

and the loopfuls of suspension were measured out with the "machine."¹ "Neutral agar" holding 1.5 percent agar-agar was employed, and the cultures were in all cases grown on the same agar as that used for the plates, for fear there might be some difference in the preparations made up at different times—although in every case the same recipe was followed. Losh has shown the need for this precaution in his experiments with iodine, which he found would act well in a neutral medium containing a trace of albumin, but not nearly so actively in an alkaline medium containing more albumin.

Preliminary experiments were, of course, necessary in order that the number of colonies grown on a plate might be suitable for counting. In the case of staphylococcus, for instance, one cc of 0.6 percent brine was added to a 24-hour-old pure culture and shaken; the second suspension was made by adding three loops of the first to 5 cc of the brine, and 10 cc agar was infected with one loop of this second suspension. The plates were so thickly sown that a count could not be made. The procedure was varied by using 5 cc salt solution for the first suspension and 10 cc for the second, but the plates were still too thickly sown. Finally, by using 10 cc for the first and 10 cc for the second, a satisfactory count of 7300 colonies was obtained; the plates had an area of 63 to 64 square centimeters, and the number of colonies on 12 cm of each plate was counted.

The spores were used instead of the vegetative forms of anthrax and potato bacillus because the former are much more resistant to phenol, and thus enabled the experiments to be made with higher concentrations of the poison. It was hoped that the innocuous potato spores might be used instead of the virulent anthrax, and preliminary experiments showed that insofar as their resistance is concerned, this is quite feasible; but it proved too difficult to prepare a uniform constant culture, while the very characteristic colonies of the anthrax made it easy to procure and maintain a pure strain, and in the end work with the potato bacillus was abandoned.

¹ Lash Miller: Jour. Phys. Chem., 24, 563 (1920).