Preparation and Use of Collodion Sacs in Exalting Micro-Organisms.

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The conception of enhancing the virulence of micro-organisms by growing them *in vivo* in a hermetically sealed permeable membrane introduced collodion sacs. By this method the organisms are enabled to develop unaffected by the action of the phagocytes and at the same time permitting their soluble injurious metabolic products to diffuse more or less out while the highly nutritive body fluids of the living animal pass in.

This idea was first attempted in 1893 by Morpurgo and Tirelli[1] in their cultivation experiments with the tubercle bacillus. The bacteria were placed in celloidin capsules which were introduced either subcutaneously or in the peritoneal cavity of rabbits.

Metchnikoff, Roux and Salimbeni[2] in 1896 really introduced the collodion sac method when they demonstrated that the toxin of the cholera germ would very readily diffuse through the walls of these sacs when placed in the peritoneal cavity of guinea-pigs. Since then this method has received a great deal of attention and notable results have been obtained in increasing the virulence of various micro-organisms.

The year of 1898 is rather memorable for its accomplishments along these lines. The germ of pleuro-pneumonia was successfully cultivated at this time by Nocard and Roux[3] by utilizing the collodion sac method. During this year by using this method Nocard[4] increased the pathogenesis of the tubercle bacillus, which was human in origin, so that it proved fatal to chickens.

At this time Vincent,[5] employing the method, pursued his research upon converting saprophytic bacilli (B. megaterium and B. mesentericus vulgatus) into pathogenic types.

Podbelsky[6] by applying the same principle showed the destruction of the spores of the Hay bacillus *in vivo*. Due to the difficulty of preparing collodion sacs he made tubes out of reeds. A reed sac, which is even more permeable than one prepared of collodion, is made from the tubular membrane lining the central canal of the bulrush.

It is prepared briefly as follows: Common bulrush reeds, if fresh, are boiled for about 15 minutes. If dry, they should be autoclaved for 60 minutes at 115° C. The end of the softened reed is sharpened as

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[1] Archives Ital. de Biologie, Vol. XVIII, p. 187—1893. Ref. in Centrabl. f. Bacteriol., Bd. XIII, p. 74—1893.

[2] Annales de L' Institut Pasteur, Vol. X, p. 261—1896.

[3] Annales de L' Institut Pasteur, Vol. XII, p. 240—1898.

[4] Annales de L' Institut Pasteur, Vol. XII, p. 564—1898.

[5] Annales de L' Institut Pasteur, Vol. XII, p. 787—1898.

[6] Annales de L' Institut Pasteur, Vol. XII, p. 431—1898.

one would sharpen a pencil, so as to expose the membrane lining the central canal. A desirable length of this membrane is denuded. One end is tied firmly and by means of a glass rod it is turned inside out. A glass tube is fitted into the open end of the sac and fastened with strong thread. The sac is filled with distilled water and sterilized. The fluid is removed from the sterile sac and replaced with a suspension of the organism. The sac is tied shut and the glass tube removed. The end is covered with melted gum lac. The thus prepared sac is placed in the peritoneal cavity of the animal.

The method of preparing collodion sacs as first carried out by the Pasteur Institute is quite different from the present-day procedure. The collodion of desirable consistency, which is in a cylinder, is inclined at a suitable angle. A glass tube of small diameter with a closed rounded end is inserted into the solution and rotated until a surface of collodion of sufficient thickness has been deposited upon the tube. This tube is then rotated in the air until the collodion has set and is no longer sticky.

With a scalpel the upper edge of the collodion layer is cut circularly. The thumb nail is used to turn back upon itself this even edge of the collodion sac. By turning the sac inside out it can be slowly peeled off like a "glove finger." The sac is then everted and distended. A small piece of glass tubing is fitted into the open end of the sac and fastened with thread which is then coated with collodion. The sac is filled with water, suspended in water in a flask and sterilized.

The water is removed from the sac with a sterile pipette and the suspension containing the germs under investigation introduced. The sac is closed with a sterile rubber stopper. This plug is dried and painted with collodion. Instead of closing the opening of the tube in this manner, the glass tube which is fitted into the sac can be drawn out into a capillary beforehand. After the sac has been sterilized and inoculated with the organism the end of the capillary tube is sealed in the flame, thus closing the sac.

In view of the fact that the sac is liable to break, especially if kept in the animal for months, Novy[7] introduced a perforated glass tube which has been drawn out into a capillary, into the sac. The sac is attached to this tube. The apparatus is sterilized, inoculated and sealed in the usual way.

McCrae,[8] and a little later Harris,[9] who slightly modified the former's method, prepared sacs by introducing the hot end of a small glass tube into a gelatin capsule. When cold the tube becomes fixed and is painted with moderately thick collodion which is allowed to dry. It is then rotated in the air so as to permit the solution to dry. This procedure is repeated several times until the desired thickness of collodion is obtained.

The gelatin inside of the collodion covering is removed by introducing hot water into the tube, and also by placing the capsule in hot

[7] Laboratory Work in Bacteriology, pp. 198, 499– 1899.
[8] Journ. Exp. Med., Vol. VI, p. 635—1901.
[9] Eyre, Bacteriological Technique, p. 358—1913.

water. The liquid gelatin is pipetted off. The apparatus is sterilized and prepared for introduction into the animal's body in the afore-described manner.

Grubbs and Francis[10] utilized the perforated tube heretofore referred to, the openings of which are obliterated with gelatin. The outside of this tube is coated with several layers of collodion and the gelatin removed by means of hot water.

The method of preparing collodion sacs as carried out by the Pasteur Institute is difficult. The difficulty is in separating the collodion casing from the glass tube. Gorsline[11] has overcome this and by his method the sac can be prepared with ease. He selects a tube, which may be a test tube, with a 2 mm. opening in its rounded end. The opening is closed with a thin film of collodion. The tube is then rotated in the collodion in the usual way. The sac is removed from the glass tube by filling the tube with water. By constant blowing the water is forced through the opening, allowing it to run between the outside wall of the tube and the inside of the sac, and thus separating the latter from the former. The top of the sac is cut loose from the tube and the sac is slipped off. It is then immersed in water. Its subsequent preparation is similar to that previously described.

The method of preparing the collodion sac for this work is that of Gorsline slightly modified. The technique of sealing the sac containing the suspension of micro-organisms, however, is entirely different from that described by other experimenters.

The procedure of preparing these sacs is as follows: Freshly prepared collodion is used, the consistency of which depends upon whether or not very thin or thick walled sacs are desired. This may be regulated by diluting with a mixture of equal parts of alcohol and ether or by exposing in the air and allowing it to evaporate. This solution must be free from bubbles. A clean tube about 300 mm. by 8, 10, 14, 16 18 mms. with one end rounded with a small opening (2-4 mm.) in the center is used. The outside of the tube is wiped perfectly dry after being moistened with a five per cent glycerin solution. This leaves a very thin film of glycerin on the wall of the tube and facilitates removing the collodion covering from it.

The opening in the end of the tube is closed by painting it over with a film of collodion and allowing it to dry for about one minute. The tube is rotated several times in the collodion, which is in a glass cylinder inclined at a desired angle. The tube is then withdrawn, care being taken that it does not come in contact with the glass container. It is rotated in a horizontal position until the collodion hardens. If a thick walled sac is to be made this process may be repeated several times. The collodion-covered tube is then held under the tap and water is run onto it. By filling the tube with water and by blowing, the sac is removed as in the Gorsline method. At this stage if the collodion

[10] Bulletin No. 7 of the Hygiene Laboratory of the U. S. Marine Hospital Service —1902.

[11] Contributions to Medical Research, dedicated to Victor Clarence Vaughan by colleagues and former students of the Department of Medicine and Surgery of the University of Michigan, pp. 391-393—1903.

cover has not perfectly solidified, bulging will occur. The sac is then immersed in water, where it is practically invisible.

A properly made sac will not rupture if one were to blow into it with all one's might. They are tested in this way before being used.

By this method sacs for dialysing purposes, as described by the author[12] in a previous article, may be prepared with ease.

The top of the sac is cut even, filled and immersed in distilled water in a cotton stoppered flask or tube. It is sterilized preferably in streaming steam or in the autoclave at 105 or 110° C. for 10 or 15 minutes.

The sac is now ready to be inoculated with a suspension of organisms. A sterile pair of forceps is used to remove the sterile sac from the container in which it was sterilized. This sac is transferred to a sterile short tube or held in an upright position with sterile filter paper. The water is removed from it by means of a sterile Pasteur bulb pipette.[13] With a similar pipette the suspension of organisms is introduced into the sac. The walls of the open end of the sac are dried with sterile filter paper and pressed together with a flat-surfaced sterile pair of forceps. Using a red-hot flattened iron wire or spatula, this surface is seared and then coated with several layers of collodion.

The supposedly hermetically sealed sac is tested by taking hold of it with sterile filter paper and applying gentle pressure. If it is found found to be satisfactory it is placed upon sterile filter paper in a sterile dish and covered.

The finished sac is now ready for insertion into the peritoneal cavity of a desired animal. For this purpose several animals are available. Guinea pigs, rabbits, rats, dogs, sheep and chickens are most frequently used. In this work the first three animals were employed.

In the case of the guinea pig and the rabbit, they may be held firmly upon their backs on an animal-board. If a suitable animal-board is not available they may be tied down in this position, as in the case with rats, by fastening cords to each leg and tying over a bridged board.

The hair is removed from the abdomen with a pair of scissors, and after lathering well with soap and water the area is shaved. Alcohol and mercuric bichloride solution are used to disinfect this surface.

After etherizing the animal a small incision along the median line is made through the skin of the upper part of the abdomen. The abdominal cavity is opened up next. With sterile pressure forceps the abdominal muscles are held up and the peritoneal cavity is exposed.

The collodion sac is picked up with sterile forceps and introduced into the cavity and pushed well back under the aponeurosis. With a curved needle and silk thread the cavity is closed and the surface disinfected. The skin is likewise sewed; also disinfected; dried with alcohol followed with sterile filter paper and finally covered with collodion.

The sac remains in the animal from forty-eight hours to several weeks and in some cases months. After the sac has remained *in vivo*

[12] Proceedings of the Indiana Academy of Science, pp. 265, 266—1916.
[13] Proceedings of the Indiana Academy of Science, pp. 266, 267—1916.

the required length of time the animal is etherized. The peritoneal cavity is opened aseptically and the sac removed.

If the sac has been in the animal body for weeks it is surrounded with a fibrous sheath. In this case the sheath is removed before the sac is opened.

The bottom of the sac is sterilized by means of a red hot searing iron, wire or glass rod. With a bulb pipette the content, which is milky in color, is removed, examined and resaced or cultivated *in vitro* or used for classroom work.

By this method the virulence of micro-organisms may be enhanced markedly and it is advantageously used to bring out capsule formation, especially when the germs are so attenuated that their pathogenesis cannot be exalted, as may be the case of Micrococcus Tetragenus, Micrococcus Pneumonia and others.

These cocci and B. Pneumonia (Friedländer's) under this condition form enormous capsules which can be used for classroom work.

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