CLXVII. THE CARBOHYDRATE AND FAT METABOLISM OF YEAST. PART II. THE INFLUENCE OF PHOSPHATES ON THE STORAGE OF FAT AND CARBOHYDRATE IN THE CELL.

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It has been clearly established that fat is formed by the yeast cell from carbohydrate; in a previous communication [1923] we brought forward evidence that the addition of alkali phosphates to a solution of carbohydrate in which yeast was incubated produced an increase in the amount of fat stored up by the yeast cell. We also found that if the proportion of sugar was varied in a medium containing a certain definite concentration of phosphate, the amount of phosphate taken up by the yeast cell varied with the concentration of the sugar in the medium.

Many years ago Harden and Young [1906] showed that the initial rate of fermentation with yeast juice was much increased by the addition of phosphates to the fermenting liquid. In the work described in our previous communication, the experiments were all carried out under carefully controlled conditions in which the incubation of the yeast in the solution under investigation was allowed to proceed for 46 hours, and the composition of the yeast then determined. It seemed possible that the phosphate might also act here, as it does in the yeast juice fermentation, by increasing the rate of change in the transformation of the carbohydrate. If this were the case the total amount of fat in a yeast incubated in a solution of sugar without phosphate would, if the experiment were continued, approach to that stored in a yeast incubated in a sugar solution to which phosphates had been added.

Incubation in glucose solution.

When yeast is incubated in a 4 % solution of a fermentable sugar, the sugar is continually removed from the medium, and at the end of three days very little sugar remains in the solution; in this case the rate of fat formation steadily decreases, most of the fat being made in the first 24 hours. If the original yeast contains a high percentage of carbohydrate a preliminary fall in carbohydrate content may take place followed by a rise; if the original carbohydrate content is low the maximum amount of carbohydrate seems to be reached in from 24 to 36 hours (see Table I).

Both in the presence and absence of phosphates in the fermenting liquid the rate of fat formation was found to be highest at the beginning of the experiment.

Table I. *Yeast incubated in a 4 % solution of glucose.*

	(G. per 12.5 g. of original yeast.)						
	(a) Without phosphate			(b) With phosphate			Mg.
Hours	Prot.	Carb.	Fat	Prot.	Carb.	Fat.	phosphorus
Original	1.30	1.03	0.075	1.34	0.82	0.091	65.4
12	1.25	1.24	0.342	1.30	1.08	0.496	78.2
24	1.22	1.25	0.514	1.15	1.36	0.665	82.0
36	1.24	1.09	0.602	1.19	1.26	1.047	101.1
Original	1.30	1.00	0.056	1.24	1.43	0.052	66.5
24	—	1.18	0.436	1.19	1.14	0.656	107.5
48	1.19	1.14	0.454	1.15	1.21	0.669	122.9
72	1.20	1.07	0.542	1.17	1.16	0.911	125.2

We then studied the rate at which fat was stored up by the yeast cell when incubated in a solution of sugar, the sugar of which was periodically renewed, so that an excess of this substance was always present. Experiments were carried out both with and without the addition of phosphates.

The method employed was to incubate for 46 hours several quantities of 12.5 g. of yeast each in a litre of 4 % glucose solution, oxygenated throughout the experiment. At the end of this time usually less than 1 % of sugar remained. The samples of yeast were then filtered off and each again incubated in a litre of sugar solution for 46 hours just as before, one sample however being retained for analysis. At the end of a further period of 46 hours the samples were again filtered off, one retained for analysis, and the others returned as before to fresh quantities of sugar solution; the process was again repeated. In this way a comparison was made of the composition of the samples after incubation for 2, 4, 6, 8 and 10 days respectively.

A similar set of experiments was carried out, in which the medium contained in addition to the 4 % of glucose a mixture of alkali phosphates (0.3692 g. di-sodium phosphate and 0.0286 g. potassium di-hydrogen phosphate %), the mixture of sugar and phosphates being renewed every 46 hours.

The analyses obtained in these experiments are shown in Table II.

Samples of yeast obtained from the brewery on different days showed considerable variations in their fat-forming power, which did not appear to be connected with the age of the yeast; we also observed that when a sample of yeast had been kept overnight in the cold room it became less active in storing fat.

The yeast used in Exp. K was one of the most efficient we tested in its power of forming fat. The fat extracted from this yeast after it had been incubated in the glucose solution smelt strongly of crotonaldehyde. The residues were left in the Soxhlet thimbles for some days exposed to air and light, and were then again extracted with ether. After evaporating off the ether on a

Table II. *Yeast incubated in glucose solution (4 %), renewed every 48 hours.*

(G. per 12.5 g. of original yeast.)								
No. of days incubated	(a) With phosphate			No. of days incubated	(b) Without phosphate			P. mg.
	Prot.	Carb.	Fat		Prot.	Carb.	Fat	
<i>Exp. A</i>								
Original	1.42	1.51	0.056	<i>Exp. F</i>	Original	1.41	0.88	0.109
2	1.34	1.36	0.292	2	1.24	1.02	0.616	92
4	1.28	1.39	0.690	4	1.20	2.02	1.080	121
6	1.30	1.67	1.080	6	1.18	2.39	1.146	135
<i>Exp. B</i>								
Original	1.41	1.48	0.095	<i>Exp. G</i>	Original	1.36	1.28	0.061
2	—	—	—	2	1.36	1.26	0.490	75
4	1.40	1.57	0.722	4	1.28	1.79	0.853	122
6	—	—	—	6	1.32	2.50	1.112	156
8	1.35	1.87	1.321	8	—	—	—	177
<i>Exp. C</i>								
Original	1.37	1.16	0.110	<i>Exp. H</i>	Original	1.37	1.16	0.110
6	1.39	1.89	1.528	6	1.20	2.93	1.730	—
8	1.19	2.08	1.661					
10	1.23	2.25	1.568					
<i>Exp. D</i>								
				<i>Exp. K</i>	Original	1.40	0.97	0.090
				4	1.36	2.12	1.308	58
				6	1.37	2.43	1.708	109
				8	1.37	2.87	2.279	135
<i>Exp. E</i>								
Original	1.21	1.00	0.088	<i>Exp. L</i>	Original	1.21	1.00	0.088
2	1.12	1.10	0.321	2	1.18	1.33	0.433	68
4	1.18	1.30	0.549	4	1.21	1.53	1.129	109
6	1.26	1.42	0.823	6	1.25	1.72	1.279	135
								137

In Exps. C and H and in E and L the same specimen of yeast was added so that the results in each pair of these experiments are strictly comparable.

water-bath a small quantity of an unsaturated aldehyde, in odour and volatility strongly resembling crotonaldehyde, was left. During a long series of some hundreds of experiments which we have carried out, we have twice noted the presence of an unsaturated lower fatty aldehyde in the fat, one in the experiment above referred to, in which a mixture of glucose with phosphate was fermented, and the other in which a fructose solution had been fermented. So far we have not been able to determine the exact conditions to which the presence of the aldehyde may be ascribed, but we are inclined to think it is associated with some slight variation in the yeast. In both cases, the yeast produced rather specially large amounts of fat. The result seems to us of considerable importance, and suggests the occurrence of an unsaturated aldehyde as an intermediate stage in the chain of reactions by which carbohydrate is converted to fat, which would be expected to occur if the fat is built up by the condensation of aldehyde with pyruvic acid [Smedley and Lubrzynska, 1913], or with other aldehydes [Magnus-Lévy, 1902].

The results obtained show that the rate of fat formation is increased by

adding phosphates to the glucose-containing medium in which the yeast is incubated. In the absence of phosphates, when the experiment is continued for several days with the addition of fresh quantities of glucose to the medium at the end of every 46 hours, the rate of increase seems to be more or less constant until a limit is reached when further incubation produces no increase of fat. If, however, the fermenting liquid contains phosphate, the rate of increase is higher at the beginning of the experiment and then diminishes.

In those experiments where the same yeast is used both with and without phosphates, the total amount of fat is always greater in the yeast which has been incubated in the phosphate-containing medium. On the whole, therefore, the evidence shows that not only is the rate of fat-formation stimulated by the addition of phosphates to the medium, but the total amount of fat deposited in the yeast is greater than that stored in the same specimen of yeast incubated in a sugar solution of similar strength, but containing no phosphate.

Incubation in fructose solution.

The influence of phosphates in increasing the total amount of fat formed is shown most strikingly when equal amounts of the same sample of yeast are incubated in a 4 % oxygenated fructose solution respectively with and without the addition of phosphates.

Table III. *Showing composition of yeast after incubation in 4 % fructose solution.*

(G. per 12.5 g. of original yeast)									
	Days	Prot.	Carb.	Fat		Prot.	Carb.	Fat	P. mg.
<i>Exp. A</i>	—	1.14	1.36	0.068	<i>Exp. C</i>	1.14	1.36	0.068	71
	2	1.09	1.15	0.273		1.11	1.43	0.587	123
	4	1.14	1.35	0.541		1.04	1.92	1.069	130
	6	1.14	1.58	0.717		1.13	2.58	1.470	143
	8	1.15	1.70	0.743		1.04	2.23	1.360	140
	10	1.08	1.78	0.988		1.12	2.62	1.974	145
<i>Exp. B</i>	—	1.40	0.47	0.101	<i>Exp. D</i>	1.40	0.47	0.101	71
	2	1.26	1.32	0.390		1.40	1.39	0.703	116
	4	1.25	1.39	0.608		1.21	2.03	1.119	134
	6	—	1.72	0.680		1.17	2.18	1.335	146

The experiment was arranged so that the medium could be renewed every 48 hours, and samples of the yeast analysed after incubation for periods of 2, 4, 6, 8 and 10 days respectively in the solutions of fructose, with and without the addition of phosphates. In each case the effect of the addition of the phosphate to the fructose solution was to double, almost exactly, the amount of fat in the yeast when compared with the control without the addition of phosphates. Although the amount of fat was greater in the yeast incubated in the glucose-phosphate solution than in the yeast incubated in the glucose solution, there was no such regular relation between these amounts as we observed when the yeasts were incubated in the corresponding fructose solutions.

The results obtained from these experiments with fructose strongly support the view that phosphate and fructose are both concerned in forming some intermediate substance which constitutes an essential stage in the conversion of sugar to fat; for the further transformation of this intermediate substance to fat, the presence of a supply of oxygen appears to be essential, and there is some evidence which suggests that an unsaturated aldehyde is formed at a later stage in the transformation.

Storage of carbohydrate.

Both with the yeast incubated in fructose and glucose solutions the addition of phosphate to the medium exercises a pronounced effect on the amount of carbohydrate stored by the cell. With each successive period of incubation in the phosphate and hexose medium, the amount of carbohydrate in the yeast increases, the amount being always greater than that contained in the yeast incubated in the hexose solution without the addition of phosphate. When the experiments are carried out in the manner now described, *i.e.* continued for periods of from 6 to 10 days with frequent renewals of the sugar in the medium, though fructose is more efficient in producing storage of fat than is a corresponding solution of glucose, no constant difference in their influence on the storage of carbohydrate could be detected.

The action of yeast on solutions of hexosephosphate.

When a solution containing sugar and sodium phosphate is fermented by yeast juice large quantities of hexosephosphate can be obtained from the fermentation liquid, but if the fermentation be carried out by living yeast the medium does not contain hexosephosphate.

Neuberg, Levite and Schwenk [1917] regard the formation of hexosephosphate as a pathological phenomenon, but the evidence which we brought forward [1923] that the absorption of phosphate by the yeast cell depends on the concentration of sugar in the medium, supports the view of Harden and Young [1905] that the formation of hexosephosphate is a normal stage in the fermentation process. From the fact that hexosephosphate is found in the fermentation liquid only when the fermentation is carried out with yeast juice, and not when it is carried out with the living yeast cell, it would appear that while the molecules of hexose and sodium phosphate can pass through the wall of the cell, the larger molecules of hexosephosphate are unable to do so. The yeast contains an enzyme capable of synthesising hexosephosphate but incapable of passing through the wall of the cell, and it is only when the cell is broken up and the enzyme obtained in the yeast juice that the hexosephosphate is found in the fermentation liquid.

In order further to test this conclusion we compared the action of yeast on solutions of sodium hexosephosphate, with its action on solutions of the corresponding amounts of glucose and sodium phosphate. In a 4.8 % solution

the action of hexosediphosphate was found to be deleterious to the yeast cell, a loss of carbohydrate and an increase of fat were noted similar to those recorded when yeast is incubated in water, but the losses of protein and of phosphorus were considerably greater than when yeast is incubated in water alone. When a more dilute solution of hexosephosphate was used there was little loss of protein, but there was no increase of phosphorus such as that obtained when yeast is incubated in a solution of similar concentration containing glucose and sodium phosphate.

Table IV. *Composition of yeast after incubation in a solution of hexosephosphate and in solutions of glucose with alkali phosphates.*

	Protein	Fat	Carbo- hydrate	P. mg.
Original yeast	1.28	0.097	1.01	63.5
Na hexosediphosphate (4.8 %)	0.90	0.164	0.28	32.1
Corresponding glucose (2 %) and phosphate	1.30	0.416	1.05	81.3
Original yeast	1.34	0.085	0.83	65.9
Na hexosediphosphate (1.8 %)	1.32	0.218	0.16	62.5
Corresponding glucose (0.8 %) and phosphate	1.28	0.356	0.55	75.5
Original yeast	1.25	0.098	0.40	73.7
Na hexosediphosphate (1.16 %)	1.07	0.175	0.33	73.5
Corresponding glucose (0.48 %) and phosphate	1.24	0.268	0.49	74.1
Original yeast	1.36	0.079	0.40	61.0
Na hexosemonophosphate (1.2 %)	1.24	0.137	0.15	55.0
Corresponding glucose (0.75 %) and phosphate	1.34	0.482	0.60	77.0
Phosphate alone (0.37 % NaH_2PO_4) (0.03 % K_2HPO_4)	1.06	0.129	0.19	—

The increase of fat observed after the yeast had been incubated in the dilute hexosephosphate solution, was much less than after incubation in a solution of a mixture of glucose and phosphate of similar concentration. The amount of fat after incubation in the dilute hexosephosphate solution was of the same order as that obtained after incubation in a solution of sodium phosphate alone, and probably came therefore from the carbohydrate originally stored in the cell but which disappears during the period of incubation.

These experiments support the view that the hexosephosphate molecules are not able to pass through the wall of the yeast cell but that glucose and phosphate molecules pass separately into the cell and are there combined.

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