The apparatus fulfils all the ordinary requirements of a miniature bacteria grinder. It grinds in an aseptic manner, requires no artificial cooling, is entirely devoid of danger, and is simple to use. It has the advantage of being easily constructed as most of the working parts are to be obtained in a well-equipped laboratory, and it is thus inexpensive.

7528 C

Cultivation of Leishmania Donovani in Media of Embryonic Chick Tissues.

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For the cultivation of *Leishmania donovani* a number of media generally containing blood have been advocated. In such media only flagellate forms are found. It is of interest to record the growth of Leishmania flagellates in media of various embryonic chick tissues which have not hitherto been used for the purpose.

The chick embryos employed were fertilized hen's eggs incubated at 37°C. for a varying period of 4 to 20 days. The embryonic chick brain, heart, liver and intestines were removed separately and chipped into fine pieces with sterile scissors in different dishes. They were then suspended in sterile Tyrode's solution having a pH 8.0 in approximate concentration of 0.5 gm. of tissue to 10 cc. of fluid. In some experiments the whole chick embryo from 4 to 7 days old minced finely and suspended in Tyrode's solution was used. With a sterile capillary pipette one drop (about 0.15 cc.) of the embryonic tissue suspension was placed in the center of sterile cover-glass. To this was added a similar drop of Tyrode's solution containing *Leishmania donovani* from the spleen of an infected hamster. A sterile hollow glass slide measuring 7.5 cm. long, 2.5 cm. wide, and 0.7 cm. thick, with a central pit or well, 1.8 cm. in diameter and 0.4 cm. deep, was smeared with sterile vaseline around the mouth of its well. The hollow glass slide was then inverted over the cover-glass in such a way that the inoculated media fluid on the cover-glass faced the center of the well of the hollow glass. The whole slide together with the cover-glass was then turned over by quick motion so that the hanging drop did not spread to the per-

iphery but remained in the center of the under-surface of the cover-glass. Pressure was gently applied to the latter to ensure airtight sealing of the hanging drop inside the hollow glass. Many such hanging drop preparations were made and incubated at 37°C. and at 20°C. in separate lots. The advantage of such hanging drop cultures is obvious. It permits direct microscopic examinatiion at frequent intervals without contamination.

Growths of flagellates were easily demonstrable in the chick brain, heart and whole embryonic tissue preparations from the fifth day on when the cultures were kept at 20°C. Similar preparations kept at 37°C. consistently yielded negative results. In number and size the flagellates reached their maximum at the end of 2 weeks, beyond which the number began to diminish. Generally after 5 weeks it became difficult to demonstrate the presence of the well preserved flagellates due apparently to the exhaustion of the nutriment in the hanging drop or possibly to the accumulation of toxic metabolic products.

Multiplication of the Leishmania flagellates in these embryonic chick tissue media were shown by the successful subcultures from the first positive hanging drop to a fresh hanging drop preparation. Such subcultures at 20°C. generally showed increase of flagellates in large numbers, which became obvious from the 7th day on but there was no growth at 37°C. Attempts to subculture in hanging drops the flagellates from N.N.N. media were also successful only at 20°C.

Of interest is the fact that the media containing either embryonic chick liver or intestine alone repeatedly showed no growth of flagellates. When the tissue suspension was made up of equal parts of chick liver and chick brain, the flagellates also failed to grow.

7529 C

Flagellation of Leishmania Donovani in Blood from Normal and Infected Hamsters.

HUEI-LAN CHUNG. (Introduced by F. R. Dieuaide.)

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Hitherto the cultivation of *Leishmania donovani* has been on various artificially prepared media usually with rabbit's blood as one of the ingredients. It is of some interest to record the flagella-