# XLIX. DECOMPOSITION OF HEXOSEPHOSPHATES BY *B. COLI COMMUNIS*, ESCHERICH.

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Both hexosemonophosphoric and hexosediphosphoric acids are decomposed by *B. coli communis*, Esch. with production of acid and gas. The following experiments were made to ascertain whether the products of decomposition of these acids differed in any marked way from those formed by the action of the same organism on glucose and fructose. The barium salts of the acids were prepared by the method described by Robison [1922].

On account of the toxicity of the barium salts, as well as of the slight solubility of the barium hexosediphosphate, the sodium salts were formed and used in the experiments. These were decomposed by *B. coli communis* in atmospheres of oxygen and of nitrogen, respectively, at 37° for periods of seven to ten days and the resultant liquids then analysed. The products of decomposition were found to be carbon dioxide, alcohol, formic, acetic, lactic and succinic acids, whether the decomposition took place in presence or absence of oxygen[1], this being in agreement with the observations of Grey, when decomposing glucose by *B. coli communis* [1919, 1920; Grey and Young, 1921].

The more rapid the bubbling of oxygen through the bacterial digest the greater was the amount of the sugar decomposed completely to carbon dioxide. In the anaerobic digest the amounts of alcohol and of all the acids were markedly greater than in the aerobic, the carbon dioxide being correspondingly less.

### *Purification of barium hexosediphosphate.*

256 g. of crude barium hexosediphosphate were carefully triturated with 2560 cc. water and filtered. The residue was again triturated twice in succession very carefully with 400 cc. water, filtered and the combined filtrates were used for the preparation of the barium hexosemonophosphate. The residue containing insoluble inorganic phosphates and barium hexosediphosphate was now ground up carefully over a period of two hours with 100 parts of water and from this solution by twice precipitating with lead acetate as described by Robison [1922] the purified barium hexosediphosphate used in the experiments was finally obtained.

[1] No estimation of gaseous hydrogen was made.

*Method of experiment.*

Ten grams of barium hexosediphosphate were ground up with 4·7 g. of sodium sulphate in 250 cc. water. The barium sulphate was filtered off and washed with 50 cc. water. The combined washings and filtrate were now sterilised by filtration through a Chamberland candle and to the solution of sodium hexosediphosphate thus obtained the bacterial suspension was added. To prepare this 2 cc. of a 24-hour bouillon culture of *B. coli* were added to each of ten Roux bottles and incubated for two days. The bacteria were then washed off by using 500 cc. of a sterile solution containing 6 g. potassium sulphate and 0·5 g. magnesium sulphate per litre. Filtered oxygen was bubbled slowly into the liquid contained in a 1500 cc. flask to which was connected an absorption tube containing alkali for the retention of carbon dioxide, and the whole kept at 37° for ten days. At the end of that time washed air was bubbled through vigorously for four hours and the analysis carried through as subsequently described.

In the case of the anaerobic fermentation of the sodium hexosediphosphate, nitrogen was bubbled through the liquid in the digestion flask for two hours before the carbon dioxide absorbing apparatus was attached. The outlet of this was placed below mercury and the whole left in a warm room for one week. At the end of the fermentation period nitrogen was passed through the apparatus for four hours with the delivery tube of the nitrogen well below the surface of the liquid.

*Analytical methods.*

*Carbon dioxide.* In determining the carbon dioxide formed, the amount absorbed by the alkali in the absorbing apparatus was determined by double titration, using methyl orange and phenolphthalein. Some carbon dioxide was at times retained in the combined form in the decomposition mixture as carbonate or bicarbonate; this residual carbon dioxide was determined by taking a portion of the bacterial digest, strongly acidifying with sulphuric acid and aspirating for a couple of hours into alkali.

For the determination of the alcohol and volatile acids, formic and acetic, about 500 cc. of the bacterial digest were acidified with 10 cc. phosphoric acid and distilled with steam. The volatile acids passed over quite slowly so that it was necessary to distil as much as two or three litres, titrating each successive portion of 250 cc. until a small but almost constant titre was obtained, due doubtless to the lactic acid that continued to come over in minute quantities. The separation of the volatile and non-volatile acids was therefore not very accurate.

*Alcohol.* Part of the neutralised distillate was used for the determination of the alcohol; 50 cc. were usually taken, 25 cc. distilled off and the alcohol was estimated in the distillate by the method of Pringsheim [1908]. Care was taken to keep the final volume of the sample oxidised by the dichromate constant and equal to that used in standardising the dichromate, otherwise

the readings were quite irregular. A dilution of 110 cc. was used throughout and at that dilution 1 cc. $N/20$ dichromate was found to oxidise 0·5919 mg. alcohol. As traces of alcohol were sometimes retained by the barium hexose-diphosphate and barium monophosphate, the alcohol content of these was estimated by dissolving the barium salts in water or decomposing them by means of sodium sulphate, filtering, distilling and estimating the alcohol in the distillate. A little alcohol was sometimes carried over from the digest into the carbon dioxide absorbing apparatus and this also had to be estimated.

*Volatile acids.* The total neutralised steam distillate was evaporated to dryness on a water-bath and extracted with water and filtered from various wax-like substances invariably present. The formic acid was estimated in this extract by the method of Blank and Finkenbeiner [1898] and the acetic acid by difference from the total titre.

*Succinic and lactic acids.* These were estimated by the method described by Grey [1917].

*Carbohydrate.* Any residual reducing sugar left at the end of the bacterial digest was estimated by precipitating the protein from a sample of the digest with a slight excess of Patein's mercuric nitrate solution, and using Bertrand's method to determine the hexose in the clear filtrate. Usually no residual carbohydrate was found in aerobic fermentation unless the oxygen supply was limited, but under anaerobic conditions as much as 10 % of the hexose was still unfermented, although the hydrolysis of the sodium hexose-diphosphate had been nearly complete. The carbohydrate was found not to be a free hexose for the most part but rather a non-reducing polysaccharide, which was estimated by taking 20 cc. of the filtrate, acidifying with 4 cc. dilute sulphuric acid, heating in a boiling water-bath for 1–2 hours, neutralising, and then determining the hexose by Bertrand's method.

*Inorganic and organic phosphorus.* The phosphorus present both as free phosphate and combined in the hexosephosphates was determined by Briggs' modification of the Bell-Doisy method [1922]. This was best done by adding 5 cc. of an 8 % solution of trichloroacetic acid to 10 cc. of the digest and filtering. 10 cc. of the clear filtrate were diluted to 100 cc. and this solution was used for inorganic and organic phosphorus determinations.

During the process of bacterial digestion both aerobically and anaerobically, practically all the hexosephosphates were decomposed so that only a small amount of the phosphorus was found not to be present as inorganic phosphate. From the increase in the inorganic phosphate, the amount of the hexosephosphate that had been decomposed was calculated and from this the carbon for the carbon balance.

*Products of decomposition of sodium hexosediphosphate by* B. coli.

The amounts of the various substances formed by aerobic and anaerobic decomposition were as follows:

Table I.

| | Aerobic decomposition | | Anaerobic decomposition | |
|---|---|---|---|---|
| | Weight g. | C atoms per mol. of sugar fermented | Weight g. | C atoms per mol. of sugar fermented |
| Carbon dioxide ... | 3·1930 | 5·62 | 0·5632 | 1·26 |
| Alcohol ... ... | 0·0900 | 0·30 | 0·2393 | 1·03 |
| Acetic acid ... ... | 0·0579 | 0·15 | 0·5860 | 1·92 |
| Formic acid ... ... | 0·0021 | 0·003 | 0·1352 | 0·29 |
| Lactic acid ... ... | 0·0218 | 0·06 | 0·0478 | 0·16 |
| Succinic acid ... ... | 0·0102 | 0·03 | 0·3960 | 1·32 |
| Residual carbohydrate | — | — | 0·3200 | — |
| | 3·3750 | 6·163 | 2·2875 | 5·98 |
| Original carbohydrate | 2·3230 | — | 2·1460 | — |

*Preparation of barium hexosemonophosphate.*

The combined filtrates from the extraction of the crude barium hexosediphosphate were united and treated with a solution of basic lead acetate until precipitation was complete. The lead was removed by suspending the precipitated lead salts in water and treating with hydrogen sulphide. To the filtrate from the lead sulphide, hot baryta and an equal volume of alcohol were added to precipitate the crude barium hexosemonophosphate.

To purify the barium hexosemonophosphate it was extracted with 10 % alcohol, filtered, and reprecipitated with an equal volume of alcohol, washed frequently with absolute alcohol and dried *in vacuo*. On analysis of 1 g. of this product, which dissolves quite easily in water, giving a clear solution, its glucose equivalent was found to be 0·2720 g. There was no trace of inorganic phosphate and the combined phosphorus was 0·0709 g. (calculated 0·0785).

*Decomposition of sodium hexosemonophosphate by* B. coli.

This partially purified product was converted into sodium salt and subjected to the action of the *B. coli* under aerobic and anaerobic conditions in the way employed in the experiments with the hexosediphosphate. For the aerobic decomposition 9 g. of barium hexosemonophosphate were employed and for the anaerobic decomposition 6 g.

Table II.

| | Aerobic decomposition. Weight (g.) per 1 g. of Ba salt used | Anaerobic decomposition. Weight (g.) per 1 g. of Ba salt used |
|---|---|---|
| Carbon dioxide ... ... | 0·184 | 0·117 |
| Alcohol ... ... ... | 0·028 | 0·052 |
| Acetic acid ... ... | 0·152 | 0·085 |
| Formic acid ... ... | 0·005 | 0·009 |
| Lactic acid ... ... | 0·004 | 0·056 |
| Succinic acid ... ... | 0·002 | 0·038 |
| Residual carbohydrate ... | 0·019 | 0·024 |

In each case there was some residual carbohydrate unfermented and a small amount of phosphorus still in organic combination.

From the above tables it appears that both with the mono- and the di-phosphates oxygenation results in a marked decrease in the formation of the acids as well as of the alcohol, and a corresponding increase in carbon dioxide.

The high acetic acid content of the products of aerobic fermentation of sodium hexosemonophosphate would probably have been markedly diminished and the carbon dioxide formed increased if the oxygen supply had been more rapid. Acetic acid may possibly be one of the last stages in the decomposition of hexose by *B. coli*. In this regard the results are not similar to those found by Grey for the action of *B. coli* on glucose, who states that in this case the effect of introducing oxygen in the fermentation is to increase the lactic, acetic and succinic acids and to diminish the hydrogen, carbon dioxide and formic acid but to leave the alcohol unchanged. On the other hand the decomposition under anaerobic conditions is of the same character as that of the free sugars.

REFERENCES.

Blank and Finkenbeiner (1898). *Ber. deutsch. chem. Ges.* **31**, 2980.
Briggs (1922). *J. Biol. Chem.* **53**, 13.
Grey (1917). *Biochem. J.* **11**, 134.
—— (1919). *Proc. Roy. Soc. Lond.* B, **90**, 75, 92.
—— (1920). *Proc. Roy. Soc. Lond.* B, **91**, 294.
Grey and Young (1921). *Proc. Roy. Soc. Lond.* B, **92**, 135.
Pringsheim (1908). *Biochem. Z.* **12**, 155.
Robison (1922). *Biochem. J.* **16**, 809.