THE INFLUENCE OF CARBOHYDRATES ON HYDROGEN SULPHIDE PRODUCTION BY *BACILLUS AERTRYCKE* (MUTTON).

By HARRI HEAP and BESSIE H. E. CADNESS.

(*Department of Bacteriology and Preventive Medicine, Victoria University of Manchester.*)

(With 4 Charts.)

An important aspect of the investigation of bacterial metabolism is the study of the substrate and its changes and of the resulting products; together with an examination of the rate of change. The present report deals with the influence of certain carbohydrates in modifying the action of one species of bacteria upon the protein molecule.

Hirschler (1886) found that sugars inhibited the production of indol and other phenolic bodies, and preferred the explanation that sugars were more easily decomposed than proteins. He showed that in mixed cultures proteins are not attacked when sugars are present, although the acids formed were neutralised by calcium carbonate.

Smith and also Peckham (1897) concluded that non-production of indol in sugar media was due to acid formation. This seemed to be supported by the work of Glenn (1911), who found that 0·5 per cent. of added lactic acid prevented indol formation, and also that the presence of sugars prevented the liquefaction of gelatine. It has, indeed, frequently been stated that organisms growing on a sugar-protein medium do not form indol, liquefy gelatine nor digest casein, and that ammonia production is greatly lessened. Acid production from sugars has been put forward as an explanation for this inhibition of the production of protein decomposition products.

Fischer (1915) found that neither hydrion concentration nor total acidity influenced indol production; and suggested that glucose inhibited the action of the proteolytic enzyme.

Kendall, Day and Walker (1913) adopted a very similar hypothesis, in stating that "protein sparing" is not due to acids produced from sugars, but that sugars serve the energy requirements more readily than do proteins, and that the activity of the organisms is diverted from the protein to the sugar, so that protein decomposition products are found wanting. They state that the slight ammonia production, which occurs, indicates the small amount of protein decomposition necessary for structural requirements. It is generally stated that the production of acid from sugar inhibits the liquefaction of gelatine by *B. proteus*, but Kendall and Walker (1915) found that enzymes from this organism reacted in the presence of glucose or of organic acids as

https://doi.org/10.1017/S0022172400008482 Published online by Cambridge University Press

easily as in their absence. In dextrose gelatine, however, no proteolytic ferment was formed until the dextrose had been completely utilised.

Jordan (1917) states that the hydrion concentration of the medium is not an explanation of the protein sparing effect, and is only of importance in so far as it influences the general conditions of growth.

Logie (1919) found that the production of indol was decreased in the presence of sugars, and increased in amount in the absence of sugars, and suggested that this was due to the increased utilisation of indol in the presence of sugars.

Jones (1916) found that, with varying amounts of glucose, *B. proteus* produced proteolytic enzymes first in the cultures containing the smaller amounts of carbohydrate. He believed that the absence of protein-splitting in sugar media was due to the fact that the proteolytic ferment does not appear till the utilisable sugar has undergone complete hydrolysis, or that the ferment is unable to act in an acid medium.

Berman and Rettger (1918) found that the presence of sugar did not affect the nitrogen metabolism, the concentration of the amino nitrogen remaining unchanged both in plain, and in glucose media inoculated with *B. coli*.

De Bord (1923) found that the total amount of amino nitrogen may be increased to but a slight extent during bacterial growth in plain medium of peptone free from glucose, while in the presence of glucose a marked increase in amino nitrogen takes place, using Folin's amino nitrogen method and not Sörensen's method of estimation. He found the production of amino nitrogen was always greater in the presence of glucose, but was unable to correlate the amino nitrogen figures with the number of living cells. His explanation was that the amino nitrogen in plain media was used as rapidly as formed, but that in sugar media the production is in excess of the amounts being used, so that the concentration of amino nitrogen in the culture is increased. He suggests also that the presence of sugar may increase the availability of nitrogen products other than amino nitrogen. A material increase in amino nitrogen he regards as an index of proteolysis, clearly shown in sugar media, while in non-sugar media the amino nitrogen cannot serve as an index of proteolysis, except in those cases in which a definite increase occurs.

In De Bord's experiments the presence of a sugar resulted in lessened ammonia production, and this has been claimed as being due to a decreased proteolysis. Gordon (1917) found that *B. coli* utilised nitrogen from ammonium salts when a sugar was present. It seems, therefore, that decreased ammonia production does not necessarily indicate lessened proteolysis. It may indicate greater ammonia utilisation.

So far most attempts at the elucidation of this problem have been made by a consideration of the fate of the nitrogen complex in the protein molecule. Our attempt has been directed to the sulphur containing fraction of the protein molecule, and with results of no little interest, if not at the moment capable of easy explanation.

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We have attempted to estimate the extent of proteolysis by measuring the hydrogen sulphide formation, and to show how this is influenced by the presence of sugars and phosphates. Our attention has been confined to one organism, a known hydrogen sulphide producer—*B. aertrycke* (Mutton).

Previously hydrogen sulphide production has been detected by the blackening of lead solution either in the medium, or on papers suspended over it, and the amount of blackening has been used as an index of the amount of sulphide. This method has been used for various purposes such as:

The differentiation of *Bacillus paratyphosus* A and *paratyphosus* B (Burnet and Weissenbach, 1915), and for the measurement of the pollution of water, Schardinger (1894) and Dunham (1879).

Sasaki and Otsuka (1912) added various sulphur compounds to Fränkel's artificial medium, and found that the majority of the strains of bacteria they investigated produced hydrogen sulphide from cystine and from sulphur, a few from sodium thiosulphate, one or two from sodium sulphite, and none from sodium sulphate, or from taurine. These results were substantiated by the findings of Myers (1920) and Tanner (1917) who also stated that no hydrogen sulphide was produced from 2-thiohydantoin or magnesium sulphate.

Seiffert (1909) investigated the effect of sugars on hydrogen sulphide production. Working with strains of the Salmonella group he found that this substance was evolved in very varying amounts, and that the addition of sugar to the peptone water greatly increased the production, as judged by the blackening of lead acetate paper. He used 0·5 per cent. sugar in the media, and sterilised it by steaming for one hour. This may account for some of his results, as the higher sugars would probably be hydrolysed to some extent, and the lower ones possibly destroyed. He found that hydrogen sulphide production was greatest for cane sugar, less for laevulose and galactose, while after twenty-four hours' incubation in the presence of dextrose there was no hydrogen sulphide, and only traces in the presence of lactose.

Myers (1920) found no marked, nor constant effect, with the different carbohydrates he employed, though he refuted Seiffert's results for dextrose and lactose, which sugars he found to have no inhibitory influence on hydrogen sulphide production.

Tilly (1923) observed a curious correlation between sulphide production in lead-acetate agar, and ability to grow in synthetic medium composed of 0·2 per cent. sodium ammonium hydrogen phosphate and 1 per cent. glucose. Out of more than 150 strains of *B. suipestifer* examined, only six failed to conform to the rule that strains which produced hydrogen sulphide also grew on the synthetic medium and *vice versa*.

Wilson (1923) has suggested that the hydrogen sulphide produced during the growth of bacteria in peptone water is derived, at least in part, from sulphites, present as an impurity, and accounts in this way for the very varying results obtained with different brands of commercial peptone.

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Experimental.

1. To ensure that all the sulphur present in the Witte peptone media used was present as organic sulphur, an attempt was made to detect sulphite in the peptone water by means of Votoçek's method (1907).

The effect of adding 5, 10 and 15 drops of a 0·00025 per cent. solution of fuchsin to water, and to an equal volume (10 c.c.) of 3 per cent. peptone water was compared. The peptone water did not decolourise the fuchsin, thus indicating the probable absence of sulphite in this particular Witte peptone.

2. In order to obtain a roughly quantitative idea of the amounts of hydrogen sulphide, produced by *B. aertrycke* (Mutton) in peptone water under various conditions, a series of tube experiments was carried out in which an expression of gas production was estimated by measuring the blackening produced on lead acetate paper suspended in a narrow tube, which passed into the experimental tube through a waxed cork. The medium employed was the basal medium used throughout the whole series of experiments, and consisted of 3 per cent. Witte peptone dissolved by steaming in a watery 0·25 per cent. solution of sodium chloride. This was filtered, its reaction adjusted to *p*H 7·6 and then autoclaved at 115° C. for 20 minutes. 2 per cent. of glucose (sterilised by steaming as a 50 per cent. solution in water) was added to half the tubes, and control tubes were prepared containing varying amounts of iodine, in order to further eliminate the possibility of sulphites in the media. The tubes were inoculated with one loopful of the standard inoculum, used throughout the experiments. This consisted of a four hours' growth of *B. aertrycke* (Mutton) in 5 c.c. of 1 per cent. peptone water, which in its turn had been inoculated with one standard loopful from a stock culture. The tubes were incubated at 37° C., and the hydrogen sulphide produced estimated by measurement of the blackening of lead papers.

Iodine proved to have no effect on the hydrogen sulphide production provided it was not present in a concentration high enough to inhibit growth, thus indicating that the peptone itself was the only source of sulphur.

The results obtained in the glucose and non-glucose media are shown in Table I.

Table I. *Hydrogen sulphide production by* B. aertrycke *(Mutton) in the presence and absence of 2 per cent. of glucose; as measured by the blackening of lead acetate paper.*

Period of growth	Length in mm. of lead acetate paper blackened	
	In the absence of glucose	In the presence of 2 % glucose
24 hours	0·5 mm.	11·0 mm.
48	2·3	12·7
72	3·0	12·7
96	4·0	12·7
120	5·0	12·7
144	5·0	12·7
240	5·0	12·7

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The fact that glucose appeared to accelerate hydrogen sulphide production, led us to devise a more accurate method for its estimation.

250 c.c. of medium was employed, contained in a special flask with a ground glass stopper, through which two tubes passed in such a way that gas could be bubbled through the culture at the end of a period of growth, in order to drive off the hydrogen sulphide produced. This was connected with a series of four Dreschel towers, containing alternately standard iodine solutions and dilute starch paste solutions, the latter to catch any iodine that might be blown over. In a large number of experiments, a fifth tower containing standard sodium thiosulphate solution proved that the amount of iodine blown over further than the fourth tower was negligible.

A stream of carbon dioxide (washed by passing through lead acetate, potash, and water bulbs) was used to distil off the hydrogen sulphide, which in the majority of experiments was small enough in quantity to be entirely oxidised in the first tower, the three subsequent towers serving as controls.

After one hour's exposure to the stream of carbon dioxide, the iodine in the towers was titrated against a standard solution of sodium thiosulphate, and from the figures obtained the volume of hydrogen sulphide produced in certain definite times could be calculated.

The results throughout this paper are expressed in terms of $N/1000$ hydrogen sulphide, though experiment proved that $N/100$ iodine solutions were more convenient for use.

Many control experiments were carried out with uninoculated media, and these showed that no iodine of any significance was lost during the gas distillation.

During the early part of the work the medium, after growth and gas distillation, was tested for residual hydrogen sulphide by heat distillation with and without the addition of acid, and examination of the gaseous distillates. Hydrolysis, however, took place to such an extent as to render the figures useless for our purpose, and this method was discarded.

In many experiments 0·42 per cent. of disodium hydrogen phosphate was added to buffer the medium. Experiment proved, however, that the buffering effect of the peptone itself, in the high concentration employed, rendered the effect of added phosphate almost negligible. Nevertheless the results of the phosphate experiments are included here, as they serve to confirm the plain peptone experiments; though, on the whole, the figures obtained tend to be slightly higher.

The second set of experiments consists of a series of quantitative determinations of hydrogen sulphide after various periods of growth. Each figure obtained represents the hydrogen sulphide in one 250 c.c. culture after the stated period of growth, except the figures marked (*a*) which represent the average of the two figures and those marked (*b*) which represent the average of three.

1 c.c. of the standard inoculum was employed, and a parallel series of

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cultures was grown in the incubator, and from this a record was obtained each day of the hydrogen-ion concentration of the media during growth.

The results obtained are summarised in Table II, and are shown graphically in Chart I.

Table II. *Hydrogen sulphide production by* B. aertrycke (*Mutton*) *in the presence and absence of* 2 *per cent. of glucose and* 0·42 *per cent. of phosphate.*

Period of growth Hours	3 % peptone (Witte) in 0·25 % sodium chloride solution together with							
	No addition		2 % glucose		0·42 % phosphate		2 % glucose + 0·42 % phosphate	
	c.c. $N/1000$ H_2S	Hydrion concentration	c.c. $N/1000$ H_2S	Hydrion concentration	c.c. $N/1000$ H_2S	Hydrion concentration	c.c. $N/1000$ H_2S	Hydrion concentration
4	—	—	—	—	—	—	10·5	7·5
6	—	—	—	—	—	—	15·1	7·4
8	—	—	—	—	—	—	19·6	7·35
14	—	—	—	—	—	—	177·5	5·05
15	—	—	—	—	—	—	178·0	5·0
16	—	—	—	—	—	—	171·4	4·95
18	27·1	7·3	170·5	5·1	27·3 (*a*)	7·5	173·5 (*b*)	4·4
24	—	—	—	—	—	—	—	—
42	34·4 (*a*)	7·25	215·5	4·8	27·3	7·6	236·5	4·8
48	—	—	—	—	—	—	—	—
66	36·7	7·35	131·7	4·7	—	—	232·0	4·75
72	—	—	—	—	31·7	7·8	—	—
90	42·3	7·4	123·7	4·7	—	—	196·0	4·7
96	—	—	—	—	41·5	7·8	—	—
114	48·9	—	—	—	—	—	145·0	4·7
120	—	—	—	—	57·7	7·9	—	—
138	—	—	100·0	4·7	—	—	—	—
186	—	—	—	—	85·0	8·0	—	—
234	177·3 (*a*)	8·0	—	—	—	—	—	—
240	—	—	—	—	112·5 (*b*)	8·0	—	—
258	—	—	112·2	4·7	—	—	—	—
282	115·2	8·0	—	—	—	—	123·3	4·7
345	—	—	101·1	4·7	—	—	—	—

These results seem to show that glucose definitely stimulates hydrogen sulphide production by *B. aertrycke* (Mutton), and it appeared of interest to investigate the effect of varying the concentration of glucose in the media.

A series of lead-acetate paper trial tubes was set up, and to the basal phosphate medium the following amounts of glucose were added: 0·1, 0·25, 0·5, 0·75, 1·0, 1·1, 1·25, 1·5, 1·75, 2·0, 2·5, 3·0, 4·0, 5·0 per cent.

The areas of lead-acetate paper blackened were practically identical in all of the tubes after 18 hours, and again on the second day of growth, after which no more blackening took place.

Using the quantitative method of hydrogen sulphide estimation, 18 hours was the period of growth chosen to demonstrate stimulation of the hydrogen sulphide production, and amounts of glucose varying from 2 per cent. to 0·0001 per cent. were added to the phosphate medium with the results shown in Table III and Chart II. In this experiment the titrateable acidity of the media, at the end of the period of growth, and after the distillation, was determined by neutralising with caustic soda, to the neutral point of phenolphthalein.

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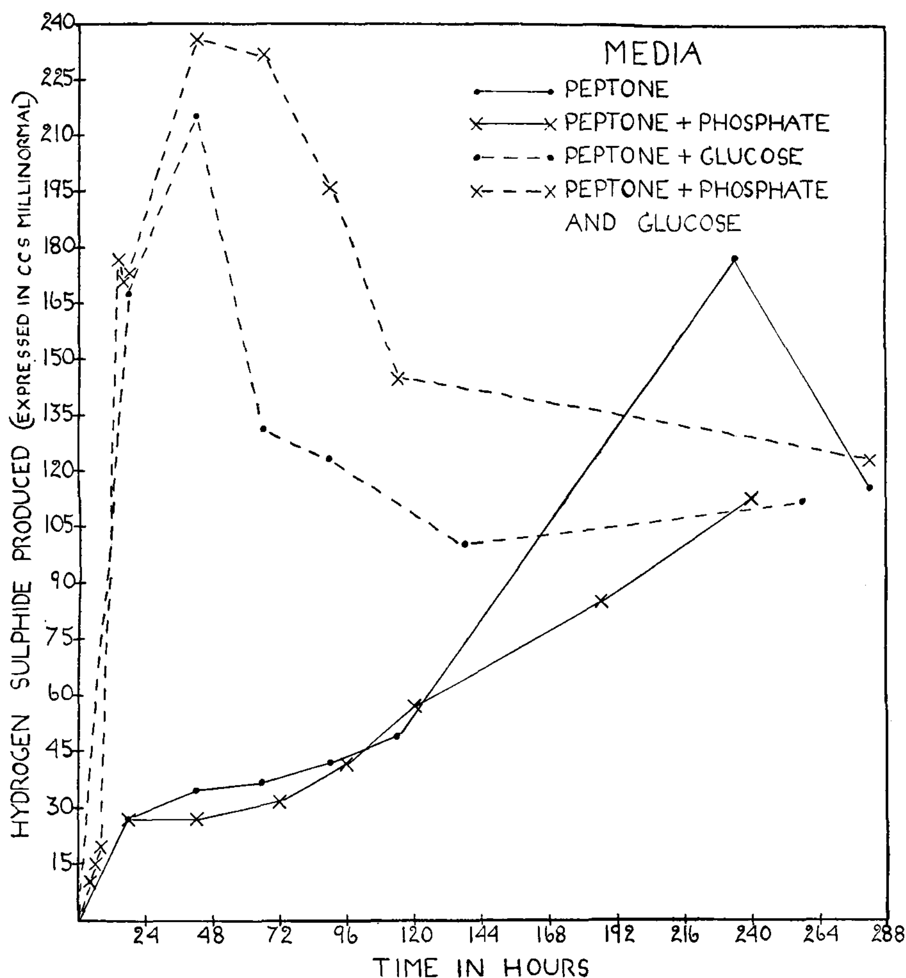

Chart I. Hydrogen sulphide production by *B. aertrycke* (M.) in the presence and absence of 2 % of glucose.

Table III. *Hydrogen sulphide produced in* 18 *hours by* B. aertrycke (*Mutton*) *in the presence of various amounts of glucose.*

% of glucose	c.c. $N/1000$ H_2S produced	Vol. N.NaOH to neutralise 250 c.c. media after CO_2 dist.
2·0	173·5 (*b*)	20·0
1·5	179·0	—
1·0	159·0	19·6
0·5	177·0	—
0·25	142·0	16·3
0·1	173·9 (*a*)	14·5
0·05	173·5	13·8
0·025	173·7	13·2
0·01	64·25	13·9
0·001	34·46	13·8
0·0001	29·3	12·0
0	29·3	11·8

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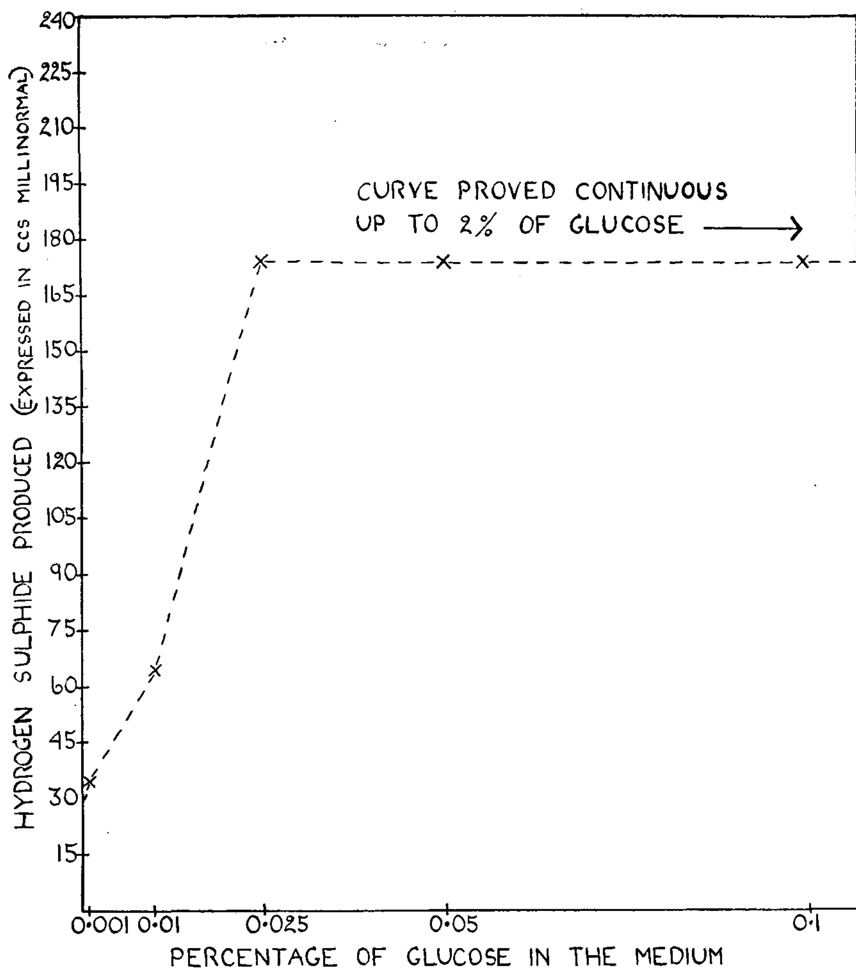

Chart II. Hydrogen sulphide produced in 18 hours by *B. aertrycke* (M.) in the presence of various amounts of glucose.

It will be seen that 0·025 per cent. of glucose proved to be sufficient to produce a maximum acceleration of the hydrogen sulphide production.

On turning to the literature we found a publication by Ayers and Mudge (1922) who, working on Vitamines, found that the stimulating effect of adding cabbage extracts to cultures of a streptococcus was due to the minute amounts of glucose present in it. They found 0·014 per cent. of glucose to have a stimulating effect on the growth (as measured by turbidity) of their streptococcus in Difco peptone medium.

Our last results led to two series of longer growth experiments using 0·05 per cent. glucose in the plain and phosphated peptone media.

A few experiments were gas-distilled with nitrogen instead of carbon dioxide, but this did not appear to affect the results, which are set out in Table IV and Chart III.

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Table IV. *Production of hydrogen sulphide by* B. aertrycke (*Mutton*) *in peptone solution containing* 0·05 *per cent. of glucose with and without added di-sodium hydrogen phosphate.*

Period of growth Hours	3 % peptone in 0·25 % NaCl solution together with: 0·05 % glucose: c.c. $N/1000$ H_2S	0·05 % glucose: Hydrion concentration	0·05 % glucose + 0·42 % phosphate: c.c. $N/1000$ H_2S	0·05 % glucose + 0·42 % phosphate: Hydrion concentration
18	137·2	6·8	173·5	7·0
42	174·3	—	200·0	—
66	189·0	8·2	215·8	7·6
90	175·2	8·4	191·8	7·9
114	172·5	8·3	202·6	8·0
162	166·5	8·6	162·4	8·2
234	170·7	8·6	203·6	8·2
Nitrogen distilled experiments				
18	—	—	201·8	—
162	—	—	167·0	—
234	—	—	238·5	—

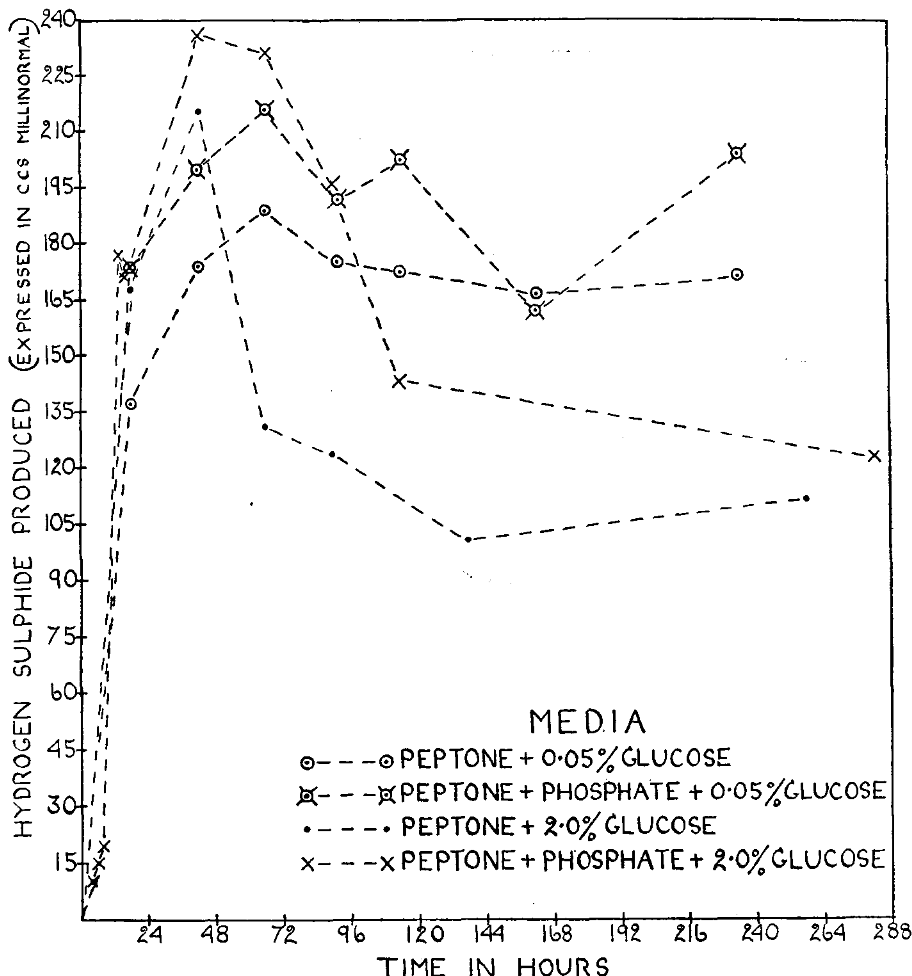

Chart III. The effect of 0·05 % and 2·0 % glucose on the hydrogen sulphide produced by *B. aertrycke* (M.) ± 0·42 % phosphate.

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From the later results in this experiment, which are high in H_2S value in cultures containing small amounts of glucose, it would appear that the disappearance of some of the free hydrogen sulphide from the longer 2 per cent. glucose cultures was due to the presence of comparatively large amounts of glucose decomposition products.

The early parts of the glucose curves being the most interesting, they were investigated by means of continuous gas distillation with nitrogen, as a continuous stream of CO_2 through the medium would have rendered it too acid for the growth of *B. aertrycke* (Mutton). The outlet of the culture flask was connected to a two-way tap so that the gases could be diverted to either of two series of iodine and starch towers, so that at intervals of the distillation, the towers could be renewed without interference with the gas flow.

The results obtained, using the 2 per cent. glucose + 0·42 per cent. phosphate medium, are shown in Table V.

Table V. *Hydrogen sulphide production by* B. aertrycke (*Mutton*) *in the presence of* 2 *per cent. of glucose, during continuous gas distillation.*

Period of growth	$N/1000$ H_2S c.c.	Rate of H_2S production per hour
6 hours	11·3	1·88
Between 6th and 12th hours	226·5	37·75
,, 12th and 18th hours	100·0	16·67
,, 18th and 24th hours	32·8	5·47
,, 24th and 48th hours	17·2	0·96

To eliminate the possibility that the increased hydrogen sulphide production in the presence of glucose is due to the formation of acid and its action on the peptone, we carried out two short series of experiments. One of these consisted in allowing growth to take place in the glucose media in the presence of limestone, so that the hydrogen-ion concentration of the culture was never below pH 7·2. The hydrogen sulphide formed in all cases reached its normal high figure in the usual time.

The other series of experiments consisted in incubating uninoculated media in the presence of varying amounts of acid. In no case did gas distillation reveal the presence of any hydrogen sulphide.

Working with a synthetic medium composed of:

Sodium chloride	5 grms.
Disodium hydrogen phosphate ...	4·2
Sodium citrate	6
N caustic soda to adjust reaction to pH	7·6
Distilled water	1000
Cystine	0·2

we found that glucose exerted a similar influence to that which it does in a glucose peptone medium.

In order to ascertain the number of viable organisms at different periods of growth, counts were made according to the method of G. S. Wilson (1922), the dilutions being made with calibrated dropping pipettes, and the colonies

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counted in roll-tubes. Table VI gives the results of a typical experiment, with the numbers and also the logarithms of the numbers of viable organisms in one c.c. of 3 per cent. peptone water + 0·25 per cent. sodium chloride. In 2 per cent. glucose media the viable count increases rapidly during the first 16 hours, whereas in non-glucose media the count steadily increases, but does not attain to such a high figure even in prolonged periods of growth.

Table VI. *Viable counts of* B. aertrycke (*Mutton*) *in the presence and absence of* 2 *per cent. glucose and* 0·42 *per cent. phosphate.*

	3 % peptone (Witte) in 0·25 % sodium chloride solution together with							
	No addition Viable count per c.c.		2 % glucose Viable count per c.c.		0·42 % phosphate Viable count per c.c.		2 % glucose + 0·42 % phosphate Viable count per c.c.	
Period of growth Hours	Actual	Log.	Actual	Log.	Actual	Log.	Actual	Log.
4	17,700	4·25	25,550	4·41	18,900	4·28	34,650	4·54
8	8,800,000	6·94	120,500,000	8·08	3,170,000	6·50	120,500,000	8·08
12	51,000,000	7·71	213,000,000	8·33	65,000,000	7·81	194,000,000	8·29
16	51,000,000	7·71	377,750,000	8·58	87,750,000	7·94	397,000,000	8·60
20	84,000,000	7·92	251,500,000	8·40	96,500,000	7·98	369,500,000	8·57
24	86,500,000	7·93	78,000,000	7·89	71,500,000	7·85	85,500,000	7·93
42	112,000,000	8·05	46,000,000	7·66	137,000,000	8·14	1,160	3·06
66	185,000,000	8·27	10,250	4·01	121,500,000	8·08	—	—
90	140,000,000	8·15	3,290	3·52	87,000,000	7·94	—	—
114	143,000,000	8·16	438	2·64	148,000,000	8·17	—	—
138	158,500,000	8·20	—	—	149,500,000	8·17	—	—

On varying the amounts of glucose as follows:

nil, 0·025 per cent., 0·05 per cent. and 2·0 per cent.

we found that 0·05 per cent. gave a maximum viable count in 66 hours, which slowly decreased. 0·025 per cent. glucose seemed to have very little effect on the count, despite the fact that it had a pronounced effect on the hydrogen sulphide production (see Table VII and Chart IV).

This suggests that the glucose is serving a purpose other than that of a source of energy.

These results led to a series of confirmatory experiments in which viable counts and hydrogen sulphide estimations were carried out on parallel cultures incubated together (see Table VIII).

Finally we made a series of amino-acid determinations by the Sörensen method of formol titration, and found, as previous workers have, that in the presence of 2 per cent. glucose, the amino-acid nitrogen figure thus obtained remained unchanged during growth. However, the presence of 0·025 per cent. glucose in the cultures only appeared to keep the figure stationary during the first 66 hours, after which time it began to slowly increase, just as it did during the whole growth period in peptone containing no glucose (see Table IX).

Having summarised the reaction of *B. aertrycke* (Mutton) in the presence of glucose, we next investigated its reaction with various other sugars; maltose, which is the only disaccharide fermented by it; xylose, which may be taken as typical of the three pentoses fermented by it; and sucrose, which it

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Table VII. *Viable counts of* B. aertrycke (*Mutton*) *in the presence of various amounts of glucose.*

Period of growth Hours	3 % peptone (Witte) in 0·25 % sodium chloride solution together with: No addition Viable count per c.c. Actual	Log.	0·025 % glucose Viable count per c.c. Actual	Log.	0·05 % glucose Viable count per c.c. Actual	Log.	2·0 % glucose Viable count per c.c. Actual	Log.
16	—	—	—	—	—	—	377,750,000	8·58
18	46,480,000	7·67	58,000,000	7·76	217,500,000	8·34	279,000,000	8·45
20	84,000,000	7·92	—	—	—	—	251,000,000	8·40
42	112,000,000	8·05	78,000,000	7·89	280,500,000	8·45	46,000,000	7·66
66	185,000,000	8·27	131,500,000	8·12	295,000,000	8·47	10,250	4·01
90	140,000,000	8·15	—	—	—	—	3,290	3·52
114	143,000,000	8·16	149,000,000	8·17	140,500,000	8·15	438	2·64
138	158,500,000	8·20	—	—	—	—	—	—
186	—	—	137,000,000	8·14	52,500,000	7·72	—	—

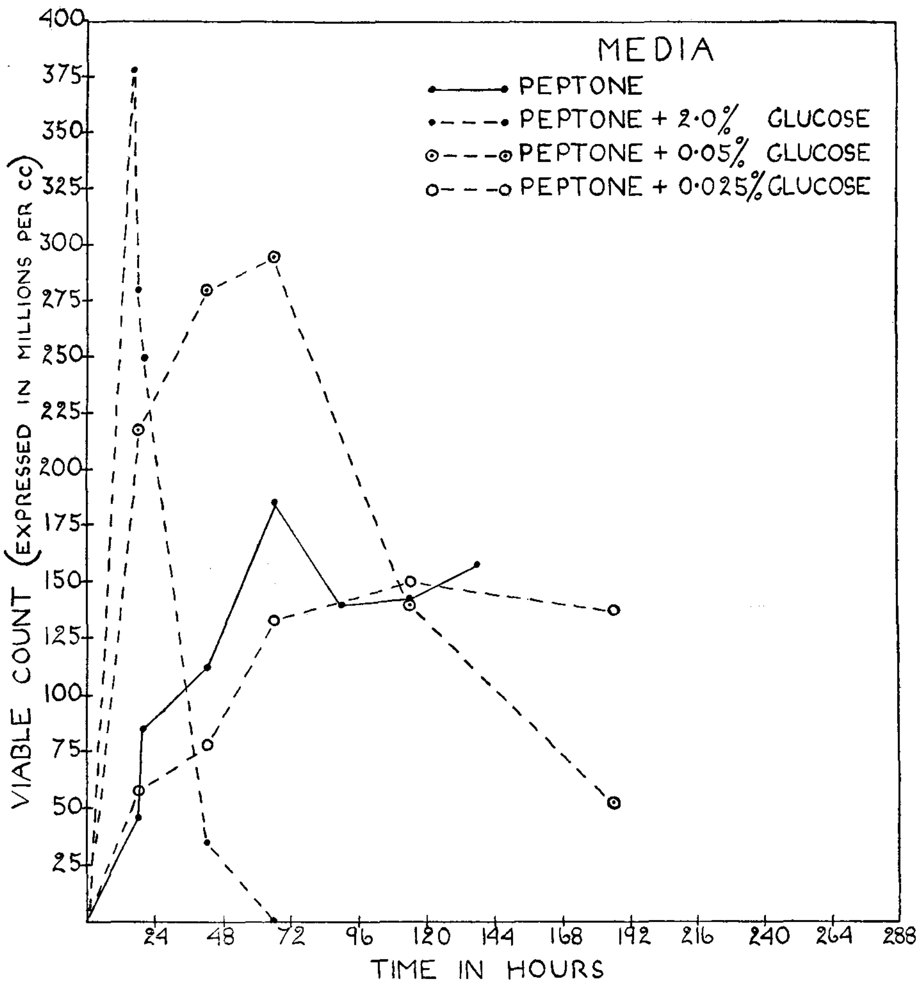

Chart IV. Viable counts of *B. aertrycke* (M.) in the presence of various amounts of glucose.

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Table VIII. *Viable counts of, and hydrogen sulphide production by,* B. aertrycke *(Mutton), in* 18 *hours in the presence of various amounts of glucose.*

Medium 3 % peptone (Witte) in 0·25 % NaCl solution together with	18 hours' growth: Viable count per c.c.	18 hours' growth: c.c. $N/1000$ H_2S
No addition	32,000,000*	—
	46,480,000	—
	55,000,000	27·1
0·42 % phosphate	32,000,000*	—
	55,000,000	—
	61,500,000	27·3
0·025 % glucose	32,000,000*	—
	58,000,000	—
	62,000,000	144·4
0·025 % glucose + 0·42 % phosphate	67,000,000*	162·5
	71,500,000	105·6
	73,000,000	164·7
0·05 % glucose	217,500,000	137·2
0·05 % glucose + 0·42 % phosphate	369,500,000	173·5
2·0 % glucose	279,000,000	170·5
2·0 % glucose + 0·42 % phosphate	322,600,000	173·5

* Incubated at 31° C.

Table IX. *Amino-acid nitrogen determinations in cultures of* B. aertrycke *(Mutton) containing no added glucose,* 2 *per cent. glucose, and* 0·025 *per cent. glucose.*

Period of growth	3 % peptone (Witte) in 0·25 % sodium chloride solution together with		
	0·42 % phosphate	2 % glucose + 0·42 % phosphate	0·025 % glucose
	Amino-acid nitrogen	Amino-acid nitrogen	Amino-acid nitrogen
Hours	mgm. per 250 c.c.	mgm. per 250 c.c.	mgm. per 250 c.c.
0	70·0	70·0	78·75
18	74·38	70·0	78·75
42	78·75	70·0	74·38
66	78·75	52·5	78·75
138	96·25	70·0	96·25
210	113·75	70·0	113·75
402	157·50	70·0	148·75

does not ferment. We were compelled to add the sugars in the dry state without sterilisation, as the trace of glucose inevitably produced by sterilising would have been sufficient to cloak all other reactions.

The large amount of hydrogen sulphide produced by cultures of *B. aertrycke* (Mutton) in 3 per cent. peptone water to which varying quantities of maltose had been added soon convinced us that the reaction in the presence of maltose was similar to the glucose reaction, though possibly the acceleration was slightly greater (see Table X).

Table X. *Hydrogen sulphide production by* B. aertrycke *(Mutton) in* 18 *hours in the presence of various percentages of maltose.*

% maltose	c.c. $N/1000$ H_2S
2·0	229·3
0·05	258·6
0·25	166·2
0·01	59·6

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With xylose the reaction proved slightly different, 2 per cent. of xylose gave a fairly high figure in 18 hours, but 0·05 per cent. and 0·1 per cent. showed a lag period, during which the xylose appeared to have no influence on the hydrogen sulphide production, and it was not until between the 42nd and 66th hours of growth that the hydrogen sulphide production appeared to be accelerated (see Table XI).

Table XI. *Hydrogen sulphide production by* B. aertrycke (*Mutton*) *in the presence of various percentages of xylose.*

Period of growth	0·05 %	0·1 %	2 %
18 hours	20·5 c.c.	23·23 c.c.	115 c.c.
42	38·0	27·0	
66	178·0	181·8	
234	129·6	238·7	

With sucrose we experienced great difficulty in obtaining a sample absolutely free from glucose.

We found that laboratory "pure" sucrose gave a maximum acceleration of the hydrogen sulphide production, though the amount of acid produced in the cultures was not sufficient to be detected by indication with Andrade reagent or phenol red; the hydrogen-ion concentration by colour comparison being the same as those of cultures containing no added sucrose.

The results led us to believe that the acceleration was due to the presence of traces of glucose, and to eliminate this we endeavoured to prepare pure sucrose by recrystallisation from absolute alcohol, finally obtaining a sample which did not accelerate the hydrogen sulphide production, though after a prolonged period it rose to a higher figure than we had obtained in the absence of added sugar (possibly this was due to the slow hydrolysis of the sucrose in alkaline solution) (see Table XII).

Table XII. *Hydrogen sulphide production by* B. aertrycke (*Mutton*) *in the presence of* 2 *per cent. pure sucrose.*

Period of growth	c.c. $N/1000$ H_2S
18 hours	12·0
42	23·5
66	47·0
234	202·0
354	235·0

Discussion.

The results obtained show that there is a considerable degree of parallelism between the influence of carbohydrates on bacterial multiplication and their influence on the production of hydrogen sulphide.

In peptone media, without glucose, bacterial multiplication passes through its well-known phases, and is succeeded by a balance between the generation-time and death-rate of bacteria, such that the viable count is maintained at a high figure over several days. This phase of equilibrium gradually gives place

https://doi.org/10.1017/S0022172400008482 Published online by Cambridge University Press

to a stage in which the death-rate of bacteria exceeds the multiplication-rate, so that the viable count decreases.

In the presence of glucose, in amounts which are relatively small, but which exceed a definite limit, bacterial multiplication is markedly accelerated in its earlier phases, but is soon retarded, and replaced by a rapidly increasing death-rate; so that the viable count decreases to a very low value, and the culture soon becomes sterile.

The rate of production of hydrogen sulphide varies in a very similar way, according to the presence or absence of a fermentable carbohydrate. In the presence of 2 per cent. glucose, for instance, the rate of production is markedly accelerated during the earlier phases of the experiment, but practically ceases after the 24th hour. In peptone media without glucose the rate of production of hydrogen sulphide is far slower during the earlier phases, but continues for a much longer period.

An obvious suggestion is that this correlation is the expression of a common underlying cause; that the glucose provides a ready source of energy for bacterial growth and activity, and that the consequent acceleration of bacterial growth and metabolism leads to increased production of hydrogen sulphide as the result of protein cleavage. We are, however, precluded from accepting this hypothesis, as affording any complete description of the course of events, by the results obtained in those experiments in which the amount of glucose in the medium was varied over a wide range. These results showed clearly that amounts of glucose, too small to influence the rate of bacterial multiplication, sufficed markedly to accelerate the rate of hydrogen sulphide production.

In plain peptone, both the hydrogen sulphide and the amino nitrogen steadily increase. This shows that both the nitrogen and the sulphur fractions of the protein complex are suffering degradation. The increase in the amino nitrogen may be due to the fact that the production is in excess of the amount utilisable, or to the fact that it is an end-product of bacterial metabolism under these conditions, in which case an increase in the amino nitrogen will indicate increased proteolysis proportionately to the amount of amino nitrogen found.

In the presence of glucose the production of hydrogen sulphide is increased, whilst the amino nitrogen remains almost stationary in amount. This may be due to increased utilisation of amino nitrogen, thus indicating increased proteolysis along this line of cleavage, or it may be that the reaction as regards nitrogen splitting is decreasing at the expense of an accelerated decomposition of the sulphur-containing fraction of the protein.

With very small amounts of glucose present, hydrogen sulphide is still formed rapidly and in large amount, whilst the amino nitrogen is stationary as determined by the Sörensen method, but if the amount of glucose be still further decreased a position is reached where a fall in hydrogen sulphide production is associated with an increase in the amino nitrogen figures. From this it seems that the glucose stimulates the attack on the sulphur fraction of the protein, and so causes a diversion of the original reaction.

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Thus it seems that in a plain peptone medium both the nitrogen and sulphur fraction of the complex are attacked slowly, but on addition of glucose the sulphur complex is more vigorously attacked; so that, as measured by the degradation of the sulphur complex, the addition of glucose appears to increase proteolysis. Whether the actual rate of proteolysis is influenced by the presence of carbohydrates, cannot be determined from these results.

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(*MS. received for publication* 15. v. 1924.—Ed.)

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