INDOL PRODUCTION BY BACTERIA

JOHN F. NORTON AND MARY V. SAWYER

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

Received for publication January 11, 1921

The formation of indol in certain culture media has long been considered an important differential characteristic for the idenification of bacteria. Special interest in the test has been recently aroused in connection with investigations on respiratory diseases because Pfeiffer's bacillus appears to be practically the only mouth organism producing indol (Jordan, 1919, Malone, 1920); in consequence of which Malone has suggested that the test be used as an index of the presence of this organism without actually isolating it. Rivers (1920) has made a similar suggestion for diagnosis of influenzal meningitis. For this paper we have attempted to collect from the literature the more recent information, both positive and negative, concerning indol production by bacteria. On account of the uncertainty of indol tests much of the older work is unreliable. This information has been supplemented by tests on over 180 strains of bacteria, most of which are being carried as stock cultures in this laboratory and have been collected from a great variety of sources. Incidentally we have made a comprehensive comparison of three recommended media and also determined the effect of the incubation period on indol production.

MEDIA

Indol is a disintegration product of proteins containing the tryptophane group. The ideal medium would therefore be one to which pure trytophane had been added but on account of the difficulty of obtaining the material it is impractical, although Zipfel (1913) used such a medium. Dunham's pepton is the easiest medium to prepare and has been most frequently used.

Sicre (1909) and Porcher (1911) have studied the use of various peptons finding that some already contain indol and that with others indol is never produced by bacteria, so that it is necessary not only to make blank tests on any chosen medium but also to control experiments with a known indol-producing organism. In each instance our tests were controlled by inoculating one tube of medium with *Bact. coli*, an indol-forming organism, and another with *Bact. typhosum*, a non-idol-former, and we regarded such controls as essential. For media, besides Dunham's pepton and the tryptophane medium of Zipfel, Rivas has suggested trypsinized pepton (Rivas, 1912) and Cannon (1916), instead of preparing pure tryptophane from casein as was done by Zipfel, used hydrolyzed casein as the basis for his medium.

Homer (1916) believes that tryptophane is necessary for bacterial life and if not present will be synthesized by the organisms. Logie (1920) has used synthetic media containing ammonium lactate with asparagin or sodium asparaginate and claims that indol producing organisms possess an enzyme which enables them to split off and utilize part of the tryptophane molecule. It is possible that many organisms may be capable of synthesizing indol but make use of it in their metabolism.

For the hemophilic group a heated blood broth has been used (Jordan, 1919).

It is well known that the addition of glucose to a medium interferes with the indol test. Fischer (1915) reports that lactose, galactose, maltose or fructose are without effect. He believes that this action of glucose is due not to acid production, as had been supposed, but to the inactivation of the proteolytic enzyme concerned in splitting the tryptophane. Logie (1920) found that if glucose was added to a living culture of *Bact. coli* in which indol had already been produced the latter rapidly disappeared. From this he concluded that glucose caused an increased demand in the organism for indol. Homer (1916) explains the effect of the presence of glucose either on the basis of the preference of the organism for glucose over tryptophane or by assuming the formation of a chemical compound between glucose and tryptophane which is relatively stable.

We have used three media. (1) Dunham's pepton solution. One per cent of pepton (Armour's) and 0.5 per cent sodium chloride were dissolved in distilled water and the reaction adjusted to +1 to phenolphthalein. (2) Rivas' trypsinized pepton (Rivas 1912). Ten grams of pepton (Armour's) were dissolved in 200 cc. distilled water. To this was added a solution of 0.5 gram trypsin in 10 cc. of water (trypsin dissolved by shaking and gentle heating not over 40°C.) and digestion allowed to continue for three hours at 37°C., with frequent stirring. The solution was then made up to 1 liter and reaction adjusted to + 1 to phenolphthalein. (3) Cannon's casein medium (Cannon, 1916). Twenty grams of chemically pure casein were added to 250 cc. distilled water and the whole made alkaline to phenolphthalein with sodium carbonate. One-half gram of trypsin was added and the casein allowed to digest for six hours. The medium was then autoclaved and 5 grams each of asparagin and ammonium lactate, 2 grams of dipotassium phosphate and 0.2 gram magnesium sulphate were added. The solution was made up to 1 liter and reaction adjusted to +1 to phenolphthalein.

A large number of comparative tests were made on these three media. In no case did the final indol test vary but a positive reaction was obtained more quickly, and the color tests were stronger, with the trypsinized casein or pepton than with Dunham's pepton solution. Positive tests with the trypsinized pepton were noted after six hours incubation with *Bact. coli* and color production with Ehrlich's reagent was at its maximum at the end of twenty-four hours. With Dunham's solution the maximum was obtained only after four days. After six days the indol began to disappear. As most of our tests were made simultaneously on all three media we used the four day period, although forty-eight hours is sufficient for the trypsinized media.

The influence of oxygen supply on indol formation has been studied by Porcher and Panisset (1911). They found that growing cultures of the colon bacillus and of proteus anaerobically decreased the amount of indol formed, while if a current of oxygen was kept going through the flask, the amount was increased. However, they were unable to provoke the formation

of indol by *Bact. typhosum* by an oxygen current. Our cultures were incubated aerobically, except in the case of the strict anaerobes.

INDOL REAGENTS

Numerous tests for indol have been suggested and used. Nelson (1916) gives four: (1) dimethylamine, glycolic acid, glyceric aldehyde and sulphuric acid, giving a pink color; (2) peruvic aldehyde, sulphuric acid and ferric sulphate, giving a violet color; (3) vanillin and an acid, giving an orange color; (4) Salkowski test—sulphuric acid and potassium nitrite, giving a pink to red ring. Escallon (1908) recommends furfural. This, in the presence of hydrochloric acid, gives an orange yellow color. It is claimed that this test is sensitive to 1 part in 800,000. Baudisch (1915) describes a reaction using nitromethane. By far the most satisfactory test is that suggested by Ehrlich (1901). The reagent is prepared by dissolving 4 grams of paradimethylamido-benzaldehyde in 380 cc. of alcohol and adding 80 cc. of concentrated hydrochloric acid. A red color is formed in the presence of indol at the junction of the reagent and the liquid to be tested if the former is added so that it forms a layer on top. A solution of potassium persulphate is sometimes added to bring out the color more clearly but we have found the reagent quite satisfactory without this. In making our tests, if a red color appeared on adding the Ehrlich reagent, 1 cc. of amyl alcohol was added and the tube shaken. The red coloring matter, if due to indol, is soluble in amyl alcohol.

SUMMARY

In the following table we have summarized the results of our tests together with those we have been able to find in the literature. Owing to the uncertainty of results obtained by use of the older methods of testing for indol, only relatively recent work has been included. The organisms are divided into two groups: (I) those which may pretty definitely be regarded as giving negative tests and (II) those for which positive results have been reported. It should be noted that in every instance where

any considerable number of strains of an organism in group II have been examined, negative as well as positive results have been reported, with the exception of the cholera vibrio.

From this summary we must conclude that whereas the indol test may serve as a valuable aid in differentiating bacteria, it cannot be regarded as an absolute criterion. A positive test may give definite information but a negative test must be interpreted with caution.

We should also like to emphasize the necessity for a standard reagent for the indol test and suggest the use of Ehrlich's dimethylamidobenzaldehyde solution for this purpose.

In this table we have used the classification adopted by the Society of American Bacteriologists (Winslow, 1920).

Group I. Indol negative

Bcaillus anthracis* (Zipfel)
subtilis*
Actinomyces asteroides*
bovis*
graminaris*
Bacterium abortum (Weeter)
cloacae (5 strains*, Kligler)
enteritidis (3 strains*, Crossonini, Porcher and Panisset, Zipfel, Nonnotte and Demanche)
fecalis-alcaligenes (3 strains*)
icteroides (Crossonini)
mucosum-capsulatum* (Hiss and Zinsser)
paratyphosum A (2 strains*, Zipfel, Jordan, Nonnotte and Demanche)
paratyphosum B (13 strains*, Zipfel, Jordan, Nonnotte and Demanche)
pullorum (5 strains*, Mulsow)
rhinoscleromatis*
sanguinarium (Mulsow)
suipestifer (4 strains*, Zipfel, Crossonini)
typhosum (15 strains*, all investigators)
typhi-murium (Nonnotte and Demanche)
Clostridium botulinum (6 strains*)
chauvei*
Welchii*
Corynebacterium Hoffmannii (3 stains*)
pseudodiphtheriae (4 strains*)
xerosis (2 strains*)

* Our tests.

Diphtheroids (Malone)
Diplococcus pneumoniae (Jordan, Malone)
Erythrobacillus miniaceus*
mycoides-roseus*
mycoides-corallinus*
prodigiosus (4 strains*, Crossonini, Zipfel)
Myobacterium leprae*
Moelleri (2 strains*)
tuberculosis (4 strains*, Zipfel)
Neisseria meningitidis (Jordan)
catarrhalis (Malone)
Pseudomonas cyanea*
cyanogenes*
violacea*
Pasteurella pestis* (Zipfel)
Staphlococcus albus (8 strains*, Zipfel)
aureus (4 strains*, Zipfel)
citreus (Zipfel)
not specified (Jordan, Malone)
Streptococcus viridans (13 strains*, Jordan, Malone)
hemolyticus (2 strains*, Zipfel)
rheumaticus*
Sarcina lutea*
rosea*
Vibrio tyrogenus (Deneke)* (Zipfel, Tobey)
Zopfius zopfii (3 strains*)
Sporothrix schenkii*
Blastomycetes dermatitidis*
Sac. pastorianus*

Group II. Indol positive or negative

Bacterium aerogenes* (Kligler, Chen and Rettger)†
coli* (Kligler, Chen and Rettger)‡
dysenteriae* (Zipfel, Kolle and Wassermann)§
Clostridium sporogenes (edematis)* (Bertrand)
tetani (Hall)
Corynebacterium diphtheriae (Escallon, Zipfel)
Hemophilus influenzae (Rhein, Jordan, Malone)±

---

* Our tests.

† Chen and Rettger found 141 strains +, 306 −.

‡ Chen and Rettger found from feces 173 strains +, 0 −; from soil 15 strains + 5 −.

§ We found as + Flexner, Hiss-Russel, Shiga, 110, 12 U. S., as − Hoffmanni, 177. Zipfel found Flexner andY +, Shiga −. Kolle and Wassermann give Strong −.

± Rhein found 7 strains +, 1 −; Jordan 18 +, 7 −; Malone found 92 per cent +, 8 per cent −.

Pasteurella aviseptica (Mulsow, Kolle and Wassermann)
Proteus group (Bengston, Horowitz, Kligler, Larson and Bell, Rhein, Sicre)
Pseudomonas pyocyanea* (Jordan)¶
Vibrio cholerae (2 strains*, Crossonini, Baudisch, Zipfel, Tobey)
finklerei (Crossonini, Tobey, Zipfel)
metschnikovi* (Crossonini, Steensma, Tobey)
protea*

---

* Our tests.

¶ We found 13 strains −, 6 of them freshly isolated; Jordan reported both + and −; see also Lartigau (1898)

REFERENCES

BAUDISCH 1915 Über eine neue Indolreaktion, Zeitschr. Physiol. Chem., **94**, 132.

BENGSTON 1919 The proteus group. Jour. Infect. Dis., **24**, 445.

BERTRAND 1913 Influence des régime alimentaire sur la formation de l'indol dans l'organisme. Ann. Inst. Pasteur, **27**, 76.

CANNON 1916 A simple and rapid indol test. Jour. Bact., **1**, 535.

CHEN AND RETTGER 1920. A correlation study of the colon-aerogenes group of bacteria, with special reference to the organisms occurring in the soil. Jour. Bact., **5**, 253.

CROSSONINI 1910 Über den Nachweis von Indol. Arch. Hyg., **72**, 161.

EHRLICH 1901 Über die Dimethyl-Amidobenzaldehyde Reaction. Berl. Med. Wochensch., **1**, 151

ESCALLON 1908 Reserche de l'indol dans les cultures microbiennes a l'aide du furfural. Compt. rend. soc. biol., **65**, 507.

FISCHER 1915 Hemmung der Indolbildung bei *Bact. coli* in Kulturen mit Zuckerzusatz. Biochem. Zeitschr., **70**, 105.

HALL 1920 Unpublished communication.

HISS AND ZINSSER 1917 Textbook of Bacteriology.

HOMER 1916 A suggestion as to the cause of lessened production of indol in media containing glucose. Jour. Hygiene, **15**, 401.

HOROWITZ 1916 Contribution a l'étude du genre Proteus Vulgaris. Ann. Inst. Pasteur, **30**, 307.

JORDAN 1899 Bacillus pyocyaneus and its pigments. Jour. Exp. Med., **4**, 627.

JORDAN 1917 Differentiation of the paratyphoid-enteriditis group I. Jour. Infect. Dis., **20**, 7.

JORDAN 1919 Production of indol by strains of Pfeiffer's bacilli. Jour. Am. Med. Assn., **72**, 1542.

JORDAN 1920 Biology of the Pfeiffer bacillus. Am. Jour. Pub. Health, **10**, 648.

KLIGLER 1914 Observation on indol production in the Colon-typhoid group. Jour. Infect. Dis., **14**, 81.

KOLLE AND WASSERMANN 1913 Handb. d. pathogen. Mikroorganismen.

LARSON AND BELL 1913 A study of the lesions produced by Bacillus proteus. Jour. Infect. Dis., **13**, 510.

LARTIGAU 1898 Epidemic dysentery caused by Bacillus pyocyaneus. Jour. Exp. Med., **3**, 604.

LOGHEM AND LOGHEM-POUW. 1912 Beiträge zur Differenzierung des Proteus Groupe. Centrabl. Bakt., Orig., I. Abt., **66**, 19.

LOGIE 1920 Synthesis of tryptophane by certain bacteria and the nature of indol formation. Jour. Path. and Bact., **23**, 224.

MALONE 1920 The production of indol by Pfeiffer's bacillus. Indian Jour. Med. Res., **7**, 519.

MULSOW 1919 Avian paratyphoid bacilli. Jour. Infect. Dis., **25**, 135.

NELSON 1916 Some color reactions for indole and scatole. Jour. Biol. Chem., **24**, 527.

NONNOTTE AND DEMANCHE 1908 Sur la recherche de l'indol dans les cultures microbiennes. Compt. rend. soc. biol., **64**, 658.

PORCHER 1911 Les diverses peptone et la formation de l'indol. Compt. rend. soc. biol., **70**, 464.

PORCHER AND PANISSET 1909 Indol dans les bouillons microbiens. La présence dans las cultures du cholera des poules. Compt. rend. soc. biol., **66**, 624.

PORCHER AND PANISSET 1911 La formation de l'indol dans les cultures en milien aerobes et en milien anaerobes. Compt. rend. soc. biol., **70**, 436.

RHEIN 1919 Sur la production d'indol per le bacille de Pfeiffer. Compt. rend. soc. biol., **82**, 138.

RIVAS 1912 Studies on indol. Centralbl. Bakt., Orig., I. Abt., **63**, 547.

RIVERS 1920 Indol test on the spinal fluid for rapid diagnosis of influenzal meningitis. J. Am. Med. Assn., **75**, 1495.

SICRE 1909 Sur la reserche de l'indol dans les cultures microbiennes a l'aide des nouveaux réactife. Compt. rend. soc. biol., **67**, 76.

STEENSMA 1906 Über den Nachweis von Indol und die Bildung von Indol vortausehenden Soffen in Bakterien Kulturen. Centralbl. Bakt., Orig., I. Abt., **41**, 295.

TOBEY 1906 Cholera red and indol reaction. Jour. Med. Res., **15**, 305.

WEETER 1920 Unpublished communication.

ZIPFEL 1913 Weitere Beiträge zur Kenntniss der Indolreaktion. Centralbl. Bakt., Orig., I. Abt., **67**, 583.

WINSLOW AND OTHERS 1920 The Families and Genera of the Bacteria. Jour. Bact., **5**, 191.