STUDIES ON THE BACTERIAL METABOLISM OF SULFUR

I. FORMATION OF HYDROGEN SULFIDE FROM CERTAIN SULFUR COMPOUNDS UNDER AEROBIC CONDITIONS

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Hydrogen sulfide is a decomposition product of many sulfur-containing compounds, and its formation by bacteria from both organic and inorganic substances has received much attention. An interesting paper on this subject is that of Sasaki and Otsuka, (1912). These investigators used Fränkel's protein-free medium as the substrate to which were added cystine, neutral sulfur, taurine, sodium thiosulfate, sodium sulfite, and sodium sulfate. All of the 21 pure cultures which they used formed hydrogen sulfide from cystine, with the exception of *Ps. pyocyaneus* and *Ps. fluorescens*. The pyogenic cocci were able to reduce neutral sulfur only. Burger (1914) observed hydrogen sulfide formation from cystine by a few common bacteria, but none of their strains produced it from taurine, peptone or sodium thiosulfate. Incubation periods of 12, 24 and 48 hours were used.

EXPERIMENTAL

In the course of some work that was being done with the fluorescent group of bacteria the action of these organisms, together with other strains of common bacteria, was tested upon cystine and other sulfur-containing compounds. The fluorescent bacteria used in this investigation were isolated from water. The other forms were secured from the American Museum of

Natural History, with the exception of the Bacillus of winter cholera.[1]

Fränkel's solution was used for the substrate. This was made up according to the following formula.

Sodium chloride	5 grams
Monocalcium phosphate	2 grams
Ammonium lactate	6 grams
Asparagin	4 grams
Distilled water	1000 cc.
$\frac{N}{1}$ NaOH	20 cc.

After the various sulfur compounds had been added the media were placed in test tubes and sterilized in the Arnold steam sterilizer. Inoculations were made with small portions of growth from a young slant agar culture.

To detect the formation of hydrogen sulfide, strips of bibulous paper which had been saturated with lead acetate solution containing glycerol were suspended in the top of each culture tube. The tubes were then plugged with cotton and incubated at 25°C. for thirty days, at the end of which time observations were made for darkening of the lead acetate paper. A full set of control tubes was employed. In no instance did the control tubes darken the indicator paper, which points to the fact that the hydrogen sulfide, when present in the culture tubes, came from the sulfur compounds in the substrate.

PEPTONE

Fränkel's medium with 4 per cent added peptone was used. Sixty-two of the 97 fluorescent bacteria formed hydrogen sulfide from peptone. Four of these strains produced it from peptone and not from cystine. This may be taken as evidence of other sulfur linkings in protein than that of cystine. Practically all of the pure cultures were able to split hydrogen sulfide from peptone.

[1] This organism was received from Mr. A. J. Hinkelmann, Galesburg, Illinois, and is described in the Illinois Medical Journal, November, 1915.

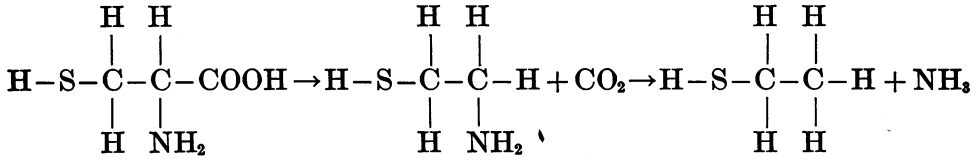
CYSTINE

The cystine used in this experiment was prepared from wool, according to Folin's method (Folin, 1910) and purified by repeated recrystallizations.[2] It was used in the strength of 6 per cent in Fränkel's medium. On account of the small supply of cystine, small culture tubes holding about 5 cc. were employed.

After incubation for thirty days 86 of the 97 strains of fluorescent bacteria had formed hydrogen sulfide from cystine. At the same time 34 strains of other common bacteria were inoculated into this medium, most of which formed hydrogen sulfide. The results are indicated in the table. *B. paratyphi "B," B. enteritidis*, the Bacillus of winter cholera, and *B. coli* formed large amounts of hydrogen sulfide from cystine in much less time than 30 days.

These data differ from those reported by Sasaki and Otsuka (1912). The fluorescent bacteria in their investigation formed no hydrogen sulfide. The discrepancy between the results of Sasaki and Otsuka and those reported in this paper may be due to the period of incubation. The former adopted one week as the incubation period, while in this investigation it seemed best to lengthen the period to 30 days. Many of the fluorescent bacteria isolated from water produced no hydrogen sulfide until after the fifteenth day. Most of the intestinal bacteria produced it in less than a week.

The decomposition of cystine has been studied from a chemical and biochemical viewpoint, but very few definite data are available. Mathews (1915) believes that in digestion in the intestine cystine is first formed, and that this later undergoes decarboxylation and deaminization to yield ethyl mercaptan, according to the following equation.



[2] A portion of this was secured from Dr. H. B. Lewis of the division of physiological chemistry. The rest was prepared directly from wool.

The above changes are thought to be probable on account of the presence of mercaptans in the feces. Neuberg and Asher (1907), upon the dry distillation of cystine, obtained amino-ethyl-disulfide, $S_2(CH_2-CH_2-NH_2)_2$. It is improbable that this

TABLE 1

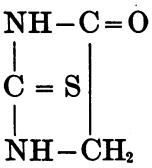
NAME OF BACTERIUM	NUMBER OF STRAINS	HYDROGEN SULFIDE FORMATION IN															
		Peptone		Cystine		2 thiohydantoin		Thiourea		Taurine		$Na_2S_2O_3$		$MgSO_4$		Na_2SO_3	
		+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Fluorescent bacteria	97	62	35	85	12		10*	62	35		97	42	55		97		97
B. coli	2	2	−	2	−	0	0	−	2	−	2	2	−	−	2	−	2
B. paracoli	1	1	−	1	−	0	0	1	−	−	1	1	−	−	1	−	1
B. enteritidis	1	1	−	1	−	−	1	1	−	−	1	1	−	−	1	−	1
B. alkaligines	1	1	−	1	−	0	0	1	−	−	1	−	1	−	1	−	1
B. cloacae	1	1	−	1	−	0	0	1	−	−	1	1	−	+	1	−	1
B. aerogenes	1	1	−	1	−	0	0	−	1	−	1	1	−	−	1	−	1
B. pyogenes-fecalis	1	1	−	1	−	0	0	−	1	−	1	1	−	−	1	−	1
B. paratyphi A	1	1	−	−	1	0	0	1	−	−	1	−	1	−	1	−	1
B. paratyphi B	1	1	−	1	−	−	1	−	1	−	1	1	−	−	1	−	1
B. typhi	1	1	−	1	−	0	0	−	1	−	1	−	1	−	1	−	1
B. capsulatus	3	3	−	3	−	0	0	1	2	−	1	3	−	−	3	−	3
Staph. albus	1	1	−	−	1	0	0	−	1	−	1	−	1	−	1	−	1
Staph. citreus	1	−	1	−	1	0	0	−	1	−	1	−	1	−	1	−	1
M. ureae	1	1	−	1	−	0	0	1	−	−	1	1	−	−	1	−	1
B. proteus	1	1	−	1	−	0	0	1	−	−	1	1	−	−	1	−	1
Clam bacillus	1	1	−	−	1	0	0	−	1	−	1	−	1	−	1	−	1
Sarcina ventriculi	1	−	1	−	1	0	0	−	1	−	1	−	1	−	1	−	1
B. butyricus	1	1	−	−	1	0	0	−	1	−	1	−	1	−	1	−	1
Bacillus of winter cholera	1	1	−	1	−	0	0	1	−	−	1	1	−	−	1	−	1
B. subtilis	1	−	1	−	1	0	0	−	1	−	1	−	1	−	1	−	1
Ps. pyocyaneus	1	−	1	+	−	0	0	−	1	−	1	−	1	−	1	−	1
M. tetragenus	1	−	1	−	1	0	0	1	−	−	1	−	1	−	1	−	1
B. cyaneus	1	1	−	−	1	0	0	−	1	−	1	−	1	−	1	−	1
Ps. phosphorescens	1	−	1	−	1	0	0	−	1	−	1	−	1	−	1	−	1
B. smegmae	1	1	−	−	1	0	0	−	1	−	1	−	1	−	1	−	1
B. arborescens	1	1	−	−	1	0	0	−	1	−	1	−	1	−	1	−	1
B. cereus	1	1	−	1	−	0	0	−	1	−	1	−	1	−	1	−	1
B. granulosus	1	−	1	−	1	0	0	1	−	−	1	−	1	−	1	−	1
B. mesentericus	1	1	−	1	−	0	0	1	−	−	1	1	−	−	1	−	1
M. flavus	1	−	1	−	1	0	0	−	1	−	1	−	1	−	1	−	1
B. dysenteriae	1	1	−	1	−	0	0	−	1	−	1	1	−	−	1	−	1

* Only the first ten strains were used in a 2 thiohydantoin medium.

compound would be formed in cellular metabolism, because the sulfur in cystine seems to be split off with little difficulty. Wohlgemuth (1905) reported that cystine which had been fed to rabbits in the diet appeared as increased sulfates in the urine and as taurine in the bile. Some of the hydrogen sulfide which many investigators have found in the urine may have come from cystine as well as sulfates.

The question of sulfur linkages in protein has received much attention. Johnson (1911) has given a good resumé of this subject. Many investigators have produced evidence which indicated that the sulfur in protein may not be fully accounted for by the cystine linkage. Bacteria may have a selective action, and may be able to attack only the sulfur in protein which is loosely combined. Johnson has prepared some thioamides of amino acids which offer more possibilities for the linkage of sulfur in protein.

2-THIOHYDANTOIN



In this compound sulfur has replaced the oxygen of the hydantoin nucleus. It has been studied by Johnson (1911, 1912) and his co-workers. Lewis (1912) has shown that the hydantoin nucleus is excreted as such when introduced into the organism of the cat, rabbit, or dog. In a later paper (Lewis, 1913) the same author reports a study of the behavior of 2-thiohydantoin when injected subcutaneously into rabbits. It was found "that approximately 0.125 gram per kilo body weight is the lethal dose for 2-thiohydantoin, while amounts of over 1.5 grams of hydantoin have been fed to rabbits without any toxic effects. The difference in reaction toward the animal body seems to rest in the sulfur of the 2-thiohydantoin.

Through the kindness of Dr. H. B. Lewis, a small amount of

2-thiohydantoin was secured upon which to try the action of bacteria. This was prepared from ammonium thiocyanate and hippuric acid, as described by Johnson and Nicolet (1911). The 2-thiohydantoin was dissolved in sterile water to yield a saturated solution. One cubic centimeter of this was added to 5 cc. of Fränkel's solution. The small amount of this compound limited the experiment to a few bacteria. The results are indicated in the table.

None of the bacteria formed the slightest amount of hydrogen sulfide detectable by the method used. The color of the solution changed upon incubation from the reddish orange of a solution of 2-thiohydantoin to a pale yellow. This may be regarded as indirect evidence that the compound was in some way broken down. Growth in all cases took place with the formation of a thick pellicle and a heavy sediment. This lack of toxicity for bacteria is of interest, in view of the marked toxicity for higher forms of life already referred to.

THIOUREA

A 10 per cent solution of this compound in Fränkel's medium was used. Thirty-four of the 97 fluorescent strains formed no hydrogen sulfide from this agent. Results with the other bacteria which were used may be found in the table. The formation of hydrogen sulfide from thiourea is of some interest, since none of the strains formed hydrogen sulfide from 2-thiohydantoin, a compound having the same sulfur linking, bivalent sulfur replacing bivalent oxygen. In peptone and thiourea substrates the fluorescent bacteria are correlated with regard to the number of strains forming hydrogen sulfide. This apparent correlation is probably of no significance. A strain which reduced the sulfur in peptone to hydrogen sulfide often failed to show this ability with regard to thiourea.

TAURINE

This was prepared from desiccated ox-bile and purified by repeated recrystallization (Hawk, 1916). None of the bacteria

used in this investigation reduced taurine to hydrogen sulfide, as determined by the method here employed. These results agree with those of Sasaki and Otsuka.

SODIUM THIOSULFATE

A 0.3 per cent solution of this compound in Fränkel's medium was used. Fifty-seven of the 97 fluorescent bacteria reduced the sulfur in sodium thiosulfate to hydrogen sulfide. The amount was not always large, but sufficient to cause a distinct change in the color of the lead acetate paper. The results for the other bacteria are indicated in the table. Lederer (1913) secured the formation of hydrogen sulfide when sodium thiosulfate was used in the place of sodium sulfate. Similar results have been reported by others.

MAGNESIUM SULFATE

To determine this property, Fränkel's solution was not used, but one proposed by Sullivan (1905) as being especially favorable to the development of fluorescent bacteria. It has the following composition.

Asparagin	10 grams
Magnesium sulfate	2 grams
K_2HPO_4	1 gram
Distilled water	1000 cc.

None of the bacteria reduced magnesium sulfate to hydrogen sulfide. This is in accord with the work of Sasaki and Otsuka and that of Lederer, and is probably due to the aerobic methods of culturing which were used. Beijerinck (1895, 1896,—) has shown that oxygen must be absent in order to secure formation of hydrogen sulfide from sulfate. An organism isolated by Zelinski (1893) seems to be the only one to which has been attributed the ability to form hydrogen sulfide from sulfate under both aerobic and anaerobic conditions.

SODIUM SULFITE

A 3 per cent solution of this compound was used in Fränkel's medium. None of the strains employed in this study reduced the

compound. This may have been due to the fact that very little growth was secured in the medium. Two of the strains, however, gave good growth, but no hydrogen sulfide. Sodium sulfite is known to inhibit the enzymes of the intestinal tract, and it may have acted in the same way with regard to the bacteria.

SUMMARY

The ability to form hydrogen sulfide from peptone is a rather wide-spread characteristic of the bacteria used in this study, including the fluorescent bacteria. With regard to cystine, the ability to liberate sulfur was possessed by almost all of the organisms used. The fluorescent bacteria and a few other forms are able to split hydrogen sulfide from thiourea with apparent ease, but do not attack 2-thiohydantoin to yield hydrogen sulfide. No hydrogen sulfide was formed from taurine and magnesium sulfate, both of which contain sulfur in more highly oxidized forms. The results with taurine and magnesium sulfate are in accord with those of Sasaki and Otsuka, indicating that bacteria may be unable to reduce highly oxidized sulfur with the formation of hydrogen sulfide. The results with sodium thiosulfate differ slightly from those reported by other investigators. With regard to sodium sulfite, no strain reduced sulfur to hydrogen sulfide, but this may be due to the concentration used. More work on this subject is planned.

I wish to acknowledge my indebtedness to Dr. Howard B. Lewis for his personal interest and timely suggestions.

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