THE RELATION BETWEEN CHEMICAL COMPOSITION OF PEPTONES AND HYDROGEN SULPHIDE PRODUCTION BY BACTERIA

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Received for publication August 19, 1922

In a paper recently published in this journal (Tilley, 1923) the writer showed that variations in hydrogen sulphide production by bacteria in lead acetate agar may be due on the one hand to differences in the hydrogen sulphide producing power of different strains of the same organism and on the other hand to the use of different varieties of peptone in the lead acetate agar. As explained in that paper the writer had expected to find that the value of the various peptones for H_2S production depended upon the amount of cystin present, just as the value of peptones for indol production depends upon the tryptophan content (Tilley, 1921). Preliminary experiments, however, indicated that the problem was more complicated than at first supposed so further work was undertaken, the results of which are reported in the present paper.

EXPERIMENTAL WORK

In view of the great complexity of the composition of peptones it was not considered advisable to attempt to make complete and exact analyses of the various peptones used. For the purposes of the work here reported it was thought sufficient to ascertain roughly the approximate amounts of "unoxidized," "partly oxidized" and "oxidized" sulphur in the six peptones employed in the work reported in the previous paper.

The term "unoxidized sulphur" will be used in this paper to denote sulphur as it occurs in proteins and protein derivatives

loosely combined with carbon and hydrogen. Upon heating with caustic alkali hydrogen sulphide is liberated and as it is detected by means of lead acetate solution or lead acetate paper this form of sulphur is often called "lead blackening sulphur." Cystin is an example of this form of sulphur. The term "partly oxidized sulphur" will be used to denote sulphur combined in such a form that upon distillation with phosphoric acid sulphur dioxide is liberated. The term "oxidized sulphur" will be used to denote sulphur as it occurs in sulphates or other similar compounds.

Unoxidized sulphur was estimated in the following manner: 5 cc. of a strong (1:1) solution of potassium hydroxide were

TABLE 1

Relation between chemical composition of peptones and H_2S production by bacteria

PEPTONE NUMBER	UNOXIDIZED SULPHUR	PARTLY OXIDIZED SULPHUR	OXIDIZED SULPHUR	H_2S BY BACTERIA
1	+	+	+	+
2	−	+++	+++	+++
3	++	−	−	−
4	++	++	−	++
5	+++	+	+++	++
6	−	+++	+++	+++

− = none; + = small amount; ++ = moderate amount; +++ =large amount.

mixed with an equal quantity of a filtered 3 per cent solution of peptone in water or in 20 per cent hydrochloric acid; a few drops of a 10 per cent aqueous solution of lead acetate were added and the mixture boiled vigorously. The relative amounts of unoxidized sulphur present were estimated roughly by the amount of blackening which resulted.

The estimation of partly oxidized sulphur was made by Mr. R. R. Henley of this Division, to whom I am also much indebted for advice and assistance so far as the chemical aspects of this work are concerned. The details of the method employed may be found in Bureau of Chemistry Bulletin No. 107 (1908). Oxidized sulphur was estimated by precipitation with barium chloride in an acid solution.

The relation between chemical composition of the various peptones, as shown by the previously described tests, and hydrogen sulphide production by bacteria in lead acetate agar containing these peptones is shown in table 1. It should be clearly understood that the relative amounts indicated are only rough approximations. In order to avoid any suggestion that the differences shown represent exact quantitative results arbitrary symbols are employed instead of any statement of amounts.

The results shown in table 1 indicated that partly oxidized sulphur is the form of sulphur which yields the largest amount of hydrogen sulphide. Further experiments were, however,

TABLE 2

Relative values of oxidized and partly oxidized sulphur for H_2S production

ORGANISM AND STRAIN	H_2S PRODUCTION BY BACTERIA IN MEDIA CONTAINING		
	No added sulphur	Ammonium sulphate	Sodium thiosulphate
Proteus 4	+	+	+++
Bact. suipestifer 416	+	+	+++
Bact. suipestifer 420	+	+	+++
Bact. enteritidis G 4	+	+	+++
Bact. typhosum A	+	+	++
Bact. typhosum C	+	+	++
Bact. typhosum D	−	−	−

− = no H_2S; + = small amount; ++ = moderate amount; +++ = large amount. Results shown are after twenty-four to forty-eight hours incubation.

undertaken to determine more exactly the relative value of the different forms of sulphur for hydrogen sulphide production. In these experiments various sulphur compounds were added to lead acetate agar and observations made upon the relative amounts of hydrogen sulphide produced by various bacteria.

In the first place an attempt was made to show the relative values of oxidized sulphur in the form of sulphate and partly oxidized sulphur in the form of thiosulphate. These compounds were added to lead acetate agar prepared according to the directions of Jordan and Victorson (1917) except that the reaction was adjusted to pH 7.2 instead of to +1 to phenolphthalein. The amount of sulphate or thiosulphate added was 0.25 per cent.

The peptone used in the preparation of the lead acetate agar was peptone 1 (see table 1). The results are given in table 2.

The results shown in table 2 indicate that oxidized sulphur in the form of sulphate does not yield any appreciable amount of hydrogen sulphide while partly oxidized sulphur in the form of thiosulphate seems to be especially suitable for hydrogen sulphide production by bacteria.

TABLE 3

Comparative effect of different compounds containing partly oxidized sulphur

ORGANISM AND STRAIN	H_2S PRODUCTION BY BACTERIA IN MEDIA CONTAINING		
	No added sulphur	Sodium thiosulphate	Sodium sulphite
Bact. suipestifer 360	−	−	+++
Bact. suipestifer 416	−	+++	+
Bact. coli C	−	−	+
Bact. cloacae T 2	−	−	+
Proteus 4	−	+++	+
Proteus 11	−	+	−
Bact. typhosum A	−	+++	+++
Bact. typhosum K 110	−	−	++
Bact. dysenteriae Y	−	−	+
Bact. dysenteriae Shiga	−	−	−
Paratyphoids	−		
A. K A 28	−	−	++
B. K B 31	−	+++	+++
C. K C 1	−	+++	+++

− = no H_2S; + = small amount; ++ = moderate amount; +++ = large amount.

In the remaining experiments instead of following the directions of Jordan and Victorson, lead acetate agar was prepared by adding lead acetate solution to ordinary beef infusion agar containing 1 per cent of peptone 1. In the experiment shown above in table 3, sodium thiosulphate or sodium sulphite was added to this medium in the proportion of 0.25 per cent.

The results shown above indicate that, while both sodium thiosulphate and sodium sulphite readily yield hydrogen sulphide, sodium thiosulphate, on account of clearer distinctions between organisms and strains, would give the better results when used

in a culture medium intended for diagnostic purposes to distinguish between different species and strains of bacteria.

In table 4 there are shown the results of an experiment with representatives of the two classes of organic compounds containing sulphur designated by Hawk (1918, p. 108) as unoxidized and oxidized. Cystin was selected as containing unoxidized organic sulphur and taurin as containing oxidized organic sulphur.

TABLE 4

Comparative value of different organic sulphur compounds for H_2S production

ORGANISM AND STRAIN	H_2S PRODUCTION BY BACTERIA IN MEDIA CONTAINING		
	No added sulphur	Cystin	Taurin
Bact. suipestifer 360	−	+	−
Bact. suipestifer 416	−	++	−
Bact. coli C	−	+++	−
Bact. cloacae T 2	−	++	−
Proteus 4	−	+++	−
Proteus 11	−	+++	−
Bact. typhosum A	−	++	−
Bact. typhosum K 110	−	+	−
Bact. dysenteriae Y	−	+	−
Bact. dysenteriae Shiga	−	+	−
Paratyphoids	−		
A. K A 28	−	++	−
B. K B 31	−	+++	−
C. K C 1	−	+++	−

− = no H_2S; + = small amount; ++ = moderate amount; +++ = large amount.

The technique employed was similar to that used in experiment III except that the amount of cystin used was 0.1 per cent and special technique was employed in adding it to the culture medium. The cystin was partly dissolved and partly suspended in N/10 HCl and after sterilization in the autoclave was added to the culture medium with a sterile pipette. Just previous to this there was added enough sterile N/10 NaOH to neutralize the N/10 HCl in which the cystin was carried, thus leaving the reaction of the culture medium as far as possible unchanged.

The results given in table 4 indicate that taurin is not utilized by bacteria for H_2S production while cystin gives rise to an abundant production of H_2S, so much so that differences between the various species and strains are largely obliterated. This, and the fact that organisms usually classed as "lead negative" are rendered "lead positive," make it inadvisable to use cystin to correct deficiencies in the sulphur content of peptones used in lead acetate agar.

The results of table 4 taken together with those of table 1 bring up a very interesting question. In table 4 we see that unoxidized sulphur in the form of cystin yields a large amount of H_2S and yet in table 1 we see that although peptone 3 contains apparently as much unoxidized sulphur as any of the peptones except no. 5, no appreciable amount of H_2S is formed in lead acetate agar made with peptone 3.

The most plausible explanation of this discrepancy would seem to be that the unoxidized sulphur of the various peptones is not all free cystin.

In order to test this explanation the six peptones previously employed were examined for free cystin in the following manner, the technique employed being based on that given by Hawk (1918, p. 87) for the separation of cystin formed by the action of hydrochloric acid on wool; 5 grams of peptone were dissolved in 25 cc. of 20 per cent hydrochloric acid, the solution being kept cool to avoid hydrolytic action as far as possible, and the resultant solution filtered. To this filtered solution sodium acetate was added in excess, as shown by a negative reaction for mineral acid with Congo red. Whatever precipitate was formed was separated out by filtration through a hardened filter paper and then dissolved in hot dilute (5 per cent) hydrochloric acid. Then this dissolved precipitate, and also the filtrate from the original solution, were tested for unoxidized sulphur by the method already given in the first part of the paper. Free cystin being insoluble in acetic acid should appear in the precipitate while any unoxidized sulphur found in the filtrate would be regarded as something other than free cystin. The results are shown in table 5.

The results shown below indicate that none of the peptones

except no. 5 contain any really appreciable amount of free cystin. The presence of cystin in no. 5 is no doubt the reason for the results shown in table 1 where it is seen that although peptone 5 contains very little partly oxidized sulphur it yielded a fairly large amount of hydrogen sulphide. The fact that the cystin served to bring up the amount of hydrogen sulphide only to a moderate degree is presumably due to its relative insolubility. In spite of the large amount in the peptone only a small amount was dissolved by the culture media.

TABLE 5

Relative amounts of free cystin and other unoxidized sulphur in peptones

PEPTONE NUMBER	UNOXIDIZED SULPHUR	
	Precipitate	Filtrate
1	None	Present
2	None	Present
3	None	Present
4	A trace	Present
5	Present	Present
6	None	Present

DISCUSSION

It is interesting, in the first place, to note that the results shown in tables 2, 3 and 4 agree very well with those reported by Myers (1920) and by Sasaki and Otsuka (1912). These investigators worked with various sulphur compounds in fluid media containing no peptone but their results correspond very closely to those obtained by the writer with the same compounds in lead acetate agar.

It seems evident from the experimental results herein reported that commercial peptones contain unoxidized, partly oxidized and oxidized sulphur in varying proportions. On account of differences in the amount and availability of these various forms of sulphur in the peptones it is inevitable that variable results should be obtained with lead acetate agar made with the different peptones unless precautions are taken to prevent such results.

Variable results could be avoided by testing the peptones

employed, either by chemical methods similar to those described in this paper, provided the necessary apparatus is available, or by actual trial in media with known strains of known organisms.

The use of sodium thiosulphate as a usual ingredient of lead acetate agar would, however, obviate the necessity for testing the peptone employed and in the writer's judgment, the results obtained would be entirely comparable with those usually obtained with the best peptones. It is quite possible that with organisms other than those used by the writer, or with different strains of the same organisms, this might not hold true but on the other hand the use of a poor peptone will give wholly misleading results, and even with the best peptones there may be variations in the composition of different samples and consequent variations in the results obtained.

It is therefore recommended that sodium thiosulphate be used as a regular ingredient of lead acetate agar. If used in a synthetic medium containing no other source of sulphur it would no doubt give clearer distinctions between different organisms and strains than in a peptone medium. But so far as the writer is aware, there is no synthetic medium which will support as vigorous growth by as many different organisms as the usual peptone media.

In the absence of such a synthetic medium it seems advisable to use lead acetate agar prepared according to the directions of Jordan and Victorson (1917) except for the following changes: the use of 1 per cent instead of 3 per cent of peptone, adjustment of reaction by the hydrogen ion method instead of by titration, and the addition of sodium thiosulphate. The smaller amount of peptone is sufficient to support the growth of bacteria and would lessen possible interference by sulphur compounds already present in the peptone. The writer has in his own work adjusted the reaction to pH 7.2 and used 0.25 per cent of sodium thiosulphate.

CONCLUSIONS

1. Commercial peptones have been shown to contain unoxidized, partly oxidized and oxidized sulphur in varying proportions.

2. Bacteriological tests with media containing various sulphur compounds showed that no hydrogen sulphide was liberated by bacteria from compounds containing oxidized sulphur while it was given off freely from those containing partly oxidized sulphur.

Unoxidized sulphur in the form of cystin yielded an abundance of hydrogen sulphide but experimental evidence indicated that the unoxidized sulphur of commercial peptones may consist largely of some compound, or compounds, other than cystin and not utilized by bacteria for the production of hydrogen sulphide.

3. On account of the qualitative and quantitative differences in the sulphur content of the various commercial peptones, there are resulting variations in hydrogen sulphide production by bacteria in media containing these peptones.

4. To insure uniform results in testing hydrogen sulphide production by bacteria in lead acetate agar the peptone used should be suitable for the purpose, as shown by chemical or bacteriological tests; or, preferably, sodium thiosulphate should be used as an ingredient of the lead acetate agar.

REFERENCES

Bureau of Chemistry Bulletin No. 107 1908 Official and Provisional Methods of Analysis. Association of Official Agricultural Chemists, p. 187.

HAWK, P. B. 1918 Practical Physiological Chemistry. 6th edition.

JORDAN, E. O., AND VICTORSON, RUTH 1917 Differentiation of the paratyphoid enteritidis group. II. Lead acetate agar. Jour. Inf. Dis., **21**, 554–555.

MYERS, J. T. 1920 The production of hydrogen sulphide by bacteria. Jour. Bact., **5**, 231–252.

SASAKI, T., AND OTSUKA, I. 1912 Experimentelle Untersuchungen über die Schwefelwasserstoffentwicklung der Bakterien aus Cystin und sonstigen Schwefelverbindungen. Biochem. Ztschr., **39**, 208–215.

TILLEY, F. W. 1921 Influence of peptone on indol formation by *Bacillus coli*. Amer. Jour. Pub. Health, **11**, 834–836.

TILLEY, F. W. 1923 Variations in hydrogen sulphide production by bacteria. Jour. Bact., **8**, 115.