XXII. THE ENZYMES OF WASHED ZYMIN AND DRIED YEAST (LEBEDEW). I. CARBOXYLASE.

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When zymin is thoroughly washed with water a residue is left, which no longer has the power of fermenting glucose, but regains this power when the washings are added to it, even when the latter have been boiled [Harden and Young, 1910]. The dried yeast used by Lebedew behaves in a precisely similar manner [Euler and Bäckström, 1912].

It is therefore a matter of considerable interest to ascertain how far this treatment affects the various enzymes which are known to exist in zymin and dried yeast. It can thus be ascertained whether these enzymes are themselves soluble and whether if insoluble they require for their action the presence of a soluble substance of the nature of a coenzyme.

It is also probable that some light may be thrown on the possible function of some of these enzymes in the process of alcoholic fermentation.

Carboxylase.

Since the discovery by Neuberg and Hildesheimer [1911] of the unexpected fact that yeast, yeast juice and zymin readily and rapidly decompose pyruvic acid and other α-ketonic acids with evolution of carbon dioxide and formation of an aldehyde, the opinion has been expressed in many quarters that pyruvic acid may form a stage in the enzymatic decomposition of glucose into alcohol and carbon dioxide [Neubauer and Fromherz, 1911; Neuberg and Kerb, 1912; Kostytschew, 1912; Lebedew 1912].

On the other hand it is possible that carboxylase is quite independent of the enzymes of alcoholic fermentation, its function being that of decomposing the α-ketonic acids formed by the deaminisation of the α-amino-acids (see Neubauer and Fromherz, 1911).

In order to examine the action of washed yeast preparations on pyruvic acid, experiments were made not only on the free acid but also on the

sodium salt in presence of weak inorganic acids, by the aid of which the acidity was diminished, whilst at the same time the whole or almost the whole of the carbon dioxide produced was evolved.

The evolution of carbon dioxide was observed by the aid of the apparatus previously described [Harden, Thompson and Young, 1910]. No quantitative estimations of acetaldehyde were made, but its presence was proved by the reaction with Schiff's reagent in all cases in which an evolution of carbon dioxide was observed. Control experiments were at the same time carried out with glucose and phosphate. It was thus found that the residue obtained by washing zymin and dried yeast (Lebedew) until they could no longer ferment glucose, was capable of decomposing pyruvic acid quite readily. The interesting fact is thus ascertained that carboxylase does not require the presence of a coenzyme removable by washing in order to exert its characteristic reaction on pyruvic acid. This result does not however allow any definite conclusion to be drawn as to the possible function of carboxylase in alcoholic fermentation. It can only be concluded that if the decomposition of pyruvic acid actually be a stage in the alcoholic fermentation of glucose, the soluble coenzyme is required for some change precedent to this, so that in its absence the production of pyruvic acid cannot be effected. The following experiments illustrate the results obtained. The solutions throughout were saturated with carbon dioxide at the temperature of the bath before the commencement of incubation.

Exp. 1. A sample of Schroder's dried yeast (nach Lebedew) was washed 3 times on the centrifuge with water and then brought into acetone and dried. The following experiments were then made.

(*a*) 2 g. washed and acetoned yeast + 10 c.c. 1 per cent. pyruvic acid + 40 c.c. H_2O.

(*b*) 2 g. washed and acetoned yeast + 25 c.c. 1 per cent. pyruvic acid + 25 c.c. H_2O.

(*c*) 2 g. washed and acetoned yeast + 25 c.c. 1 per cent. pyruvic acid + 5 c.c. 0·3 molar Na_2HPO_4 + 20 c.c. H_2O.

(*d*) 2 g. washed and acetoned yeast + 0 pyruvic acid + 5 c.c. 0·3 molar Na_2HPO_4 + 45 c.c. H_2O + 2 g. glucose.

These were incubated at 25° and the evolution of CO_2 measured.

Time	(*a*)	(*b*)	(*c*)	(*d*)
1 hr. 50 mins.	3·5	1·6	18·6	1·9

The action of the washed yeast on glucose was extremely small (*d*) and very little fermentation of the free pyruvic acid (*a* and *b*) occurred. In presence of sodium phosphate however a considerable evolution of CO_2

occurred (18·6 c.c.) and a strong reaction for acetaldehyde was given by the filtrate. The small effect on free pyruvic acid is ascribed to the effect of the acidity of the solution on the enzyme; in presence of sodium phosphate, on the other hand, the acidity is mainly due to NaH_2PO_4 formed by interaction of the pyruvic acid with the Na_2HPO_4.

Exp. 2. A similar experiment was made with similar results using the same washed and acetoned yeast, but washing it again 3 times to remove the last trace of coenzyme.

10 g. of acetoned yeast were washed and made up to 85 c.c.

(*a*) 25 c.c. yeast suspension + 21 c.c. 1 per cent. pyruvic acid + 7 c.c. H_2O.

(*b*) 25 c.c. yeast suspension + 21 c.c. 1 per cent. pyruvic acid + 7 c.c. 0·3 molar Na_2HPO_4 (approx. equivalent to the pyruvic acid).

(*c*) 25 c.c. yeast suspension + 21 c.c. H_2O + 7 c.c. 0·3 molar Na_2HPO_4 + 2 g. glucose.

Time	(*a*)	(*b*)	(*c*)
55 mins.	4·1	22·0	1·2 c.c.

Here again the action on the free acid is small, whereas a considerable evolution of CO_2 occurs in presence of phosphate. The amount produced is really greater than that evolved since when pyruvic acid and sodium phosphate are present in equivalent amount a portion of the CO_2 is retained as $NaHCO_3$ in equilibrium with NaH_2PO_4.

$$CH_3 \cdot CO \cdot COOH + Na_2HPO_4 \rightarrow CH_3 \cdot CO \cdot COONa + NaH_2PO_4 \rightarrow$$
$$CH_3 \cdot CHO + NaHCO_3 + NaH_2PO_4 \rightleftarrows CH_3 \cdot CHO + Na_2HPO_4 + CO_2 + H_2O.$$

Accordingly, the combined CO_2 was determined after incubation and, in a separate sample, before incubation and it was thus found that 12·9 c.c. of CO_2 were retained and should be added to the amount observed in (*b*), making a total production of 34·9 c.c.

Exp. 3. The effects of neutral potassium citrate and of excess of free boric acid in presence of the sodium salt of pyruvic acid were tried. The yeast employed was a similar preparation to that used in Exp. 2.

(*a*) 25 c.c. yeast suspension + 21 c.c. 1 per cent. pyruvic acid + 7 c.c. 0·3 M. potassium citrate neutral to phenolphthalein.

(*b*) 25 c.c. yeast suspension + 21 c.c. H_2O + 7 c.c. 0·3 M. sodium phosphate + 2 g. glucose.

(*c*) 25 c.c. yeast suspension + 21 c.c. H_2O + 7 c.c. 0·3 M.K. citrate.

(*d*) „ „ + 21 c.c. 1 per cent. pyruvic acid + 2·4 c.c. N. KHO + 3 g. H_3BO_3.

Time	(*a*)	(*b*)	(*c*)	(*d*)
1 hr. 25 mins.	4·5	1·3	1·0	25·6

The sample of yeast had practically no action on glucose (*b*), comparatively little on pyruvic acid in presence of citrate (*a*) and a large action in presence of free boric acid (*d*). This result therefore is in agreement with the idea that the action of the enzyme is greatly inhibited by acid, citric acid being much stronger than boric acid.

Exp. 4. 10 g. dried yeast was washed 3 times and made to 100 c.c.

(*a*) 25 c.c. yeast suspension + 25 c.c. 1 per cent. pyruvic acid + 2·5 c.c. N. KHO + 3 g. boric acid + toluene.

(*b*) 25 c.c. yeast suspension + 21 c.c. H_2O + 5 c.c. 0·3 M. Na_2HPO_4 + 2 g. glucose + toluene.

Time	(*a*)	(*b*)
1 hour	21·04	0·2 c.c.

Here again a good fermentation of pyruvic acid is produced by a sample of washed yeast incapable of fermenting glucose, the total evolution from which in 18 hours was only 0·4 c.c.

Exp. 5. 20 g. zymin (Schroder) were washed 3 times and made to 100 c.c.

(*a*) 25 c.c. yeast suspension + 22·5 c.c. H_2O + 2 g. glucose + 5 c.c. 0·3 M. Na_2HPO_4.

(*b*) „ „ + 25 c.c. 1 per cent. pyruvic acid + 2·5 c.c. N. KHO + 3 g. boric acid.

Time	(*a*)	(*b*)
43 mins.	17·8	0·8
18 hours	22·7	0·8

Washed zymin therefore has the same action as washed dried yeast.

Summary.

Zymin and dried yeast (Lebedew) after being freed from coenzyme by washing and thus rendered incapable of fermenting glucose, readily decompose pyruvic acid into carbon dioxide and acetaldehyde, provided that the acidity of the solution is kept low.

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