ON THE SURVIVAL OF BACTERIUM TYPHOSUM
INTRAPERITONEALLY IMPLANTED IN
COLLODION SACS

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In the course of certain experiments on the permeability of
collodion membranes, it was found that the body fluids could
furnish diffusible nutrient substances to bacteria in intraperi-
toneally implanted collodion sacs (Gates, 1921). Conversely,
although sterile collodion sacs were practically inert, an active
proliferative cell reaction occurred around sacs containing living
bacteria, thus demonstrating the passage of irritating bacterial
products in the opposite direction. These reactions were of in-
terest in relation to the survival of bacteria in vivo and to anti-
body formation as a result of chronic local infection. The re-
action of the body to a localized infection with Bacterium ty-
phosum, enclosed in collodion sacs, was therefore observed in
a series of 8 rabbits, 4 of which were killed for examination after
from twenty-four to forty-five days, and the others after a
survival of from fifteen to nineteen months.

EXPERIMENTAL

Under ether anesthesia, each rabbit was implanted intraperi-
toneally with 3 or 4 collodion sacs of about 5 cc. capacity (Gates,
1921), containing distilled water or 0.85 per cent salt solution and
inoculated with a strain of Bact. typhosum originally obtained by
Dr. C. G. Bull from an ampoule of Besredka’s sensitized typhoid
vaccine. Two of the rabbits carried uninoculated control sacs also.
The effects of implantation on the host

The presence of the sacs had no observable effect upon the health of the animals. No variations from normal were observed in temperature, weight, or appetite. Shortly after implantation, sacs containing living microorganisms became covered with a mantle of fibrin and wandering cells. Later this exudate became organized and replaced by ingrowth of connective tissue cells and vascular elements until the sac, or group of sacs, formed the center of a dense pedunculated mass of fibrous tissue with vascular walls from 1 to 3 mm. in thickness. There was never any evidence of intestinal obstruction or other interference with function due to the presence of these masses. They were usually attached to the omentum. In all the rabbits of this series the abdominal organs appeared normal, or showed only the lesions of coccidiosis which are frequently encountered in stock animals.

Collodion sacs do not withstand intraperitoneal conditions indefinitely. The material gradually deteriorates, losing its transparency and elasticity, so that after a time the sacs break and disintegrate. After twenty-four days, 8 sacs in 2 rabbits were found intact, as were 3 sacs in another rabbit after 41 days incubation. After forty-five days, however, 2 of the 3 sacs in a fourth rabbit were broken and all of the sacs in the 4 rabbits examined more than a year later had collapsed. It seems probable that in these animals the sacs did not remain intact longer than one or two months and thereafter served only as the focus of a localized infection. At the time that the sacs broke they were undoubtedly already surrounded by a capsule which prevented a wide dissemination of the liberated organisms. At autopsy no other foci of typhoid infection were discovered. Cultures of the blood, urine, and bile were negative for Bacterium typhosum.

Examination of sac contents after intraperitoneal incubation

In the shorter experiments (twenty-four or forty-one days) when the sacs were still intact, they were surrounded by a small amount of sterile seropurulent fluid. The sacs themselves were
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filled with a milky material which separated, on standing, into a cloudy, straw-colored fluid of pH 7.4 to 7.6 on the Sörens en scale, and a cream-colored sediment whose volume was from \( \frac{1}{4} \) to \( \frac{1}{3} \) of the whole. This heavy deposit consisted of amorphous débris and a variety of bizarre-shaped Gram-negative bodies, coccal and bacillary, which took bacterial stains with all degrees of intensity and often were stained more heavily at the poles. Some forms were typical of Bacterium typhosum. Larger, oval and spherical, deeply stained bodies such as are often called "involution forms" were also observed. The amount of this material was far in excess of what is ever formed in old cultures of Bacterium typhosum, in vitro. Multiplication had proceeded long after growth would have ceased in an artificial medium. Obviously the continued growth was due to a persisting diffusion of fresh nutrient material through the sac wall. From the contents of these sacs, intact after twenty-four or forty-one days incubation, pure cultures of typical Bacterium typhosum were obtained.

The filtered supernatant fluid from two sacs which had been in the peritoneal cavity of Rabbit 5 for forty-one days proved somewhat toxic for a normal rabbit only when injected intravenously in a dose of 5 cc. It caused a transient rise in temperature and a temporary depression.

The sacs which had broken during forty-five days incubation contained a milky fluid and sediment made up of bacterial débris and flakes of pus. Films showed many degenerated leucocytes and some typical monocytes and polynuclear cells. No phagocytosis was observed. The unbroken sac contained the same bacterial forms but no leucocytes. The contents of the sacs produced confluent growths of Bacterium typhosum in pure culture.

The next observations were made on the 4 rabbits which were allowed to survive for periods of fifteen to nineteen months. The findings were practically identical throughout. A section of the dense, tough capsules of fibrous tissue disclosed a mass of thick, white, creamy or cheesy material containing the broken remnants of a collodion sac. This material consisted of amorphous, diffusely and irregularly staining débris, in which no wandering cells could
be identified. Many "shadows" of bacillary shape made blank spaces in the background. Recognizable stained organisms were rarely encountered. Nevertheless, each pocket in the capsular masses yielded a luxuriant, confluent growth of *Bacterium typhosum*. Some of the sacs had been inoculated with cultures derived from sacs of the earlier series and so represented a second period of incubation *in vivo*. Cultures from the capsules were compared with the original strain, kept on nutrient agar in the laboratory. Even after long periods of survival in the animal body in more or less intimate contact with wandering cells and tissue fluids, no change whatever in the morphology, staining reactions, antigenic properties or functional activity of *Bacterium typhosum* could be observed.

Zinsser and Raymond (1921) recently published a note which is of interest in this connection. Celloidin capsules made on candy cores and emptied by dialysis were punctured with a needle and inoculated with streptococci in agar. The puncture hole was left open. Rabbits harboring such celloidin sacs in the peritoneal cavity usually lived for months. Some gradually emaciated, others developed agglutinins. After four months the capsules were found to contain living streptococci. The organisms recovered were morphologically and culturally identical with those implanted. The authors consider that their experiments furnish "some evidence, at least, against the mutations of streptococci in the animal body."

**Antibody production**

The serum of the rabbits tested agglutinated *Bacterium typhosum* in dilutions of from 1:32 to 1:100 after the twelfth to sixteenth day, when the sacs were certainly or supposedly intact. On the forty-first day when 1 rabbit was killed and found to harbor unbroken sacs, the agglutinin titer of the animal's serum was 1:160. Apparently there was some passage of agglutinogen through the sac walls. After the sacs had almost certainly broken, higher titers were found in the serum of the rabbits which were allowed to survive. Even so, the antibody formation was slight in comparison to that readily obtained with small doses of
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_Bacterium typhosum_ injected intravenously. The highest titer observed was 1:800 in the serum of one rabbit six months after the sac implantation. The titers of serum specimens from the other 3 rabbits ranged from 1:25 to 1:500, six to nine months after operation. Thereafter the agglutinin content of the sera fell off gradually until the tests made after a year showed agglutination only in serum dilutions of 1:4 to 1:8. Such figures indicate that practically no agglutinogen was being produced in the capsules, or that the foreign material was so completely walled off by the fibrous tissue that no absorption of agglutinogen was taking place.

In 1901 McCrae (1901) reported somewhat similar results from the intraperitoneal implantation of bacteria of the colon group contained in celloidin capsules. In one instance an agglutination titer of 1:1000 was obtained against _Bacterium enteritidis_ on the twenty-first day. After the capsule had been removed on the twenty-sixth day the serum titer had dropped to 1:500 by the thirty-first day. McCrae concluded that agglutinins in the serum are strictly associated with the existence of bacteria in a living state in the body, and noted that his observations might explain long-continued reactions in the serum of persons who had recovered from typhoid fever. He referred to the persistence of typhoid bacilli in the gall bladder and in abscesses, which proves that the microorganisms may survive for long periods "either lying latent or proliferating very slowly in some one or other region of the body."

**Summary**

Eight rabbits, intraperitoneally implanted with collodion sacs containing _Bacterium typhosum_ in salt solution or distilled water, furnished nutrient materials to the organisms which promoted luxuriant growth and maintained the bacilli in a viable condition for periods up to nineteen months, the longest interval tested. The bacteria developed in many bizarre shapes and sizes, but these forms were either not viable, or reverted to normal on transfer to a more favorable medium. Even after a sojourn of many months _in vivo_, no permanent change in morphological, cultural, or antigenic properties could be detected.
The rabbits themselves were unaffected by the implantations and maintained their health and rate of growth unimpaired. The sacs were at first covered with a mantle of fibrin and wandering cells and later isolated by dense connective tissue capsules which closely confined their contents after the sacs had broken. No evidence was found of the invasion of other tissues by the microorganisms, and the slight absorption of antigenic material from sacs and fibrous pockets was only sufficient to maintain a relatively low antibody titer in the blood serum.

The conditions produced bear some similarity to the typhoid carrier state and show experimentally the possibility of an indefinite parenteral survival of _Bacterium typhosum._

REFERENCES