PREPARATION OF COLLODION SACS FOR USE IN BACTERIOLOGY.

By FREDERICK L. GATES, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, June 28, 1920.)

Metchnikoff, Roux, and Taurelli-Salimbeni[1] state that Fick in 1856 was the first to suggest the use of semipermeable collodion membranes in biology. Certainly a general interest in the properties of these membranes from the bacteriological standpoint dates from their own report in 1896 of the intoxication of guinea pigs from the diffusion of cholera toxin through the walls of collodion sacs containing cultures of the vibrio and placed in the peritoneal cavity. Since then a considerable number of articles on collodion sacs have appeared, which it is not within the province of this paper to review. It suffices here to note that the use of collodion sacs has been almost constantly attended with difficulties in manufacture, standardization, and mode of employment which have precluded their widespread use in bacteriology. There is at present no established bacteriological technique involving the intraperitoneal implantation of collodion sacs.

Heretofore most of the studies, whatever their primary object, have come at last to deal mainly with the properties of collodion sacs themselves, especially their permeability to a variety of substances, rather than with their use as an instrument for the furtherance of bacteriological research. The exceptions, however, are noteworthy. In addition to the demonstration of a soluble diffusible toxin of the cholera vibrio, noted above, mention may be made of Vincent's[2] enhancement of the virulence of saprophytes, *B. megaterium* and *B. mesentericus*, by collodion sac passage through rabbits; of Nocard and Roux's[3] demonstration of changes in collodion sac contents suggestive of the multiplication of inoculated material from pleuropneumonia of cattle; and of Nocard's[4] studies on the relation of human to avian tuberculosis by the growth of the human bacilli in sacs in the peritoneum of the chicken. All these papers appeared in 1896 or 1898 from the Pasteur Institute and with a few exceptions represent the total of the contributions made to bacteriology by the intraperitoneal use of collodion sacs.

[1] Metchnikoff, E., Roux, E., and Taurelli-Salimbeni, *Ann. Inst. Pasteur*, 1896, x, 257.

[2] Vincent, M. H., *Ann. Inst. Pasteur*, 1898, xii, 785.

[3] Nocard, M., and Roux, E., *Ann. Inst. Pasteur*, 1898, xii, 240.

[4] Nocard, M., *Ann. Inst. Pasteur*, 1898, xii, 561.

Collodion sacs seem to afford a general method for the cultivation of bacteria in pure culture under conditions approaching the invasion of a host; the bacteria are free to yield soluble diffusible products which might affect the animal and to obtain nutritive substances from the animal tissues, yet they are protected against phagocytic attack and, to some extent certainly, from exposure to antibodies. The successful transmission of one human infection after another to animals previously supposed to be immune emphasizes the importance of new methods and new channels of infection, and, in view of the experiments from the Pasteur Institute already mentioned, suggests the further use of collodion sacs for the maintenance of bacterial growth intraperitoneally.

It was with these considerations in mind, and especially with the object of developing another method by which the inciting agent in certain diseases of unknown origin might be sought, that we have tried to obtain a standardized technique for the manufacture and manipulation of collodion sacs. The requirements are that sacs of suitable size and shape may be easily made in large numbers, that they shall be uniformly strong and highly permeable, and that they may be easily sterilized and handled without danger of contamination before and after incubation in the animal body. In the method to be described these problems have been given especial consideration. While methods formerly in use have been freely drawn upon, certain essential modifications have been found necessary, and it appears desirable to report the technique in detail so that it may be followed with exactness.

Following the method of Prudden and McCrae,[5] as modified by Harris,[6] the collodion sacs here described were made on a gelatin capsule foundation which was then dissolved out with hot water. The procedure is as follows:

Preparation of the Sacs.

A small piece of thin walled glass tubing 5 by 0.5 cm., the edges of which have been smoothed in the flame, is heated slightly at one end and pressed against the rounded end of the cap of a large veterinary

[5] McCrae, J., *J. Exp. Med.*, 1900–01, v, 635.

[6] Harris, N. MacL., *Centr. Bakt.*, *1te Abt.*, *Orig.*, 1902, xxxii, 74.

capsule (4.8 by 1.6 cm.),[7] to which it adheres. With a hot wire a hole is burned through the cap into the glass neck. The open end of the body of the capsule is momentarily dipped just beneath the surface of very hot water, applied to the cap, and thus sealed in with an overlap of about 2 mm. Slight suction with the lips discloses whether the joint is tight. If not, a drop of hot water is touched to the leak. After drying, this framework is ready for the first dip in collodion.

Collodion solutions of two different consistencies are required, one for reinforcement, the other for the permeable membrane. After some experiment Squibb's collodion U. S. P. IX was found satisfactory, though a 4 per cent collodion, as a basis, may be made up from the formula, absolute alcohol 25 parts, ether 75 parts, pyroxylin 4 parts. Squibb's 4 per cent collodion or this solution of pyroxylin is too thin to give a proper membrane with one dip, but higher percentages of pyroxylin do not readily dissolve, and it is found most convenient to obtain the thicker solutions by evaporation. This is accomplished under vacuum with the application of gentle heat (water bath). The collodion boils rapidly, and evaporation is measured by the loss in volume.

The 12 per cent collodion required for the permeable membrane and the 14 to 15 per cent collodion required for reinforcement are obtained by evaporating 4 per cent collodion to one-third or less of its original volume. For evaporating and for dipping, museum jars 20 by 6.5 cm.,[8] with clamped tops and rubber gaskets, are used.

Of the entire surface of the gelatin capsule, the body is to be covered by the permeable membrane of the sac, and the cap by the heavier impermeable wall of collodion which serves to strengthen and support the membrane. This impermeable part is made first by dipping the inverted gelatin capsule (held by wedging the somewhat conical body into the end of a test-tube) neck downward into the 15 per cent collodion to a depth that just covers the joint between body and cap.[9] The capsule is dipped and withdrawn slowly to avoid air bubbles in

[7] Parke, Davis and Company, Detroit, Mich., Empty Capsules No. 12. A capsule of any suitable size may be used.

[8] Whitall Tatum and Company, Philadelphia, Pa.

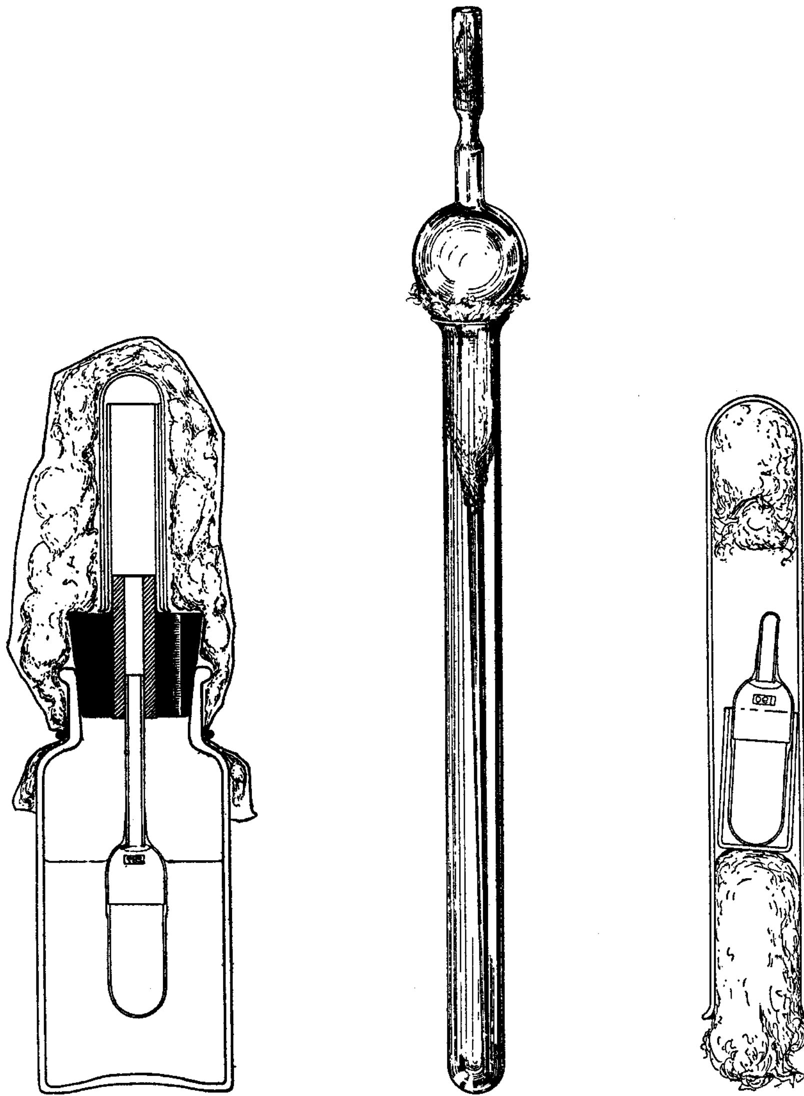
[9] The open end of the glass neck may be covered with a bit of adhesive plaster.

the angle between neck and shoulder and to allow excess collodion to run down the neck. After a few moments the collodion sets and ceases to flow, and the capsule, still held in the test-tube mouth, is inverted and set aside to dry. Before drying is complete the excess collodion is cut away from the glass neck with a knife, leaving a collar 7 mm. high that effectually joins sac and neck together when it dries. This heavy, dry collodion membrane seals and reinforces the joints between body and cap and between cap and glass neck.

The sacs may be identified by means of small numbered paper labels sealed into the wall. Tiny rectangles of very thin paper are numbered with India ink. These are cut apart, curved slightly on a blotter with the rounded end of a test-tube, dipped with forceps into thin collodion and applied to the dry membrane just below the shoulder, where they adhere and become a permanent part of the wall (Text-fig. 1).

The capsules are now ready for the final dip. They are to be handled, after the sacs are made, in 120 cc. (4 ounce) wide mouth bottles with rubber stoppers. These stoppers have one hole centrally placed for the glass neck, and a second peripherally, which equalizes pressure in the bottle and the sac. On account of its greater elasticity and resistance to autoclaving, a length of stethoscope tubing makes a good core for the central hole in the rubber stopper. The hole is cut with a cork borer slightly smaller than the tubing, which is seized with forceps, pulled through with a projection of 1 cm. on the upper side of the stopper, and cut off flush with the bottom. The use of the projection will be described later.

The neck of the reinforced capsule is inserted into the rubber stopper. The capsule is then slowly lowered into 12 per cent collodion in the jar with a rotary to and fro motion which reduces the incidence of air bubbles. It is immersed to the neck in the collodion, so that the joints at neck and body are reinforced. On withdrawal, the coated capsule is suspended above the collodion by means of a slotted card slipped under the stopper and is allowed to drain a definite length of time, as will be described. The membrane dries very little during this draining because of the ether-alcohol tension above the collodion. If a drop hangs from the capsule at the end of the drainage time it is removed by touching it to the surface of the collodion solution. The



TEXT-FIG. 1. TEXT-FIG. 2. TEXT-FIG. 3.

TEXT-FIG. 1. Cross-section of a completed collodion sac in its protecting bottle. The various steps in the preparation of the sac are explained in the text.

TEXT-FIG. 2. A Pasteur pipette prepared for dry sterilization.

TEXT-FIG. 3. A sac in its cup and test-tube, after the neck has been sealed off in a small blast flame.

capsule is rapidly withdrawn from the jar and inverted to dry in the air for 1 to 2 minutes. It is then lowered into 95 per cent alcohol in a 4 ounce wide mouth bottle, where the collodion membrane finally sets.

The capsule with its collodion coating may remain in the alcohol indefinitely, but a few minutes are sufficient to extract the remaining ether and saturate the membrane with 95 per cent alcohol. As the viscosity of the collodion and the drainage determine the thickness of the sac, so the drying and the alcohol treatment determine its permeability.

After treatment in 95 per cent alcohol the capsule is plunged into cold water until the alcohol is removed and the gelatin capsule framework softens, a matter of an hour or less. The gelatin is then thoroughly washed out with a stream of hot water. For this purpose we have used a board with holes to accommodate twelve stoppers, resting on a basin of hot water. From the faucet, distributing tubes, ending in glass capillaries which are just long enough to reach into the sacs but not to pierce their bottoms, carry in the hot water and so wash the dissolved gelatin out through the neck. An hour or so of washing leaves the completed sac, attached to its glass neck by the collar of heavy collodion. It is then emptied with a Pasteur pipette and tested for strength and imperfections. Each sac must withstand an internal pressure of at least 25 cm. of mercury (5 pounds per square inch). The empty sac is immersed in distilled water in a 4 ounce bottle, and the second hole in the stopper is connected by a stub of glass tubing with a vacuum pump and gauge. The air in the bottle is exhausted 25 cm. A stream of air bubbles from the surface of the sac reveals any leak. If bubbles in the collodion were carefully avoided a leak is rare. Imperfect sacs are not worth repairing and are discarded.

The sac is then filled to the neck with distilled water and is ready for the autoclave. The expansion of water on autoclaving is sometimes sufficient to cause an overflow, and consequent loss of water on cooling. The projection of the stethoscope tubing above the stopper is therefore fitted with a short length of glass tubing open at the top. This forms an expansion chamber, from which the water drains back into the sac on cooling. To prevent contamination the glass tube is capped by a short, loosely fitting, inverted test-tube.

The top of the bottle is swathed in raw cotton and covered with paper, tied on. In this way contamination of either the inside or outside of the sac is avoided, and the sac is protected indefinitely for future use. Ten or twelve sacs are thus prepared at one time and then autoclaved at 15 pounds pressure for 30 minutes (Text-fig. 1).

Mode of Use.

Before discussing permeability, which is the vital factor in the collodion sac technique, the mode of inoculation and peritoneal implantation may be described briefly.

The most convenient instrument for filling and emptying the sacs and preserving their contents is a Pasteur pipette. Those which we use are made in quantities in the laboratory from 24 cm. lengths of rather heavy glass tubing pulled out in the middle and sealed off to make two pipettes. A bulb about 2 cm. in diameter is then blown just above the shoulder. The requisites are very hot glass and gentle pressure. The tip is cut just to reach the bottom of a Noguchi culture tube (20 by 1.5 cm.), and a wrapping of cotton below the bulb acts as a stopper for the tube, in which the pipette is dry sterilized (Text-fig. 2). With these pipettes the sacs are filled, and after incubation the sac contents are similarly withdrawn and transferred to the test-tube which has protected the pipette from contamination. Then the pipette with its cotton collar serves as a plug for the tube and is used for subsequent withdrawal of fluid for examination.

Inoculation of small quantities of material into media in the sacs is accomplished with a platinum loop or a small sterile Wright pipette plugged with cotton. Such a pipette, flamed, and bent into a V saves the use of a Pasteur pipette in emptying out the distilled water from the sterilized sac preparatory to use. The bottle is inverted, the small arm of the pipette inserted upward through the neck, and gentle breath pressure expels the distilled water without danger of air contamination.

After being filled to the neck with a suitable medium and inoculated with bacteria or suspected material, the sac is withdrawn from the protecting bottle and the neck sealed off smoothly in a small blast flame. During this process and subsequently, contamination of the outside of the sac is avoided by carrying it in a short glass cup with

a flat bottom so that it will stand upright. Those used in the present work were made from 6 dram homeopathic vials cut off with a hot wire and a plunge into cold water. They are wider than the sacs and about 3 cm. long, so that the shoulder and neck project. The cups are sterilized by dry heat in wide test-tubes. When the neck of the sac is to be sealed off, one hand holds a cup containing the sac, while the other hand manipulates the rubber stopper. The cup, with its sac, is reinserted into the inverted test-tube until the neck has cooled (Text-fig. 3). The neck is then painted with a layer of collodion, which fuses with the collodion collar and so prevents the possibility of leakage between collar and neck. After a few minutes drying the sac is ready for intraperitoneal insertion. This is done aseptically under full ether anesthesia through a short incision in the abdominal wall above the umbilicus. The surgical technique need not be described, except to say that the incision should be sewed up firmly in layers. Metal skin clips save time. A dressing of cotton and collodion is sufficient. No bandage is required.

As many as eight such sacs have been tolerated by an adult rabbit without any apparent discomfort. The tissue reactions which often occur around the sacs will be discussed later. After an appropriate incubation period the rabbit is killed by an occipital blow and the sacs are recovered through a wide abdominal incision. They are again dropped into the glass cups, opened at the shoulder with a hot wire loop, and their contents transferred to a Noguchi tube by means of its Pasteur pipette.

General Characteristics of the Sacs.

Sacs made as just described have a capacity before autoclaving of from 6.5 to 7 cc. The upper third is a tough, impermeable wall which supports the glass neck and the permeable bottom. The lower two-thirds, with a surface area of about 14 sq. cm., is a thinner, moist, elastic membrane of high permeability. It is heavy enough, however, to stand immersion in water without collapsing and to withstand an internal pressure of 5 pounds per square inch. Such sacs rarely break during incubation. The walls of the sac are colorless and transparent.

Autoclaving at 15 pounds pressure for 30 minutes causes a uniform shrinkage to a capacity of 4.7 to 4.2 cc., corresponding to a diminution in surface area of approximately 25 to 30 per cent. Permeability is somewhat diminished but remains high. The shape and transparency of the sac are unaltered. The sacs may be kept sterile in distilled water in their bottles for months without appreciable loss in permeability, but the walls tend to become more brittle and inelastic.

Permeability.

The permeability of the membranes has been tested in a number of ways. The quantitative test by which variations in materials and methods of manufacture were compared consisted in dialyzing a 2 M solution of sodium chloride within the sac against 20 or 25 volumes of distilled water and titrating a sample of the dialysate from time to time with 0.02 M silver nitrate solution, sodium chromate being used as indicator. The quantitative relations were such that when equilibrium was reached 1 cc. of the dialysate would precipitate 5 cc. (or 4 cc.) of the silver solution as silver chloride.

In 1915 Brown[10] contributed a careful study of collodion membranes and showed in particular that the permeability of membranes could be accurately controlled by complete drying, followed by immersion in ethyl alcohol of a definite dilution. Alcohol dilutions below 30 per cent confer practically no permeability on an air-dried membrane. 95 per cent alcohol produces a high degree of permeability. In preliminary experiments Brown's results were fully confirmed, and the value of his discovery was appreciated. His membranes, however, were prepared for chemical dialysis and did not require sterilization. On trial it was found that air-dried membranes, rendered permeable by alcohol treatment, lost their permeability again upon sterilization by heat. On the other hand, sacs which were not allowed to dry before immersion in alcohol lost little in permeability by sterilization in the Arnold sterilizer or the autoclave. Since highly permeable sacs were desired, 95 per cent alcohol was used as a routine.

Viscosity of the Collodion.—The percentage of pyroxylin in the collodion determines its viscosity, and viscosity in turn determines

[10] Brown, W., *Biochem. J.*, 1915, ix, 591.

the thickness of the collodion coating that will adhere to the dipped capsule. On account of rapid evaporation of the solvents the solution must be tested frequently and maintained at approximately the proper density. Commercial collodions vary considerably in density, and it is not a safe rule to evaporate a so called 4 per cent or U. S. P. collodion to one-third of its volume to obtain a 12 per cent solution.

The percentage of pyroxylin in the solution may be measured directly by weighing a specimen before and after evaporation to dryness. But this is not a convenient method, except as a check and to standardize a simple viscosimeter by which the thick collodions may be readily tested and compared.

The viscosimeter which we use consists merely of a 15 cm. length of Pyrex glass tubing, with square-cut ends, the internal diameter of which happens to be 0.365 cm. Near the middle of this tube file marks measure a distance of 5 cm. The tube is dipped into the collodion to be tested, a column is drawn up by suction well beyond the upper file mark, and the tube is withdrawn. As the column is then released in the vertical tube a stop-watch is used to time the meniscus over the measured distance of 5 cm. 12 per cent collodion at 20°C. requires 15± seconds to flow past the marks on the viscosimeter used.

Having standardized such a simple instrument it is easy to maintain thick collodion at the proper density by frequent tests and the addition of ether and absolute alcohol 3:1 to compensate for evaporation.

An even simpler method of determining viscosity, and one which is probably sufficiently accurate for the purpose, is to time the flow of collodion from a dipped capsule until the stream breaks and is succeeded by a series of drops. This time interval increases rapidly with the density of the collodion. In one test, for example, it increased from 12 seconds with a 10 per cent collodion to 1 minute, 15 seconds with the same collodion evaporated to approximately 12.7 per cent. The stream should flow about 1 minute before breaking.

The heavier collodion used for the neck and shoulder of the sac need not be standardized. We use it so thick that it will just flow smoothly, with no tendency to "jell." Collodion that has been used for permeable membranes until it has lost its clear transparency will serve the purpose.

Determination of the Draining Time.—The length of time that drainage is allowed to proceed influences the thickness of the collodion

coating and the evenness of its distribution over the surface of the capsule. Provided the first few drops are permitted to fall after the stream of collodion has broken, little is gained by prolonging the drainage. As will be shown later, slight variations in thickness are of minor importance, and sufficient uniformity is obtained by allowing a definite number of drops to form and fall.

Experiment 1.—From five sacs, after dipping, an unbroken stream of collodion flowed for an average of 1 minute, 16 seconds. Successive drops then fell after 1 minute, 26 seconds; 1 minute, 52 seconds; 2 minutes, 32 seconds; and 3 minutes, 29 seconds on the average. After 2 drops had fallen, 40 seconds were required to reduce the thickness of the membrane by the amount of 1 drop of collodion, and 57 seconds were required for the following drop to form.

It is hardly profitable to prolong the drainage time beyond the separation of the first few drops.

Effect of the Drying Time on Permeability.—

Experiment 2.—Five collodion sacs were made on No. 12 gelatin capsules by dipping once in 12 per cent collodion, draining 2 minutes, and drying for various intervals, as shown in Table I, before immersion in 95 per cent alcohol for 1 hour.

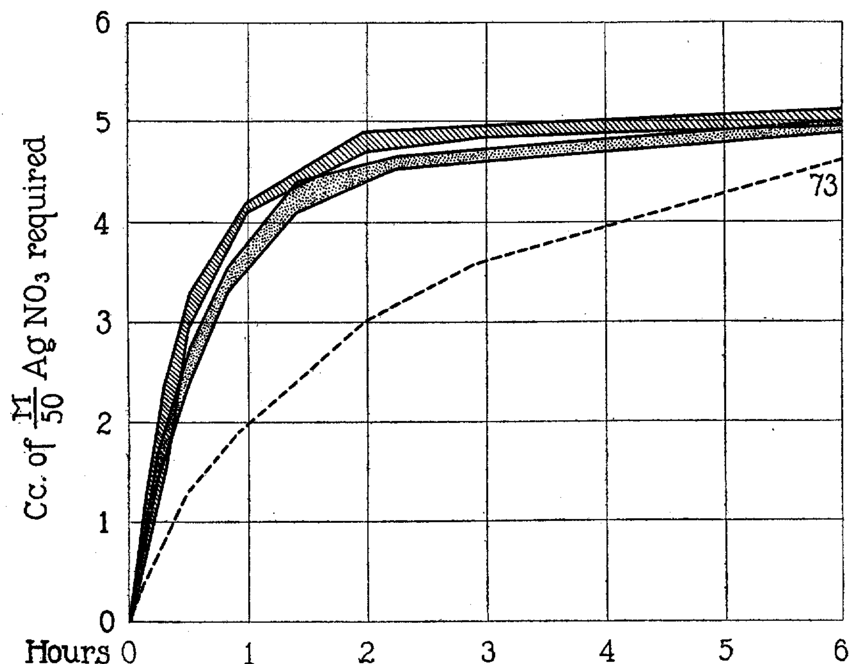
TABLE I.

Sac No.	Drying time.	Sterilization.	Capacity.	
			Before sterilization.	After sterilization.
			cc.	*cc.*
81	0	Arnold sterilizer, 3 hrs.	6.4	4.6
76	15 sec.	Autoclave, 30 min.	6.2	4.8
82	30 "	Arnold sterilizer, 3 hrs.	6.4	4.7
80	1 min.	Autoclave, 30 min.	6.1	4.6
73	1 hr.*	Arnold sterilizer, 3 hrs.	7.3	6.2

* Completely dry in 1 hour.

The permeability of the sacs to sodium chloride was determined both before and after sterilization and is shown in Text-fig. 4. Sodium chloride diffused much more rapidly through the sacs which were not completely air-dried before the alcohol treatment. Sterilization, whether in the Arnold sterilizer at 100°C. or in the autoclave at 15 pounds pressure, did not greatly impair the permeability, notwithstanding the shrinkage which accompanied the process. The air-dried sac,

No. 73, on the other hand, shrank less during sterilization, but its permeability was so far lost in the Arnold sterilizer that no measurable amount of sodium chloride had passed through it in 6 hours, and only 1.30 units (on a scale of 5) had passed in 48 hours.

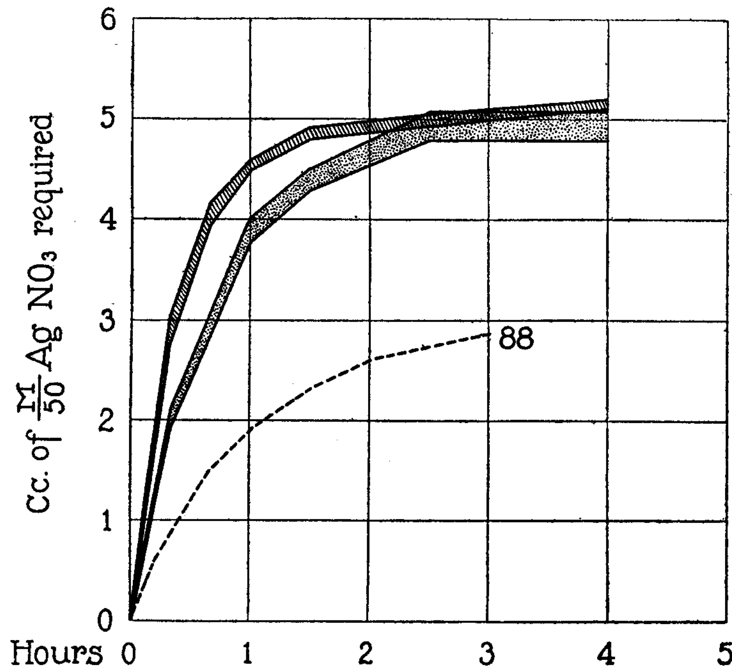


TEXT-FIG. 4. Experiment 2. The cross-hatched area covers variations in permeability of Sacs 81, 76, 82, and 80 (Table I) before sterilization. The stippled area covers variations in permeability of these sacs after sterilization. The broken line is the permeability curve of Sac 73, dried before the alcohol treatment. Sac 73, after sterilization, passed no measurable amount of sodium chloride in 6 hours.

Experiment 3.—In a similar experiment four sacs were dipped once, drained 3 minutes, dried 1, 2, 3, and 4 minutes respectively, and treated with 95 per cent alcohol over night. Their capacities before sterilization were 6.3, 6.1, 6.0, and 6.5 cc., and they shrank to 4.9, 4.7, 4.7, and 5.3 cc. in the autoclave. Their permeability before and after autoclaving is shown in Text-fig. 5.

From these two experiments it is evident that variations in the drying time have little influence on permeability, provided the membrane is still moist when immersed in alcohol. Sacs dried for 30

seconds to 1 minute are the most satisfactory. If plunged immediately into alcohol after drainage they tend to wrinkle and to show a smoky bluish opacity, and they are not so tough and strong as the partly air-dried sacs.



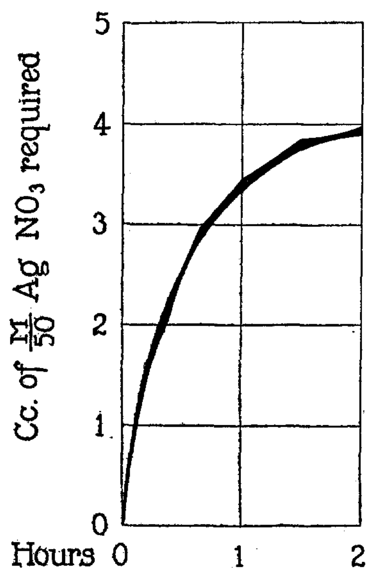
TEXT-FIG. 5. Experiment 3. The cross-hatched area covers variations in permeability of four sacs before sterilization. The stippled area covers variations in permeability of the same sacs after sterilization. The broken line is the permeability curve of Sac 88, not treated with alcohol before sterilization.

Importance of the Alcohol Treatment.—Other experiments indicate that treatment in 95 per cent alcohol for as short a time as 1 minute is sufficient to insure sacs of high permeability. It is only necessary that the alcohol should replace the ether-alcohol solvent of the collodion before the membrane is set by immersion in water. The importance of the alcohol treatment is illustrated in Text-fig. 5 by the permeability curve of Sac 88, which was drained 1.5 minutes, dried 30 seconds, and then plunged into water without the preliminary

immersion in alcohol. Although this sac was made of the same collodion as the others and shrank only from 6.9 cc. to 6.5 cc. during sterilization, its permeability to sodium chloride was much less than that of alcohol-treated sacs.

Shrinkage of the Sacs during Sterilization.—The hot water used to wash out the gelatin framework of the sacs causes an initial loss in capacity from 7+ cc. to about 6.6 cc. Sterilization by heat further shrinks the membrane to an average capacity of 4.5 cc. The loss in capacity and permeability due to heating is absolute and does not continue once the limit is reached.

Experiment 4. Effect of Re-Autoclaving.—Four sacs, dipped once in 12 per cent collodion, drained 1.5 minutes, dried 30 seconds, and immersed in 95 per cent alcohol for 1 hour, were autoclaved at 15 pounds pressure for 30 minutes. Two of the sacs were then re-autoclaved under similar conditions. The capacity of the first two sacs was 4.5 and 5.1 cc. respectively, of the second two 4.4 and 5.05 cc. All four sacs had a practically identical permeability as shown by Text-fig. 6. The curve incidentally illustrates the uniformity of collodion sacs made as described above.



TEXT-FIG. 6. Experiment 4. The solid black area covers variations in permeability of four sacs, two of which were autoclaved once, the other two autoclaved twice.

The relation between the diminution in capacity and in surface area of the sacs on sterilization is shown in the following experiment.

Experiment 5.—Eight sacs made in an identical manner from the same collodion solution were divided into two groups of four each. After volume measurements had been taken, four of the sacs were autoclaved and their capacities again determined. All the sacs were then cut open so that they could be flattened out, shadowgraphs were made on photographic paper, and the areas measured with a planimeter. The average measurements are shown in Table II, in which are included similar figures for two sacs that were completely dried before the alcohol treatment.

TABLE II.

Condition and No. of sacs.	Volume.				Surface.			
	Before autoclaving.		After autoclaving.		Before autoclaving.		After autoclaving.	
	Body.	Total.	Body.	Total.	Body.	Total.	Body.	Total.
	cc.	*cc.*	*cc.*	*cc.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*
Two dried sacs.			4.9	6.95			14.3	21.9
Four undried sacs; not autoclaved.	4.5	6.72			14.0	21.6		
Four undried sacs; autoclaved.	4.45	6.67	2.72	4.5			9.6	15.8
Capacity loss.			39 per cent.	33 per cent.			31 per cent.	27 per cent.

It is seen from Table II that sterilization of the permeable sacs caused a loss of 33 per cent in capacity, corresponding to a surface shrinkage of 27 per cent. The losses were not proportionately distributed between the impermeable cap and the permeable body, which lost 39 per cent in capacity and 31 per cent in surface area.

This shrinkage on heating is due largely to the alcohol treatment of undried sacs. Dried sacs, even though treated subsequently with alcohol, shrink but little in the alcohol, as Brown[10] has shown, or in the autoclave (Table II). Undried sacs, not treated with alcohol, also shrink but little on sterilization. The part played by shrinkage in decreasing permeability has probably been overestimated. That the shrinkage itself is of minor importance is shown by a comparison

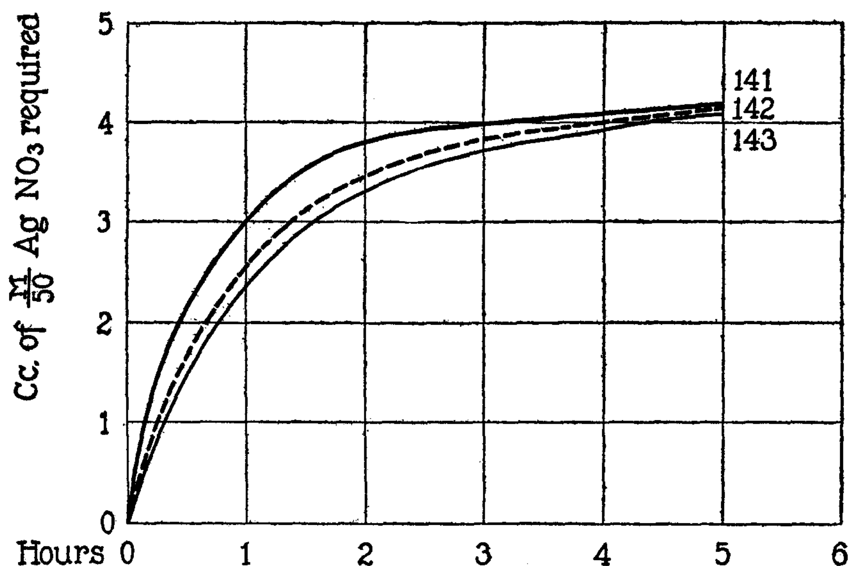
of the permeability of alcohol-treated undried sacs with that of sacs prepared by other methods.

In this connection it may be pointed out that titrations with sodium chloride do not give complete information with regard to the permeability of the sac walls. The sodium chloride molecules and their constituent atoms are of relatively small size, and the rate at which they diffuse through the permeable membranes suggests that the pores of the membrane must be several times the size of the molecules to permit such rapid passage. The volume shrinkage on sterilization is approximately 33 per cent, which corresponds to a diminution in permeable surface area of 31 per cent. Assuming that the pores diminish correspondingly, it is conceivable that while small molecules might still pass through with ease, other molecules which more nearly approached the unshrunken pores in size might now be entirely held back. So far, however, among the substances tested none has been found which passes through the unheated, but not through the heated membranes.

Relation of Thickness to Permeability.—Another factor emphasized in permeability experiments with collodion membranes is thickness. In the present method, within limits, this factor also is found to be secondary in importance to the alcohol treatment. The viscosity of the collodion solution and the drainage and drying time are the variables in determining thickness and have been experimentally controlled (Experiments 1, 2, and 3). A 12 per cent solution forms a sac of sufficient strength for intraperitoneal incubation. Thicker sacs, however, may be used without great loss in permeability.

Experiment 6.—Three collodion sacs were made on a gelatin capsule framework. No. 141 was dipped once in 12 per cent collodion, No. 142 twice, and No. 143 three times. The sacs were drained 2 minutes and dried 45 seconds between each dip. All were immersed in 95 per cent alcohol for 1 hour, washed, and autoclaved. Sac 141 was thin and transparent. Sac 142 was of medium thickness, with a slightly smoky opacity. Sac 143 was very thick and tough, with a smoky opacity. Capacities before autoclaving, 6.8, 6.1, and 6.4 cc. respectively; after autoclaving, 4.7, 4.0, and 4.2 cc. Such sacs in their thinnest area, just below the joint, were found to measure 0.09, 0.31, and 0.52 mm. in thickness after sterilization.

The results of permeability tests with 4 cc. of 2 M sodium chloride against 96 cc. of distilled water are shown in Text-fig. 7.



TEXT-FIG. 7. Experiment 6. The permeability curves, after sterilization, of three sacs of widely different thicknesses. Sac 141 dipped once in 12 per cent collodion, Sac 142 dipped twice, and Sac 143 dipped three times. In this experiment equilibrium in dialysis is represented by 4 cc. of silver nitrate. The error in the final readings is due to the removal of successive portions of dialysate for test. This loss in volume was not compensated during the experiment.

The difference between the sacs is less than would be expected from the comparative thickness of their walls and indicates that slight variations in thickness would be of no practical significance.

Qualitative Tests of Permeability with Other Substances.—In addition to the titrations of permeability with sodium chloride a number of qualitative tests with other substances have been made. These tests were incidental to other experiments and are therefore incomplete, but the results are useful in indicating the relative permeability of the sacs.

1. Various inorganic salts passed through the sacs with an ease approaching that of sodium chloride. With such substances the endosmotic pressure of a concentrated solution is so quickly lowered by passage through the sac that the level of liquid within the sac is hardly raised significantly before equilibrium is established.

2. Oxygen in solution rapidly diffuses through the sac wall and restores the color to methylene blue reduced by the action of dextrose broth. This fact probably explains the consistent failure of representative strict anaerobes to grow in permeable sacs intraperitoneally implanted. Haggard and Henderson[11] estimate the oxygen tension of the peritoneal cavity at about 45 mm. So far no method has been devised by which this handicap may be overcome.

3. During intraperitoneal incubation the hydrogen ion concentration of sac contents tends to come to an equilibrium with that of the peritoneal fluid. Ascitic fluid, dilute rabbit serum, and broth, for example, whatever their initial reaction, and $\frac{M}{15}$ phosphate mixtures, in proportions to give various pH concentrations, have all been reduced to a pH of 7.4 to 7.5. *In vitro* it is found that the primary and secondary phosphates diffuse through the walls with equal facility. The dialysate comes almost immediately to the pH concentration of the phosphate mixture used.

4. Among the simpler organic compounds, dextrose alone has been tested. It soon shows its presence on the opposite side of the membrane, but diffuses more slowly than do inorganic salts and so gives opportunity for a considerable endosmosis before equilibrium is established.

5. Nutrient materials of meat infusion broth pass through the sacs with sufficient rapidity to promote a luxuriant growth of bacteria (*B. typhosus*, *B. pyocyaneus*) inoculated into distilled water. The straw-colored pigments of the broth are likewise diffusible. Nutrient materials are obtainable from body fluids in a similar manner. Heavy growths of *B. pyocyaneus*, *B. typhosus*, and Type I pneumococcus were obtained by incubating inoculated sacs of distilled water over night in a rabbit. *B. pfeifferi*, lacking hemoglobin, did not grow under similar conditions.

6. The diffusible products of bacterial metabolism have not been determined by analysis. That the sacs are permeable to these products is shown by the tissue reactions which occur around actively growing cultures. The sacs themselves are practically inert, and it is common to find uninoculated control sacs lying free among the intestines without any observable irritation of the surrounding tissues. Sacs which contain living bacteria, on the other hand, are usually the center of an active proliferative process. They are found wrapped in folds of thickened and injected omentum or among intestinal adhesions, sections of which show fibrin deposits, accumulations of leucocytes, rarely to the extent of pus formation, localized hemorrhages, infiltration with fibroblasts, and newly formed capillaries; in short, the various elements and stages of a degenerative and regenerative tissue reaction.

7. No evidence has been observed that hemoglobin or other unsplit proteins will pass through the sacs. In two experiments in which highly potent antimeningococcic serum was surrounded by a suspension of meningococci no agglutination occurred.

---

[11] Haggard, H. W., and Henderson, Y., *J. Biol. Chem.*, 1919, xxxviii, 71.

In summary, it may be said that the sacs as described are permeable to gases in solution, to inorganic salts, to dextrose, to certain protein-split products which are nutritive to bacteria, and to certain toxic products of bacterial metabolism, but they hold back antibodies, unsplit proteins, and formed elements such as bacteria and body cells.

SUMMARY.

A standardized method is described in detail by which collodion sacs suitable for intraperitoneal incubation and for other bacteriological experiments may be produced in large numbers, sterilized, and handled with convenience and the minimum danger of contamination. Various factors influencing permeability have been subjected to experiment. Like Brown, we found that immersion in alcohol is the most important factor, but the high permeability conferred by alcohol treatment is lost during heat sterilization if the membrane was previously allowed to dry. Quantitative experiments on the dialysis of sodium chloride, and simple tests with other substances indicate the general character of the membranes and their probable field of usefulness in bacteriology.