This important question can be solved only by the actual test on human beings. It seems to us, however, that this objection is partially obviated by the observation that for many years the attenuated calf vaccine has been found satisfactory as regards its "take."

7527 C

A Simple Method for Aseptic Grinding of Bacteria.

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For the mechanical disintegration of bacteria Macfadyen and Rowland[1] employed a highly-complicated machine in which the organisms are pulverized in a frozen condition between a rapidly rotating metal pestle and a stationary metal receptacle immersed in liquid air. The bacteria are rendered very brittle by this intense chilling and are rapidly disintegrated by the grinding process. The amount of bacteria dealt with is not large, varying from 0.5 to 1 gm. of the washed organism. Barnard and Hewlett[2] introduced a ball mill capable of grinding bacteria in a moist pasty condition. Thomson[3] devised a vaccine churn that is capable of smashing bacteria in emulsion form with some success. Recently, Krueger[4] described a type of ball mill for disintegrating bacteria and tissues under aseptic conditions.

The grinding machines above described are generally very costly to construct, and are not all uniformly successful. For grinding small quantities of bacteria it appears that an inexpensive and equally efficient apparatus may be used with advantage. A simple method in which bacteria may be disintegrated in a moist condition is here described.

The grinding chamber consists of a large-size pyrex test tube measuring approximately 20 cm. long and 3 cm. wide. Into this are placed a number of rustless steel balls, 8 mm. in diameter, sufficient to fill one-third of the test tube. The tube is tightly closed, plugged by means of a rubber cork and inserted into an outer metal tube 23 cm. long and 3.2 cm. wide. The test tube is held firmly in position

1 Macfadyen, A., and Rowland, S., *Centralbl. f. Bakt.*, 1903, **34**, 765.

2 Barnard, J. E., and Hewlett, R. T., *Proc. Roy. Soc. B.*, 1912, **84**, 57.

3 Thomson, D., *Annals Pickett-Thomson Res. Lab.*, 1924, **1**, 275.

4 Krueger, A. P., *J. Inf. Dis.*, 1933, **53**, 195.

inside the metal tube by means of a rubber cork as shown in Fig. 1. The test tube will now move with the metal tube when the latter is rotated. The outer rubber cork so effectually seals up the metal tube that in case of breakage (which practically never happens) the bacterial contents cannot escape.

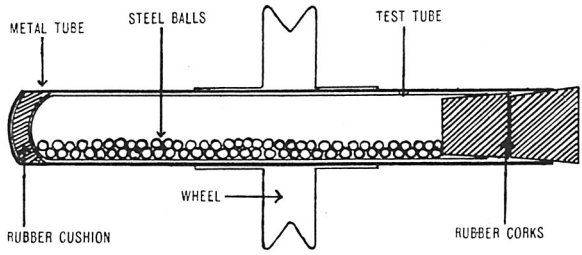

FIG. 1.
Cross section of the grinding apparatus.

Into the middle of the metal tube a wooden pulley wheel, 12 cm. in diameter, is fixed by means of a screw. The grinder is supported by means of a pair of metal rings on a retort stand so that it is capable of rotating in a horizontal axis (Fig. 2).

Power is derived from an electric motor, developing 1/8 H.P. such as is found in most laboratories. It should preferably be fitted with a speed regulator, but failing this, the grooved wheel of the motor may be replaced by one with a very narrow diameter, altering the gear ratio to such an extent that the grinder will describe approximately 350 rotations a minute.

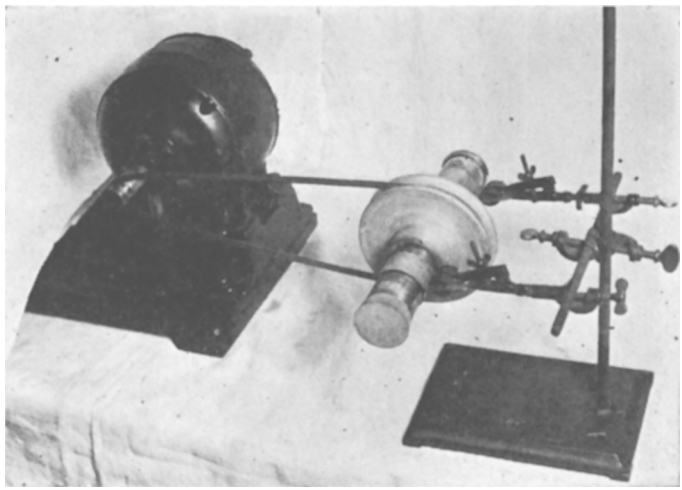

FIG. 2.
Showing the grinding apparatus in operation.

The retort stand is tightly clamped to the table to minimize vibrations, and the moving parts are well-oiled to reduce friction.

Fig. 2 represents the apparatus properly fitted up for grinding (clamp not shown in photo). Bacteria from 15 or more Kolle flasks are washed repeatedly with saline in a centrifuge tube and then emulsified in 15 cc. of saline. The emulsion is pipetted with aseptic technic into the sterile grinding chamber which is then held in position inside the metal tube. Grinding is continued at 350 rotations a minute for from 20 to 70 hours. The period of grinding may be shortened or lengthened at will, depending on the thickness of the emulsion and the resistance of the organisms to grinding. When the organisms have been efficiently ground, as judged by smear examination, a sterile extract may be made by high speed centrifuging to remove intact bacteria, or by filtration through a sterile candle. It may be necessary to dilute the extract a few times to facilitate filtration, as the volume of extract is small.

To obviate the trouble of drying the bacteria we have attempted to grind them in a moist condition. Disintegration in emulsion form, is, according to Thomson, much cleaner and more convenient than dry disintegration, and our experience supports this view. The organisms are introduced into the grinder in the form of a thick emulsion so as just to fill the interspaces between the steel balls. Larger volumes retard the grinding as the bacteria at the surface of the emulsion are not subjected to the tumbling effect of the balls.

However, the apparatus is also capable of grinding bacteria in a dry powdery condition. This has the advantage of being able to deal with a larger quantity of bacteria.

Artificial cooling is not necessary with this apparatus. There is but little heat formation during the grinding process, and at no time has the temperature risen above 37° C.

Staphylococcus aureus, B. diphtheriae, B. tuberculosis, B. proteus, and yeast cells have been disintegrated by this method. Yeast cells are very easily ruptured, and 5 hours' grinding removes about 95% of the intact cells from sight. A microscopic examination at this time shows but a few intact cells per field and an abundance of gram-negative debris derived from the ruptured cells. Staphylococci are much more resistant and a much longer grinding is necessary in order to bring about a satisfactory result. Grinding for 40 hours removes about 85% of the intact cocci as judged by microscopic examination. A 20 hour grinding is sufficient to cause 50% of the staphylococcal proteins to go into colloidal solution as found by the Van Slyke method.

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-