Stainless Steel Grade F	35.3	37.0	18.8	19.3
I.D 6	Porous Stainless Steel Grade E	59.3	53.6	50.8	46.2
I.D 7	Porous Stainless Steel Grade E		210.0		110.0
I.D 8	Porous Stainless Steel Grade D	188.1	218.0	161.6	173.0
I.D 9	Porous Stainless Steel Grade D	148.1		131.1	
I.D10	Porous Stainless Steel Grade D	122.7		104.8	
I.D11	Permutit Porous Bronze Grade 4 (Super-Fine)	40.8		40.8	
I.D12	Permutit Porous Bronze Grade 4 (Super-Fine)	45.4		42.4	
I.D13	Permutit Porous Bronze Grade 4 (Super-Fine)		121.0	82.0	102.0
I.D14	Permutit Porous Bronze Grade 3 (Fine)	95.3		83.9	
I.D15	Permutit Porous Bronze Grade 3 (Fine)	86.5		80.5	• • • •
I.D16	Permutit Porous Bronze Grade 3 (Fine)			83.5	
I.D17	Permutit Porous Bronze	322.0		205.0	
I.D18	Fisher Porous Bronze Grade—"Standard"	100.6		92.0	

pore size obtained from the 1-bubble determination. The test results indicate that there is a wide discrepancy in the 1- and 5-bubble maximum pore diameters even with different septums of the same mean pore size. For example, septums I.D.-8, -9, and -10 were made using porous stainless steel septums composed of the same 65 micron material. The 1-bubble maximum pore sizes determined using xylene were 188.1 microns, 148.1 microns, and 122.7 microns, respectively. Their 5-bubble maximum pore sizes were 161.6 microns, 131.1 microns and 104.8 microns, respectively. Obviously, the variations in the average size of the maximum pore would influence considerably the usefulness of that material for cyst removal.

Decrease in pore size. Table 6 lists the results of the mean pore size determinations for the septums designed for use in the cyst filtration tests. In each case, the pore size of the septum was determined immediately before the making of the cyst-filtration test in which that septum was used. The only exception to this procedure was followed with the septums whose I. D. numbers were 11 and 12. These septums were fabricated from a piece of porous bronze tubing 6 in. long. The tube was cut into 3-in. pieces which were fitted with end plates as shown in Fig. 7, detail A. The mean pore sizes of the septums were found to be 18.6 and 20.1 microns, respectively, before either had been used in a cystfiltration test. Immediately after cyst-filtration test #20 had been completed with septum I.D.-12, the septum was sterilized in an autoclave, and the pore size was again determined. The mean pore size decreased during the test from 20.1 microns to 9.6 microns, indicating that the porosity of the septum was being decreased due to the clogging by the suspended material in the water, or by cysts and rice starch. A similar clogging tendency was observed during all of the cyst-filtration tests with porous bronze septums. In all tests, the head loss across the bronze septum increased rapidly as the small rice particles in the raw water clogged the septum pores. Such rapid loss of head was not observed with stainless steel septums of comparable mean pore size.

Discrepancies in pore-size determinations. An inspection of mean pore size of septums I.D.-8, -9, -10 indicates the possibility of a wide difference in the mean pore size of two septums of similar material. The three septums were made from a piece of Grade D porous stainless steel whose mean pore size was theoretically 65 microns. The only explanation which can be offered to explain the discrepancy is concerned with the irregularity and nonuniformity of the pore size of stainless steel septums.

The maximum pore-size results included in Table 5 were determined using water or xylene as the liquid forced through the capillary. In calcu-

Tab	le 6					
Description of and Mean Pore	e Sizes of Some Fil	ter Septums				
Septum Identity Number and Description	Mean Pore Diameter					
	Manufacturer's Designation	Experimental Determination				
I.D 1 PSS* No. 34 Grade H I.D 2 PSS* No. 34 Grade H I.D 3 PSS* No. 33 Grade G	5 5 10	$4.1 \\ 4.4 \\ 8.9 \\ 150 \\ 100 $				
1.D 4 PS5* No. 32 Grade F I.D 5 PS5* No. 32 Grade F I.D 6 PS5* No. 31 Grade E I.D. 7 PS5* No. 31 Grade E	20 20 35	15.0 15.9 18.1 20.1				
I.D 8 PSS* No. 30 Grade D I.D 9 PSS* No. 30 Grade D I.D 9 PSS* No. 30 Grade D	65 65 65	$     18.75 \\     34.4 \\     35.8 $				
I.D11 PPB† similar to Septum 47. Grade 4	Super-Fine	18.6				
I.D12 PPB† similar to Septum 47. Grade 4	Super-Fine	20.11 9.6§				
I.D13 PPB† similar to Septum 47. Grade 4	Super-Fine	19.4				
I.D14 PPB† similar to Septum 47. Grade 3	Fine	46.8				
I.D15 PPB† similar to Septum 47. Grade 3	Fine	47.6				
I.D16 PPB <sup>†</sup> similar to Septum 47. Grade 3	Fine	42.1				
I.D. 17 PPB† similar to Septum	Regular	102.4				
I.D18 PPB† similar to Septum 48, Grade—Standard	Standard	38.1				
* Refers to Porous Stainless Steel Ser	otum.					

Before Cyst Filtration Test #20. Immediately After Cyst Filtration Test #20.

Septum

lating the pore diameter no allowance was made for the fact that the angle of contact between water and stainless steel or bronze is not zero. For that reason, the pore diameters resulting from the use of water are not comparable between materials, and the pore diameters for any one material are in error by an undetermined constant factor. Most organic liquids are reported to have a zero angle of contact with metals because of their low surface tension. Xylene has been used in similar testing procedures in other laboratories and has been found to have a zero angle of contact with metal. For this reason, the determination of the 1-bubble and 5-bubble maximum pore diameter for several septums was made using xylene to obtain a zero contact angle.

Cyst-filtration tests. Some of the results of the cyst-filtration tests are given in Tables 7 and 8. Table 7 shows a compilation of the cyst-filtration data pertaining to each individual test. The number of cysts in the influent prefixed with the letter A was determined by counting the approximate number of cysts in the cyst culture and dividing by the volume of influent water used in the test. The number of cysts in the influent prefixed by the letter B was determined from the cyst count made at the completion of a cyst filtration test. In general, the percentage removal of cysts was based on the B number of cysts recovered in the influent, when the B number was available. Otherwise, the A number of cysts in the influent was used in calculating the percentage removals.

Table 8 shows a compilation of cyst-filtration test data correlated, in so far as possible, with the maximum and mean pore size of the porous septums tested. Most of the maximum pore sizes in this table were determined using xylene as the test liquid. Those determinations made using water as the test fluid are marked (water) in Table 8.

An inspection of the results indicated that 100 percent cyst removal was obtained with all septums whose mean pore size was 18.6 microns or less. Imperfect cyst removal was obtained with most septums whose 5-bubble maximum pore size was as large as 80.2 microns in the case of porous bronze and 50.8 microns in the case of porous stainless steel. Imperfect removals were obtained with one septum whose 5-bubble maximum pore size was as low as 42.5 microns. In cyst-filtration test #20, the average size of the cysts recovered in the effluent was 9.3 microns. The maximum size was 10.5 microns. The cysts in the influent, however, ranged in size up to 17 microns, with a majority between 11 and 17 microns. It appears that this septum effectively removed the cysts above about 10.5 microns in diameter. The average pore size of the septum was 20.1 microns at the start of the test and decreased to 9.6 microns at the end of the test.

The biological filtration tests indicate that the pore size of porous-metal filters can be controlled so that amoebic cysts will be removed. In most cases, the cyst filtration tests were made with a rate of filtration of about 2 gpm per sq ft of filter, the recommended filter rate for use with diatomite filters. Several filtration tests were made with Grade D, E, and F porous stainless steel septums at rates of flow from 2 to 5.08 gpm per sq ft. The filtration tests of the Grade D porous stainless steel

				Some I	Biological	Filtering	Efficienc	y Tests					
Group No.	Test Run	Date	Septum No. and Description	Rate Filtra Max. gpm	e of Min. $/ft^2$	App Press in. of hg	$\stackrel{ m lied}{{}_{{sure}}}{psi}$	Filte Tir Min	ering me Sec	$ \begin{array}{c} \mathrm{Ml} \\ \mathrm{of} \\ \mathrm{Flow} \\ \pm 5\% \end{array} $	Cysts in In- fluent/cc	Cysts in Ef- * fluent/cc	% Removal
7 7 6	··· ·3	$3/12/49 \\ 3/12/49 \\ 4/12/49$	1, Mandler-Regular 3, Mandler-Prelim. 31, PSS, Grade E I.D6	${}^{0.52}_{0.63}_{3.32}$	${}^{0.47}_{0.63}_{2.97}$	$\begin{array}{c} 16\\ 22 \end{array}$	26	$\begin{smallmatrix}&3\\&2\\20\end{smallmatrix}$	$\begin{smallmatrix}14\\0\\00\end{smallmatrix}$	750 800	A-435 A-435 A-450 B-371	0 0 0	$100 \\ 100 \\ 100$
6	7	5/31/49	30, PSS, Grade D I.D8	$2.0 \\ 5.0$	$2.0 \\ 5.0$		$\frac{26}{26}$	$10 \\ 10$	$15 \\ 15$		A-92 A-92	$9 \\ 14$	$90.2 \\ 84.8$
5	8	5/31/49	29, Porous Carbon	2.0	2.0		26	10	00		A-92	0	100
6	11	4/24/50	48, Fisher Bronze I.D18	2.0	2.0		14.7	4	00	840	A-415 B-270	118	56.0
2	14	5/18/50	51, Aloxite-Perm 20	2.0	2.0		14.8	3	00	1,750	A-490 B-462	298	35.0
2	15	5/18/50	52, Aloxite-Perm 60	2.0	2.0		14.8	3	00	1,750	A-490 B-462	461	0.0
6	18	11/18/50	47, Permutit Porous Bronze, Grade 3	2.0	2.0		14.0	••		2,000	A-530 B-925	250 1,000	73
6	22	5/9/51	31, PSS, Grade E I.D5	2.0	2.0		14	••	•••	3,100	A-420 B-400	0	100
6	23	5/9/51	31, PSS, Grade E, I.D7	2.0	2.0		14	••	••	3,500	A-420 B-400	121	70

Table 7

\* A-applied; B-recovered in counting.

septum, Run #7, were made at rates of 2.0 and 5.0 gpm per sq ft. With an influent concentration of 92 cysts per cc, the filter removed 90.2 percent of the cysts at the 2.0 gpm rate. When the flow was increased to the higher rate, the percentage removal dropped to 84.8. Obviously, therefore, the rate of flow through a septum will affect its ability to remove amoebic cysts. In this study, however, most of the tests were made at the 2 gpm per sq ft rate of filtration.

Tests #9 and #10 were both made on porous bronze filter septums using cysts which had been stored in a refrigerator for about 7 to 10 days be-

Table 8 Summary of Some Cyst-Filtration Tests

Test No.	Date	Type of Septum	Approxi- mate % Removal of Cysts	One- Bubble Max. Pore Size <i>microns</i>	Five- Bubble Max. Pore Size microns	Mean Pore Size microns
3	4/12/49	P.S.S., Grade E,	100	59.3	50.8	18.1
7	5/31/49	P.S.S., Grade D, I.D8	$90.2 \\ 84.8$	188.1	161.6	18.75
8	5/31/49	Porous Carbon	100			
11	4/24/50	Fisher Bronze, I.D18	56	100.6	92.0	38.1
14	5/18/50	Aloxite, Permeability 20	35			
15	5/18/50	Aloxite, Permeability 60	0			
18	11/18/50	Permutit Porous Bronze, Grade	5 73	95.3	83.9	46.8
22	5/9/51	P.S.S., Grade E,	100	35.3	18.8	15.9
23	5/9/51	P.S.S., Grade E, I.D7	, 70	210.0 (water)	110.0 (water)	30.1

fore the test. After the test, another 10 days was required before the cyst counts were completed. The influent was dosed with an average of 22 cysts per ml of water, but the influent count under a microscope disclosed that it was impossible to make an accurate count of the cysts recovered in the influent. Many of the cyst walls had been broken due to the agitation of the cysts in the storage tank and their passage through the filter. The effluent samples were examined and no cysts or definite particles of cyst walls were found.

Tests #11 and #12 were made using the same septums utilized in Tests #9 and #10. Both influent samples were dosed with sufficient living cysts to provide a concentration of about 415 per ml in the influent. The method of counting provided the detection of about 270 per ml in the influent samples taken periodically during the test.

The Fisher porous bronze septum, I.D.-18, consisted of a septum  $1\frac{1}{4}$  in. O. D. x 6 in. long which had open ends. The septum, as originally supplied by the manufacturer, contacted metal at each end and resulted in poor filtration due to leakage past the filter at this point. Before this cyst test, a graphite paper gasket was cut to size and placed at each end of the septum. Evidently, however, leakage still existed as is evidenced by the passage of more cysts through this septum than were passed through the more porous Permutit septum. It is believed, therefore, that an improved housing installation would result in better septum performance.

Criterion for testing septums. On the basis of the cyst-filtration test results, it appears the mean pore size is the best criterion to determine whether a septum will provide positive protection against the passage of amoebic cysts. However, the 5bubble maximum pore size should be checked and given considerable weight in the final analysis. The tests indicate that the mean pore size should not exceed a value of about 17 or 18 microns and that the 5-bubble maximum pore size should not exceed about 35 to 40 microns or roughly twice the mean. Additional verification tests on a larger scale must be conducted to narrow these limits before they are used for full-scale field specifications. The tests have served to indicate the feasibility of the methods used to set and check septum specifications.

#### 16. Discussion of Test Results

A comparison of the results of the bacteriological filtering efficiency tests (Table 4), the biological filtering efficiency tests (Table 7), and the pore-size determination tests (Table 6) indicated that a relationship exists between the septum pore size, the cyst removal, and the bacteria removal.

Table 9 shows the percentage of cyst removal against the 5-bubble maximum pore size. The data indicate that with porous stainless steel septums a complete cyst removal will be obtained with a 5bubble maximum pore size as large as 50.8 microns. The tests using porous bronze septums indicate that complete cyst removal will be attained with a 5-bubble maximum pore size of about 40 microns.

An attempt was made to correlate the percentage removal of *Serratia marcescens* and amoebic cysts with mean pore diameter. Although the bacterial filtration tests indicate a wide range of bacteria removals, even with the same septum, the results indicate that a septum that will effect a removal of roughly 75 to 80 percent of the applied bacteria will remove also 100 percent of the applied cysts. A rough check of such a conclusion is indicated by the fact that the porous carbon septum (septum 29), which will remove 100 percent of the

		BASED ON MI	EAN PORE SIZE	BASED ON 5-BUBBLE MAXIMUM PORE SIZE					
Porous Stainless Steel microns % removal		Porous Bronze Permutit microns % removal		Porous Bronze Fisher microns % removal		Porous Stainless Steel microns % removal		Porous Bronze Permutit microns % removal	
$\begin{array}{r} 4.1 \\ 4.4 \\ 8.9 \\ 15.6 \\ 15.9 \\ 18.1 \\ 18.1 \\ 18.1 \\ 18.7 \\ 30.1 \end{array}$	$100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 84.8 to 90.2 \\ 70$	18.619.420.142.146.847.6102.4	$100 \\ 100 \\ 95 to 100 \\ 97 \\ 73 \\ 80.5 \\ 72$	38.1 38.1	100 56	$17.5 \\ 18.8 \\ 19.9 \\ 26.6 \\ 33.9 \\ 50.8 \\ 50.8 \\ 50.8 \\ 110.0 \\ 161.6 \\ 1.6 $	$100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 70 \\ 84.8 \ to \ 90.2$	$\begin{array}{c} 40.8\\ 42.4\\ 80.5\\ 82.0\\ 83.5\\ 83.9\\ 92.0\\ 205.0\end{array}$	$100 \\ 95 \text{ to } 100 \\ 80.5 \\ 100 \\ 97 \\ 73 \\ 100 \\ 72$

Table 9 Relation Between Cyst Removal and Septum Pore Size

cysts, was found to remove at least 73.0 percent of the *Serratia marcescens* in the influent in two separate tests.

The biological and bacteriological filtration tests have served to provide some of the information required for the assignment of the various septums into their proper grade classifications. Briefly, all of the cloth and wire cloth septums may be classed as retention grade 4 or grade 5. The group 2 porous aloxite septums, depending upon their permeability, may be classed as retention grade 3 or grade 4. The septums in groups 3 and 4 may be classed as retention grade 3 or grade 4, but because of their construction would be unsatisfactory for field use. The septums which are in groups 5 and 6 may be classed in retention grade 2 or 3 depending on their maximum pore size. The three septum materials which fall into this group are porous carbon, porous stainless steel, and porous bronze. The cyst removal tests indicate that any of these materials can be manufactured so that the pore size is small enough to remove cysts. The three materials are, therefore, worthy of special consideration with respect to their advantages for use in field equipment.

The porous carbon material, in addition to its ability to remove cysts, would also provide a medium for the removal of taste and odors in the water. The thicknesses of the material required to provide a septum of reasonable strength and durability, however, result in initial head losses that are considerably higher than the head loss through porous stainless steel or porous bronze. See Figs. 10 and 16. The use of filter aid as a precoat, however, would serve to keep the septum head loss relatively constant.

The porous metal materials are manufactured in a number of porosities and are to be preferred in a field unit. The high strength and ability to be formed in many sizes and shapes make porous stainless steel desirable for filter service. It can be made in thicknesses of  $\frac{1}{8}$ -in. and can be formed in any desired shape. The cyst tests indicate that, in general, the F, G, or H Grades will adequately remove cysts from water. In view of the higher head losses through the G or H septums, consideration should be given only to the use of the F Grade. Before any accurate determination can be made of the maximum pore size that will just retain cysts, a wide range of small porous stainless steel septums must be obtained and tested so that a minimum of 50 to 75 cyst removal tests are completed for each material. In view of the fact that such tests have not yet been completed, the tentative conclusion may be made that any porous stainless steel septum which has a 5-bubble maximum pore diameter less than 46.2 microns will remove pathogenic amoebic cysts. It is possible that the 5-bubble maximum pore size may be increased as additional cyst tests are completed.

In view of the fact that only the Grade F porous stainless steel septum was found to have a 5-bubble pore size consistently below 46.2 microns, 5 septums of that grade were obtained and used in head loss vs. rate of flow tests. Figure 16 shows the initial resistance of the grade with various precoats of commercial C-535 filter aid. The head losses obtained, in comparison to those obtained with filter septums now used, are not excessively high when considering the positive filtration protection provided.

The porous bronze septums, which can be made in thicknesses down to  $\frac{1}{4}$ -in. in large cylindrical septums, must be considered for filter use. The manufacturing process restricts available septum shapes to those which can be molded, or in other words, probably to cylinders. The path through the pores is more uniform than through stainless steel due to the fact that the individual particles are more spherical than are the particles of stainless steel.

All group 7 septums, including diatomite and porcelain filter candles, remove all of the cysts and *Serratia marcescens* bacteria from water and therefore may be classed as retention grade 1 septums.

## IV. CONCLUSIONS AND RECOMMENDATIONS

1. The type of diatomite filter now in use for potable water purification does not provide positive protection against the passage of pathogenic cysts of E. histolytica through the filter under all types of operation. The effectiveness of such filters in the removal of pathogenic cysts of E. histolytica depends on the quality of the precoat formation. Since this quality is dependent, in part, on the skill and attention of the filter operator, the possibility of the delivery of a contaminated effluent is present. The tests in this investigation show that it is possible to design the filter so that no biologically-contaminated water can pass as a result of improper operation so long as the septum is in place.

2. Three materials, porous carbon, porous stainless steel and porous bronze, are satisfactory for use in the manufacture of septums to remove pathogenic cysts of E. histolytica. These materials are satisfactory for commercial use, and the pore sizes of the materials can be controlled in the manufacturing process to provide septums of any desired permeability within practicable limits.

3. No satisfactory specification limiting the maximum septum pore size has been devised because insufficient tests were made. However, the tests made indicate that with further study such a specification might be prepared.

4. A satisfactory procedure has been developed for the cultivation and counting of cysts of E. histolytica in water both before and after filtration.

5. A satisfactory method has been devised for the measurement of the porosity of filter septums.

6. A study should be made to determine chemical and physical means of cleaning porous metal septums should they become clogged in operation.

7. Further study should be made of the size, cyst-removal and bacteria-removal relationships in porous metal septums to permit the design and specification of filters and of filter septums beyond the ranges of the satisfactory septums found in these tests.

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