NOTES UPON THE AGGLUTINATIONS OBTAINED BY INTRAPERITONEAL INSERTION OF CELLOIDIN CAPSULES CONTAINING BACILLI AND UPON A MODE OF PREPARING SUCH CAPSULES.

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While carrying out some studies upon agglutinations by the usual method of injecting bacilli, living or dead, into the animal tissues, I was led to try a method of enclosing the bacilli in capsules, which were inserted into the abdominal cavity of the animal, and I found that by this method also the serum gained agglutinative power. The extent and the other characters of such agglutinations will be dealt with in later paragraphs, but before recording my results, it is well, I think, that a few words should be said with regard to the preparation of the capsules. For although Nocard and Roux's[1] work on the micro-organism of pleuro-pneumonia of cattle, and Nocard's[2] experiments on the transformation of human into avian tubercle bacilli, by placing them in sealed capsules within the fowl's peritoneal cavity, have brought increased attention to this method of growing bacteria within the body, free from the intervention of the body cells, there does not exist, to my knowledge, in bacteriological literature, any detailed account of a satisfactory method of preparation of the same, and if I mistake not, the difficulty in making capsules which will not rupture nor leak, has been found so considerable that the method has by many been taken up only to be abandoned..

The idea of using collodion or celloidin capsules is, bacteriologically speaking, of comparative antiquity; quite early in the nineties, a somewhat primitive capsule was employed in the Pasteur Institute by

[1] *Ann. de l'Institut Pasteur*, 1898, xii, p. 270.
[2] Ibid., 1898, xii, p. 561.

Metchnikoff and others. This was formed by taking a glass rod or pencil, dipping it into collodion until the desired thickness of coating was obtained; the capsule was then stripped off the rod and the culture inserted, the mouth tied, and finally, the interstices at the neck were cemented over with collodion. The procedure was first mentioned in an article by Metchnikoff, Roux and Salimbeni.[3]

The defects of this method are obvious: namely, the amount of manipulation required, the long time the capsule has to be kept exposed to contamination while the mouth is being sealed up and the liability for the new coat of collodion at the neck not to adhere thoroughly to the previously dried material. Gradually, it would seem, the method of making these capsules was improved in Paris, though I can find no clear statement of how the capsules were made by Metchnikoff, Roux, and Nocard in their later work.

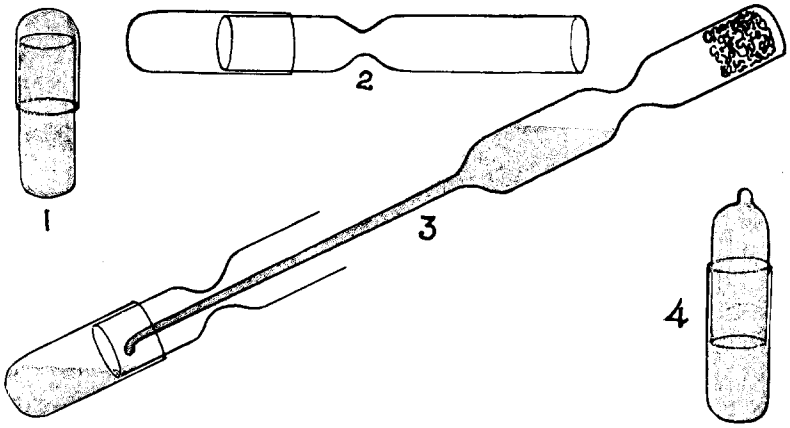
A distinct step in advance was made upon this side of the Atlantic some two years back, so far as I have been able to trace, by Dr. Prudden and others in the Laboratory of the College of Physicians and Surgeons of New York. It consists in employing the gelatin capsules now obtainable at any druggist's, and used for the administration of unpleasant drugs. These capsules are taken as a framework and coated with celloidin; next, the gelatin is dissolved out in a sterile test tube, the cap being then luted upon the body of the capsule by means of painting with thin celloidin. We obtained a knowledge of this method from Drs. Trudeau and Baldwin at the Saranac Lake Laboratory, and, if we are not mistaken, yet further advance was there made by replacing the gelatin cap with a length of glass tubing to which the body of the capsule was luted, the tube being sealed in the flame after filling the capsule. At Saranac Lake they employ a bulb-shaped capsule, and from what I learn from Dr. Baldwin, some little difficulty has been experienced owing to the tendency of capsules so made to undergo rupture within the body and thus set up a general infection.

The method described below has proved so simple and at the same time so successful that a detailed account of it may furnish means to

[3] Toxine et antitoxine cholérique. *Ann. de l'Institut Pasteur*, 1896, x, p. 257.

others of adopting this manner of passing bacteria through the animal body without coming into direct contact with the tissues. The early methods have been touched upon lest I be thought to claim in the slightest degree the credit due to originality.

Celloidin is especially adapted to this work, as it prevents the escape of the organisms while it allows osmosis of the fluids to go on freely. Enclosed in the celloidin capsule the bacilli lie exposed to the body fluids, their soluble products have free egress to the tissues, and, should observations upon the bacilli themselves be desired, the capsule can be removed with a certain knowledge that it contains the form that was originally introduced.



1. Gelatine capsule, natural size.
2. Diagram of glass tube with adherent capsule.
3. Method of filling the capsule.
4. Capsule filled and sealed ready for introduction into the peritoneal cavity; natural size.

A piece of glass tubing, 1 cm. in external diameter and 6 to 8 cm. long, is taken and a narrow and rather abrupt neck is drawn upon it about 3 cm. from one end. It is well, as a matter of precaution, to round off the edges of this end in the flame, lest any sharp edge should later, on manipulation or movement of the capsule within the body, cut through the celloidin. Then over this end, after heating it slightly, the body of a gelatin capsule is fitted, the top being discarded; the hot glass melts the gelatin and there is immediate adhesion. The accompanying diagram 1 gives the exact shape and

size of this capsule with its top. The advantage of this form over the previously mentioned globular forms with neck is that in the latter there is danger of rupture where the neck joins the body, and this danger is done away with here. The glass tubing should pass without difficulty into the capsule (2 in the figure), thereby providing to some extent against the slight shrinkage which may occur during sterilization. The capsule is now repeatedly dipped in thin celloidin, care being taken to dip well beyond the upper edge of the capsule, so that the celloidin adheres directly to the glass; between each fresh coat the capsule is allowed to dry, the dipping being continued until the layer of celloidin is judged to be sufficiently thick.

It is now necessary to melt out the gelatin. I used to accomplish this by first pouring some water into the capsule and then placing the whole in a sterile test tube, with the open end of the glass tube downwards, and heating it in the autoclave for 20 minutes. Lest the melted gelatin refuse to run down the sides of the capsule, a thin wire or fine broom straw may be inserted through the neck of the tube. By this method I obtained a thoroughly practicable capsule and could keep it under sterile conditions without difficulty until it was needed for use. The capsules did, however, exhibit some tendency to shrink, and as shown to me by Dr. C. H. Higgins (who in this laboratory has employed the capsules in his studies upon the tuberculosis of cattle), this shrinkage may be prevented. The modification is as follows:

The capsules are filled with water and placed in test tubes themselves half filled with water, and these are then heated in the autoclave or steam sterilizer. Capsules so treated retain their form admirably, and after emptying out the melted gelatin and half filling with water they may be sterilized and kept, with the open end upwards, in a sterilized test tube containing either water or broth until they are needed.

Into the capsule thus prepared a culture is inserted by means of a fine Pasteur pipette, which is sufficiently small for the capillary tube of the same to pass through the narrow neck (3 in figure). Care must be taken that the inside of the neck is not wetted by the culture,

for if it should be the glass will probably crack during the sealing of the capsule. This step is accomplished by removing the capsule from the test tube by means of a pair of sterile forceps and fusing the narrow neck of the tube rapidly in the blow-pipe flame (4 in figure). The capsule thus sealed must be replaced in the sterile test tube until needed for use. By this wet method of keeping the capsules there is so little danger of contamination that they may immediately be inserted into the peritoneal cavity or elsewhere. When using the dry method, to make sure that the capsule is intact, it is well to keep the sealed capsule in a broth tube for 24 hours, when, if the broth remains clear, the operation may be performed. Nevertheless, one becomes so proficient, that after the first few attempts I was able to use the capsules immediately, and upon subsequent removal from the body found that they were entirely free from any evidence of bacterial growth externally. Until a worker has perfected his technique, it certainly must be laid down that the filled capsule should be preserved in a broth test tube for 24 hours before being placed within the tissues.

The abdomen of the animal is opened antiseptically, the capsule inserted and the wound closed. I have generally found on removal that the capsule has slipped around freely in the cavity; sometimes, however, it is surrounded by adhesions. If it is desired to remove the capsule without killing the animal, the chief practical difficulty lies in finding and recognizing it through the small wound by touch. When found it can be placed in a sterile tube and opened by sterile forceps or scissors.

The agglutinative results, spoken of above, were observed while passing through rabbits some forms of the paracolon group, obtained from Drs. Harvey Cushing, Gwyn and Harris, of the Johns Hopkins Pathological Laboratory and Hospital, Baltimore; their cultural characteristics were tested and found to correspond to Bacillus O (Cushing),[4] B. paracolon (Gwyn),[5] and B. enteritidis (Gärtner). They were inserted in the capsules as 24- or 48-hour bouillon cultures.

The fact elicited in the course of these experiments was that for a

[4] *Bulletin of the Johns Hopkins Hospital*, 1900, xi, p. 156.

[5] Ibid., 1898, ix, p. 54.

few days after the insertion of the capsule, no agglutinative reaction of the serum appeared, even in dilutions of 1 in 10; but on the 10th to the 15th day it began to appear, first in dilutions of 1 in 10 and 1 in 20, gaining in potency daily, until by the 19th to 21st day it would attain a potency of 1 in 1000. Upon the removal of the capsule the agglutinative power decreased day by day, with the same speed with which it had arisen. The agglutinative power of the serum was restricted to the variety of bacillus contained in the capsule, and did not extend to the other, apparently closely related, forms, except in one single instance. If two capsules containing different organisms were put in the same animal, the animal's serum was found to have an agglutinative power over both at the same time, and to about the same degree of potency.

The facts of the experiments are here briefly summarized:

(1) *Rabbit,* with intraperitoneal capsule containing B. paracolon (Gwyn).

Serum: Positive to B. paracolon (Gwyn), on 8th day, 1 in 10, increasing to 1 in 80 on 15th day. Negative to Bacillus O, B. chol. suis, B. icteroides (Sanarelli), B. icteroides (Reed), B. enteritidis (Gärtner), B. morbificans bovis.

(2) *Rabbit,* with intraperitoneal capsule, 1st B. paracolon (Gwyn), 2d (after removal of 1st), B. icteroides (Reed).

Serum: Positive to B. paracolon (Gwyn) and B. icteroides (Reed), and negative to all the others.

(3) *Rabbit,* with capsule of B. enteritidis.

Serum: Positive to B. enteritidis, 1 in 10, on 11th day, increasing to 1 in 1000 on 21st day; capsule removed on 26th day; agglutinative reaction had fallen to 1 in 500 on 31st day. Serum negative throughout to all the other above-named forms.

(4) *Rabbit,* with capsule of Bacillus O.

Serum: Positive to Bacillus O on 13th day; increasing. Positive to B. icteroides (Sanarelli) in 1 in 10; apparently not increasing. Negative throughout to others.

These results will be seen to bear out Cushing's results in his experiments upon Bacillus O, that inter-agglutinations do not necessarily occur between closely related varieties of bacilli.

The above observations do not represent all my studies upon the effects of introducing bacteria in capsules into the peritoneal cavities

of rabbits. They give, however, the results obtained with reference to the agglutinating properties of the blood serum of these animals. I had intended to make the series more complete, but my departure to South Africa arrested my work along these lines at this point. So far as they go, the results obtained by me were so constant and so definite that I feel that I am justified in publishing this note and even in drawing certain conclusions from the results obtained.

Of these results, that which is most obvious is that agglutination, however produced, would appear to be strictly associated with the existence of the bacteria in a living state within the body, for otherwise we cannot explain the fact that removal of the encapsulated bacteria from the peritoneal cavity is followed by the steady disappearance of the agglutinating property of the serum of the animal.

So far as these observations go, they would appear to explain the continued existence of the reaction for months and years in some individuals following upon an attack of typhoid and the rapid disappearance of the reaction in other cases, and this by the continued existence of the bacteria within the tissues in one set of cases and by their complete destruction in the other series. A few years ago this conclusion would have seemed impossible, but now-a-days we are, I think, prepared to accept it, for it is now a familiar experience that typhoid bacteria may be obtained from the gall bladder many months after the patient has apparently wholly recovered from the acute disease, while similarly, long months after the patient has been subjected to the disease we occasionally encounter the bacilli in pure culture in abscesses in the neighborhood of joints and other lesions, observations which prove absolutely that the bacilli may continue for long periods, either lying latent or proliferating very slowly in some one or other region of the body.

In attempting to form a satisfactory theory of agglutination, we possess data which lead us to suppose that the bacteria in culture form certain agglutinins which unite with certain other agglutinins which are the product of the tissues. (The normal serum will, in certain cases, produce in low dilutions an agglutination; but this power is probably not an inherent quality of tissues or serum, but is

caused by the presence of a subinfection by a bacillus nearly related to the bacillus with which the agglutination is made. So rarely does this agglutination by apparently normal serum occur that it may be disregarded.) The fact remains that the tissues, reacted upon by an infection, respond by the production of an agglutinin. By the capsule method we are able to assert that the agglutinin produced by the tissues is not a reaction to the bacillary bodies, nor yet a reaction of the kind called inflammatory, but a reaction to the chemical bacillary products or a combination of the serum with these chemical products.

SUMMARY.

1. Capsules made as described above allow dialysis, when placed in the peritoneal cavity.

2. The normal tissues, unstimulated, do not possess the power of causing agglutination; they do not require to be stimulated by the presence of the bacterial bodies, but will produce their share of the agglutinins when acted upon by the bacillary products.

3. Agglutination follows the insertion, in the peritoneal cavity, of "capsuled" bacilli; it gradually increases in degree, and on the removal of the capsule containing the bacilli, begins to disappear.

4. Varieties of bacilli, related closely in morphology and cultural reactions, do not, as a rule, produce serums which inter-agglutinate.