DETECTION OF HYDROGEN SULPHIDE IN CULTURES

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For the past two years papers on the use of bismuth for the detection of hydrogen sulphide have been presented before the Academy. However, certain difficulties developed in the preparation of the media and during the past year a large number of experiments have been carried out on the whole problem. It is desired to present our recent information relative to the preparation of the bismuth medium before considering the production of hydrogen sulphide by members of the enteric group.

In considering the detection of hydrogen sulphide produced by cultures two main problems present themselves. First, the selection of a sensitive indicator of low toxicity and distinct color change. Second, the selection of a medium suitable for the growth of the organisms, and containing an available source of sulphur. An indicator may be of very low toxicity but when added to a medium it may combine with some of the ingredients producing a precipitate, thereby increasing the toxicity and decreasing the sensitivity.

All of the heavy metals will react with hydrogen sulphide but only a few are desirable for bacteriological work. Those commonly used as indicators are lead and iron salts. Theoretically, iron is more sensitive to hydrogen sulphide in an alkaline menstruum than in an acid one and as most of the culture media are slightly acid or neutral, it follows that the sensitivity of iron is decreased and minute quantities of hydrogen sulphide will not be detected. Therefore, an indicator which will be sensitive in the acid range is desirable.

Theoretically, bismuth is a metal which is sensitive in an acid menstruum. The difficulty in the use of bismuth is that most bismuth compounds are insoluble and therefore not applicable to bacteriological work. There are, however, several soluble bismuth compounds which are of low toxicity and which are good indicators for the detection of hydrogen sulphide.

A soluble bismuth compound, suitable for bacteriological work and commonly known as "bismuth liquor" is prepared as follows:

Place 3 grams bismuth citrate, Merck U. S. P. VIII, in a glass stoppered bottle and add about 5 cc. of distilled

water. Mix well and add approximately 1 cc. of ammonium hydroxide sp. gr. 0.90. The bismuth citrate dissolves quickly forming a clear, colorless solution. If it does not clear up readily gentle heat will facilitate. Add distilled water to bring the volume up to 100 cc. (0.5 cc. of the bismuth liquor is added to each 100 cc. of medium.)

Several experiments were performed to determine the relative sensitivity of ferric ammonium citrate and bismuth liquor. A series of test tubes were prepared containing 5 cc. of various buffers ranging from pH 6.0 to 9.0 in steps of 0.5 pH. To each tube of the buffer series was added 2.5 m. of ferric ammonium citrate and to each tube of a second series 0.125 cc. of bismuth liquor. The tubes in each series were then titrated, to a definite brownish-black color, with a hydrogen sulphide solution containing a known number of millimols of hydrogen sulphide per cc.

Similar titrations were performed using a 2% tryptone phosphate agar adjusted to the same H-ion concentration as the buffers employed in the previous experiment.

Some difficulty was encountered in the titration of the ferric ammonium citrate solutions. The iron compound reacts very slowly with hydrogen sulphide to form iron sulphide, therefore, it was found necessary to run several series of these tubes and after the addition of a known amount of hydrogen sulphide the tubes were allowed to stand five or ten minutes before readings were made. This clearly shows that iron is not as sensitive to hydrogen sulphide as some other metals, for, in the case of bismuth the formation of the sulphide was instantaneous.

The results of these experiments are given in the following table:

SENSITIVITY OF BI & FE AS SHOWN BY THE NUMBER OF MILLIMOLS OF H_2S REQUIRED TO SHOW THE PRESENCE OF SULPHIDES AT VARIOUS H CONCENTRATIONS

	BUFFERED SOLUTIONS			NUTRIENT AGAR		
pH	Bismuth	Iron	Sensitivity of Bi over Fe	Bismuth	Iron	Sensitivity of Bi over Fe
6.0	0.00481	0.101	21X	0.00240	0.0577	24X
6.5	0.00481	0.0481	10X	0.00240	0.0481	20X
7.0	0.00481	0.0481	10X	0.00240	0.0481	20X
7.5	0.00481	0.0481	10X	0.00240	0.0384	16X
8.0	0.00481	0.02885	6X	0.00240	0.02885	12X
8.5	0.00481	0.02885	6X	0.00240	0.02885	12X
9.0	0.00481	0.02885	6X	0.00240	0.0192	8X

The data presented here do not agree with the results reported by Zobell and Feltham (1934). They found that ferrous iron was more sensitive than bismuth salts. It is, however, difficult to compare data, due to several factors. First, these investigators used bismuth carbonate which is an insoluble form of bismuth, and therefore the sensitivity to sulphides was markedly reduced. Second, the titration of the medium containing the indicator was with sodium sulphide which is a basic compound and, due to the hydrolytic dissociation, would tend to make the medium more alkaline, in which case iron becomes more sensitive to the sulphide.

The results presented here not only agree with the theoretical consideration of these indicators, but definitely show the superiority of bismuth over iron in detecting hydrogen sulphide irrespective of the pH.

The second phase of work was to produce a medium incorporating a soluble bismuth compound as an indicator. This really developed into a problem of considerable magnitude. Soluble bismuth compounds are very active and react with many substances producing insoluble compounds under certain conditions. During the past year experiments have been performed with over 650 media, some being of no value and others showing definite promise. Time does not permit a discussion of all the results. Several media have been developed which are satisfactory but only one will be stated:

Tryptone	7 gms.
K_2HPO_4	0.3 gms.
Agar	5.0 gms.
Water	1000 cc.
Boil to dissolve and then add	
Na_2SO_3 (20%)	10 cc.
Bismuth liquor	5 cc.
Mannitol	5 mgs.

In comparing the bismuth medium with peptone iron it was found, as should be expected from the theoretical consideration of the indicators, that the bismuth medium would detect hydrogen sulphide formation in a larger percentage of cultures than would peptone iron. Organisms which are normally classified as non-hydrogen sulphide formers gave a definite reaction in the bismuth medium. In one series of experiments in which 80 stock cultures were innoculated into peptone iron, peptone iron and bismuth, and

to bismuth sulphite medium it was found that after 38 hours 19 or 23.7% were positive in peptone iron, and 46 or 57.5% were positive in bismuth sulphite medium.

In another series of experiments with over 70 members of the dysentery and salmonella group, the superiority of the bismuth sulphite medium was definitely proven. Lead acetate agar, peptone iron agar and bismuth sulphite medium were inoculated with these organisms and results recorded after 24 and 48 hours incubation at 37°C. The results are as follows:

Organisms	Lead Acetate	Peptone Iron	Bismuth Sulphite
S. dysentery (38 cultures)	0	0	+
S. paratyphi (25 cultures)	3 +	1 trace	(24+) (1-0)
S. schottmulleri (8 cultures)	++	++	++++

Members of the dysentery group and of the Salmonella paratyphi are stated in the literature as not producing hydrogen sulphide. Our results show that all the dysentery cultures which we have tested are negative hydrogen sulphide producers as determined by lead acetate and peptone iron but with the bismuth sulphite all produced hydrogen sulphite. In the case of the paratyphi cultures with a few exceptions they did not produce hydrogen sulphide using lead acetate and peptone iron media. However, with the bismuth sulphite medium all but one culture showed that they liberated hydrogen sulphide. The results with the schottmulleri cultures showed the presence of hydrogen sulphide with all media but the blackening was much stronger than with the other media. This group of organisms is listed as strong hydrogen sulphide producers. These results definitely show that members of the dysentery and paratyphi group are capable of producing hydrogen sulphide but the quantity is so small that lead and iron will not detect it, but when using such a sensitive indicator as bismuth the presence of hydrogen sulphide can be easily demonstrated.