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Differential counting of living and dead cells of bacteria.

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By means of the procedure here described it is possible to determine with considerable accuracy the number of bacterial cells in a suspension and at the same time to determine the size and form of the cells and to differentiate the living and dead cells. The method makes use of the principle of the Breed and Brew[1] method of counting bacteria in milk, and the negative staining method of Benians.[2]

A measured quantity of bacterial suspension is mixed thoroughly with an equal quantity of 2 per cent. aqueous Congo red solution; the mixture is allowed to stand ten minutes. After again shaking the mixture, 0.01 c.c. is removed by means of a capillary pipette of that capacity and discharged on to a clean slide which has been clamped to the table over a piece of white paper on which a 2 cm. square has been ruled. By means of a stiff wire the drop of liquid is spread as evenly as possible over this area. After it has become thoroughly dry the slide is immersed a moment in a 1 per cent. solution of hydrochloric acid in 95 per cent. alcohol. This turns the dye blue and also fixes the film. If covered with a layer of cedar oil the slides will keep indefinitely, but if exposed to the air they fade considerably.

Cells which were alive at the time of staining are unstained and appear as white spots on a blue ground. While the cells themselves may shrink considerably after fixation and drying, a number of comparative measurements have shown that the clear space in the film faithfully reproduces the size and form of the living wet cells. With favorable material flagella may be demonstrated by this stain.

Seiffert[3] has observed that when bacteria are suspended in a weak Congo red solution and examined in hanging drops that

1 Technical Bulletin No. 49, New York Agricultural Experiment Station.

2 *British Medical Journal*, 1917, ii, 722.

3 *Centralbl. fur. Bakt.*, etc., Abt. I, Orig., 1922, lxxxviii, 151.

the dead cells are stained whereas the living cells are not. The differentiation by this method is, however, not so sharp as when Benians' technique is used. In the latter case the dead cells appear a distinct blue color, usually deeper in tint than the surrounding film of dye. I have found that with suspensions killed by heat the staining is not intense immediately after killing, but that the cells stain more deeply if allowed to stand several hours after heating; and that they stain more readily after heating to 60° than when heated to 100°. When killed and preserved in formalin the cells do not stain; but dead cells preserved in formalin retain their staining properties. It would seem that autolysis must commence before the cells can stain.

The metachromatic granules of some diphtheroids and the sporogenous granules of some bacilli are stained by the Congo red even in the living cells. The differentiation of the living and dead cells can not, therefore, be attributed to changes in the permeability of the cell membrane. The stain not only colors the protoplasm of the dead cells but is also concentrated in the film about them. It is possible that the staining may be explained by a loss or change of electrical charge in the cells.

The films dry rather unevenly, being denser in the middle. It is best to prepare a number of slides and to choose for counting those which show the most uniform films. At least five slides should be counted to compensate for the uneven distribution. The counting is done by means of an eye-piece micrometer ruled in squares and calibrated against a stage micrometer. With suspensions of ten million per c.c. or over I count twenty fields of .01 sq. mm. each from each of five slides. The average deviation of such counts is usually less than 10 per cent. A growth curve obtained by this method of counting was much smoother than those obtained by other methods. A series of comparative counts of a yeast cell suspension by the method described and by the use of a counting chamber of the Helber type showed that the two procedures were about equally accurate. But with small bacteria the cells can be seen so much more distinctly in the negatively stained film that a much larger number can be counted without fatigue or eyestrain than is possible with the counting chamber; and I believe that the method will prove correspondingly more accurate with such organisms.

Broth and peptone solution precipitate the Congo red. The method can not be used with such cultures unless they are first

centrifuged and the sediment is resuspended in a measured volume of water or salt solution. This is not true of the so-called synthetic media which contain no protein substances.

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Demonstration of an instrument for taking repeated blood pressures in rabbits, with report of some experiments.

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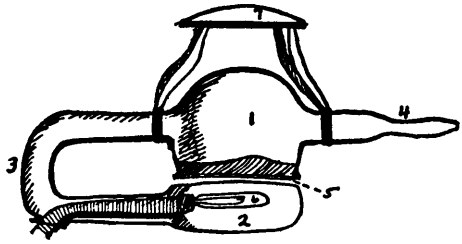
In connection with a study of renal insufficiency in rabbits,[1] it became desirable to take a series of blood pressures on the animals. An instrument has been perfected by means of which the blood pressure can be taken in the central artery of the ear. The instrument is composed essentially of three parts; namely, a pressure piece, a "U" tube containing mercury, and a rubber bulb with which to make pressure.



The pressure piece is made of glass. It is composed of an open cup (1) (see drawing) an apposing smooth, slightly convex stage (2), a connecting curved arm (3), and a short glass point (4) by means of which the cup may be connected with the "U" tube. The mouth of the cup is covered by a rubber membrane (5) which, when pressure is made within the cup, bulges against the stage. The rabbit's ear is slipped between the stage and rubber membrane. The stage contains a light

[1] To be reported at a later date.