

The above mentioned authors do not state in their preliminary paper how old their cultures were when they made their observations and if they have distinguished between cultures of fat containing bone marrow, nearly fatless bone marrow and bone marrow with large amounts of red blood corpuscles. All these facts may alter the conclusions because if the bone marrow contains a large amount of fat then many "Riesenzellen" are present and phagotization can be observed in a very considerable degree. If fatless bone marrow is used, phagotization appears to go on in a remarkable degree only in the second outlined period of culture life, because only then the cells near the bone marrow network begin to migrate in the plasma clot, to phagotize and to assume different types of connective tissue cells.

67 (1245)

On the isolation of streptococci from rabbits.

EDGAR T. H. TSEN (*by invitation*).

[*From the Department of Bacteriology of the College of Physicians and Surgeons, New York.*]

In our work for the purpose of investigating the relation of streptococci to poliomyelitis as claimed by Dr. E. C. Rosenow, we have made cultures of the brains of 6 monkeys and 20 rabbits. Our technique was as follows:

The animals were etherized just before death and, when anesthetized, were fastened to an autopsy-board—abdomen downward. With animals that died during the night this part of the procedure could not, of course, be carried out. Our purpose was to make sure, whenever possible, that any organisms cultivated from the brains were not post-mortem invaders, but were present during the life of the animal. A median incision was made through the skin over the skull running from the tip of the nose to the back of the neck, and the skin dissected back on both sides of the head. The skull was disinfected with tincture of iodine, and the head and body covered with 3 layers of gauze soaked in lysol. A small hole was made in the gauze so as to expose the upper

part of the skull. The skull was next opened, and, to further insure sterility, the surface of the brain was seared with a scalpel. A big piece, and sometimes one-half of the brain was removed with a pair of long forceps and then immediately put into a Rosenow sterile air chamber, where it was emulsified. (The chamber was sterilized in the hot-air sterilizer for one hour at 160° C.) In some cases it was possible to remove considerable parts of the brain while the animal was still alive. The emulsion was poured into a large tube from which different media were inoculated with sterile pipettes. The tubes were examined after two days and, if they were found to be sterile, were again examined two or three days later. The cultures were all kept at 37.5° C.

The media used were plain broth, ascites broth, glucose broth, glucose ascites broth, plain broth with tissue, ascites broth with tissue, glucose broth with tissue, glucose ascites broth with tissue, glucose agar, and glucose ascites agar. The amount of glucose in the broth was 0.2 per cent. and that in the agar 1 per cent. The reaction of all the media was 0.6 + to 0.8 +.

A part of the tubes inoculated in each case was prepared by methods simulating as exactly as possible those employed by Dr. E. C. Rosenow.

All the six monkeys were inoculated with glycerinated poliomyelitis virus—4 intracerebrally, 1 subcutaneously, and 1 both intracerebrally and intraperitoneally. Both anærobic and aerobic cultures of the brain and cord emulsions were made on ascites glucose agar slants at the time of inoculation. We found no growth in any of the tubes. These monkeys were found dead or were etherized from 4 to 16 days after inoculation. Those 4 which were inoculated intracerebrally showed typical changes of poliomyelitis and the other two did not.

From all these 6 monkeys we have isolated streptococci. On November 12, 1916, two rabbits were inoculated intravenously with a 20-hour culture of streptococci isolated from the brain of a monkey which showed typical changes of poliomyelitis. Before inoculation this culture was stated by Dr. Rosenow to be similar to his. Rabbit 1151 was inoculated with the growth from 22.5 c.c. of ascites glucose broth suspended in 1.5 c.c. of sterile salt

solution, and rabbit 1152 with that from 30 c.c. of the same medium suspended in 2 c.c. of sterile salt solution. Cultures of the bacterial suspension were made at the time of inoculation, and the streptococci were found to be alive and free from contamination. On December 2, 1916, rabbit 1151 was again inoculated with a bigger dose. These two rabbits are still in normal condition today—more than 4 months after inoculation.

Of the 51 rabbits with which we have worked 11 were inoculated with glycerinated poliomyelitis virus—7 died and 4 are living; 9 were inoculated with the brain emulsions of rabbits that died after inoculations with poliomyelitis virus—2 died and 7 are living; 6 were inoculated with streptococci from monkeys—3 died and 3 are living; 13 were inoculated with streptococci from rabbits that died after inoculations with poliomyelitis virus—5 died and 8 are living; 3 were inoculated with streptococci from rabbits that died after inoculations with streptococci—1 died and 2 are living; 2 were syphilitic, and 7 were normal.

Of the 18 rabbits that died after inoculations with poliomyelitis virus or streptococci, none showed any of the changes usually thought characteristic of poliomyelitis of man and monkey. Nevertheless, we have isolated streptococci from the brains of all the 11 on which we have made autopsies. Seven were inoculated with poliomyelitis virus.

We have also isolated streptococci from the brains of the two syphilitic rabbits and 5 out of the 7 normal rabbits. Or in other words, only the brains of two normal rabbits remained sterile.

It therefore appears from our experiments that streptococci are probably saprophytic microorganisms in the normal body which, under conditions of lowered resistance in the course of disease from other cause, acquire the power of more extensively invading the tissue. They do not seem to have any etiological relationship to poliomyelitis.

We are continuing this work with more special attention to a study of the tissue sections and of the cultivation of the globoid bodies.