XL.—*The Bacterial Decomposition of Formic Acid into Carbon Dioxide and Hydrogen.*

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THE action of the lower organisms on chemical substances has been frequently investigated, in order, if possible, to discover a case of bacterial decomposition of a chemically simple nature, which might serve to elucidate the action of bacteria on organic compounds in general; that this end has so rarely been obtained we are inclined to attribute to the fact that comparatively complex substances, such as the sugars, have hitherto formed the main subjects of investigation. We therefore instituted a search for compounds of very simple molecular composition which should be attacked by the commoner organisms, and have succeeded in proving definitely that various bacteria in pure culture decompose formic acid directly into equal volumes of carbon dioxide and hydrogen.

Hoppe-Seyler observed (*Zeit. physiol. Chem.*, 1887, **11**, 566) that on bacterially infecting a calcium formate solution with river mud, equal volumes of carbon dioxide and hydrogen were evolved. At a later date, P. F. Frankland and his pupils independently concluded (Trans., 1892, **61**, 270, 432, 737) that a similar decomposition occurs as one stage during the action of the *Bacillus ethaceticus* and Friedländer's *Pneumobacillus* on mannitol.

Since the *Bacillus coli communis* and others of the bacteria with which we have been working are frequently found in river mud, our present results are in a measure confirmatory of those of Hoppe-Seyler.

Kitasato has observed that the addition of sodium formate to media containing *d*-glucose materially assists the growth of certain bacteria under anaerobic conditions; he, however, gives no details as to the nature of the gaseous products. We have confirmed his results

and made the further observation that the gases produced from media containing *d*-glucose and sodium formate differ both in volume and composition from those evolved from a medium differing only from the former in that it contains no sodium formate.

The gases produced by the action of the *Bacillus coli communis* upon *d*-glucose in a solution of meat extractives containing peptone have been examined by Th. Smith, Chantemesse, Lehmann and Neumann, and others, who agree that the constituents are carbon dioxide, hydrogen, and nitrogen, but differ widely as to the value of the ratio $CO_2 : H_2$. The proportion of carbon dioxide in the mixture is variously stated to be from 25 to 50 per cent. by volume.

On analysing the gases produced from *d*-glucose, using the method of collection and analysis previously described (Pakes and Jollyman, this vol., p. 322), we find that the amount of gas evolved per 100 c.c. of medium depends upon the particular organism dealt with. The medium ("M") used for the following experiments was made by extracting lean meat with ordinary tap water, coagulating the proteid by heat, and filtering; 1 per cent. of Witte's peptone and 0·5 per cent. of sodium chloride are subsequently added. After addition of the other constituents mentioned in each series of experiments, the whole is heated in the steamer and neutralised with *N* caustic soda.

Series I. "M" with 2 per cent. of *d*-glucose.

Organism.	Total gas evolved from 100 c.c. of medium.	Percentage volume of carbon dioxide.
B. coli communis	32·8 c.c.	31·5 per cent.
B. enteritidis of Gärtner	68·6	35·4 ,,
Pneumobacillus of Friedländer	10·9	22·2 ,,
B. maligni œdematis	36·4	40·0 ,,

The medium "M," alone, yields but a small quantity of gas when planted with these organisms; thus the *B. coli communis* gave 5·7 c.c. of gas per 100 c.c. of medium and this contained 17·8 per cent. of carbon dioxide, 58·2 per cent. of hydrogen, and 23·9 per cent. of nitrogen.

The addition of sodium formate to the medium "M" containing 2 per cent. of *d*-glucose gave the following results:

Series II. "M" with *d*-glucose and sodium formate.

(i) October 15th.—Four hundred and ninety c.c. of the medium "M" containing 2 per cent. of *d*-glucose and 0·5 per cent. of sodium formate were inoculated with the *B. coli communis*, attached to the gas receiver of the apparatus already described (*loc. cit.*) and incubated under strictly anaërobic conditions. The gas evolved was drawn off day by day and analysed, with the following results:

Oct.	16th.	Gas produced	390 c.c.	containing	34·2 per cent. CO_2.
,,	17th.	,,	298	,,	— ,,
,,	18th.	,,	399	,,	48·8 ,,
,,	19th.	,,	146	,,	56·0 ,,
	20th.	,,	169·6	,,	57·2 ,,

e residual gas was found to be hydrogen with a daily decreasing quantity of nitrogen. After the termination of the experiment the medium was very acid to litmus and therefore all the carbon dioxide had been evolved as gas.

(ii) November 5th.—A flask containing 425 c.c. of the same medium containing *d*-glucose and 0·4 per cent. of sodium formate was inoculated with the *B. coli communis* and joined to the gas receiver.

Nov.	6th.	Gas produced	473 c.c.	containing	36·4 per cent. CO_2.
,,	7th.	,,	428	,,	44·0 ,,
,,	8th.	,,	292	,,	54·0 ,,
,,	9th.	,,	226·8	,,	57·3 ,,
,,	10th.	,,	97·9	,,	56·4 ,,
,,	12th.	,,	92·0	,,	58·0 ,,
,,	21st.	,,	65·0	,,	52·3 ,,

As before, the residual gas was hydrogen and a small amount of nitrogen. At the end of the experiment, the medium was very acid to litmus.

Similar results were obtained when the same medium was inoculated with the *B. enteritidis* of Gärtner, the *Pneumobacillus* of Friedländer, and the *Bacillus* of malignant œdema.

The increasing percentages of carbon dioxide in the evolved gases led to the conclusion that a certain amount of alkali was produced—probably from the formate—which neutralised the acids produced by the bacteria from the sugar. This neutralisation allowed the bacteria to attack more of the sugar and so to produce more gas. Similar results can be obtained by placing about 1 per cent. of calcium carbonate in the medium.

In order to determine whether the alkali was derived from the formate, we omitted the *d*-glucose from the medium and substituted 1 per cent. of sodium formate. This was placed in test-tubes which were severally inoculated with the above-named bacteria and incubated. At the end of 24 hours, there was a definite froth upon the surface, and at the end of 48 hours the medium was distinctly alkaline.

Many experiments have been performed with this medium, but the following will serve as an example.

Series III. "M" with sodium formate.

October 22nd.—A flask containing 490 c.c. of meat extract in water

with 1 per cent. of sodium formate, and neutralised, was inoculated with the *B. enteritidis* of Gärtner and connected to the gas receiver.

Oct.	23rd.	Gas produced	66·8	c.c. containing	12·0	per cent. CO_2.
,,	24th.	,,	104·4	,,	19·9	,,
,,	,,	,,	97·8	,,	20·8	,,
,,	25th.	,,	121·0	,,	21·3	,,
,,	26th.	,,	101·0	,,	21·1	,,
,,	27th.	,,	44·4	,,	21·8	,,
,,	28th.	,,	27·0	,,	20·0	,,
Total (exclusive of 1st)			495·6	Average	20·7	,,

The fact that the percentage of carbon dioxide remained so constant throughout the experiment suggested that the formate was decomposed in one stage, no secondary reactions occurring, and although it was impossible to determine the alkalinity of the medium during the progress of the experiment, we determined subsequently that it does increase day by day until no further gas is evolved.

In order to determine the amount of carbon dioxide retained in the medium as bicarbonate, the flask was first carefully disconnected from the receiver. A second receiver, full of mercury, was then connected to a small flask fitted with a rubber cork carrying a piece of glass tubing bent at right angles and a thistle tube descending to the bottom of the flask. By means of a pipette, 20 c.c. of the medium were transferred to the small flask, the cork replaced, and an excess of pure sulphuric acid was run into the fluid through the funnel. The contents of the flask were then well boiled and all the air and gas driven over into the receiver; while the contents of the flask were still boiling, the tap of the receiver was turned and the flask quickly disconnected. The gas in the receiver was allowed to cool and the carbon dioxide present determined. The determination was several times repeated and the average result found to be that each 20 c.c. of medium retained 14·3 c.c. of carbon dioxide.

There were therefore 350·3 c.c. of carbon dioxide in the medium and 111·3 c.c. evolved as gas, making a total of 461·6 c.c. Control experiments were made with the medium before inoculation, but no trace of carbon dioxide could be found. This gas can therefore only have been produced from the sodium formate.

The total amount of hydrogen produced was found to be 451·1 c.c.

Within the limit of experimental error, the ratio $CO_2 : H_2$ is therefore 1 : 1.

This leads us to the conclusion that these bacteria decompose sodium formate according to the equation:

$$H \cdot CO_2Na + H_2O = NaHCO_3 + H_2$$

or that the formic acid is decomposed thus :

$$H \cdot CO_2H = H_2 + CO_2.$$

This decomposition of formic acid is thus similar to that induced by platinum black or powdered ruthenium, iridium, or rhodium.

The use of such a medium as meat extract is open to the objection that it itself yields a small amount of gas. In subsequent experiments, we have therefore substituted for this medium a 2 per cent. solution of peptone in water. This, when inoculated with the various bacteria mentioned above, yields no trace of gas.

On inoculating the 2 per cent. solution of peptone after adding 0·5 per cent. of sodium chloride and varying percentages of sodium formate, vigorous evolution of gas occurs and the analytical examination of the gases produced gave results exactly similar to those described above in detail.

In estimating the amount of formate decomposed we have been unable to find any satisfactory method of determining the amount of formate present in the medium, we have therefore calculated the amount decomposed from the volume of hydrogen evolved. From this it seems that never more than from 25—30 per cent. of the formate present is decomposed: this certainly applies to proportions of from 0·2 to 2·0 per cent. of formate. Many of the bacteria will grow in media containing as much as 5 or even 10 per cent. of formate, but we have as yet made no estimations of the gases produced under these conditions.

Since the power of decomposing sodium formate might possibly serve as a useful means of diagnosis in the identification of bacteria, we have made some preliminary observations with a few of the better known organisms.

The following bacteria have given positive results :

The *B. coli communis* (80 different strains), *B. enteritidis* of Gärtner (2 strains), *Pneumobacillus* of Friedländer (2 strains), *B. lactis aerogenes* (2 strains), *B. cloacæ*, *B. choleræ gallinarum*, *Proteus vulgaris*, *B. prodigiosus*, and *B. rouge de Kiel.*

The following have failed to decompose the sodium formate :

B. typhi abdominalis (11 strains), *B. rhinoscleromæ*, *B. pyocyaneus* (2 strains), *B. denitrificans*, *B. anthracis* (2 strains), *B. subtilis*, *B. mesentericus vulgatus*, *B. mycoides*, *B. ramosus*, *B. arborescens*, *B. fluorescens non liquefaciens*, *B. janinthus*, *B. megatherium*, *B. buccalis maximus* *B. choleræ suis*, *Vibrio Metschnikovi*, *V. choleræ*, *Vibrio* of Finkler and Prior, *Vibrio* of Deneke, *Spirillum rubrum*, *Micrococcus cereus flavus*, *M. luteus*, *Staphylococcus aureus* (2 strains), *Staph. citreus*, *Staph. albus*, *Sarcina lutea*, *S. aurantia*, and all the common *Saccharomycetes.*

It will be observed that all the bacteria mentioned above which

produce gas from sodium formate also produce it from *d*-glucose, whilst those which do not produce gas from sodium formate do not produce it from *d*-glucose; the yeasts, however, it is important to note, do not produce gas from the formate, although they readily decompose *d*-glucose.

We are extending this work on the action of bacteria to other substances of a similar nature, and hope shortly to publish the results obtained with acetic and oxalic acids.