A COLLODION SAC FOR USE IN ANIMAL EXPERIMENTATION[1]

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Sanarelli (1891) is believed to have been the first to employ collodion sacs in animal experimentation. Subsequently, sacs of different types have been used in various *in vivo* experiments by a number of investigators (see bibliography). Alcohol-ether collodion was employed in each case for the preparation of the semipermeable membranes. In order to withstand the distorting effect of the intestinal movements, the collodion had to be of rather high concentration, with correspondingly low permeability. In undertaking the present work, it was felt that certain investigations could be made much more satisfactorily if sacs could be prepared that were more permeable and at the same time more rugged than those that had been previously described. According to Elford (1931), acetic-acid collodion membranes are not only of a higher order of permeability than those of alcohol-ether collodion, but they are also highly heteroporous. The use of this fragile material necessitated the finding of a practical support. Fouard (1909) impregnated wire gauze with collodion. This type of support was tried and was found to be highly practical. The description by Gates (1921) was used as the point of departure in trying to prepare a highly permeable yet sturdy modification of his type of sac. After a considerable number of failures, a satisfactory sac has been evolved which is quite different from that of Gates. However, many of his practices, particularly those concerning the handling of sacs, have been incorporated.

[1] Presented before Eastern New York Branch of the Society of American Bacteriologists, December 2, 1938, Albany, New York.

In brief, the sac consists of a semipermeable membrane supported by a cylinder of stainless-steel wire gauze closed at one end with molded carnauba wax and capped at the other end also by wax, through which a glass tube passes. The technic of manufacturing the framework is an empirical one, the detailed procedures having been adopted after trial and error.[2]

MATERIALS AND METHODS

Materials

The equipment used in making the framework of the sac is displayed in plate 1. Carnauba wax is melted in a glass beaker, ready to be pipetted to the two glass molds. The first mold consists of 9-mm. glass tubing that widens at the top to form a glass cup 2 cm. in diameter and 1.5 cm. high. The tube is filled with melted paraffin to within 2.5 cm. of the bottom of the cup. The paraffin hardens and forms a permanent plug. The mold thus prepared is held in a vertical position by means of clamps. The second mold, of the same dimensions as the cup of the first, is made by severing the bottom part of a test tube with a hot wire. The tubes that form the openings into the collodion sacs are made of 6-mm. glass tubing cut into 3-cm. lengths and fire-polished. Rectangles of 100-mesh stainless-steel wire gauze, 4 by 3 cm., to be used as supports for the semipermeable membranes are scrupulously cleaned in boiling 25 per cent sodium hydroxide, rinsed in water, dipped in acetone, and dried in air. They are curved into cylinders with the long dimension as circumference.

Manufacture of framework

Before pipetting the wax into the molds, the inner surfaces are wiped with vaseline to prevent the wax from adhering at any point. One of the 3-cm. glass tubes is lowered into the first mold so that one end rests on the paraffin plug and the other projects up into the glass cup. Enough water is now delivered into the tube portion of the mold to fill it up to the bottom of the cup. Next, carnauba wax is allowed to run into the mold from a pipette

[2] The work was done with the assistance of Charles A. Clark.

until it all but reaches the top of the 3-cm. glass tube. It will not run down into the tube portion of the mold for it is lighter than the water there. A wire-gauze cylinder is immediately lowered into the cooling wax, which, solidifying rapidly, contracts so that the approximating edges of the gauze are pressed tightly together. After cooling, the wax is eased out of the mold, together with the attached glass tube and gauze cylinder, as shown in plate 2.

Using the second mold, the wax bottom of the framework is affixed in a comparable manner. The wax is shaped further by means of a hot spatula so that the edges become smooth and almost flush with the gauze cylinder. Although it does not tend to spatter during this procedure, provided it is dry, care must be taken to prevent wax from running down the gauze lest the future dialyzing surface of the sac be unnecessarily reduced. The line of approximation of the gauze is sealed by allowing melted wax to flow into the adjacent interstices from a capillary pipette. An identification number is printed on the wax of each frame in indelible ink. The frames are now ready to be reinforced with heavy alcohol-ether collodion; the percentage of nitrocellulose in this collodion is immaterial as long as it just flows smoothly. To prevent the collodion from running up into the short glass tube when the inverted sac is dipped, the tip of the tube is first touched to the collodion and then set aside for a short time to dry. During this time the bottom of the sac can be coated by lowering it into a jar of heavy collodion until the junction of the wax and wire gauze is barely submerged. After the excess collodion has drained off, it is allowed to dry. The upper end of the sac is coated in the same manner, the 3-cm. glass tube being completely submerged during the process. Using a scalpel, the distal portion of the 3-cm. tube is bared of collodion. Foreign material clogging any of the interstices of the gauze is flicked out with the point of the scalpel or a needle.

Semipermeable membrane

After several hours, when the heavy alcohol-ether collodion has become thoroughly hard but not so dry that it is very brittle or

tends to peel, the sac is dipped into acetic-acid collodion of whatever strength is required. Tests have been performed with sacs made with from 4.5 to 10.3 per cent Parlodion, by weight, in glacial acetic acid. A description of the experiments for which collodion solutions of different strengths have been used is not germane to this article, but it may be said that the higher concentrations of collodion have proved to be practical and of greater usefulness. The handling of the sac is facilitated by the use of a short piece of rubber tubing drawn through the hole of a no. 5 rubber stopper so that it projects a short distance from each end. The rubber tubing is of adequate size to hold the glass tube lightly but firmly. After the sac has been attached, it is lowered into the acetic-acid collodion in a wide-mouthed bottle. If the collodion is too cool, it will not cover the gauze smoothly. Slight rotation of the sac serves to dispel any bubbles which may cling to the surface of the gauze. As soon as it has been submerged up to the lower portion of the 3-cm. tube, it is slowly withdrawn. The excess collodion is allowed to drip into a Petri dish. While the sac is submerged, the collodion flows through the interstices and down the inner surface of the gauze. As it collects on the bottom, it is aspirated by a special capillary pipette, depicted in plate 3. The drawn-out end of the pipette must be large enough to allow the collodion to be aspirated without clogging, yet small enough to allow plenty of air space around it when it is lowered through the rubber and glass tubing to the bottom of the sac; otherwise the negative pressure established in the sac may rupture the membrane. Aspiration must be controlled so that collodion and not air is sucked up; the drawing of a vigorous stream of air into the sac is to be avoided. Frequent flushing of the pipette with glacial acetic acid helps to prevent clogging. After the sac has drained for an arbitrary but constant period (60 seconds), it is plunged into a bottle of stone-filtered tap water. The aspirating is continued, however, until the collodion on the inside bottom has been removed as completely as possible. A stiff wire bent into the shape of a hairpin is slid under the rubber stopper and allowed to rest on the edges of the bottle, thus suspending the sac in the water. The sac is filled with water by

means of a capillary pipette. The water is changed about every half hour or hour, for from four to six hours, the water on the inside being changed at the same time. A longitudinal section of the completed sac is schematized in figure 1.

Testing the sac

The method of testing porosity and intactness was suggested by the work of Asheshov (1933). The sac and attached rubber tubing are filled to overflowing with filtered tap water and a

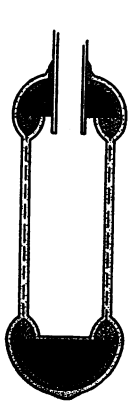

FIG. 1

FIG. 2

FIG. 1. SCHEMATIC DIAGRAM OF SAC

The glass tube at the top of the sac passes through the wax cap, represented by shading. The wire gauze is indicated by interrupted lines. The heavy stippling reveals the portion of the framework covered by heavy alcohol-ether collodion and the light stippling shows coverage by the acetic-acid collodion.

FIG. 2. RINSING BOTTLE

filled 10-ml. pipette is thrust firmly into the upper end of the rubber tubing. The pipette is held in a vertical position, as shown in plate 4, and the level of the water in which the sac is suspended is adjusted so that for each test it is the same distance (2 cm.) above the upper margin of the semipermeable membrane. The length of time required for the meniscus to descend from the 0-ml. to the 1-ml. mark is recorded. The temperature of the room and the barometric pressure are noted. Any marked increase in speed of filtration above the average for the percentage

of collodion used generally indicates the presence of a flaw in the sac. As a further check on the intactness of the sac, it is withdrawn from the water with the pipette still attached and the surface gently brushed with filter paper. If drops immediately and persistently appear at any one spot after each brushing, the presence of a flaw is indicated and the sac is discarded.

Sterilizing the sac

The sac is sterilized in "B.K." solution,[3] 1 part in 100 of distilled water, for about eighteen hours at room temperature. Except for spore-forming bacilli, which on rare occasions have subsequently proliferated, contaminating bacteria are always destroyed by this method. The sterilizing solution is then washed out with 3 liters of sterile distilled water by means of the sterile rinsing device shown in plate 5. After the stoppers are inserted in the bottles, they may be swathed in cotton soaked in cresol solution as a precaution against contamination. The water is delivered by syphon to the rinsing bottle which is diagrammed in figure 2. The water flows into the sac through the water inlet tube and the capillary tube, spills out of the mouth of the sac, runs down the outside, and causes the water level in the bottle to rise slowly until it reaches the cotton-plugged air vent. If the stopper is tightly inserted in the bottle, the water will not reach its under surface but will rise in the vent and at the same time spill over through the outlet tube, establishing a syphon which, because of the large size of the outlet tube, promptly drains the bottle; thus the syphon is broken. The continuous, fine stream of water entering through the capillary tube now causes the water level to rise again, and the automatic rinsing continues. The water inlet tube may be clamped off from time to time, thus allowing the sac to soak. It has been found that the syphon will break more readily if the inner surface of the outlet tube is coated once with a thin layer of paraffin.

[3] A sodium hypochlorite solution marketed by the Pennsylvania Salt Manufacturing Company of Philadelphia. Quigley and Sickles (J. Bact., 1937, **33**, 110–111) recommended it for sterilizing collodion membranes.

Filling the sac

A test-tube holder fitted with extra strands of wire and sterilized in boiling water serves as a satisfactory tool for handling the sterile sac. The water is expelled from the inverted sac by introducing into it a gentle stream of air through a cotton-plugged sterile capillary pipette. A flat-bottomed glass cup, made from a small vial by cutting off the upper portion with a hot wire, serves to hold the sac from this point on. Before use, the cup is put, open end first, into a large test tube and sterilized. In order to introduce the sac, the test tube is inverted and the cotton plug and the cup are withdrawn together. The sac is then deposited in the cup, and the cup and plug are thrust back into the inverted test tube as shown in plate 6. The sac is allowed to remain empty for only a short time, lest drying alter the permeability. When the sac is to be filled, the cup is removed again, and the glass tube of the sac thoroughly dried in a Bunsen flame, care being taken not to crack it by too rapid heating. The test fluid is introduced as shown in plate 7. Great caution must be exercised to prevent any fluid from running down the outside of the glass tube; as an added precaution, the tube is thoroughly flamed again after the pipette has been withdrawn. A few drops of sterile, melted paraffin serve to plug the glass tube. A coating of alcohol-ether collodion is applied to the outside of the tube; thus the whole surface of the sac is sealed with collodion.

Implantation in animal

The sac is now ready to be introduced into the peritoneal cavity of the experimental animal. The operative technic need not be described here. When the sac is removed at the conclusion of the experiment, a hot wire is thrust through the collodion and paraffin, thus providing a hole through which the contents can be aspirated with a Pasteur pipette. Plate 8 shows two sacs after their removal from an experimental animal. The wall of one is clean and the wire mesh is plainly visible through the film of collodion. The other sac is covered with a heavy coating of fibrin.

The results of experiments entailing the use of the described collodion sacs will be published subsequently.

SUMMARY

A review is presented of the development of the collodion-sac technic for use in animal experimentation.

A method of making a sturdy, yet permeable, sac is detailed.

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PLATE 1

MATERIALS USED IN MAKING THE FRAMEWORK OF SACS

PLATE 2

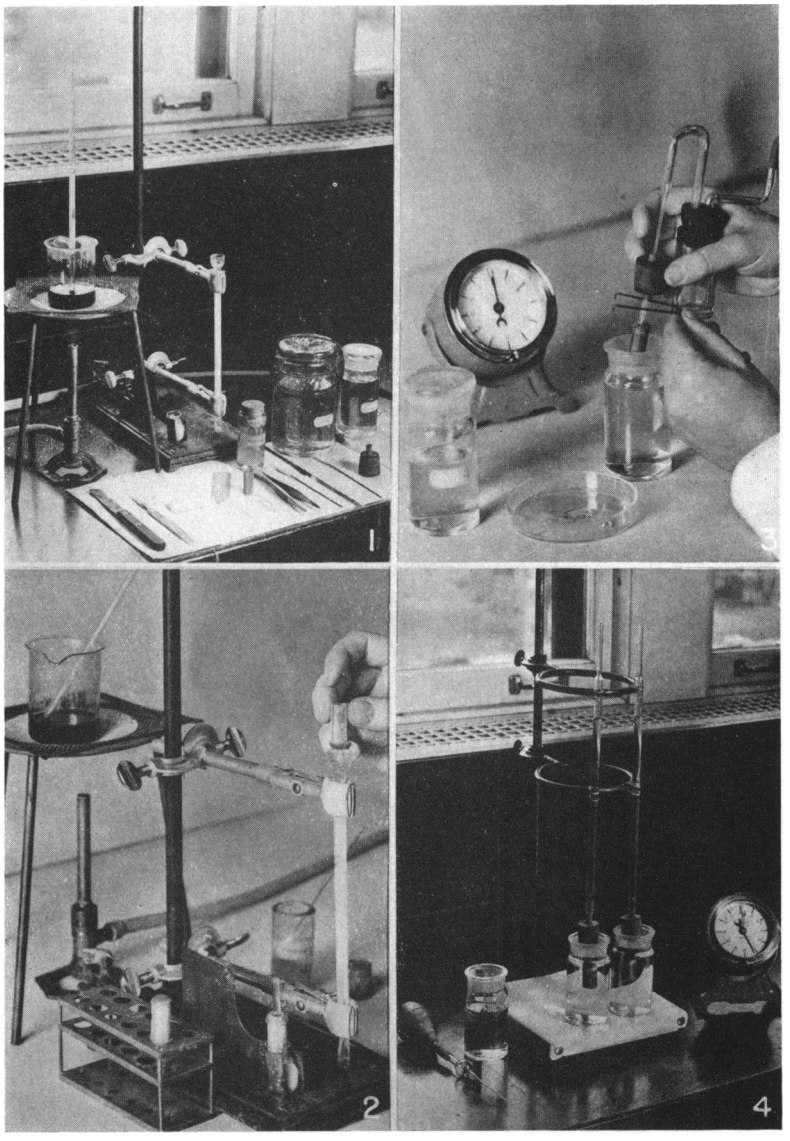
REMOVING A PARTLY FINISHED SAC FROM THE FIRST MOLD

PLATE 3

ASPIRATING EXCESS COLLODION IN A SAC, AND GELLING THE COLLODION IN WATER

PLATE 4

TESTING THE RATE OF WATER FLOW THROUGH SACS



(A. H. Harris: Collodion Sac for Animal Experimentation)

PLATE 5

RINSING OUT THE STERILIZING SOLUTION

PLATE 6

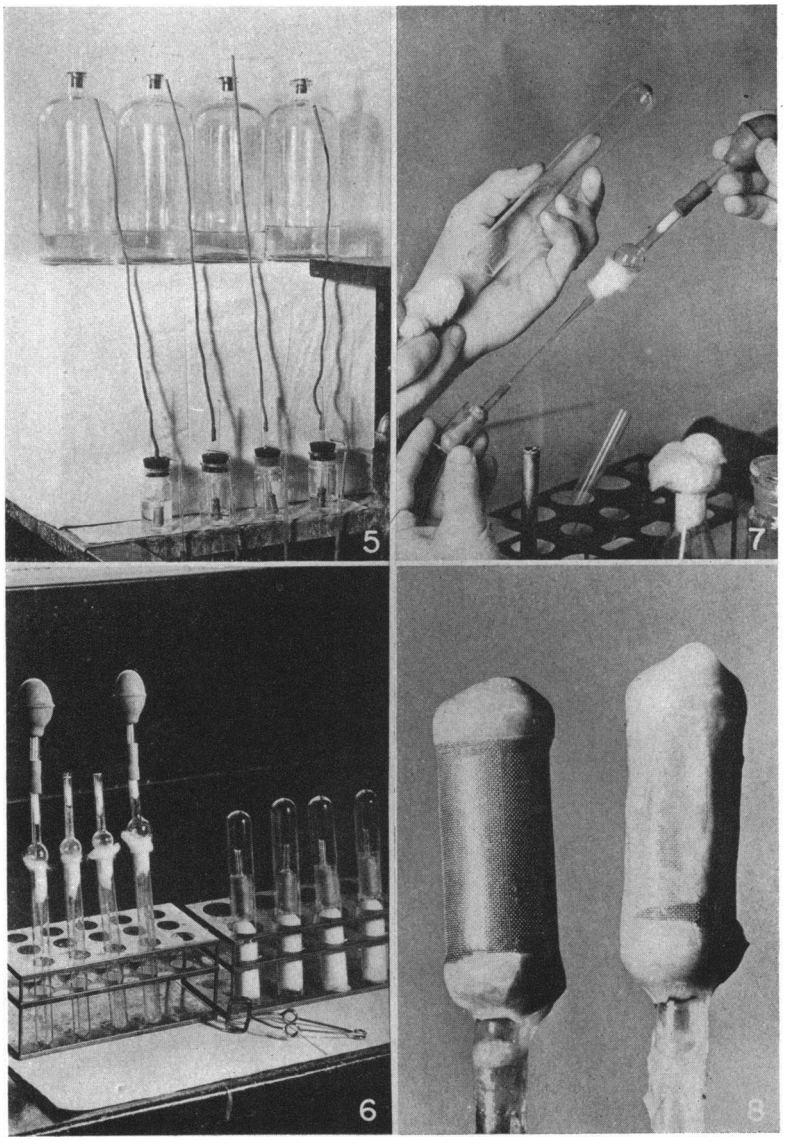
PASTEUR PIPETTES, AND SACS READY TO BE FILLED

PLATE 7

FILLING A SAC

PLATE 8

SACS AFTER REMOVAL FROM THE PERITONEAL CAVITY OF A RABBIT. NOTE FIBRIN DEPOSITION ON ONE



(A. H. Harris: Collodion Sac for Animal Experimentation)