CCLXII. THE SYNTHESIS OF RESERVE CARBOHYDRATE BY YEAST.

II. THE EFFECT OF FLUORIDE.

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WHEN studying the incubation of yeast in glucose solutions to which phosphate had been added we found in one series of experiments a low glycogen content which was difficult to reconcile with the results of other experiments apparently carried out under similar conditions. Subsequent investigation revealed that the alkali phosphate used in these particular experiments was contaminated by a considerable amount of fluoride. We were thus led to make a study of the effect on glycogen storage of the addition of sodium fluoride at different concentrations to the glucose and maltose media in which the yeast was incubated both in the presence and absence of added phosphate.

The method of experiment was similar to that already described by us [1935]. In order to be able to compare a larger number of media using the same sample of yeast, the experiments were carried out on a smaller scale, 2 g. of yeast being incubated in 125 ml. of the various solutions to be tested: the only estimations made were those of the glycogen content of the yeast and of the amount of reducing sugar which had disappeared from the medium.

The concentrations of sodium fluoride compared were in most experiments 0.01% (0.0024 *M*), 0.02% (0.0048 *M*) and 0.03% (0.0071 *M*); intermediate and somewhat greater concentrations were also used in a few cases.

The effect of the addition of fluoride on glycogen storage.

When yeast was incubated in a 5% solution of glucose or maltose at 25° the addition of even 0.01% NaF to the medium caused a marked inhibition of glycogen storage; after addition of the fluoride to a 5% solution of glucose or maltose, the glycogen content of a similar sample of yeast incubated in either medium was identical, although without this addition the maltose produced much more glycogen. If however 0.05% phosphate was also added to the medium the effect of the fluoride was considerably modified. The addition of 0.01% NaF to the glucose-phosphate medium did not diminish but markedly increased the amount of glycogen stored: with further increase of the fluoride concentration to 0.03% the glycogen content steadily diminished.

In Fig. 1 the amounts of glycogen stored by 10 g. yeast after incubation in the sugar solution are plotted against the concentration of fluoride. The variability of different samples of yeast obtained on different days from the same brewery has already been pointed out [1935] and it happened that in most of these experiments the glycogen content of the yeast after storage in the glucose-phosphate medium was unusually low and the stimulating effect of the fluoride was marked: in the only sample which after incubation in the glucose-phosphate medium gave a high glycogen value, the increase usually produced by the fluoride was not apparent.

With the samples used for incubation in the maltose-phosphate medium, glycogen storage was particularly efficient, showing a marked increase over the values after incubation in the pure maltose medium: the addition of 0.01 % NaF to the maltose-phosphate medium caused no further increase of glycogen storage but sometimes a diminution. The addition of 0.01 % NaF to the pure maltose medium however always produced a marked diminution of glycogen content; increase above 0.01 % of the fluoride produced a diminished storage both in the presence and absence of phosphates.

Fig. 1. *A*, maltose + 0.10 % PO_4 ; *B*, maltose + 0.05 % PO_4 ; *C*, glucose + 0.10 % PO_4 ; *D*, glucose + 0.05 % PO_4 ; *E*, glucose; *F*, maltose.

Since the action of the phosphate seemed to a certain extent to inhibit the action of the fluoride, we carried out a series of experiments in which the effect of increased phosphate concentration in the presence of fluoride was observed. The significant rise in glycogen when 0.01 % fluoride was added to the glucose medium containing 0.05 % phosphate was not always apparent when the phosphate concentration was doubled. In the maltose media, increased phosphate concentration, in comparable experiments, showed a diminution of the effect of fluoride in lowering the glycogen content. After incubation in the glucose-phosphate medium, the increase in glycogen storage at a low fluoride concentration (Fig. 1) followed by a decrease at higher fluoride concentrations may be explained on the assumption that two inhibitory processes are at work, one

Table I. *Glycogen per 10 g. of original yeast as g. of glucose.*

% NaF...	After incubation in 5% glucose solution				After incubation in 5% glucose + 0.05% PO ₄ solution				After incubation in 5% glucose + 0.10% PO ₄ solution			
	0.00	0.01	0.02	0.03	0.00	0.01	0.02	0.03	0.00	0.01	0.02	0.03
No.												
1	—	—	—	—	0.51	0.86	0.64	0.48	—	—	—	—
2*	—	—	—	—	0.32	0.77	0.66	0.53	—	—	—	—
3	—	—	—	—	0.25	0.39	0.39	0.34	—	—	—	—
4	—	—	—	—	0.45	0.55	0.40	0.36	—	—	—	—
5	0.42	0.37	0.31	0.27	—	—	—	—	0.59	0.74	0.71	0.60
6	—	—	—	—	0.36	0.69	0.41	0.37	0.36	0.46	0.41	0.34
7	—	—	—	—	0.54	0.65	0.35	0.32	0.69	0.67	0.65	0.57
8	0.55	0.29	0.25	0.21	†1.11	1.05	—	—	†0.86	0.91	—	—
Means	0.48	0.33	0.28	0.24	0.40	0.65	0.47	0.40	0.55	0.62	0.59	0.50

% NaF...	After incubation in 5% maltose solution				After incubation in 5% maltose + 0.05% PO ₄ solution				After incubation in 5% maltose + 0.10% PO ₄ solution			
	0.00	0.01	0.02	0.03	0.00	0.01	0.02	0.03	0.00	0.01	0.02	0.03
No.												
3	0.69	0.26	0.21	0.12	—	—	—	—	—	—	—	—
9	0.82	0.51	0.29	0.30	1.04	1.00	0.82	0.50	—	—	—	—
4	—	—	—	—	0.90	0.74	0.57	0.39	—	—	—	—
10	†0.79	0.37	—	—	1.02	0.64	0.80	0.65	†—	1.02	1.02	—
11	—	—	—	—	1.75	1.77	1.30	1.00	2.07	1.99	1.77	1.56
12	—	—	—	—	1.32	0.99	0.84	0.65	1.52	1.57	1.27	1.04
Means	0.75	0.38	0.25	0.21	1.21	1.03	0.87	0.64	1.79	1.78	1.52	1.30

* Measurements were made at NaF concentrations of 0.005, 0.015, 0.025, 0.035%, the results given were read off from a curve.

† Incomplete series not included in calculation of means.

inhibiting the synthesis, the other the breakdown of glycogen. At low concentrations of fluoride, the breakdown would seem to be most affected whilst at higher concentrations the inhibition of breakdown is counteracted by the inhibition of synthesis.

Further the fact that no such preliminary rise in the glycogen content occurs after incubation in the maltose-phosphate medium at a concentration of 0.01% NaF may be explained by assuming that the inhibition of glycogen synthesis is proportional to the rate of synthesis. The amount synthesised being greater in the maltose than in the glucose medium, the diminution in the amount of glycogen synthesised when fluoride is added will be greater in the maltose solution. Thus if the rates of decomposition of glycogen are the same in both media, it is possible that at a concentration of 0.01% NaF, in the glucose-phosphate solution, the increase due to the inhibition of decomposition is greater than the decrease due to the inhibition of synthesis and a rise in the curve takes place, whilst in the maltose-phosphate solution, the inhibition of synthesis is equal to or greater than the inhibition of breakdown of the glycogen and the curve remains at a constant level or falls.

The influence of the addition of phosphate and fluoride on the total amount of sugar fermented.

It is noteworthy that in all experiments in which incubation in the medium has been continued for 48 hours the sugar which has disappeared from the medium when the same sample of yeast has been incubated in glucose and in

glucose-phosphate media respectively has always been greater in the phosphate-containing medium. This is particularly interesting since no such difference seems to have been observed in experiments of shorter duration. Apparently on prolonged incubation phosphate exercises a beneficial effect on the fermentation of glucose by living yeast. With maltose however no such difference was apparent.

Both for maltose and glucose the addition of fluoride to the medium diminished the amount of sugar removed by the yeast cell, but the effect was largely counteracted by the addition of phosphate to the media, 0.1 % phosphate being more effective than 0.05 %. The results of these experiments are given in Table II.

Table II. *g. sugar used up by 2 g. of yeast in 2 days in 125 ml. of medium.*

% NaF...	5% glucose solution				5% glucose + 0.05% PO ₄ solution				5% glucose + 0.10% PO ₄ solution			
	0.00	0.01	0.02	0.03	0.00	0.01	0.02	0.03	0.00	0.01	0.02	0.03
No.												
2*	—	—	—	—	6.20	5.91	5.54	5.01	—	—	—	—
3	—	—	—	—	6.19	5.70	5.31	4.75	—	—	—	—
4	—	—	—	—	6.10	5.55	5.00	4.38	—	—	—	—
5	5.59	3.85	2.87	2.37	—	—	—	—	5.95	5.85	5.50	5.50
6	—	—	—	—	6.19	5.91	5.46	4.96	6.10	6.01	5.96	5.94
7	—	—	—	—	5.89	4.01	3.61	3.50	5.74	5.46	5.10	4.91
8	3.48	2.25	1.31	0.95	—	—	—	—	—	—	—	—
12	†3.50	—	—	—	†5.50	—	—	—	—	—	—	—
13	†4.75	—	—	—	†5.75	—	—	—	—	—	—	—
14	†4.19	—	—	—	†4.87	—	—	—	—	—	—	—
Means	4.53	3.05	2.09	1.66	6.09	5.42	4.98	4.52	5.93	5.77	5.52	5.45

% NaF...	5% maltose solution				5% maltose + 0.05% PO ₄ solution				5% maltose + 0.10% PO ₄ solution			
	0.00	0.01	0.02	0.03	0.00	0.01	0.02	0.03	0.00	0.01	0.02	0.03
No.												
3	5.59	4.17	3.06	2.31	—	—	—	—	—	—	—	—
9	5.54	4.42	3.25	2.55	5.64	5.14	4.59	3.92	—	—	—	—
4	—	—	—	—	5.51	5.25	4.38	3.94	—	—	—	—
10	†5.37	4.23	—	—	5.47	5.06	4.50	4.15	†—	5.37	5.38	—
11	—	—	—	—	4.31	4.27	3.15	2.31	4.19	4.25	4.20	3.79
13	†3.57	—	—	—	†3.72	—	—	—	—	—	—	—
Means	5.56	4.29	3.15	2.43	5.23	4.93	4.15	3.58	4.19	4.25	4.20	3.79

* Measurements were made at NaF concentrations of 0.005, 0.015, 0.025, 0.035 %, the result given were read off from a curve.

† Incomplete series not included in calculation of means.

Variations in the wet weight of the pressed yeast after incubation.

Table III shows the mean wet weights of 10 g. of the original yeast, filtered on a Büchner funnel under as far as possible similar conditions, after incubation in the various media. The values for individual experiments in any given medium were found to agree surprisingly well: further there appeared to be a correspondence between wet weight and total carbohydrate content of the yeast.

In view of the recent discussion as to whether a constant amount of water is held per g. of glycogen in the liver [Bridge and Bridges, 1932; Puckett and Wiley, 1932; Mackay and Bergman, 1932; 1934] it seemed of interest to compare the wet weights with the amounts of glycogen and total carbohydrate they contained. After incubation in any carbohydrate medium the ratio of the wet

Table III.

	Mean wet wt. of 10 g. original yeast g.	Mean total carbo- hydrate per 10 g. of original yeast g.	Mean glycogen per 10 g. of original yeast g.	Mean wet wt./ Mean total carbo- hydrate	Mean wet wt./ Mean glycogen	No. of experi- ments
Original yeast before incubation	10.0	0.59	0.26	16.9	38.5	8
Incubated in						
5% glucose for 2 days	8.0	0.90	0.35	8.9	22.9	8
5% glucose for 4 days	6.9	0.80	0.38	8.6	18.2	7
5% glucose for 6 days	5.8	0.64	0.27	9.1	21.5	4
5% glucose + PO ₄ for 2 days	11.9	1.24	0.66	9.6	18.0	4
5% glucose + PO ₄ for 4 days	10.8	1.31	0.81	8.2	13.3	3
5% maltose for 2 days	10.5	1.33	0.73	7.9	14.4	3
5% maltose + PO ₄ for 2 days	12.3	1.67	0.83	7.4	14.8	4
5% glucose + PO ₄ + 0.035% NaF for 2 days	10.2	1.02	0.24	10.0	42.5	3
5% glucose + PO ₄ + 0.035% NaF for 4 days	9.9	1.08	0.30	9.2	33.0	4
5% glucose + PO ₄ + 0.035% NaF for 6 days	10.1	1.20	0.24	8.4	42.1	4

weight of yeast to the total carbohydrate is surprisingly constant, but the ratio of wet weight of yeast to glycogen content varies widely. The numbers so obtained for the original yeast (taken directly from the wort) are nearly double those after incubation in the carbohydrate medium. It would appear therefore that the original yeast which had been growing in a nitrogenous medium, when transferred to the non-nitrogenous medium loses some substance other than carbohydrate which holds a considerable amount of water within the cell, but that after incubation for 48 hours in carbohydrate media the wet weight is chiefly dependent on the carbohydrate content of the yeast. As we were not concerned with this question when we planned our experiments we did not always take specimens for the determinations of the dry weights and it has not therefore been possible in a large number of the experiments to calculate the actual water content. Such data as are available show that the differences in dry weight are almost entirely accounted for by the changes in carbohydrate content. According to Daoud and Ling [1931] the insoluble cell carbohydrates consist largely of glycogen esters and Willstätter and Rohdewald [1934] have shown that in the liver part of the glycogen remains bound in the cell in combination with protein. It is possible therefore that the water held would bear a closer relationship to the glycogen content of the cell if any glycogen present in combination in the cell were also contributing to retain water.

SUMMARY.

1. Yeast was incubated in glucose, maltose, glucose-phosphate and maltose-phosphate solutions, and sodium fluoride added in concentrations ranging from 0.0024 to 0.0071 *M*.

2. Addition of fluoride to the glucose or maltose solutions always caused marked inhibition of glycogen storage.

3. Addition of 0.0024 *M* fluoride to the glucose-phosphate medium caused a marked increase in the storage of glycogen; with further increase in the concentration of fluoride the glycogen content fell. The effect of the fluoride in diminishing the glycogen storage after incubation in maltose-phosphate solutions was never marked at low concentrations but increased at higher concen-

trations: no actual increase of glycogen such as that shown in the glucose-phosphate medium on the addition of 0.0024 *M* fluoride was observed. An explanation is offered on the assumption that the effect of the fluoride is to inhibit both the synthesis and breakdown of glycogen in the cell.

4. In fermentations lasting 48 hours, addition of phosphate to glucose solutions increased the amount of sugar decomposed but produced no similar effect when added to maltose solutions.

5. Addition of fluoride to the media diminished the amount of sugar which underwent decomposition, the effect being largely counteracted by the addition of phosphate.

6. There appears to be a definite correlation between the wet weight of the yeast and its total carbohydrate content.

REFERENCES.

- Bridge and Bridges (1932). *J. Biol. Chem.* **96**, 381.
Daoud and Ling (1931). *J. Soc. Chem. Ind.* **50**, 379.
McAnally and Smedley-MacLean (1935). *Biochem. J.* **29**, 1872.
Mackay and Bergman (1932). *J. Biol. Chem.* **96**, 373.
——— (1934). *J. Biol. Chem.* **105**, 59.
Puckett and Wiley (1932). *J. Biol. Chem.* **96**, 367.
Willstätter and Rohdewald (1934). *Z. physiol. Chem.* **225**, 103.