XXXVI. THE PRODUCTION OF ACETALDEHYDE DURING THE ANAEROBIC FERMENTATION OF GLUCOSE BY *BACILLUS COLI COMMUNIS* (ESCHERICH).

By EGERTON CHARLES GREY, 1851 *Exhibition Scholar*.

From the Biochemical Laboratory of the Lister Institute.

(Received June 8th, 1913.)

Acetaldehyde has long been known as a product of alcoholic fermentation by yeast [see Roeser, 1893]. It has likewise been found to be formed by the leaves of higher plants when their metabolism is restricted to anaerobic conditions [Kostytschew, 1913].

In view of this wide distribution, it was to be expected that acetaldehyde would be a product of bacterial fermentation. This is the case.

The quantity of acetaldehyde found by Roeser in various wine musts after a fermentation lasting from five to fifteen days, varied from 20–200 milligrams per litre of the fermented fluid. In the case of *B. coli communis* acting on glucose, one litre of fluid containing originally 15 grams of the sugar yielded in 15 days when half the sugar had been fermented 2.34 mg. of acetaldehyde, while, at the same time, 1.375 grams of alcohol had been produced. Since in the case of the true alcoholic fermentation quoted above, the quantity of alcohol formed was certainly twenty times that produced in the experiment with *B. coli communis*, it will be seen that the ratio of acetaldehyde to alcohol is of the same order in the two cases.

It is not at present settled, in the case of yeast fermentation, whether acetaldehyde is a primary product or whether it results from the oxidation of preformed alcohol, the following result is therefore significant in showing that the production of acetaldehyde by *B. coli communis* is related to the formation of alcohol, carbon dioxide, and acetic acid, rather than to the other products.

The experiment consisted in comparing the production of acetaldehyde from normal *B. coli communis* (Escherich) with that from a strain derived from the normal organism by selection with sodium chloracetate [see Penfold, 1911, and Harden and Penfold, 1912]. This derived strain had completely

lost the power of producing gas from carbohydrates. That it was closely related to the original organism was demonstrated by testing the agglutinating power of both the original and the derived organism towards a rabbit serum obtained after inoculating with the former. Both organisms agglutinated completely with the serum up to a dilution of one part of the serum in 25,600 of normal saline solution.

REACTIONS EMPLOYED FOR THE DETECTION AND DETERMINATION OF ACETALDEHYDE.

(1) *Rimini's reaction* [1898].

A few drops of diethylamine are added to a similar quantity of a solution of sodium nitroprusside. A blue colour appears which rapidly fades (2-3 minutes). If this solution be now diluted with 1-2 c.c. of water a pale yellow or yellow-green solution is obtained but if diluted with 1-2 c.c. of a solution containing acetaldehyde a blue colour results characteristic of this aldehyde. Though specific for acetaldehyde the reagent is not so delicate as that of Schiff, and the coloration is moreover less permanent.

(2) *Schiff's reagent*. This reagent was used for the determination of acetaldehyde.

It is important to make sure that no alkali is present in the fluid to be tested by Schiff's reagent since even the alkalinity of tap water produces a definite coloration. The maximum intensity of colour, by simple addition of alkali, was obtained with 0.1 c.c. normal KHO, added to 50 c.c. distilled water containing 0.25 c.c. Schiff's reagent. A greater concentration of alkali destroys the colour, which is regained on the addition of acid. The solution before examination was therefore always tested with phenolphthalein paper.

Acetone is said to give a coloration with Schiff's reagent, but this effect which is not produced for many hours cannot possibly be confused with the reaction for acetaldehyde in which case the coloration reaches a maximum in about twenty minutes and has very considerably faded in less than an hour. It is important to emphasise this fact, since Mendel [1911] states that he found acetone amongst the products of the action of *B. coli communis* and certain other organisms on glucose.

DETAILS OF THE DETERMINATION OF ACETALDEHYDE.

(1) About 750 c.c. of the fluid containing the liquid and solid products of fermentation (acetaldehyde, alcohol, and calcium salts of volatile and non-volatile acids etc.) was treated with oxalic acid in excess of that necessary for

the complete precipitation of the calcium. When the precipitate of calcium oxalate had settled, as much as possible of the supernatant liquor was removed with the aid of a siphon, and distilled. The first 400–500 c.c. of the distillate was made alkaline with barium hydroxide and redistilled, using a fractionating column, until 100 c.c. had been collected. In both distillations the receivers were well cooled by means of ice.

50 c.c. of this solution were used for the determination of the acetaldehyde, the method of procedure being an adaptation of that of Ryffel [1909].

(2) A standard solution of formaldehyde was prepared as described by Ryffel. The strength of this solution was determined by comparing the colour produced from it on the addition of Schiff's reagent, with that produced on adding the same quantity of the reagent to a solution of acetaldehyde prepared by the distillation of a known weight of lactic acid.

0.02494 gram lactic acid was converted into acetaldehyde. The intensity of colour produced on adding 0.5 c.c. Schiff's reagent to 100 c.c. of this acetaldehyde solution was 1.9 times that of the standard formaldehyde colour as measured by a Duboseq tintometer, 100 c.c. of the standard formaldehyde solution was therefore equivalent to $\frac{0.02494}{1.9} = 0.01312$ gram lactic acid.

Applying the ratio determined by Ryffel empirically for these conditions, viz.: 0.4 mg. formaldehyde = 3.435 mg. lactic acid = 1.765 mg. acetaldehyde, it follows that 100 c.c. of standard formaldehyde solution contains $\frac{0.4 \times 0.01312}{3.435} = 1.528$ mg. formaldehyde, and is equivalent to $\frac{1.528 \times 1.765}{0.4} = 6.471$ mg. acetaldehyde.

(3) 50 c.c. of the solution of formaldehyde described in paragraph (2) was diluted fivefold and compared with the distillate described paragraph (1), with the result:

$$\text{The ratio of } \frac{\text{standard}}{5 \times \text{distillate}} = \frac{1}{1.33}.$$

From this it was calculated that the total amount of acetaldehyde in the original litre of fermented fluid was 2.34 mg.

DISCUSSION OF RESULTS.

In order to demonstrate the relationship between the production of acetaldehyde and that of alcohol, carbon dioxide, hydrogen and acetic acid, the analyses of the products formed by the normal and the artificially selected strain are compared below, the results being calculated to 100 grams of the sugar.

	Products from glucose	
	Normal <i>B. coli communis</i> per cent.	Strain artificially selected by the chloracetate method per cent.
Hydrogen	0.42	Nil
Carbon dioxide	16.9	Nil
Alcohol	18.1	5.3
Acetic acid	18.5	10.8
Formic acid	9.7	11.1
Lactic acid	36.8	68.0
Succinic acid	0.7	0.8
Acetaldehyde	31.345 mg.	Nil
{ Glucose decomposed	7.465 gram	7.755
{ Acetaldehyde found	2.34 mg.	Nil

It will be seen that the artificially selected organism has (as in the case of the organism examined by Harden and Penfold), besides having lost the power of producing gas, also produced less alcohol and acetic acid. Coincidentally with these changes there has been an abolition or great reduction of the yield of acetaldehyde.

In the light of these results we may consider the question of the origin of the acetaldehyde.

If this substance were a secondary product formed by the oxidation of alcohol through the activity of oxidases concerned in the cell-growth as was suggested by Roeser for the case of yeast, then since there is a large excess of alcohol in both cases, the amount of acetaldehyde formed in the two experiments should be approximately the same, or at least, of the same order. Moreover in view of the fact that not only was air excluded from the fermentation flasks, but that in the experiment in which acetaldehyde was produced, 350 c.c. of hydrogen had been evolved, such an oxidation would seem unlikely to have occurred. The strongly reducing conditions of the experiment may be well seen from the experiment in which Harden attempted to employ asparagine as a source of nitrogen. This substance, he found, was completely reduced to ammonium succinate with corresponding diminution in the hydrogen evolved.

It may be suggested therefore that the production of acetaldehyde and part of the alcohol occur simultaneously. This would agree with the view held by Kostytschew [1912] that acetaldehyde is an intermediate product in alcoholic fermentation and would moreover suggest that part of the alcohol produced by the action of *B. coli communis* on glucose, passes through at least one of the same stages as that produced by the zymase of yeast.

From a consideration of the structure of glucose and mannitol, and the

fact that *B. coli communis* produces about twice as much alcohol from the latter as from the former, Harden [1901] suggested that the terminal group $-\text{CH}(\text{OH})\cdot\text{CH}_2\text{OH}$ which occurs twice in mannitol though only once in glucose, is related to the production of alcohol.

If then the final group $-\text{CHOH}\cdot\text{CH}_2\text{OH}$ conditions the production of alcohol, the corresponding group in glucose $-\text{CHOH}\cdot\text{CHO}$ might stand in the same relation to acetaldehyde.

Clearly since an accumulation of this product would be harmful to the organism natural selection would have evolved the organism capable of transforming the aldehyde by reduction into ethyl alcohol, or oxidation to acetic acid.

Further results will be shortly forthcoming with regard to the production of acetaldehyde from other substances allied to glucose.

SUMMARY.

(1) Acetaldehyde has been detected as a product of the action of *B. coli communis* on glucose, under anaerobic conditions.

(2) By artificial selection of *B. coli communis* by means of growth on sodium chloracetate, strains of the original organism have been obtained which produce either a greatly diminished amount of acetaldehyde or none at all.

(3) It has been found that the production of acetaldehyde is related to the formation of alcohol, carbon dioxide and hydrogen rather than to the other products. This has been ascertained by a comparison of the products formed by normal *B. coli communis* with those from an artificially selected strain produced by growth on agar containing sodium chloracetate.

(4) It is therefore suggested that acetaldehyde is a primary and not a secondary product of fermentation, and also that the process of alcohol formation by *B. coli communis* is in part analogous to the alcoholic fermentation set up by the zymase of yeast and to processes which occur in the leaves of higher plants.

REFERENCES.

- Harden (1901), *J. Chem. Soc.* **79**, 610.
 — and Penfold (1912), *Proc. Roy. Soc. B.* **85**, 415.
 Kostytschew (1912), *Zeitsch. physiol. Chem.* **79**, 130.
 — (1913), *Zeitsch. physiol. Chem.* **83**, 105.
 Mendel (1911), *Centr. Bakt. Par.* **11**, **29**, 290.
 Penfold (1911), *Proc. Roy. Soc. Med.* **97**.
 Rimini (1898), *Chem. Zentr.* **2**, 277.
 Roeser (1898), *Ann. Inst. Pasteur.* **7**, 41.
 Ryffel (1909), *J. Physiol.* **39**, *Proc.* **v**.