

A RAPID AND SIMPLE INDOL TEST

PAUL R. CANNON

From the Department of Hygiene and Bacteriology, The University of Chicago

It is well known that tryptophane in an inorganic solution furnishes an excellent medium for the demonstration of indol production by bacteria. Zipfel (1) in 1912 found that indol could be demonstrated by this method at the end of twenty-four hours, giving the best reaction with p-dimethyl-amido-benzaldehyde. The test is made by adding to the culture to be tested one cubic centimeter of a solution consisting of p-dimethyl-amido-benzaldehyde, 4 parts; absolute alcohol, 380 parts; and concentrated hydrochloric acid, 80 parts.

The expense of preparing tryptophane and the great difficulty in obtaining it at all in the United States at the present time are drawbacks to the introduction of the tryptophane method. As a substitute, I have found that hydrolyzed casein can be used. Cow casein contains about 1.5 per cent tryptophane, which may be obtained in its amino-acid form by hydrolyzing the casein.

The method is as follows: 10 grams of casein are hydrolyzed by 200 cc. of 10 per cent sulphuric acid, the mixture being kept on the water bath for twenty-four hours. At the end of this time, the casein is completely dissolved and the solution is a dark brown. Next, the solution is neutralized by the addition of saturated barium hydrate, thus precipitating out the sulphate. The resulting solution is then evaporated until the amino-acids crystallize. Half of the crystalline mass is dissolved in 500 cc. of Zipfel's inorganic solution consisting of Asparagin and Ammonium lactate, 5 grams each; Potassium acid phosphate, 2 grams; Magnesium sulphate, 0.2 grams; and distilled water, 1000 grams. The medium is tubed and sterilized. Assuming that there are 0.15 grams of tryptophane in the cow casein, tryptophane should be present in the mixture to the extent of about 0.03 per cent.

Tubes of media prepared in this way were inoculated with known indol-forming bacteria, and, at the end of eighteen hours, the indol test was made by using p-dimethyl-amido-benzaldehyde. A pronounced red color almost instantly appeared, showing the presence of indol. The control and non-indol-formers remained a straw color after the addition of the aldehyde. It proved unnecessary in my tests to use amyl alcohol to dissolve out the color, although this may be done in case indol formation is doubtful.

The brief time—eighteen to twenty-four hours—necessary for the test by the above method, is a great improvement over the old standard peptone test, which required five days. Furthermore, hydrolyzed casein can easily be obtained or prepared, and the constituents of the inorganic solution are available in most laboratories.

REFERENCE

- (1) ZIFFEL: *Centralblatt f. Bakt., etc., Orig. Abt.* 1, 64 65, 1912.