receiving the serum of a suspected abortive case of poliomyelitis, indicating presumably a certain degree of germicidal power even in *normal* human serum.

To compare this germicidal property of normal serum with that shown by the sera from the six suspected abortive and two paralytic cases in series 1, we inoculated a third series of monkeys. In this series we tested the three normal sera which had shown neutralizing properties in series 2 using a *5 per cent.* emulsion of virus, thus repeating exactly the conditions of series 1. All three monkeys developed typical poliomyelitis in 10 to 14 days.

The sera of six out of nine (66.7 per cent.) suspected abortive cases of poliomyelitis have shown, when tested against the virus of poliomyelitis, a germicidal property greater than that shown by any one of six normal sera similarly tested. We interpret this as establishing, to a reasonable certainty, the diagnosis of poliomyelitis in these six cases, and strongly confirming the same diagnosis in a larger group of clinically similar cases observed in connection with the above.

Attention is called to the epidemiologic and prophylactic importance of establishing, by close study of such cases, some clinical criteria for their better recognition.

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Pure cultures of parasitic amebas on brain-streaked agar.

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This work was begun with the attempt to obtain cultures of bacteria-free amebas in crushed rabies brains, the idea being either that the amebas might take up the rabies organisms as food or that they might free the rabies organisms from their host cells and thus bring about, in some way, a culture of the latter, as Clegg reports having done with the *B. leprœ.*

Four cultures of amebas were selected for a first trial, 3, presumably parasitic (type, *Entameba coli*), the fourth, a saprophyte (*Ameba lirnax*). Of these four, only one, a culture from a case of

human amebic dysentery, grew with comparative ease and some abundance on agar plates streaked with rabies brains.

I then tried normal brains as controls and found that this strain of ameba grew with almost equal ease on them; so that the problem was changed for the time from that of a possible aid in determining the nature of the Negri bodies to that of the growth of pure cultures of amebas.

Only four investigators (Kartulis 1893, Casagrandi & Barbagallo 1897, Tsugitani 1898) have claimed to obtain amebas growing free from other living organisms and none of these have been quite clear in regard to their technic nor have they apparently grown their organisms for more than two or three culture generations, which, as controls show, does not rule out the factor of subsistence upon food stored up in the amebas themselves.

The majority of investigators who have tried to obtain pure cultures of amebas all make emphatic statements as to the impossibility of obtaining such cultures by any methods tried.

The present work, the details of which will be given in the full report, may be divided into 3 parts: (1) Obtaining living amebas free from other living organisms, (2) Obtaining sterile brain tissue, (3) Making successive transplants of amebas and brain tissue and proving that every transplant is free from other living organisms. Each step requires many controls.

In obtaining living amebas free from other organisms a method has been used which has been employed successfully by other investigators, differing only in detail. The stock culture of ameba and bacterium is planted in small amount in the center of a nutrient agar plate from which the water of condensation has been dried. On such a plate at 25° to 30° C., in 48 hours, the amebas usually spread beyond the bacteria. If they do not, a drop of normal salt solution will help them do it in another 24 hours, and there is then a wide-spreading zone of motile amebas minus bacteria. Platinum loopfuls of these edge amebas are transplanted to a series of agar plates, and thoroughly controlled for presence of bacteria.

The brain tissue is obtained by removing under strictly aseptic precaution the cerebrums of normal rabbits or guinea pigs, dropping them into 5 per cent. carbolic acid for one to two minutes, then placing them on agar plates and removing Ammon's horns

and corpus striatums which are spead over a series of fresh agar plates, and put in the thermostat for 24 hours. It is peculiarly important to have conditions such as to insure sterility of this tissue medium because of the possibility of the amebas using up any organism that might get into the plates before it could be detected.

This crushed sterile brain is now spread in small amounts over the plates containing the amebas, and placed, some in thermostat, and some at room temperature. If in 24 hours the amebas show slight or no growth as may be the case in the first culture generations, a loop or two of sterile normal salt solution may be added, then in 24 hours at 36° C. or 3–5 days at 20° C., there is generally an abundant growth.

To rule out the presence of extraneous organisms, at each transfer three or four plates are made. To one is added sterile normal salt solution, to another sterile broth, to another sterile blood, one is left simply with the transplanted material, and to the others are added the fresh sterile brain tissue for carrying on the culture. In the second or third culture generations, the cultures transferred on blood or broth or normal salt or plain agar encyst or die out. Spreads, making an added important control, have been made at practically every transfer and usually stained in three different ways. Two other strains of parasitic amebas are being tried and they seem to be growing, though it is too soon to make a positive statement. Liver tissue and spleen tissue are being tried, and the amebas seem to be growing on the liver tissue and not on the crushed spleen.

On the brain-streaked agar plates the one strain of ameba is now growing abundantly in the fourteenth culture generation, with normal brain and in the twentieth culture generation with rabies brain, grown at 36° C. and transplanted every 2–5 days.