XLVII. THE ACTION OF YEAST ON LACTIC ACID.

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(*Received March 2nd, 1926.*)

The fact that lactic acid occurs in small quantities in the fermentation liquors of yeast has caused it to be regarded by some investigators as an intermediate stage in the breakdown of carbohydrates during fermentation and various observers have shown that yeast can utilise lactic acid when it is directly supplied with the lactic acid in a suitable form. Slator found that yeast had little action on the free acid [1907], but three years later Buchner and Meisenheimer [1910] showed that whilst the free acid was not attacked by yeast the sodium salt was destroyed.

Fürth and Lieben have studied the question in considerable detail [1922, 1]. They found that when a plentiful supply of oxygen was given, lactic acid in the form of its sodium or lithium salt was readily destroyed by yeast. It was necessary to have a vigorous current of oxygen passing into a continually shaken yeast suspension. Under these conditions lactic acid disappeared from the medium with simultaneous development of CO_2. Fürth and Lieben recognised that some of this carbon dioxide came from the carbohydrate of the yeast. They estimated this carbohydrate before and after the yeast had been incubated in the lactate solution by hydrolysing the yeast with 2·2 % HCl for 4 hours on a water-bath. There was always a decrease of carbohydrate during the experiment. None of the lactic acid which disappeared from the solution could therefore be accounted for by formation of new hydrolysable carbohydrate in the yeast.

The destruction of lactic acid by yeast has been confirmed by Myrbäck and Everitt [1924], by Kayser [1924] and by Smedley MacLean and Hoffert [1923]. Table I contains the results of typical experiments carried out with sodium lactate and with the free acid.

Without oxygen very little change occurred in the yeast or in the lactate medium; free lactic acid was only very slightly attacked either with or without oxygen.

Fürth and Lieben were unable to account for all the lactic acid which disappeared from a solution of sodium lactate when it was acted upon by yeast: it was not built up into an easily hydrolysable carbohydrate in the yeast nor was it completely burnt to carbon dioxide, for the amount of carbon

[1] This paper contains some of the work submitted for the degree of Ph.D. of the University of London.

Table I.

(Figures are g. per 12·5 g. of yeast.)

Original yeast				Yeast after incubation in sodium lactate with current of oxygen				Yeast after incubation in sodium lactate with current of hydrogen			
Carbohydrate	Fat	Protein	Lactic acid given per 250 cc.	Carbohydrate	Fat	Protein	Loss of acid	Carbohydrate	Fat	Protein	Loss of acid
0·82	0·092	1·41	1·24	0·21	0·242	1·47	1·15	0·52	0·092	1·46	0·13
				Yeast after incubation in 1 % lactic acid with current of oxygen				Yeast after incubation in 1 % lactic acid with current of hydrogen			
0·98	0·065	1·34	2·85	0·40	0·072	1·09	0·41	0·60	0·074	1·07	0·20

dioxide was never sufficient to account for all the lactic acid which had disappeared. They therefore sought to obtain further information about the fate of the lactic acid and published the result of their investigations in a second paper [1922, 2]. The method they employed was to make a carbon balance, putting on one side of the balance sheet the carbon of the lactic acid which had disappeared and on the other the carbon in the compounds formed in the yeast suspension after incubation. They ran two experiments under identical conditions, *A*, in which yeast was incubated in sodium lactate solution and, *B*, in which the yeast was incubated in water only. They assumed that the changes which took place in the yeast in *A* were the same as those that occurred in the yeast in *B*, so that the difference between the amounts of carbon dioxide obtained in the two experiments could be attributed to the lactic acid which disappeared from *A*. In this experiment *A*, besides the carbon dioxide which was given off, some carbon dioxide was retained in the solution as bicarbonate, being bound by the soda which was set free when the lactic acid was destroyed. Fürth and Lieben generally calculated this amount of carbon dioxide from the loss of lactic acid which they found experimentally. When they made their balance based in this way on differences between the two experiments *A* and *B* with the lactic acid lost on the one side and the CO_2 found on the other, there was always a large deficit of carbon which could not be accounted for. From one or two experiments which these investigators carried out, they considered that no fat was formed in the yeast and that volatile substances, such as aldehyde, alcohol or acetic acid, occurred in such small amounts that they were negligible in comparison with the deficit.

Fürth and Lieben therefore put forward the view that a non-hydrolysable carbohydrate must be formed in the yeast from the lactic acid or else an increase in the carbon content of the yeast protein must have taken place. The dry weights of the yeasts were determined; that of the yeast in experiment *A* was larger than that in *B* and if this difference were calculated as cellulose the deficit disappeared. These experiments were regarded by the authors as providing sufficient evidence to justify their conclusions. In the experiments carried out by Smedley MacLean and Hoffert [1923], whenever

yeast was incubated in an oxygenated solution of sodium lactate, a certain amount of fat was always formed and could not be ignored in making the carbon balance sheet. Some of Fürth and Lieben's experiments were therefore repeated, certain modifications, which did not affect the conditions of the experiments but only the methods of estimating the final products, being introduced. In a typical experiment a quantity of 25 g. of yeast was first incubated for 7 hours in tap water, with vigorous shaking in oxygen. It was then left overnight and shaken the next day for 2 hours. 3·32 g. of sodium lactate solution containing 1·83 g. of lactic acid were added and the yeast again shaken with oxygen for a further 7 hours. The experiment was again left for a night and worked up on the following day. During the whole time the CO_2 given off was collected in receivers containing baryta about $N/4$ in strength, a sufficient number being connected so that the last two remained clear. A parallel experiment in which the yeast was incubated in water without the lactate was carried out at the same time.

After incubation the yeast was filtered off, well washed with CO_2-free water, and the liquid from it made up to a known volume with CO_2-free water. Aliquot parts were used for determining residual lactic acid, volatile acids, alcohol, non-volatile acids and CO_2 present in combination as bicarbonate or carbonate. The yeast was analysed by the method used by Smedley MacLean and Hoffert [1923], and hydrolysable carbohydrate, fat and protein determined both before and after incubation.

In the majority of experiments the bound CO_2 in the liquid was present as bicarbonate. It was determined (1) directly by acidifying with phosphoric acid, removing it by CO_2-free air or oxygen and collecting it in standard baryta; (2) by determining its equivalent amount of alkali; the liquid was boiled under a reflux condenser with excess of standard acid until the CO_2 was removed; the alkali thus set free neutralised an amount of acid which could be found by back titration with standard alkali.

The lactic acid was estimated by Long's modification [1924] of Clausen's oxidation method, after clearing the liquid with sodium tungstate and copper sulphate and lime. Preliminary experiments with pure zinc lactate gave an 87 % yield of the theoretical amount of lactic acid. Therefore, 100/87 was taken as the factor in reckoning the lactic acid.

The volatile acids were measured by distillation with phosphoric acid, and titration of the distillate with standard alkali. Lactic acid was determined in the distillate. From the weight of the sodium salts obtained on evaporating the neutralised distillate to dryness, the volatile acid, other than a small quantity of lactic acid carried over, was found to be acetic acid.

Pyruvic acid was determined qualitatively by testing with sodium nitroprusside and ammonia, and quantitatively by Smedley and Lubrzynska's method [1913].

Alcohol was estimated by Hamill's modification [1909] of the Nicloux method. The amount was generally so small as to be negligible.

In the experiment with sodium lactate in which lactic acid was destroyed each molecule of acid disappearing freed an atom of sodium to hold CO_2 in solution as bicarbonate or carbonate and it was therefore possible to calculate from the bound CO_2 the loss of lactic acid which had occurred. It was always found to be less than that given by actual determination of the acid by Clausen's method. The difference was accounted for by the production of other acids, *e.g.* acetic acid, pyruvic acid and traces of succinic acid, which were present in such quantities that they could not be neglected. Kayser [1924], in his investigation of the action of yeast on calcium lactate solution, found appreciable amounts of pyruvic, acetic and valeric acids in the medium.

A wet combustion of the residue from the evaporated liquids gave a small amount of carbon not accounted for by any of the above-mentioned substances. The amount was the same in the experiment with water as in that with sodium lactate.

Table II.

25 g. original yeast			25 g. yeast incubated in sodium lactate solution *A*			25 g. yeast incubated in water *B*		
	g.	Carbon g.		g.	Carbon g.		g.	Carbon g.
Carbohydrate	1·48	0·59	Carbohydrate	0·48	0·19	Carbohydrate	0·42	0·16
Fat	0·171	0·13	Fat	0·29	0·22	Fat	0·23	0·17
Protein	2·46	1·23	Protein	2·34	1·17	Protein	2·27	1·13
CO_2 (free) in tap water	0·029	0·01	CO_2 during 1st period in H_2O	0·850	—	CO_2 during 1st period	1·049	—
CO_2 (bound) in tap water			CO_2 during 2nd period with lactate	0·790	—	CO_2 during 2nd period	0·373	—
			Bound CO_2 in liquid	0·424	—	CO_2 in liquid	0·08	—
			Total	2·064	0·56	Total	1·502	0·41
Lactic acid	1·83	0·73	Acetic acid	0·14	0·06	Acetic acid	0·02	0·01
			Pyruvic acid	0·12	0·05	Pyruvic acid	0·02	0·01
			Residue of carbon in liquid	—	0·06	Residue of carbon in liquid	—	0·06
			Residual lactic acid	0·83	0·33			
Total carbon for lactate, Exp. *A*	—	2·69	Total carbon		2·64			1·95
Total carbon for water, Exp. *B*	—	1·96						

In Table II are the figures of a typical experiment carried out after numerous preliminary experiments had established the technique of the methods. In making the balance, the carbon of the carbohydrate, fat and protein present in the yeast at the beginning of the experiment and of the lactic acid used during incubation is put down on one side. On the other side are the carbon of the carbohydrate, fat and protein of the yeast after incubation, the carbon of the carbon dioxide and of all the substances which were found in the medium. It will be seen that all the carbon of the lactic acid which has been destroyed can be accounted for within the limits of experimental error. Under the conditions of Fürth and Lieben's experiments, *i.e.* the incubation of the yeast in a small bulk of somewhat concentrated solution, the amount of fat

formed is very small, much less than that produced under the conditions of the experiments carried out by Smedley MacLean and Hoffert. For the favourable development of fat, incubation of the yeast in a large volume of a dilute solution of lactate appears to be necessary. There was no evidence that any of the carbon of the lactic acid had gone to form a non-hydrolysable carbohydrate or new protein.

Table III. *Balance according to Fürth and Lieben's method of calculation.*

	g.	Carbon g.		g.	Carbon g.
Total lactic acid	1·83	0·73	Residual lactic acid	0·83	0·33
			Extra CO_2 compared with control	0·22	0·06
			Maximum retention of CO_2	0·49	0·13
		0·73			0·52
				Deficit	0·21

For comparison with Table II a balance sheet has been drawn up according to the method used by Fürth and Lieben and is given in Table III. The figure for CO_2 is obtained from the difference in free CO_2 between experiment *A* in which the yeast was incubated in lactate and *B* in which the yeast was incubated in water. The maximum retention of CO_2 is calculated, as described above, from the lactic acid which disappeared. When the balance is made in this way a deficit of 0·21 g. carbon is obtained. It seems, therefore, that the method of balancing by differences may introduce considerable error. This is supported by the fact that in one of Fürth and Lieben's experiments in which they did not balance on the differences between the control and the experiment but used the method of calculation shown in Table II they did not get their usual large deficit of carbon.

The experiment quoted above and the two balance sheets show clearly that, whilst lactic acid is undoubtedly destroyed by yeast, the evidence available does not make it necessary to assume either the formation of a non-hydrolysable carbohydrate or the formation of protein with a higher carbohydrate content.

The author wishes to thank Dr Smedley MacLean for help and criticism received while carrying out this work and to acknowledge her indebtedness to the Food Investigation Board for a grant.

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