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PRODUCTION OF HYDROGEN SULPHIDE BY MEMBERS OF THE COLON GROUP OF BACTERIA

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Not a little work has been done in the past few years in investigating the sulphur metabolism of the colon group of bacteria. So far no very definite conclusions seem to have been arrived at, and the results are rather conflicting. Myers[1] (1920) suggests that hydrogen sulphide production in the intestinal tract is due to proteolytic organisms. He attempted to use hydrogen sulphide production for water analysis, but concludes: "There is no constant relationship between the number of colon bacilli present from different animals and the amount of H_2S produced." Other workers have believed the production of hydrogen sulphide in the intestinal tract to be due to the action of B. coli on traces of systine present. B. coli is not generally considered a producer of this gas from peptone. Sasaki and Otuska[2] (1912), Berger[3] (1914), and Tanner[4] (1917) report B. coli as giving hydrogen sulphide from cystine.

Two methods have been employed in the preparation of the medium and using the indicator, lead acetate. Tanner[5] (1918), and Myers[1] (1920), after inoculating the tubes, used strips of sterile filter paper impregnated with lead acetate in the tubes to register H_2S production. Jordan and Victorson[6] (1917), Thompson[7] (1921), and Tilley[8] (1923) added a few drops of a sterile lead acetate solution to the partly cooled sterile tubes of media. None of these experimenters give any reason why the different materials cannot be put together and sterilized in the usual way.

Some workers, Jordan and Victorson[6] (1917), Myers[1] (1920), and Tilley[8] (1923) report a difference in the different brands of peptone as to their ability to support hydrogen sulphide production, and the last named claims to have found differences in different samples of the same brand.

In attempting this investigation the first thing was to find a more practical way of preparing the medium and handling the indicator. Media were prepared as described by Jordan and Victorson, using 3%, 2%, and 1% peptone, tubing, sterilizing, and adding two drops of 10% lead acetate solution to the partly cooled sterile tubes. Inoculation was made by introducing a loopful of a young dextrose broth culture between the wall of the tube and

the medium. The 3% and 2% peptone media showed no difference while the 1% sometimes gave negative results, or weak or delayed reactions which were strong and prompt in the 2% and 3% peptone media. Apparently 2% peptone is as satisfactory as 3% for routine work.

A medium was next prepared, using 2% peptone, 0.3% meat extract, Ph 7.6 and 1½% agar. These were brought into solution, then 10 c. c. of the clear supernatent liquid of a 10% solution of lead acetate was added to each liter of medium; it was then tubed in about 3 c. c. amounts and sterilized at 15 lbs. for 15 minutes. The tubes were inoculated as described by Jordan and Victorson, and incubated at 37°. All organisms tried (17 species, 9 positives and 8 negatives) gave results identical with those of the first method. This method just described is now used routinely in the S. U. I. medical bacteriology laboratory.

As to the variations in different samples of peptone that have been reported there is not yet a satisfactory explanation. In this work 111 strains of B. coli have been used. Their sources were somewhat varied, but for nearly all of them are definitely known. They are: 66 of fecal origin, man and animals; 13 from the genito-urinary tract of females with chronic gonorrhea; 24 from cases of cystitis and pyelitis; 2 from milk; 1 from water; 5 were obtained from ink of a certain manufacture. Five of the 111 gave H_2S from peptone. All were tried on five different brands of peptone; "Difco," Fairchild, Parke Davis, Roche, and Wittee. In no case was there any difference; the five that gave the gas from one brand gave the gas from all the brands. Those that gave the gas came from the following sources: 3 from fecal matter, 1 from a case of gonorrhea, and one from a case of cystitis. This suggests that some other factor may be the cause of the variations in peptones that have been reported.

The production of hydrogen sulphide from cystine by B. coli seems to be the rule rather than the exception, but there are a few that do not. Of all the 111 strains used in this work three were incapable of forming the gas. The three that gave negative results represent different sources; one came from sheep feces, one from milk, and one from cystitis. The medium used for this determination was prepared as follows:

Solution A:

Distilled water	800 c. c.
Sodium ammonium hydrogen phosphate	4 gms.
Dipotassium hydrogen phosphate	2 gms.

Solution B:

Agar-ager	15 gms.
Distilled water	200 c. c.
Lactose	10 gms.

Solutions A and B were sterilized separately 15 minutes at 15 lbs. After sterilization solution B was added to solution A; 10 c. c. of the clear liquid of a 10% solution of lead acetate, was next added; then 0.5 gm. of cystine dissolved in the least possible amount of concentrated ammonium hydroxide (about 5 c. c.) was added, and all well mixed. The medium was then poured into sterile tubes in about 3 c. c. amounts. The medium was rather alkaline, pink to phenolphthalein, but all gave good growths. Inoculation was with a loopful of young dextrose broth culture between the wall of the tube and the media, and incubation was at 37°.

When sodium sulphite was used three of the 111 strains were incapable of evolving hydrogen sulphide. Two of the three also failed to give the gas from cystine. Their sources were: one from sheep feces, and two from cases of renal cystitis. A synthetic medium prepared as follows was used:

Distilled water	1,000 c. c.
Sodium ammonium hydrogen phosphate	4 gms.
Dipotassium hydrogen phosphate	2 gms.
Lactose	10 gms.
Agar-agar	15 gms.
Sodium sulphite (0.1%)	1 gm.

This was melted, tubed in about 3 c. c. quantities, and sterilized 15 minutes at 15 lbs. Inoculation was with a loopful of young dextrose broth culture, and strips of sterile filter paper impregnated with lead acetate were put in the upper part of the tubes; control tubes, not inoculated, were also incubated, none of which gave the reaction

All the organisms used in this work gave a negative Voges-Proskauer reaction and a positive methyl red test. Those from pathological sources were in most cases found in pure culture, and were the probable cause of the infection, but their pathogenecity is not certain. Just why some B. coli are capable of producing hydrogen sulphide from peptone while others do not cannot be explained at this time; it may be due to the greater vigor of a few in attacking the traces of cystine probably present in the peptone. When the chemist has found and identified the common constituents of peptone the truth of this phenomenon may be revealed.

CONCLUSIONS

B. coli rarely produces hydrogen sulphide from peptone, and only a very few strains are incapable of giving the gas from cystine and sodium sulphite.

Cystine and sodium sulphite are of little, if any value in differentiating members of the colon typhoid groups.

At best the production of hydrogen sulphide by the colon group is rather uncertain, and does not mark those of any particular source.

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