

AN IMPROVEMENT IN THE TECHNIC OF THE INDOL TEST.

JOSEPH MCFARLAND AND J. HAMILTON SMALL.

(The Medico-Chirurgical College of Philadelphia.)

SOME years ago Dr. Dunham introduced the peptone solution to facilitate the detection of indol, recommending it on the ground that its freedom from color made it more easily possible to detect the reddish tinge of the nitroso-indol than an amber colored fluid such as broth. It was, however, later shown by Theobald Smith that peptone solution is less well adapted to purposes of culture than broth, and after a careful study of the subject Smith recommended that the peptone solution be abandoned for the use of a sugar free broth.

It is customary to test for indol by the addition of a small quantity of a weak solution of potassium nitrite (0.01 per cent solution) and some chemically pure sulphuric acid, the liquid being shaken and the presence of a red color, in case very little is formed, noticed on the whitish froth. When the quantity of indol present is very small, a considerable delicacy of color perception is required to recognize it, so that any method becomes welcome that will concentrate the color at some particular portion of the tube. It has, therefore, occurred to us to endeavor to modify the test by the formation of a color ring, a slight modification of the usual method sufficing for this purpose.

The culture to be tested receives an addition of one drop of chemically pure sulphuric acid for each c.c. of fluid, this being well shaken. In case the microorganisms produce both indol and nitrites the red color now makes its appearance, as in the cholera spirilla, etc., but when the organisms produce no nitrites, as in the case of those of the colon group, after the sulphuric acid has been mixed with the fluid the dilute solution of potassium nitrite is allowed to trickle slowly down the side of the tube and form a layer on the surface of the fluid it already contains. The red color of the nitroso-indol now makes its appearance at the line of

contact of the two fluids where it is quite easy to recognize amounts of indol that could with difficulty be recognized should the tube be shaken and the color diffused through it.

By making solutions of indol crystals in distilled water and testing by this method, we have found it possible to recognize the presence of indol in dilutions of upwards of 1: 750,000. In dilutions of 1: 1,000,000 the color was not distinctly appreciable. In all lower dilutions the color was in proportion to the intensity of the solution.

Dr. Peckham made use of known dilutions of indol for determining the probable percentage of indol in different cultures, but the color being diffused throughout the entire liquid, the more delicate tints were lost.

It is quite easy to prepare a series of such color rings as have been described for making quantitative comparisons for ordinary laboratory work. We have found it quite satisfactory to make a series of test color rings in solutions containing two per cent of gelatin, the melted gelatin containing the indol and sulphuric acid is placed in a test tube and the melted gelatin containing the dilute nitrite solution is superimposed upon it. The color forms at the line of contact, as usual, and as the gelatin rapidly solidifies such tubes are not disturbed by handling or oversetting and can be kept for comparison for from 12 to 24 hours. Beyond 24 hours, the color begins to diffuse itself through the gelatin and is gradually lost.