EFFECT OF pH ON THE SYNTHESIS OF CELL CARBOHYDRATE DURING FERMENTATION BY BAKER'S YEAST

by

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INTRODUCTION

SUSSMAN, SPIEGELMAN, AND REINER[1] reported that a baker's yeast which gave 80% of the theoretical amount of CO_2 when glucose was fermented to completion in a medium buffered to pH 4.5, gave 93% when the external pH was raised to 8.5. This they interpreted as showing a suppression of cellular carbohydrate synthesis at the higher pH; and they further considered this effect as due to a direct influence of pH on the equilibrium conditions of yeast phosphorylase. On the other hand HEVESY[2], in discussing the uptake of phosphate by yeast, which is maximal when the pH of the medium is 4 to 5, but almost zero at pH 7, states: "As pH of the cells is not influenced by changes in pH of the nutritive solution, this observation suggests that processes going on at the cell surfaces or within the cell boundary play an important part in phosphorus metabolism in yeast". The general principle implied here, applied to the results of SUSSMAN *et al.*[1] suggested to us that the phosphorylases of yeast might be located at the cell boundary. In view of the importance of such a finding we decided to repeat their experiments, using direct analysis of the yeast cell carbohydrate before and after fermentation, rather than relying solely on CO_2 output as an index of the amount of synthesis. Again, if suppression of carbohydrate formation was almost complete at pH 8.5 it seemed possible that a partial suppression should be demonstrable at an intermediate pH. Observations were therefore made also at pH 7.

EXPERIMENTAL

The evolution of CO_2 was followed in the standard Warburg manometric apparatus at 30° C with a shaking rate of 120 cycles/minute. The organism was a typical strain of baker's yeast, *Saccharomyces cerevisiae*, D.C.L. 211. As we wished to check the findings of SUSSMAN *et al.*[1] experiments were carried out under their conditions, in which phosphate was present in all cases. Since, however, phosphate uptake varies with pH, it seemed that a more reasonable comparison would be obtained in the absence of phosphate. Thus two series of experiments were run (1) under the conditions used by SUSSMAN

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et al., and (2) comparing fermentations in bicarbonate buffer at pH 7.0 and in phthalate at pH 4.5, phosphate being omitted.

1. *Fermentations in the presence of phosphate*

Four media of the following composition were used: (a) $M/15$ K_2HPO_4, pH 8.5; (b) $M/15$ KH_2PO_4, pH 4.5; (c) a solution containing $M/10$ glycine and $M/10$ Na_2HPO_4 adjusted to pH 8.5 with NaOH; and (d) a solution containing $M/10$ glycine and $M/10$ Na_2HPO_4 adjusted to pH 4.5 with HCl. Measurements of pH were made on the media at the end of the fermentations and no significant changes were detected. Each Warburg flask contained in the main compartment 1.8 ml of a suspension of 10 mg of yeast ($\equiv$ 2.5 mg dry matter) in the appropriate buffer solution, and in one side-arm 0.2 ml of 1% glucose solution. In the experiments at pH 8.5 the second side-arm contained 0.2 ml of H_3PO_4 (for medium *a*) or HCl (for medium *c*) of such strength that after mixing with the fermentation medium the final pH was 4.5. For purposes of comparison parallel fermentations were carried out at pH 4.5. After mixing the yeast suspension and glucose solution, manometric readings were taken at 7 minute intervals for 147 minutes, at which time the acid was tipped in the pH 8.5 experiments. Manometer readings were then continued until the system became stable. To correct for the small amount of carbonate pre-existing in the alkaline solution, blank experiments were carried out by adding acid to a non-fermenting yeast suspension.

The results are given graphically in Fig. 1, from which it will be seen that in both types of buffer the final output of CO_2 was lower at pH 8.5 than at pH 4.5. This would suggest that there is rather greater synthesis of carbohydrate at pH 8.5 than at pH 4.5. To confirm this observation total cell carbohydrate determinations were made, using the anthrone colour reaction (TREVELYAN AND HARRISON[3]; TREVELYAN, GAMMON, WIGGINS, AND HARRISON[4]) after centrifuging the cells immediately at the end of the test and washing with phosphate buffer solution. In all cases the carbohydrate values are expressed as glucose equivalents, *i.e.* compared with the colour developed in glucose standards. It was ascertained that traces of glycine did not interfere with the analysis. The results, given in Table I, Items 1, 2, 3 and 4, confirm the manometric finding that under our experimental conditions more carbohydrate was synthesized when the external pH was raised from 4.5 to 8.5.

The rate of fermentation at pH 8.5 in phosphate buffer (medium *a*) was followed

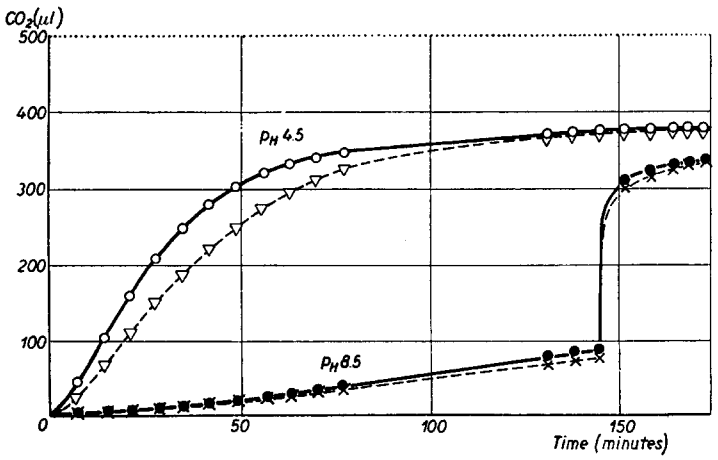

Fig. 1. Comparison of CO_2 output by yeast at pH 4.5 and at pH 8.5. 2 mg of glucose fermented by 10 mg of yeast in 2 ml. Solid lines — phosphate buffer solution; broken lines — glycine: phosphate buffer solution. Acid was tipped at 147 minutes in the experiments at pH 8.5. The dotted line represents the theoretical value for 100% recovery of 2 mg of glucose as CO_2.

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TABLE I

CARBOHYDRATE SYNTHESIS AND CO_2 OUTPUT

Fermentation of 2 mg of glucose by 10 mg of yeast in 2 ml of buffer solution at 30° C.
Theoretical possible CO_2 from 2 mg of glucose = 500 μl.
Carbohydrate expressed as glucose equivalent.

No.	*Date*	*Buffer*	*pH*	*Duration min*	*Original carbohydrate mg*	*Final carbohydrate mg*	*Carbohydrate synthesis mg*	*CO_2 µl*
1	3/ 1/51	Phosphate	4.5	91	0.88	1.08	0.20	375
2	3/ 1/51	Glycine-phosphate	4.5	91	0.88	1.10	0.22	376
3	3/ 1/51	Phosphate	8.5	91	0.88	1.21	0.33	331
4	3/ 1/51	Glycine-phosphate	8.5	91	0.88	1.14	0.26	332
5	19/12/50	Phthalate	4.5	100	0.84	1.09	0.25	349
6	19/12/50	Bicarbonate	7.0	100	0.84	1.16	0.32	347

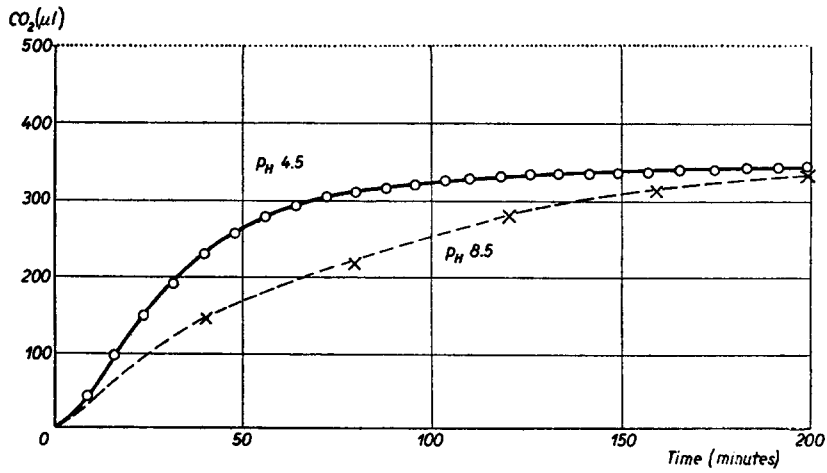

Fig. 2. Comparison of rate of CO_2 output by yeast at pH 4.5 and at pH 8.5. 2 mg of glucose fermented by 10 mg of yeast in 2 ml of phosphate buffer solution. Solid line — normal manometric measurements at pH 4.5; broken line — corrected points obtained after tipping acid in separate flasks containing buffer at pH 8.5. Dotted line represents the theoretical value for 100% recovery of 2 mg of glucose as CO_2.

by repeating the experiment described above and tipping excess acid, sufficient to stop fermentation, into individual flasks of a series, at intervals of 40 minutes. Fig. 2 shows the results plotted against time, compared with a control fermentation at pH 4.5. In medium at pH 8.5 the rate of fermentation was slower and at no point was more CO_2 evolved than in the normal fermentation at pH 4.5. The ratio of the times taken to reach a given CO_2 output was roughly constant. In other words, at any given level of glucose concentration throughout the fermentation the velocity of fermentation at pH 8.5 was a constant fraction of that at pH 4.5.

2. Fermentations in the absence of phosphate

Fermentations in media at pH 7.0 and pH 4.5 were compared. In order to avoid

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the necessity of adding acid to liberate CO_2 in the experiment at pH 7.0, a bicarbonate medium was used.

Each flask contained in the main compartment 1.8 ml of a suspension of 10 mg of yeast in $M/100$ $KHCO_3$, and in the side-arm 0.2 ml of 1% glucose in $M/100$ $KHCO_3$; the gas space was filled with a mixture of 5% CO_2 and 95% N_2 by volume. It was established that the pH did not vary during the experiment. Control runs in which 0.2 ml of 10 N H_2SO_4 was added from the second side-arm at intervals, showed that only a negligible volume of CO_2 (for which correction was made) came from the bicarbonate buffer during the fermentation, possibly due to acids produced by the yeast.

Table I, Item 6, shows the results compared with those of a fermentation in $M/100$ potassium hydrogen phthalate buffer adjusted to pH 4.5 with KOH and gassed with oxygen-free nitrogen (Table I, Item 5). Total carbohydrate figures are also given. It will be seen that there was no great difference in respect of either CO_2 output or total carbohydrate between the values obtained at pH 7.0 and 4.5. Fractionation of the yeast carbohydrates by a method described elsewhere (TREVELYAN AND HARRISON[3]) showed that the increase in carbohydrate was largely confined to the glycogen fraction. In the experiment carried out at pH 7.0 the glycogen in the system rose during fermentation from 0.24 to 0.48 mg, and at pH 4.5 to 4.0 mg, whilst the trichloroacetic-acid-soluble carbohydrate, mannan, and glucan fractions did not significantly change.

DISCUSSION

The failure to confirm the results of SUSSMAN *et al.*[1] may be due to differences in the properties of the yeasts used in the two series of experiments. However, it must be emphasized that even if they are correct in claiming to have demonstrated a virtual suppression of carbohydrate synthesis by their yeast at pH 8.5, their results and discussion provide no justification for attributing this to a direct effect of pH on phosphorylase equilibrium, for the following reasons. The rate of synthesis of glycogen from glucose-1-phosphate (G) in yeast for an intracellular orthophosphate level (P), will depend, not only on the amount of these substances and on the equilibrium constant (K), but also on the amount of enzyme (V_o), thus:

$$\text{Rate} = V_o\, \mathrm{f}(G, P, K).$$

The percentage conversion of substrate to glycogen will depend on the ratio of this rate to the rate of glucose uptake (v_g), thus:

$$S = \%\ \text{synthesis} = \frac{V_o\, \mathrm{f}(G, P, K)}{v_g} \qquad (1)$$

It should be noted here that G and P vary throughout the course of fermentations such as those of SUSSMAN *et al.* and ourselves, and in particular that the orthophosphate content, which drops immediately after the onset of fermentation, rises again as the glucose becomes exhausted (MIRSKI AND WERTHEIMER[5]). Thus expression (1) should more correctly be written, for a fermentation observed over time t:

$$S = \frac{\int_o^t V_o\, \mathrm{f}(G, P, K)\, \mathrm{d}t}{\int_o^t v_g\, \mathrm{d}t} \qquad (2)$$

Thus it is apparent that some five variables are involved. In order therefore to

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relate a change in S to K alone, as suggested by SUSSMAN *et al.*, it would be necessary to establish that the function of S was not decreased by changes in V_o, v_g, G and P. Now G and P depend in a far from simple fashion on the linking of the dozen or so enzymatic reactions of fermentation. The concentration of glucose-1-phosphate would depend on the establishment of a steady state between the (irreversible) reactions catalysed by hexokinase and phosphohexokinase, and the (reversible) reactions of phosphoglucomutase and phosphorylase: and the inorganic phosphate on nearly every reaction taking part in fermentation. If the rate of glucose uptake (v_g) were unaffected by the change of pH, it would be permissible cautiously to suppose that the concentrations of G and P had been little altered: though even this would not be strictly true, since a suppression of carbohydrate synthesis would simultaneously increase the velocity of all reactions following glucose-6-phosphate by increasing the substrate concentrations of the various enzymes. However this, by increasing the amount of combined phosphate, would be expected to favour carbohydrate synthesis: it would remain to show that V_o was not significantly affected by the alteration of pH (it may be noted that potato phosphorylase has rather a sharp pH optimum at 6.1, HANES[6]) to establish the probability of dependence of S upon changes of K. In our experiments the velocity of fermentation, and hence of glucose uptake, was seriously affected at pH 8.5. SUSSMAN *et al.* rather surprisingly do not report on the velocity of fermentation, though it may be surmised from the length of their experimental period that it was much reduced at pH 8.5. The problem is thus considerably complicated.

An alternative approach is possible. It is known that fermentation by maceration juice is very sensitive to pH. According to graphical data reproduced by NILSSON[7], yeast maceration juice has an optimum pH of 6.3, whilst the fermentation rate falls to a half at pH 7.8. The fact that in our experiments the rate of fermentation at pH 8.5 was half that at pH 4.5 or 7.0 could be explained as due to a change of internal pH from a normal value of 6.3 (CONWAY AND DOWNEY[8]) in media at pH 4.5 and 7.0, to 7.8 when the external pH was 8.5. If this is indeed the case the equilibrium constant (orthophosphate)/(glucose-1-phosphate) would change from 4.7 to 2.2, according to the relation $(HPO_4)^{--}/(C_6H_{11}O_5.O.PO_3)^{--} = 2$ at any pH (CORI, CORI AND GREEN[9]). This change could combine with simultaneous changes in V_o, G, and P to result in greater or less carbohydrate synthesis than at pH 4.5; or even result in a reversal of synthesis, *i.e.* breakdown of pre-existing cell carbohydrate. In our experiments the net combination showed no measurable change.

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SUMMARY

1. In the complete fermentation of 2 mg of glucose by 10 mg of a baker's yeast the fermentation rate and percentage conversion of glucose to cell carbohydrate were unaltered when the pH of the external medium was changed from 4.5 to 7.0.

2. At pH 8.5 the fermentation rate was inhibited to 50% of the value at pH 4.5 or 7.0, but the percentage carbohydrate synthesis was unaltered. The finding of SUSSMAN *et al.*[1], that raising the pH of the fermentation medium resulted in decreased cellular carbohydrate synthesis, is therefore not of general application.

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3. It is pointed out that the percentage conversion to carbohydrate depends on at least five factors, all of which may be altered by changes of internal pH induced by change in the pH of the medium. The discussion of SUSSMAN *et al.*, postulating a direct effect of external pH on the equilibrium constant of the yeast phosphorylase, appears to be an over-simplification of the picture.

RÉSUMÉ

1. Lors de la fermentation complète de 2 mg de glucose par la levure des boulangers, la vitesse de fermentation et le pourcentage de glucose transformé en hydrate de carbone cellulaire étaient inchangés lorsque le pH du milieu externe variaient de 4.5 à 7.0.

2. A un pH de 8.5 la vitesse de fermentation était réduite à la moitié de la valeur qu'elle avait à pH 4.5 ou 7.0, mais le pourcentage de glucose transformé en hydrate de carbone n'était pas changé. SUSSMAN *et al.*[1] ont trouvé que, lorsque le pH du milieu de fermentation augmentait, la synthèse d'hydrate de carbone cellulaire diminuait; cette découverte n'est donc pas d'une application générale.

3. Le pourcentage de conversion en hydrate de carbone dépend d'au moins cinq facteurs qui tous peuvent être altérés par des changements de pH interne induits par une variation du pH du milieu. La discussion de SUSSMAN *et al.* qui postule un effet directe du pH externe sur la constante d'équilibre de la phosphorylase de levure semble être une simplification trop poussée du phénomène.

ZUSAMMENFASSUNG

1. Bei der vollständigen Vergärung von 2 mg Glucose durch Bäckereihefe blieben die Gärungsgeschwindigkeit und die prozentuelle Umwandlung von Glucose in Zell-Kohlenhydrat unverändert, wenn das pH des äusseren Mediums von 4.5 bis 7 variierte.

2. Bei pH 8.5 war die Gärungsgeschwindigkeit auf 50% des ursprünglichen Wertes (bei pH 4.5 oder 7) herabgesetzt, aber die prozentuelle Kohlenhydrat-Synthese war unverändert. Der Befund von SUSSMAN *et al.*, dass Erhöhung des pH im Gärungsmedium die Zellkohlenhydrat-Synthese herabsetzt, kann darum keine allgemeine Gültigkeit haben.

3. Es wird darauf hingewiesen, das die prozentuelle Umwandlung in Zell-Kohlenhydrat von mindestens 5 Faktoren abhängt, die alle durch Variationen des inneren pH beeinflusst werden können, welch letztere wieder durch eine Änderung des pH im Medium hervorgerufen sind. Die Diskussion von SUSSMAN *et al.* welche einen direkten Effekt des äusseren pH auf die Gleichgewichtskonstante der Hefephosphorylase postuliert, stellt eine zu weit gehende Vereinfachung des Bildes dar.

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