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HYDROGEN SULPHIDE PRODUCTION BY BACTERIA

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There are some conflicting reports, in the investigation that has been reported, of the ability of different organisms to evolve hydrogen sulphide from various sulphur-containing compounds. One report quite at variance with a majority of others is that of Tanner [1] (1917) who has reported more exhaustive investigation than any other worker, unless it be Sasaki and Otsuka [2] (1912). Tanner, using four per cent of peptone, brand not stated, reports several organisms as producing hydrogen sulphide that other investigators report as negative. Among these are B. paratyphosus A, B. dysentery, B. aerogenes, and Staphyloccus albus. Much of the variation reported, no doubt, is due to the methods used in conducting the investigation. Each investigator has prepared his media according to his own desires, and has employed a concentration often times quite different to that used by others. While this does not make for standard methods it does suggest the extent of influence that substances and concentrations may have upon sulphur metabolism.

Practically all workers have employed lead acetate, in some manner or other, as the indicator of hydrogen sulphide production. In 1912 Sasaki and Otsuka used strips of filter paper treated with egg white, glycerol, and lead acetate; more recently Tanner [3] (1918) and Myers [4] (1920) used a modification of this method. Jordan and Victorson [5] (1917), Thompson [6] (1921), and Tilley [7] (1923) added a few drops of lead acetate solution to the partly cooled sterile tubes of media, before inoculation. In this work a little preliminary investigation showed that lead acetate is a very weak bactericide, and is a more sensitive indicator than ferric chloride.

So far no practical application seems to have been made of sulphur metabolism as a cultural test except in a few instances. Myers (1920) investigated its application to the bacteriological examinations of water. He found the test unsatisfactory because of inconstant results. Kligler [8] (1917) calls attention to the use of lead acetate agar in army camps for differentiating between B. typhosus, B. paratyphosus A, B. paratyphosus B, and B. dysen-

tery. The production of hydrogen sulphide from peptone has been generally recognized as a means of distinguishing between B. paratyphosus A, and B. paratyphosus B. Aside from these applications it seems to have no other utilitarian interest.

The results at variance with each other at least indicate that the metabolism of bacterial organisms may be disturbed by a little change in the constituents, or their concentration in the medium. Myers (1920) in his work investigated also the effect of dextrose and lactose on the production of hydrogen sulphide, but reports that little, if any, influence was shown. The variation of results also shows that if the production of hydrogen sulphide is made use of as a definite cultural reaction some standard methods of application must be adopted. So far each investigator has used his own formulae for preparing the media and the formulae vary as much as the results.

Sasaki and Otsuka (1912) found that cystine supported hydrogen sulphide production by several kinds of organisms including B. coli communis, but it seems they used only one or two strains of each kind of the organisms used. Tanner (1917) used two strains of B. coli each of which gave hydrogen sulphide from cystine; he also used ninety-seven strains of B. fluorescens, eighty-five of which gave the gas from cystine, while Sasaki and Otsuka report B. fluorescens unable to do so. Another difference in the results reported by Sasaki and Otsuka, and Tanner, is regarding the reaction in sodium sulphate. Tanner used a three per cent concentration of the salt and found none of thirty-two kinds of organisms that gave hydrogen sulphide, while Sasaki and Otsuka obtained the gas from B. coli, B. paratyphosus B, B. dysentery (Flexner), B. dysentery (Shiga), and Sp. cholera, using twenty-one kinds; of these Tanner reported the first two named, negative. Tanner suggests that his concentration may have been too great. Sasaki and Otsuka were unable to obtain the gas from sodium sulphate with any of their twenty-one organisms; Tanner got none of the gas from a two-tenths per cent solution of magnesium sulphate by any of his thirty-two kinds of organisms.

EXPERIMENTAL

In this work the production of hydrogen sulphide was detected with lead acetate. The salt was dissolved in distilled water to give a ten per cent solution. Where peptone and coagulated egg yolk media were used one cubic centimeter of the indicator was added to each one hundred cubic centimeters of the medium. The medium was then tubed and sterilized. When a synthetic broth

medium was used filter paper was impregnated with the solution, dried, sterilized, and a small strip placed in the upper part of the culture tube, just above the medium, after inoculation. All solid media were inoculated with the loop from young dextrose broth cultures; all liquid media were inoculated from young agar slants. All cultures were incubated five days unless positive results were given in less time.

Peptone medium was made as follows:

Distilled water, 1,000 c. c.

Peptone, "Difco," 20 gms.

Meat extract, 3 gms.

Agar-agar, 15 gms.

This was adjusted to P^h 7.6, the indicator added, and tubed in about four c. c. amounts and sterilized fifteen minutes at fifteen pounds pressure.

Sodium sulphate, sodium thiosulphate, sodium sulphate, and postassium sulphate plus potassium phosphate were prepared according to the following formula:

Distilled water, 1,000 c. c.

Sodium ammonium hydrogen phosphate, 4 gms.

Dipotossium phosphate, 2 gms.

Lactose, 10 gms.

Sulphur compaund (anhydrous), 1 gm.

Potassium sulphate minus potassium phosphate was prepared as follows:

Distilled water, 1,000 c. c.

Sodium ammonium hydrogen phosphate, 5 gms.

Lactose, 10 gms.

Potassium sulphate, 1 gm.

Magnesium sulphate and copper sulphate were prepared by the following method:

Solution A

Distilled water, 800 c. c.

Sodium ammonium hydrogen phosphate, 4 gms.

Dipotassium hydrogen phosphate, 2 gms.

Lactose, 10 gms.

Sodium potassium tartarate, .5 gm.

Solution B

Distilled water, 200 c. c.

Sulphate, 1 gm.

Solutions A and B were sterilized, mixed, and tubed in sterile tubes.

The cystine medium was prepared as follows:

Distilled water, 800 c. c.
Sodium ammonium hydrogen phosphate, 4 gms.
Dipotassium hydrogen phosphate, 2 gms.
Lactose, 10 gms.
Agar-agar, 15 gms.
This was sterilized, then there was added:
Ammonium hydroxide (con.), 4 c. c.
Cystine, .5 gm.
This was tubed in sterile tubes, and incubated to develop possible contaminations.

Coagulated egg yolk medium was prepared according to the following formula:
Distilled water, 1,000 c. c.
Agar-agar, 15 gms.
Lactose, 10 gms.
Egg yolks, 3.
Lead acetate solution, 10%, 10 c. c.
This was tubed and sterilized fifteen minutes at fifteen pounds.

The uncoagulated egg yolk medium was prepared according to the following method:
Distilled water, 1,000 c. c.
Lactose, 10 gms.
This solution was sterilized, then there were added aseptically three egg yolks. The medium was then tubed in sterile tubes and incubated twenty-four hours at thirty-seven degrees to develop any possible contaminations.

The accompanying table shows the kinds of organisms, and the number of strains of each, that were tried. It is interesting to note that B. proteus, B. typhosus, and B. paratyphosus B, that so readily give the gas from peptone, do not in nearly all cases attack sodium sulphite, while B. cloaceae and B. coli, which do not very frequently give the gas from peptone are quite consistent in giving it from the sulphite. In the case of B. dysentery (Flexner) the results obtained by Sasaki and Otsuka are not entirely confirmed for as shown in the table some strains give positive results.

Sodium thiosulphate seems to be more readily reduced by bacteria than is sodium sulphite, the former being reduced by some, or all strains of Staphylococcus aureura, B. proteus, B. typhosus, B. paratyphosus A, B. paratyphosus B, B. anthrax, and B. mycoides, while the latter is reduced by only some strains of these organisms, or none of them.

The sulphates that were tested and that gave negative results are sodium sulphate, confirming the results reported by Sasaki and

Otsuka, i.e., bacteria seldom, if ever, give hydrogen sulphide from sodium sulphate; magnesium sulphate, which agrees with Tanner's results, and in which many of the organisms failed to grow; copper sulphate not only gave negative results but inhibited the growth of all the organisms tested, which were the identical ones with which magnesium sulphate was tested, as shown in the table.

Since sodium sulphate fails to support the production of hydrogen sulphide it might be expected that potassium sulphate would not. As shown in the table potassium sulphate in the presence of potassium phosphate gives the gas about as readily as does sodium sulphite. It is probable that the potassium is used in the metabolism of the organism, while in the case of the sodium sulphite the oxygen is used for growth energy. This suggests that hydrogen sulphide might be more readily formed if the sulphate were the only source of potassium. As shown in the Table the omission of the potassium phosphate had no apparent effect on the amount of the gas formed, or the number of organisms forming it, except in the case of B. dysentery (Shiga) which, contrary to expectation, failed to give the gas in the absence of the potassium phosphate. At present there are insufficient results on which to base an explanation of this phenomenon.

Data relative to the reactions in cystine are too limited to warrant very definite conclusions beyond observing that B. coli seldom fails to reduce the acid and evolve hydrogen sulphide.

Since silverware is so readily tarnished by egg, and since spoiled eggs contain so much hydrogen sulphide, it would seem very probable that egg yolk would be attacked by many kinds of organisms, particularly proteolytic ones, to form hydrogen sulphide. As shown in the table none of them can so decompose egg yolk when it has been coagulated by heat. When it is uncoagulated a few are able to so attack it. It seems paradoxical that any strain of B. paratyphosus A should form hydrogen sulphide from the uncoagulated egg yolk, and not from peptone, while B. typhosus and B. paratyphosus B, which give the gas so constantly from peptone, so infrequently give the gas from uncoagulated egg yolk.

SUMMARY

There seems to be no certainty as to whether a certain bacterium will, or will not, give hydrogen sulphide from a given sulphur compound until the combination is tried. The sulphur metabolism of bacteria is easily disturbed by a change in the sulphur compound, or its concentration.

Sodium sulphate can seldom, if ever, be reduced by bacteria, while the less stable salts, sodium sulphite and sodium thiosulphate, are reduced by a number of bacteria to form hydrogen sulphide.

Potassium sulphate, unlike sodium sulphate, supports the formation of hydrogen sulphide by several kinds of bacteria, particularly of the colon-typhoid group. Potassium phosphate seems to be necessary for the production of the gas from potassium sulphate by B. dysentery (Shiga), or at least some strains.

TABLE I

	Na_2SO_3		$Na_2S_2O_3$		Na_2SO_4		$K_2SO_4 + K_2HPO_4$		$K_2SO_4 — K_2HPO_4$		$MgSO_4$		Cystine		Peptone		Egg Yolk (Coagulated)		Egg Yolk (Uncoagulated)	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Staph. aureus	3	0	1	0	0	0	1	0	3	0	3	0	0	0	1	0	1	0	1	0
Staph. albus	2	0	1	0	0	0	1	0	2	0	2	0	0	0	1	0	1	0	1	0
N. catarrhalis	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	0
B. ruber	1	1	1	1	1	0	1	1	1	*1	1	0	1	1	1	0	1	0	1	0
B. ruber deKiel	0	0	0	0	0	0	0	0	1	0	1	0	1	1	1	0	0	0	0	0
B. morganii	0	0	1	1	1	0	1	0	1	0	1	0	0	0	1	1	1	0	1	0
B. proteus	4	0	1	1	0	0	1	0	4	0	4	0	0	0	4	4	1	0	1	1
B. cloacae	2	2	0	0	0	0	2	2	2	2	2	0	0	0	1	0	1	0	1	0
B. typhosus	3	0	3	3	2	0	3	*1	3	*1	3	0	0	0	3	3	3	0	3	0
B. dysentery (Flexner)	2	1	1	0	0	0	1	0	2	0	2	0	0	0	1	0	1	0	1	0
B. dysentery (Shiga)	2	0	1	0	0	0	2	2	2	0	2	0	0	0	1	0	1	0	1	0
B. alkaligenes	1	0	1	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0	1	1
B. enteritidis	0	0	0	0	0	0	1	1	1	1	1	0	0	0	1	1	1	0	1	0
B. paratyphosus A	4	0	1	1	3	0	4	0	5	0	5	0	0	0	4	0	4	0	4	1
B. paratyphosus B	10	6	10	10	9	0	10	10	10	10	10	0	0	0	10	10	10	0	10	1
B. coli	112	109	49	48	31	0	48	40	5	5	5	0	111	108	169	14	50	0	22	1
B. (lactis) aerogenes	0	0	1	1	0	0	1	1	2	2	2	0	0	0	1	0	1	0	1	1
B. anthrax	1	0	1	1	1	0	1	0	1	0	1	0	0	0	1	0	1	0	1	0
B. subtilis	1	0	1	0	1	0	1	0	1	0	1	0	0	0	0	0	1	0	1	0
B. mycoides	1	0	1	1	1	0	1	0	1	0	1	0	0	0	0	0	1	0	1	0
B. mesentericus	1	0	1	0	1	0	0	0	1	0	1	0	0	0	0	0	1	0	1	0
B. smegma	1	0	1	0	1	0	1	*1	1	*1	1	0	0	0	1	0	1	0	1	0
Sp. cholera	1	0	1	0	1	0	1	0	1	0	1	0	0	0	1	0	1	0	1	0

* Very small amounts of hydrogen sulphide given off.

Numbers in columns numbered 1 represent the number of strains tested; those in columns numbered 2, the number that gave hydrogen sulphide.

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