

THE PRODUCTION OF HYDROGEN SULPHIDE BY BACTERIA

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Received for publication, December 11, 1919

Hydrogen sulphide formation in sewage is supposed to be due to two distinct processes, the splitting of protein by certain organisms and the reduction of inorganic sulphates by others. Lederer (1913) concludes that more hydrogen sulphide is formed under anaerobic than under aerobic conditions. He thinks that the formation of hydrogen sulphide from protein is a selective process and that it depends a great deal on the position of the sulphur radical in the protein molecule. At any rate other factors than reduction are involved in hydrogen sulphide formation. An organism may be a very strong nitrate reducer and at the same time a weak hydrogen sulphide former, although both reactions are reducing processes. Lederer is of the opinion that the reduction of inorganic sulphates may however be an important factor in hydrogen sulphide formation, and thinks it probable that the action may be due to some unknown specific organism similar to the *Spirillum desulphuricans* of Beijerinck (1895).

Several workers have observed the formation of hydrogen sulphide in large quantities in the effluent of sewage disposal plants. Barr and Buchanan (1912) report the isolation of a specific organism in one such instance. Clark (1913) and others have reported the reduction of inorganic sulphates in sewages.

Sulphur metabolism may also play a beneficial rôle in sewage disposal in that certain species of bacteria may cause the oxidation of sulphur and of hydrogen sulphide to sulphates thus reducing the amount of odor. This is a well known property of such organisms as *Beggiatoa* and *Thiothrix* (Jordan, 1918).

Another field in which bacterial sulphur metabolism may be of importance is in soil bacteriology. The part played by sulphur here is little understood. Lipman, McLean and Lent (1916) suggest that sulphur oxidation in soils may have an effect on the availability of mineral phosphates as plant food.

Again a number of workers have attempted to apply hydrogen sulphide formation to water analysis, the assumption being that the amount of hydrogen sulphide produced when water is planted in a suitable medium is proportional to the degree of pollution. Schardinger (1894) seems to have first suggested this possibility. He observed that when water polluted with fecal material was added to peptone solution and incubated, it produced a characteristic odor, and that it blackened a strip of lead acetate paper which was suspended over the liquid. Dunham in 1897 suggested the following method for the detection of polluted water. Sterilize 10 cc. of an aqueous solution of 10 per cent peptone and 5 per cent sodium chloride in a plugged Erlenmeyer flask. To this flask add 90 cc. of the water under examination, suspend a strip of filter paper impregnated with lead carbonate over the mixture, and incubate for twenty-four hours at 37°C. Dunham maintained that the colon bacillus and the organisms of putrefaction readily multiply and cause the production of hydrogen sulphide which discolors the lead carbonate paper.

Redfield (1912) studied the effect upon the speed and amount of hydrogen sulphide production when different factors were varied. Among the conditions investigated were the effect of using filtered and unfiltered peptone solutions, the effect of the concentration of the peptone, of the type of inorganic salts added to the peptone solution, of the concentration of various salts added to the medium, of the kind of bases and acids used in adjusting the initial reaction of the medium, and of the relation of the final reaction of the culture to the amount of hydrogen sulphide production. As a result of his work Redfield suggests the following method for the detection of polluted water:

Bring 700 cc. of tap water to a boil and add 300 grams of Witte peptone and 75 grams of potassium chloride. Maintain a gentle heat and

stir constantly until as much of the peptone as will do so has gone into solution. Cool rapidly and make up to 1 liter with tap water. Transfer to a flask, heat to boiling again, plug, cool rapidly, and place in the ice box for at least twenty-four hours. Filter cold through paper and distribute in 10 cc. amounts among the special flasks suggested. Sterilize the flasks in the autoclave at one atmosphere for fifteen minutes. When cool, place in the tube of each flask a strip of bibulous paper 25 mm. in length, which had previously been impregnated with lead acetate.

The top of the plugged tube was then closed by wrapping with a strip of tin foil. The flasks alluded to were of a special design. They were shaped like a Soxhlet extraction flask, were graduated at 90 cc. and 100 cc. and a ground glass cap ending in a narrow tube was fitted over the neck of each flask.

Redfield tested this method by means of an artificial sewage, prepared from human feces. This sewage was quite dilute, since it contained only twenty colon bacilli per cubic centimeter and had a total bacterial count of twenty-eight hundred. He reports a gradual increase in the amount of hydrogen sulphide produced, and in the speed of its production as the concentration of sewage increases. The same result is reported with a considerable number of untreated waters from a variety of sources. He concludes that there is a uniform relationship between the degree of pollution of a water and the amount of lead acetate paper blackened when this method is employed.

Redfield also made some quantitative determinations of the amount of hydrogen sulphide produced by sewage organisms. He compared a number of methods for the quantitative determination of sulphur in peptone solutions. He also investigated the hydrogen sulphide producing powers of several species of bacteria, and concluded that the proteolytic organisms rather than *B. coli* are responsible for its formation.

Burnet and Weissenbach (1915) suggest another use for the hydrogen sulphide producing powers of bacteria. They found that colonies of *B. paratyphosus* B, became black when grown on agar which contained a small amount of lead acetate, while colonies of *B. paratyphosus* A did not. They consider this an accurate method for the differentiation of the two organisms.

Maymone (1917) suggests a somewhat similar method for the differentiation of *B. paratyphosus* A, and *B. paratyphosus* B based on biological characteristics and the appearance of cultures on lead acetate media.

Jordan and Victorson (1917) employed a like method for *B. paratyphosus* B, and *B. paratyphosus* A. Agar tubes containing a small amount of lead acetate were inoculated between the medium and the side of the tube. All typical strains of *B. paratyphosus* B blackened the needle track. The typical *B. paratyphosus* A strains produced no blackening. Kligler (1917) suggests a simple method for the differentiation of *B. paratyphosus* A and B, *B. typhosus*, and *B. dysenteriae*, based on a double sugar medium similar to that of Russell, containing lead acetate.

Bacterial action probably plays a rôle in the formation of intestinal gases. Hydrogen sulphide is always present in intestines and is probably formed from cystin. Senator described a case in which an intoxication with hydrogen sulphide of intestinal origin occurred, but this is apparently the only case reported. (Wells 1918). Hydrogen sulphide formation by bacteria may be concerned in some of the conditions included in that vague term, autointoxication.

The formation of hydrogen sulphide by bacteria is also of interest, because of the light it may throw on the metabolism of bacteria. Sasati and Otsuka (1912) carried out some experiments with a few organisms as to the formation of hydrogen sulphide with cystin, taurin, sulphur, sodium, sulphate, and sodium sulphite. Burger (1914) compared cystin and peptone as a source of hydrogen sulphide.

Tanner (1918) published interesting data on various sulphur compounds as a source of hydrogen sulphide when acted on by bacteria. He (1918) has also contributed a valuable article on the formation of hydrogen sulphide by yeasts.

EXPERIMENTAL WORK

The application of hydrogen sulphide formation to water analysis

Experiments were undertaken to ascertain the delicacy and reliability of the hydrogen sulphide test used by Redfield to determine the potability of water.

The medium used was that suggested by Redfield with minor modifications. Sodium chloride was substituted for potassium chloride, and instead of the special flasks which Redfield employed, Erlenmeyer flasks of 150 cc. capacity and made of Pyrex glass were used. One-half per cent sodium chloride was added to all media containing peptone because it seemed to produce a clearer medium. Difco peptone made by the Digestive Ferments Company was substituted for Witte peptone since the latter is not now obtainable. Ten cubic centimeters of the medium were placed in each flask and autoclaved for ten minutes at 15 pounds pressure. A strip of filter paper 50 mm. long and 3 mm in width was suspended in the mouth of the flask in such a manner that approximately 30 mm. of the strip was exposed. The filter paper had been moistened in a 10 per cent solution of neutral lead acetate, and sterilized in the autoclave for fifteen minutes at 15 pounds pressure. It was necessary to sterilize the lead acetate paper separately because it was found that some blackening occurred when the medium was autoclaved, due probably to a slight hydrolysis of the peptone, since steam at the pressure of the autoclave ionizes more than at atmospheric pressure.

One gram of the feces under examination was weighed out. This merely served as a convenient amount from which to make dilutions since of course the moisture, bacterial content and residue varied enormously in the different samples. A 90 cc. portion of each dilution studied was placed in one of the Erlenmeyer flasks and incubated aerobically at 37°C. The approximate number of millimeters of lead acetate paper blackened was recorded at the end of twenty-four hours, forty-eight hours, seventy-two hours, and seven days. The method was of course, only approximate, but all conditions were kept as nearly identical as

possible. The total forty-eight hour 20°C. bacterial count on standard agar, the total forty-eight hour 37°C. bacterial count on standard agar, and the colon count, using the complete test fermentation tube method, were made in each case (A. P. H. A. Committee, 1912, 1917). In the fermentation tube work, ten tubes of lactose broth were inoculated with 1 cc. each of the dilution selected and several dilutions were always used in order to make sure that some one series of ten tubes would show gas in only part of the tubes. All media were prepared according to the standard method of water analysis of the American Public Health Association (1912, 1917).

The colon count was also made in each sample by plating on Endo medium and counting the colon like colonies directly. This was done as a check on the fermentation tube method and to test the possibility of making direct counts of the colon content of sewage and other materials by the use of Endo medium.

Table 1 indicates the types of feces studied and the data obtained.

The counts are expressed in millions and fractions of millions in order to make the table less bulky. Table 1 indicates that there is a fairly definite relationship between the amount of fecal material in a given solution and the amount of hydrogen sulphide formed, although occasional irregularities appear.

There were some difficulties in making a colon count directly on Endo medium, the most important being that deep colonies are at times difficult to differentiate. On the whole it would seem that the method is as accurate as the fermentation tube method and it is perhaps less cumbersome, especially when dealing with material that is heavily loaded with colon bacilli.

Table 2 gives the minimum number of colon bacilli which produced a perceptible blackening of the lead acetate paper after incubation at 37°C. for twenty-four hours, forty-eight hours, and seven days respectively. It also gives the twenty-four hour 37°C. count, and the forty-eight hour 20°C. count for purposes of comparison with the colon count. The customary 90 cc. dilution was used.

There is no constant relationship between the number of colon bacilli from different animals and the amount or rate of hydrogen sulphide production; and there is also no definite relationship between the numbers of other organisms and hydrogen sulphide

TABLE 1
Summary of examination of feces from different animals

SOURCE	37°, 24-HOUR STANDARD AGAR COUNT IN MIL- LIONS PER GRAM	20°, 48-HOUR STANDARD AGAR COUNT IN MIL- LIONS PER GRAM	COLON COUNT IN MIL- LIONS PER GRAM ON ENDO MEDIUM	COLON COUNT IN MIL- LIONS PER GRAM BY FERMENTATION TUBE	APPROXIMATE NUMBER OF MILLIMETERS OF LEAD ACETATE PAPER BLACKENED											
					Dilution = 1:100000			Dilution = 1:1000000			Dilu- = tion 1:10000000			Dilution = 1:100000000		
					24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days
Human.....	39.8	20.9	33.0	30.0	2	30	30	1	5	30	0	0	0	0	0	0
	27.5	6.1	8.0	11.0	0.5	7	30	0	7	30	1	2	4	0	0	0
Bovine.....	1.74	1.25	0.01	0.1	1	3	5	0.5	1	1	0	0	0	0	0	0
	2.5	1.8	7.5	5.0	4	10	30	1	5	30	0	1	6	0	0	0
Horse.....	0.31	0.25	0.07	0.1	7	30	30	3	10	25	0	1	2	0	0	0
	55.0	1.0	0.06	0.08	1	30	30	0	12	30	0	1	4	0	0	0
Sheep.....	90.0	11.0	10.0	5.0	10	15	30	2	7	10	2	4	3	0	1	4
	224.0	215.0	210.0	90.0	3	3	20	1	2	6	0	0	1	0	0	0
Pig.....	288.0	281.0	233.0	90.0	—	—	—	10	30	20	4	10	30	0.5	1	2
	90.0	50.0	10.0	61.0	2	30	30	1	30	30	9	2	2	0	0	0
Chicken.....	126.0	26,000.0	20.0	10.0	—	—	—	10	30	30	10	15	30	1	2	5
	28.0	32.0	2.9	4.0	10	30	30	3	20	30	0	15	30	0	0	10
Rabbit*.....	3.2	2.23	0.01	0.003	1	4	30	1	5	5	0	0	0	0	0	0
	17.0	20.9	0.04	0.01	1	20	30	0	1	30	0	15	30	0	10	30
Dog.....	53.0	48.0	47.0	50.0	10	15	20	10	15	30	5	20	30	0	20	30
	35.0	34.0	11.0	6.0	—	—	—	5	20	30	5	20	30	0	20	30

* The dilution is one-tenth lower than that given in the column heading.

formation. Certain types of animal feces seem to produce hydrogen sulphide in even higher dilutions than human feces. Therefore this test applied to the direct examination of water would have no value since it is too delicate. It is to be expected

TABLE 2
Relation between the number of bacteria, in the 90 cc. portion of the highest dilution of various types of feces, which produced hydrogen sulphide

SOURCE	COLON BACILLI			37°, 24-HOUR COUNT ON STANDARD AGAR			20°, 48-HOUR COUNT ON STANDARD AGAR		
	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days
Human.....	2,700 99	2,700 99	2,700 10	3,580 22,750	3,580 2,275	3,580 227	1,880 5,490	1,880 549	1,880 55
Bovine.....	9 450	9 45	9 45	157 225	157 22	157 22	110 162	110 16	110 16
Horse.....	1 1	0 0	0 0	28 4,950	3 4,950	3 4,950	23 90	2 90	2 90
Sheep.....	45 8,100	5 8,100	1 810	810 20,160	81 20,160	1 2,016	100 19,350	10 19,350	1 1,935
Pig.....	5,490 210	549 2	549 2	8,100 160	810 2	810 2	4,500 153	450 2	450 2
Chicken.....	2 360	0 36	0 4	113 2,520	1 252	1 25	23,000 280	230 28	230 3
Rabbit.....	30 90	3 90	3 90	28,800 158,000	2,880 158,000	2,880 158,000	20,000 18,800	2,000 18,800	2,000 18,800
Dog.....	4 54	4 —	4 —	5 315	5 —	5 —	4 306	4 —	4 —

that results of this type would be irregular since many other bacteria aside from colon bacilli are capable of producing hydrogen sulphide. It would be interesting to determine whether there is any difference in the hydrogen sulphide forming power of pure cultures of colon bacilli coming from different sources.

Table 3 summarizes the results of a rather complete bacteriological analysis of samples of water from various sources, the purpose being to compare the hydrogen sulphide test with standard methods.

The samples marked "University of Chicago tap" were specimens from the Chicago mains. Those marked "University of Chicago filtered" were taken from the University of Chicago drinking fountains, the water having been passed through a special filter belonging to the University. It will be noted that the condition of these waters was very good according to the usual standards, yet a small amount of hydrogen sulphide appeared in one of the filtered samples.

The samples marked Omaha House 1, 2, 3, 4, 5, and 6, were taken from the taps of houses in a certain district of Omaha, where several cases of typhoid fever had occurred. These analyses were made to determine the condition of the city water supply. Two series of examinations were made about two weeks apart. There was no evidence of contamination according to standard methods yet hydrogen sulphide was formed in two instances in twenty-four hours, and in most other samples there was an appreciable amount of blackening in forty-eight hours.

These results would indicate that this test is too delicate to be of value in water analysis. Hydrogen sulphide was formed by every contaminated water and by some waters in which there was no evidence of contamination by the usual methods of examination. This agrees with the results obtained by examination of feces from various animals.

TABLE 3
Summary of water analyses

SOURCE	37°, 24-HOUR STAND- ARD AGAR COUNT- PER CUBIC CENTI- METER		20°, 48-HOUR STAND- ARD AGAR COUNT- PER CUBIC CENTI- METER		COLON COUNT PER CUBIC CENTIMETER BY PERMENTATION TUBE METHOD	COLON COUNT ON EN- DO'S MEDIUM	APPROXIMATE NUMBER OF MILLIMETERS OF LEAD ACETATE PAPER BLACKENED																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
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Effect of the variety of commercial peptone, and of the presence of glucose and sucrose on hydrogen sulphide formation

The hydrogen sulphide producing power of a number of common bacteria was studied in several media. Witte's peptone, "Difco" peptone made by the Digestive Ferments Company, and Fairchild peptone were compared. Four different media were prepared from each brand of peptone, a 3 per cent solution of the peptone containing 0.5 per cent of NaCl, a 3 per cent solution of the peptone containing 0.5 per cent NaCl and 0.5 per cent glucose, a 3 per cent solution of the peptone containing 0.5 per cent NaCl and 0.5 per cent sucrose, and standard nutrient broth. Glucose and sucrose were chosen because Seifert (1909) reports that glucose decreases and sucrose increases hydrogen sulphide formation by *B. paratyphosus* B. Table 4 summarizes the results.

Witte's peptone was the most favorable of the three brands in regard to hydrogen sulphide formation, and Fairchild's was more favorable than Difco. Variable amounts of hydrogen sulphide were formed in Witte and not in Difco peptone media by *Sarcina lutea*, *Staphylococcus albus*, *Staphylococcus aureus*, *B. prodigiosus*, *B. pyocyaneus*, *Mic. tetragenus*, a laboratory strain of streptococcus, *B. cloacae*, *B. subtilis*, *B. anthracis*, *B. avisepticus*, *B. bovisepiticus*, *Sp. metchnikovii*, *Sp. cholerae*, *B. lactis-aerogenes*, and *B. mucosus-capsulatus*. Larger amounts of hydrogen sulphide were formed in Witte than in Difco peptone by some of the other organisms.

Hydrogen sulphide was produced in Fairchild and not in Difco media by *B. lactis aerogenes* and *B. cloacae*. It seemed to make no difference which of the four media prepared from each peptone was used. Ordinary broth was as good as any. Glucose and sucrose did not influence the rate or amount of hydrogen sulphide formation.

It was a striking fact that many species of bacteria produced hydrogen sulphide from Witte's peptone and not from the American brands. This was of course due to difference in chemical constituents. Witte's peptone contains more amino

TABLE 4
Relative amount of hydrogen sulphide produced by various bacteria in Witte
peptone media

ORGANISM	3% PEPTONE 0.5% NaCl			3% PEPTONE 0.5% NaCl 0.5% GLUCOSE			3% PEPTONE 0.5% NaCl 0.5% SUCROSE			BROTH		
	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days
Sarcina lutea.....	0	0	1	0	0	0	0	0	2	0	1	1
Staph. aureus.....	0	0	1	0	7	30	0	7	30	1	4	22
Staph. albus.....	2	5	12	3	5	10	3	5	8	8	10	20
B. prodigiosus.....	2	3	12	2	4	7	14	22	30	1	2	12
B. pyocyaneus.....	0	0	1	0	0	0	0	0	1	0	1	2
Mic. tetragenus.....	0	0	0	0	0	0	0	0	0	0	1	3
Strep. pyogenes.....	2	2	2	25	27	27	17	25	30	2	2	2
B. coli.....	5	15	30	19	20	20	19	22	30	15	25	28
B. lactis aerogenes.....	2	2	10	19	25	27	15	25	30	3	6	20
B. mucosus capsulatus..	0	2	5	6	12	15	4	20	30	3	3	5
B. enteritidis.....	9	15	27	13	15	20	8	17	30	12	30	30
B. para-typhosus B.....	9	18	22	7	11	15	7	9	15	15	20	25
B. para-typhosus A.....	0	0	1	0	0	1	0	0	2	0	0	3
B. typhosus.....	5	9	15	4	12	15	5	15	25	15	25	30
B. dysenteriae Shiga....	0	0	0	0	0	0	0	0	1	0	1	2
B. dysenteriae Flexner..	0	0	1	1	1	3	0	0	1	0	1	3
B. proteus-vulgarus.....	22	27	30	22	25	30	18	27	28	30	30	30
B. cloacae.....	5	20	27	15	25	30	17	30	30	4	11	25
B. subtilis.....	30	30	30	22	27	30	22	27	30	25	27	27
B. anthracis.....	30	30	30	25	30	30	25	30	30	30	30	30
B. moelleri.....	0	0	0	0	0	2	0	1	2	0	1	3
B. fecalis-alkaligenes...	0	1	2	0	1	2	0	1	3	4	6	14
B. bovissepticus.....	3	3	3	16	18	30	19	25	30	3	3	3
B. avisepticus.....	2	4	6	12	22	25	0	1	3	9	18	23
Sp. metchnikovii.....	0	12	25	0	0	0	0	1	25	8	22	27
Sp. cholerae.....	6	25	28	7	8	9	19	22	30	13	27	30
B. hofmanni.....	0	0	0	0	0	0	0	0	0	0	0	1

Relative amount of hydrogen sulphide produced by various bacteria in "Difco"
peptone media

Sarcina lutea.....	0	0	0	0	0	0	0	0	0	0	0	0
Staph. aureus.....	0	0	0	0	0	0	0	0	0	0	0	0
Staph. albus.....	0	0	0	0	0	0	0	0	0	0	0	1
B. prodigiosus.....	0	0	0	0	0	0	0	0	2	0	0	0
B. pyocyaneus.....	0	0	0	0	0	0	0	0	0	0	0	0
Mic. tetragenus.....	0	0	0	0	0	0	0	0	0	0	0	0
Strep. pyogenes.....	0	0	0	0	0	0	0	0	0	0	0	0
B. coli.....	0	1	1	1	1	2	1	1	2	1	1	1
B. lactis aerogenes.....	0	0	0	0	0	0	0	0	0	0	0	0
B. mucosus capsulatus..	0	0	0	0	0	0	0	0	0	1	1	1
B. enteritidis.....	15	30	30	4	20	20	4	4	4	22	30	30
B. para typhosus B.....	10	30	20	12	25	25	6	15	15	22	27	27
B. para typhosus A.....	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 4—Continued

ORGANISM	3% PEPTONE 0.5% NaCl			3% PEPTONE 0.5% NaCl 0.5% GLUCOSE			3% PEPTONE 0.5% NaCl 0.5% SUCROSE			BROTH		
	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days
<i>B. typhosus</i>	25	30	30	0	0	0	4	6	7	25	30	30
<i>B. dysenteriae</i> Shiga....	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. dysenteriae</i> Flexner..	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. proteus vulgaris</i>	5	10	20	7	10	10	5	6	6	25	30	30
<i>B. cloacae</i>	0	0	0	0	0	0	0	0	1	0	0	1
<i>B. subtilis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. anthracis</i>	0	0	0	0	0	0	0	0	0	1	1	1
<i>B. moelleri</i>	0	0	5	0	0	0	0	0	0	0	0	0
<i>B. fecalis alkaligenes</i>	0	0	0	0	0	0	0	0	1	0	0	1
<i>B. bovissepticus</i>	0	0	0	0	0	0	0	0	1	0	0	0
<i>B. avisepticus</i>	0	0	0	0	0	0	0	0	0	20	25	25
<i>Sp. metchnikovii</i>	0	0	0	0	0	0	0	0	1	0	0	0
<i>Sp. cholerae</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. hofmanni</i>	0	0	0	0	0	0	0	0	0	0	0	0

Relative amount of hydrogen sulphide produced by various bacteria in Fairchild peptone media

<i>Sarcina lutea</i>	0	1	5	0	0	2	0	0	0	0	0	0
<i>Staph. aureus</i>	0	1	1	0	0	1	0	0	0	0	0	1
<i>Staph. albus</i>	0	0	0	0	0	1	0	0	0	1	1	4
<i>B. prodigiosus</i>	0	0	2	0	0	1	0	0	1	0	1	2
<i>B. pyocyaneus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mic. tetragenus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Strep. pyogenes</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. coli</i>	2	2	3	1	1	2	0	0	1	1	1	2
<i>B. lactis-aerogenes</i>	1	2	3	0	1	2	0	0	1	0	1	6
<i>B. mucosus-capsulatus</i> ..	0	0	0	0	0	0	0	0	1	0	0	0
<i>B. enteritidis</i>	22	30	30	1	2	3	11	20	27	3	20	30
<i>B. para-typhosus</i> B....	25	30	30	26	30	30	15	20	27	15	18	27
<i>B. para-typhosus</i> A....	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. typhosus</i>	15	22	25	1	1	2	15	20	27	1	1	15
<i>B. dysenteriae</i> Shiga....	0	0	1	0	0	0	0	0	0	0	0	0
<i>B. dysenteriae</i> Flexner..	0	0	1	0	0	0	0	0	0	0	0	0
<i>B. proteus vulgaris</i>	25	27	30	25	27	27	11	17	27	1	5	15
<i>B. cloacae</i>	1	2	5	0	1	4	0	0	1	1	2	2
<i>B. subtilis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. anthracis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. moelleri</i>	0	1	1	1	1	3	0	0	5	0	0	0
<i>B. fecalis-alkaligenes</i> ...	0	0	0	0	0	1	0	0	0	0	1	1
<i>B. bovissepticus</i>	2	2	2	0	0	0	0	0	0	0	0	0
<i>B. avisepticus</i>	0	5	5	0	0	0	0	0	0	0	0	0
<i>Sp. metchnikovii</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sp. cholerae</i>	0	0	0	0	0	0	0	0	0	0	0	0

acids than the American products. It probably also has a higher percentage of sulphur bearing constituents, possibly cystin. It would be interesting to determine the relative amounts of cystin in each type of peptone, but this has not yet been done.

A series of experiments was carried out to study the effect of various carbohydrates on hydrogen sulphide formation, by *B. paratyphosus* B. A medium made up of 3 per cent. Witte peptone, 0.5 per cent NaCl and 0.5 per cent of the carbohydrate was used. It was sterilized for twenty minutes in the Arnold on three consecutive days. Three monosaccharides, glucose, levulose and galactose; two disaccharides, lactose and sucrose; and a glucoside salicin were tried. No marked constant effect of these different carbohydrates on hydrogen sulphide formation was noted; this is not quite in accord with the observations of Seifert (1909) who found that the presence of glucose and lactose in peptone media decreased the amount of hydrogen sulphide formed.

Hydrogen sulphide production by B. Paratyphosus A and B. Paratyphosus B. and B. typhosus and B. dysenteriae

A number of strains of *B. paratyphosus* A, *B. paratyphosus* B, *B. typhosus*, and *B. dysenteriae*, were procured. Some of the cultures were obtained from the University of Chicago, some from the University of Kansas, some from the American Museum of Natural History, New York City, and some were freshly isolated from blood or stools at the University of Nebraska, College of Medicine. Their power of producing hydrogen sulphide was tested in a 3 per cent solution of Witte's peptone containing 0.5 per cent of NaCl, the same medium made from Difco and from Fairchild's peptones, respectively, and broth prepared from Difco peptone. All media were sterilized in the Arnold. The results are recorded in tables 5 and 6.

The tables need but little comment. So far as the cultures investigated were concerned *B. typhosus* always produced large amounts of hydrogen sulphide in twenty-four hours or less, while

B. dysenteriae never produced appreciable quantities in twenty-four hours and only a few strains produced traces after a week's incubation. The same distinction held for *B. paratyphosus* A and *B. paratyphosus* B. B always forming hydrogen sulphide in twenty-four hours or less and A forming practically none even

TABLE 5
Relative amount of hydrogen sulphide production by various strains of
B. paratyphosus A, and *B. paratyphosus* B

ORGANISM	1% WITTE PEP- TONE 0.5% NaCl			1% FAIRCHILD PEPTONE 0.5% NaCl			1% DIFCO PEP- TONE 0.5 NaCl			DIFCO BROTH		
	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days
<i>B. paratyphosus</i> B. 8...	15	20	30	22	27	30	30	30	30	25	25	30
<i>B. paratyphosus</i> B. 12...	7	10	30	22	25	30	27	30	30	22	30	30
<i>B. paratyphosus</i> B. 179.	7	7	12	5	8	10	3	5	8	10	12	12
<i>B. paratyphosus</i> B. 101.	12	20	25	25	27	30	30	30	30	20	22	25
<i>B. paratyphosus</i> B. 180.				22	27	30	30	30	30	25	25	28
<i>B. paratyphosus</i> B. 323.	10	20	30	22	30	30	30	30	30	27	30	30
<i>B. paratyphosus</i> B. 22...	8	10	15	20	25	25	30	30	30	30	30	30
<i>B. paratyphosus</i> B. 138.	9	17	25	15	22	30	30	30	30	25	30	30
<i>B. paratyphosus</i> A. 3...	0	1	2	0	0	0	0	0	0	0	0	0
<i>B. paratyphosus</i> A. 158.	0	1	1	0	0	0	0	0	1	0	0	0
<i>B. paratyphosus</i> A. 9...	0	1	1	0	0	0	0	0	0	0	0	0
<i>B. paratyphosus</i> A. 294.	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. paratyphosus</i> A. 16...				0	0	0	0	0	0	0	0	1
<i>B. paratyphosus</i> A. 322.	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. paratyphosus</i> A. 295.	0	0	1	0	0	0	0	0	0	0	0	0
<i>B. paratyphosus</i> A. 10...	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. paratyphosus</i> A. 4...	0	0	0	0	0	1	0	0	0	0	0	0

after a week's incubation. This property, combined with the use of Endo and Russell's medium, should be of considerable value in differentiating these organisms, especially when the detection of carriers is the object in view. Ordinary broth is as satisfactory for this purpose as any medium tried. Sterilization in the autoclave instead of in the Arnold is satisfactory.

TABLE 6

Relative amount of hydrogen sulphide production by different strains of typhoid and dysentery bacilli

ORGANISM	1% WILTS PEPTONE 0.5% NaCl			1% FAIRCHILD PEPTONE 0.5% NaCl			1% DIFCO PEPTONE 0.5% NaCl			DIFCO BROTH		
	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days	24 hours	48 hours	7 days
Typhosus, Hopkins	8	18	25	15	20	30	27	27	30	15	20	20
Typhosus, No. 190	10	20	25	12	22	23	30	30	30	22	22	22
Typhosus, Omaha No. 1.	7	15	20	8	12	15	25	25	25	25	25	25
Typhosus, Omaha No. 2.	12	16	23	10	15	20	20	25	30	20	25	25
Typhosus, Omaha No. 3.	9	13	20	7	15	15	25	27	27	22	25	27
Typhosus, Omaha No. 4.	10	16	22	9	15	20	27	27	27	18	25	30
Typhosus, Omaha No. 5.	12	20	25	10	15	20	25	30	30	15	25	27
Typhosus, Omaha No. 6.	10	18	23	10	15	22	22	25	25	20	25	25
Typhosus, No. 189	9	17	25	12	18	18	27	27	30	20	22	25
Typhosus, No. 197	9	20	22	14	17	22	30	30	30	17	20	27
Typhosus, No. 607				5	15	15	20	25	25	20	25	25
Typhosus, No. 11	10	15	20	9	11	15	30	30	30	15	20	25
Typhosus, No. 608				7	12	12	30	30	30	18	20	20
Typhosus, 5	9	20	25	10	15	15	30	30	30	18	20	22
Typhosus, Cary	12	18	25	15	18	20	20	25	25	20	22	25
B. dysenteriae, Flexner C.	0	0	1	0	0	0	0	0	0	0	0	0
B. dysenteriae, Hofmann C.	0	0	2	0	0	0	0	0	0	0	0	1
B. dysenteriae Shiga. W.	0	0	0	0	0	0	0	0	0	0	0	0
B. dysenteriae Shiga. C.	0	0	1	0	0	0	0	0	0	0	0	0
B. dysenteriae Strong.	0	0	0	0	0	0	0	0	0	0	0	0
B. dysenteriae, Flexner W.	0	0	1	0	0	0	0	0	0	0	0	0
B. dysenteriae, 78 W.	0	0	1	0	0	0	0	0	0	0	0	0
B. dysenteriae, Omaha No. 1	0	0	1	0	0	0	0	0	0	0	0	0

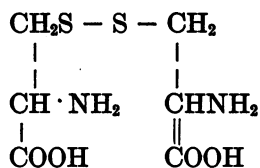
The relationship between chemical structure and hydrogen sulphide formation

In order to study the relationship between the state of oxidation of sulphur compounds and the ease with which they may be attacked by bacteria with resulting hydrogen sulphide formation, three compounds were selected. Sodium sulphate was chosen as a representative inorganic sulphur compound in which

the sulphur is fully oxidized. Taurin was taken as a representative of the sulphur components of proteins in which the sulphur is fully oxidized. This compound is amino ethyl

sulphonic acid $\begin{array}{c} \text{CH}_2\text{NH}_2 \\ | \\ \text{CH}_2\text{SO}_3\text{H} \end{array}$ analogous to a sulphate except that

one oxygen is replaced by an H. It is also an amino acid. Cystin the disulphide of diamino thiolactic acid was selected because it is an amino acid.



Here the sulphur is in the reduced state. Cystin is the usual sulphur-containing amino acid found in proteins. Cystin and taurin are analogous except as to the state of oxidation of the sulphur.

A medium was prepared as follows: ammonium tartrate 10 grams, anhydrous sodium sulphate, 10 grams, and distilled water to 1000 cc. The mixture was put in culture tubes and autoclaved for ten minutes at 15 pounds pressure. Five organisms were used, *B. coli* and *B. paratyphosus* B, because they are hydrogen sulphide formers which have no proteolytic action when tested in the ordinary media, and *B. fluorescens-liquifaciens*, *B. cloacae*, and *B. proteus-vulgaris*, because they are organisms, with varying degrees of proteolytic activity. Duplicate cultures were made, strips of sterile lead acetate paper were inserted in the mouth of the tubes, and they were placed in a moist 37° incubator. No growth appeared in any of the tubes at the end of seven days incubation under aerobic conditions.

An exactly similar medium to the one given above was made, except that 10 grams of chemically pure glucose was added. After seventy-two hours incubation a vigorous growth appeared in all of the tubes but even after fourteen days incubation there was no perceptible blackening of the lead acetate paper. Evi-

dently carbohydrate was a necessary source of carbon for the metabolism of the bacteria. It was omitted in the first medium in order to learn whether the carbon in the ammonium tartrate could serve this purpose.

Taurin was prepared by the method of Hawk (1918). Its purity was determined by making duplicate analyses for sulphur using the method of Redfield (1912). Theory called for 26.15 per cent and we found 25.90 per cent.

A 0.5 per cent solution of taurin was made in distilled water. It was sterilized with a Berkefeld filter because sterilization by means of heat caused blackening of lead acetate paper, and pointed toward a chemical change in the taurin. Filtration obviated any such possibility.

Duplicate tubes were inoculated with the same series of organisms which were used with the sodium sulphate medium; these were incubated for one week under aerobic conditions at 37°C. without growth.

Another medium was prepared, identical with the above except that 1 per cent of chemically pure glucose was added. After one week's incubation a slight growth appeared in the tubes inoculated with *B. proteus-vulgaris*, and *B. fluorescens-liquifaciens*, but not in the others. No blackening of the paper occurred in any instance. This would indicate that taurin is not readily attacked by bacteria.

Cystin was prepared by the method of Matthews and Walker (1909).

Its purity was ascertained by determining the percentage of sulphur by the same method used for the taurin. Theory called for 26.72 per cent; we found, 27.04 per cent.

An attempt was made to prepare the ammonium salt of cystin by dissolving the cystin in ammonium hydroxide and evaporating the excess ammonia. The cystin precipitated as soon as the fumes of the ammonia disappeared. Evidently the ammonium salt was easily hydrolyzed.

The citrate of cystin acted in the same way. In order to keep it in solution an excess of citric acid was required which was sufficient to interfere with bacterial growth.

Cystin plus tartaric acid behaved in a similar manner.

Finally a 0.5 per cent. solution of cystin was prepared by adding just enough sodium carbonate to keep the cystin in solution. The medium was sterilized by filtration because heat caused hydrogen sulphide formation. It was placed in sterile tubes and tested for sterility. Tubes were inoculated with the series of organisms used with the sodium sulphate and taurin mediums. After a week's incubation no growth appeared.

Another series of tubes were inoculated with the same bacteria used previously. A bit of sterile litmus paper was added to each tube and sterile 5 per cent. hydrochloric acid added till the litmus was faintly red. The cystin was precipitated as the neutral

TABLE 7

Formation of hydrogen sulphide from a solution of cystin, in millimeters of lead acetate paper blackened

ORGANISM	24 HOURS	48 HOURS	72 HOURS	7 DAYS
<i>B. paratyphosus</i> B.....	0	2	7	7
<i>B. typhosus</i>	0	0	0	0
<i>B. cloacae</i>	0	0	0	0
<i>B. proteus</i>	1	2	4	25
<i>B. fluorescens liquifaciens</i>	0	0	15	25

point was approached but this was ignored. After three days incubation the litmus turned blue and sterile acid was again added till it turned faintly red. Growth appeared in all the tubes and hydrogen sulphide was formed in some of them. Table 7 gives the results.

This experiment was repeated except that the cystin was dissolved in the smallest possible amount of tenth normal hydrochloric acid and tenth normal sodium carbonate was added till a precipitate formed and the medium was only slightly acid, the exact reverse of the preceding method. The results were the same.

Cystin could doubtless have been sterilized by dissolving it in an organic solvent, evaporating this off and adding sterile distilled water but this was not tried.

Probably nearly neutral solutions of cystin in citric acid, in tartaric acid, or in ammonium hydroxide, prepared as in the case of the sodium carbonate solution, would have given similar results but these methods were not tried. The work done seemed to indicate that organic sulphur in the partially reduced condition rather than in the oxidized forms gives rise to hydrogen sulphide when ordinary bacteria act on protein.

SUMMARY

The examination of a number of samples of feces showed no constant difference between the amount of hydrogen sulphide produced by human and animal fecal material. This indicates that a test of samples of water for hydrogen sulphide production would be of no value in distinguishing between human and animal contamination.

The hydrogen sulphide test is too delicate for use in examination of water for the detection of fecal contamination of any type. All contaminated waters examined were positive to this test and many were positive which gave no evidence of contamination by the usual criteria.

A considerable number of common bacteria were able to produce hydrogen sulphide from Witte peptone and not from Difco peptone and some other kinds were able to produce it in larger amounts. Fairchild's peptone yielded more hydrogen sulphide than did Difco peptone. This emphasizes the need for uniform media.

Glucose and lactose had little effect on hydrogen sulphide formation. There was little difference between 3 per cent. peptone solutions and standard beef extract broth.

All strains of *B. paratyphosus* B examined, produced hydrogen sulphide in twenty-four hours or less, while none of the strains of *B. paratyphosus* A were able to do this.

All strains of *B. typhosus* studied produced hydrogen sulphide in twenty-four hours or less and none of the strains of *B. dysenteriae* had this power.

Sodium sulphate was not a source of hydrogen sulphide in the limited number of experiments made.

Taurin was attacked only to a very limited extent by the bacteria used, and none of them were able to split it to the extent of formation of hydrogen sulphide.

Cystin in distilled water was able to support bacterial growth in the case of some of the common bacteria, and even in some instances to permit of hydrogen sulphide formation. This indicates that oxidized sulphur is not readily attacked by bacteria while partially reduced sulphur is completely reduced to hydrogen sulphide.

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ERRATUM

J. Bact. 5, 231. The Production of Hydrogen Sulphide by Bacteria. John T. Myers. From the Department of Hygiene and Bacteriology, the University of Chicago.

A portion of the work recorded in this paper was done in the laboratories of the University of Nebraska, College of Medicine, Department of Pathology and Bacteriology, Omaha, Nebraska.