AN IMPROVED TECHNIC FOR THE VOGES-PROSKAUER TEST

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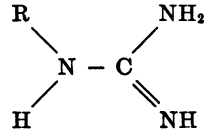
Voges and Proskauer (1898) described the production of an eosin-like coloration in glucose peptone cultures of certain organisms to which had been added a 10 per cent solution of potassium hydroxide. The reaction is generally referred to as the V-P test and serves as a valuable aid to the identification and differentiation of microörganisms.

The reaction depends upon the production of acetoin (acetylmethyl carbinol), a non-volatile reducing substance. According to Harden (1905) acetylmethyl carbinol is oxidized upon the addition of KOH and in the presence of peptone there is imparted an eosin-like coloration to the medium. Later Harden and Norris (1911) reported that diacetyl in the presence of strong KOH solution reacts with proteins to give a pink coloration with a green fluorescence. The coloration appears to depend on the group



although the significance of the radical R is uncertain. The Voges-Proskauer reaction may be indicated as follows:

$$C_6H_{12}O_6 \rightarrow CH_3 \cdot CO \cdot CHOH \cdot CH_3 + KOH \rightarrow$$

glucose acetylmethyl carbinol

$$CH_3 \cdot CO - CO \cdot CH_3 + \text{peptone} \rightarrow \text{pink coloration}$$

diacetyl

Harden and Norris (1912) explained the production of acetylmethyl carbinol and 2,3-butylene-glycol by assuming a condensation of intermediately formed acetaldehyde. Subsequently Neuberg and Reinfürth (1923) showed that if acetaldehyde is added to a sugar-containing medium undergoing fermentation by yeast, acyloin condensation occurs, one molecule of added aldehyde uniting with one molecule of aldehyde produced by the yeast. The added aldehyde is thus converted practically completely into acetylmethyl carbinol. Neuberg sees in this aldehyde condensation, a carbon-coupling action of yeast under the influence of an enzyme which he terms carboligase.

The eosin coloration of the positive V-P test is apparent only after a period of an hour or longer, and in the case of a number of microörganisms is faint and indistinct, making reading of the test uncertain and unreliable, especially in colored media. Among the attempts which have been made to improve the method of conducting the test, may be mentioned that of West (1909) who conducted air through the medium to hasten the reaction. Chen and Rettger (1920) suggested shaking and incubating at 30°C. for one to three hours and again shaking. Attempts also have been made to add oxidizing agents to increase the rate of oxidation of the acetylmethyl carbinol to diacetyl. Levine, Weldin, and Johnson (1917) after a study of various oxidizing agents, recommended the addition of two or three drops of hydrogen peroxide to 6 cc. of the alkaline medium heated in a boiling water bath for two minutes. The use of hydrogen peroxide was not entirely satisfactory when the medium was colored as was the case when a glucose or galactose medium was used. The difficulty with a glucose medium is especially important since the V-P test applies particularly to a glucose medium.

With sucrose, mannitol, raffinose, and salicin, the hydrogen peroxide method was found to give results as good as were obtained by reading the standard test after twenty-four hours. Bedford (1929) used sodium peroxide, adding 10 mgm. sodium peroxide to 2.5 cc. of the culture medium. One cubic centimeter of 40 per cent NaOH was then added and the culture tube placed in boiling water for one minute and then shaken.

An important difficulty experienced in the use of oxidizing agents is the transitory nature of the coloration of the positive cultures. Any method which results in the rapid disappearance of the coloration is inconvenient and likely to prove unsatisfactory, particularly since the eosin coloration is rather delicate.

The method to be described utilizes ferric chloride as a catalyst and avoids the disadvantages of the original V-P test or its modifications. The positive test is indicated by a deep copper coloration which appears at the surface after a few minutes and extends to the bottom of the tube. The color remains for several days and even after a week or longer it is clearly visible. The test may be positive for cultures of organisms belonging to the genus *Aerogenes* after three days' incubation at 30°C., but four-day cultures are recommended for standard procedure. Two drops of a 2 per cent solution of ferric chloride are added to 5 cc. of the culture. Five cubic centimeters of a 10 per cent solution of NaOH are now added and the tube shaken. The solution of ferric chloride must be added before addition of the sodium hydroxide solution; addition after the alkali results in a marked flocculation.

The reaction may be accelerated by heating the culture for one minute in boiling water. The advantages are not great and it is doubtful whether heating is to be recommended. Only the original culture, or the culture with ferric chloride, should be heated. Addition of the alkali before heating may result in browning if unfermented glucose is present.

In the positive tube, two distinct layers of coloration may be observed. The upper is a copper color; the lower layer is the eosin pink of the standard V-P test. The copper color soon extends to the bottom of the tube. Acetylmethyl carbinol may be detected in three-day cultures when the standard technic fails to show its presence. Since the color of a positive test is that of bright copper, reading of the test is definite and certain. The color remains for days and sometimes as long as two or three weeks. There is no danger of failure to observe the positive test.

The ferric chloride hastens the oxidation of the acetylmethyl

carbinol to diacetyl which reacts with peptone in an alkaline solution to produce the copper coloration. The ferric chloride appears to catalyze the oxidation of the acetylmethyl carbinol since the reaction does not take place in the absence of a hydrogen acceptor. Thus the reaction does not occur in the absence of atmospheric oxygen if no other suitable hydrogen acceptor has been provided. Apparently, this is the first time an improvement of the V-P test has been sought in the use of a chemical catalyst. Former attempts have used oxidizing agents which resulted in rapid oxidation and the transitory appearance of the eosin coloration.

Ferric chloride will not result in the oxidation of 2,3-butyleneglycol under the conditions of the test, and any organism which may have reduced the acetylmethyl carbinol to 2, 3-butyleneglycol will not give a false Voges-Proskauer reaction.

To show this, a medium of 2,3-butylene-glycol[1] (0.2 per cent), $(NH_4)_2$ So_4 (0.2 per cent), K_2HPO_4 (0.1 per cent) sterilized for fifteen minutes at 20 pounds pressure, was prepared in tubes and inoculated with strains of *Aerobacter aerogenes*, *A. faeni*, *A. pectinovorum*, *A. motorium*, *A. mitificans* and *A. indologenes*. Acetylmethyl carbinol was detected in cultures of all the organisms on the second day by the use of ferric chloride. Uninoculated tubes to which ferric chloride and KOH had been added did not give positive tests. Cultures from which atmospheric oxygen was excluded by boiling the constituents were negative.

This modification has been used during the past three years in comparison with the standard procedure by graduate students on over 600 cultures, some negative, others positive, and no discrepancies have been found. More than 40 species of bacteria have been represented in these tests.

REFERENCES

BEDFORD, R. H. 1929 A rapid method for obtaining the Voges-Proskauer reaction. Jour. Bacteriol., **18**, 93–4.

CHEN, CHEN CHONG AND RETTGER, LEO F. 1920. A correlation study of the colon aerogenes group of bacteria, with special reference to the organisms occurring in the soil. Jour. Bacteriol., **5**, 253–99.

[1] Breden, C. R., Fulmer, E. I., Werkman, C. H., and Hixon, R. M., Jour. Bacteriol., **19**, 41, (1930). Prepared by the action of *Aerobacter faeni* on glucose.

HARDEN, A., AND NORRIS, D. 1912 The bacterial production of acetylmethyl carbinol and 2,3-butylene-glycol from various substances. Proc. Roy. Soc. (B), **85**, 73–8.

HARDEN, A., AND NORRIS, D. 1911 The diacetyl reaction for proteins. Jour. Physiol., **42**, 332–6.

LEVINE, MAX, WELDIN, J. C., AND JOHNSON, BERYL R. 1917 The Voges-Proskauer and correlated reactions of coli-like bacteria. Jour. Infect. Dis., **21**, 39–46.

NEUBERG, C., AND REINFÜRTH, E. 1923 Eine neue form der Unwandlung des Acetaldehyde durch gärende Hefe. Biochem. Zeitschr., **143**, 553–65.

VOGES, O., AND PROSKAUER, B. 1898 Beiträge zur Ernährungsphysiologie und zur differential diagnose der Bakterien der hemmorrhagischen septicamie. Zeit. für Hyg., **28**, 20–32.

WEST, F. D. 1909 Notes on the Voges and Proskauer reaction for *Bacillus coli co-mmunis*. Amer. Jour. Pub. Hyg., **19**, 227.