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Enzyme Formation in *Saccharomyces fragilis*

1. INVERTASE AND RAFFINASE

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(*Received* 26 *February* 1953)

The phenotype of an organism is a variable property which is dependent upon an interaction between its genotype and its environment and can be altered by changes in either of these. A necessary corollary of this is that the genotype can only be reliably deduced from the phenotype when either the environment remains constant or the influence of the environment on the phenotype is accurately known. Experimentally, it is no simple matter to maintain growing cells in a constant environment, and a detailed knowledge of the effect of the environment on the phenotype would appear to be an essential prerequisite for an understanding of the mechanism of the genic control of enzyme formation. It was with this aim in view that investigations into the factors affecting enzyme formation in *Saccharomyces fragilis* were begun. This paper will be chiefly concerned with the anaerobic utilization of sucrose and raffinose by this yeast.

Willstätter, Lowry & Schneider (1925) and De Ley & Vandamme (1951) have described increases in the invertase content of brewer's yeast following continuous addition of sucrose to the washed yeast suspended in a salt solution containing ammonium, potassium, magnesium, nitrate and phosphate ions. The former authors used as their starting material a yeast in which invertase activity had become attenuated during repeated fermentations in the brewery and they showed that the slow addition of sucrose gave a much bigger stimulation than a large single addition at the start. Glucose and maltose were almost as effective if added in the same way. The maximum increase in invertase activity which they obtained was about 14-fold. De Ley & Vandamme (1951) showed that invertase activity of the cells was greatest during the stationary phase of growth on a beer-wort medium and that the ability to form extra invertase on slow addition of sucrose was also greatest for cells harvested during the stationary phase. Omission of ammonium ions or phosphate resulted in a marked fall in the amount of invertase formed, whereas replacement of ammonium ions by asparagine resulted in increased invertase formation.

It is possible that the glucose-fructose linkage in raffinose is hydrolysed by invertase. However, the rate of hydrolysis is only about one-tenth that for sucrose by the same enzyme preparations. Adams, Richtmeyer & Hudson (1943), among others, considerably purified invertase from brewer's yeast and tested the preparations for ability to hydrolyse a number of di-, oligo-, and poly-saccharides. Their most highly purified preparations possessed β-fructofuranosidase, α-D-galactosidase, β-D-glucosidase, β-D-mannosidase and inulase activities. The ratio (rate of sucrose hydrolysis)/(rate of raffinose hydrolysis) varied in these experiments from 4·11 to 8·59, yet despite the fact that their preparations were obviously impure, the authors were of the opinion that sucrose and raffinose were hydrolysed by the same enzyme. There is, therefore, as has been stated by Neuberg & Mandl (1950), no certainty that hydrolysis of the glucose-fructose linkage in sucrose and raffinose is due to the activity of invertase. Recently, however, Gilliland (1949) and Winge & Roberts (1952) have shown that *Saccharomyces italicus* has three polymeric genes controlling the ability to ferment raffinose, and that the ability to ferment sucrose appears to be controlled by the same three genes. A considerable number of yeast hybrids was examined, and in every case fermentation of raffinose and rapid fermentation of sucrose

occurred together. Failure to ferment raffinose entailed failure to ferment sucrose rapidly. The raffinose non-fermenters could ferment sucrose very slowly, but this latter activity was correlated with the presence of a gene M_1 controlling the ability to ferment maltose by means of a non-specific α-glucosidase. Winge & Roberts (1952) appear to believe that a single enzyme, β-fructofuranosidase, is involved in both the rapid fermentation of sucrose and fermentation of raffinose. However, Hestrin & Lindegren (1950*a*, *b*) state that certain hybrid saccharomycetes yield extracts containing a sucrase which is inactive on raffinose and on maltose, and they are of the opinion that there may be a specific gluco-sucrase.

S. fragilis is a lactose fermenter and is completely unable to utilize maltose for growth, either aerobically or anaerobically. It would appear unlikely, therefore, that sucrose utilization by this yeast would be complicated by the possibility of hydrolysis by means of non-specific α-glucosidases, and this yeast should therefore be suitable material with which to investigate the relationship between sucrose and raffinose hydrolysis. The fermentation of raffinose by *S. fragilis* seems to be an adaptive property (Karström, 1930), whereas fermentation of sucrose is normally a constitutive property. A study of the conditions under which the ability to hydrolyse sucrose and raffinose can be acquired might, therefore, be expected to yield information concerning the identity or otherwise of the enzymes involved.

METHODS

Organism, growth media and cell suspensions. The organism used throughout this work was a strain of *S. fragilis* Jörgensen obtained from the Centraalbureau voor Schimmelcultures at Delft. The basal medium was that of Davies, Falkiner, Wilkinson & Peel (1951) and contained ammonium and potassium phosphates, trace metals and Difco yeast extract. To this was added an appropriate source of carbon as indicated in the following paragraphs. The basal medium and glucose, fructose and galactose solutions were sterilized in the autoclave; sucrose, lactose, raffinose and melibiose were sterilized by filtration. In one experiment (Fig. 1) a synthetic medium was used having a composition similar to that of the basal medium but with the Difco yeast extract replaced by the following amounts of growth factors/ml. medium: biotin, 10^{-4} μg.; calcium pantothenate, 0·03 μg.; pyridoxine, nicotinic acid, inositol and thiamine, 0·33 μg. With the exception of thiamine, which was sterilized by filtration, the growth factors were added to the rest of the medium before sterilization. For growth, the medium was dispensed into Roux bottles (150 ml./bottle), inoculated with 0·5 ml. of a 24 hr. culture grown on basal medium plus 2% (w/v) glucose (unless stated otherwise), and the bottles incubated on their sides at 25°. For the 'age of culture' experiments two sets of seven Roux bottles each were inoculated from the same culture with an interval of 12 hr. between each set. The stock culture was held at 4° in the interval between the two sowings. After incubating for the required time a suitable number of bottles was removed and the yeast harvested.

For enzymic tests the yeast was harvested on the centrifuge, washed twice with distilled water, and suspended in distilled water to a suitable density. The optical densities of the suspensions were measured with a Hilger absorptiometer and converted to mg. dry matter/ml. by means of a calibration curve. All experiments were carried out at 25°. Where concentrations of solutes are given as % this means % (w/v) in all cases.

Estimation of invertase and raffinase activity. This was usually determined by measuring the increase in reducing sugar when cell suspensions or extracts were incubated in test tubes at 25° with sucrose or raffinose in the following system: 1·5 ml. K_2HPO_4-citric acid buffer, pH 4·5 (prepared according to the formula of McIlvaine (1921) but using K_2HPO_4 in place of Na_2HPO_4), 1·0 ml. 10% sucrose, 0·25 ml. 1% cetyltrimethylammonium bromide (CTAB), cell suspension or preparation and distilled water to a total volume of 4·0 ml. Duplicate tubes were set up containing all the components except the sugar, and at zero time the sugar was added to one tube. At the end of the incubation period, usually 30 min., 1·0 ml. of 5% $ZnSO_4$, $7H_2O$ and 1·0 ml. of 0·3 N-$Ba(OH)_2$ were added to both tubes and the sugar to the control. The precipitate was removed immediately by centrifugation and a sample of the supernatant analysed for reducing sugar. Conditions were so chosen that the amount of reducing sugar formed did not exceed 2 μmoles/30 min.; under these conditions the rate of formation of reducing sugar is proportional to the amount of enzyme present. Enzyme activities are expressed as μmoles sugar hydrolysed/mg. dry wt. yeast/hr., and one enzyme unit is defined as that amount of enzyme which will hydrolyse 1·0 μmole of sucrose or raffinose in 1 hr. at 25° under the above conditions.

When determining the enzyme content of whole cells there is always the possibility that permeability effects may be a controlling factor and the total enzyme content of the cell may not be estimated. CTAB at a suitable concentration removes these permeability barriers (Salton, 1951). At the CTAB concentration used in the tests described above fermentation of sucrose and raffinose is abolished but hydrolysis is unaffected. Although in this particular case the presence of CTAB has little effect on the measured invertase activity, other enzymic activities (e.g. lactase activity in *S. fragilis*, Davies, unpublished) are greatly increased in the presence of CTAB. Consequently, CTAB has been added as a routine to the invertase test system. In some of the earlier work iodoacetate at a final concentration of 0·01 M was used to inhibit fermentation, but it was subsequently found to cause some inhibition (10%) of hydrolysis. Two experiments are described in which iodoacetate was used for invertase estimations (Table 2 and Fig. 4), but the fact that it is somewhat inhibitory does not affect the conclusions drawn from the experiments.

Estimation of reducing sugar. The methods of Shaffer & Somogyi (1933) and of Nelson (1944) were used. In the case of Nelson's method the heating time was increased to 45 min., since 20 min. was found to be insufficient for maximum reduction. Iodoacetate and CTAB were found to be without effect on the estimations at the concentrations used.

Cell preparations. Preparations of broken cells were made by (*a*) shaking with glass beads (Ballotini no. 12) in the Mickle disintegrator (Mickle, 1948), (*b*) grinding with alumina according to McIlwain (1948). Extracts were pre-

pared by centrifuging the broken cell preparations at 1000 *g* for 15 min. Acetone powders were prepared by the method of Epps (1944).

Adaptation in washed-cell suspensions. Invertase and raffinase formation in washed-cell suspensions was studied by incubating 2 vol. of yeast suspension (about 10 mg. dry wt. cells/ml.), 3 vol. of 0·2 M-K_2HPO_4-citric acid buffer (usually pH 4·5) and 2 vol. of 10% sugar in 6 × 1 in. test tubes or 100 ml. centrifuge tubes, depending on the volume of the mixture. The gas phase was air. Replacement of the air by N_2 had no effect on either the rate or the extent of the adaptation. At the end of the adaptation period the cells were recovered by centrifugation, washed twice with distilled water and suspended in distilled water to a suitable density.

Fermentation rates. These were measured by the usual Warburg manometric techniques in N_2 at 25° as described by Umbreit, Burris & Stauffer (1949), and are expressed throughout as $Q^{N_2}_{CO_2}$ = μl. CO_2/mg. dry wt. cells/hr.

Sugars. With the exception of melibiose (Kebo, Stockholm), Kerfoot's bacteriological sugars were used throughout without purification.

RESULTS

Adaptive fermentation of sugars by Saccharomyces fragilis

Washed-cell suspensions prepared from 20 hr. cultures of *S. fragilis* in basal medium plus 2% glucose ferment only glucose and sucrose when incubated in phosphate buffer at pH 5·0. Galactose, lactose and raffinose are not fermented even after 6 hr. incubation. If either galactose or lactose is substituted for glucose in the growth medium then both galactose and lactose, in addition to glucose and sucrose, are fermented by the suspensions, but raffinose is fermented only very slowly. If raffinose is substituted for glucose in the growth medium then suspensions of cells prepared therefrom rapidly ferment raffinose but not galactose or lactose (Table 1). The ability to ferment galactose, lactose or raffinose would seem to be an adaptive property (Karström, 1930), whereas ability to ferment glucose or sucrose is a constitutive property in *S. fragilis*. However, Fig. 1 shows that the fermentation of sucrose by cells grown in basal medium plus 2% glucose does not parallel that of glucose with respect to the attainment of maximum rate. For glucose this is achieved within 10 min., but for sucrose the time required is about 2 hr. Similar results were obtained with cells grown in synthetic medium containing 10% glucose. If sucrose replaces glucose in the growth medium then the maximum rate of sucrose fermentation is achieved within 10 min. also (Fig. 1). This suggests that the

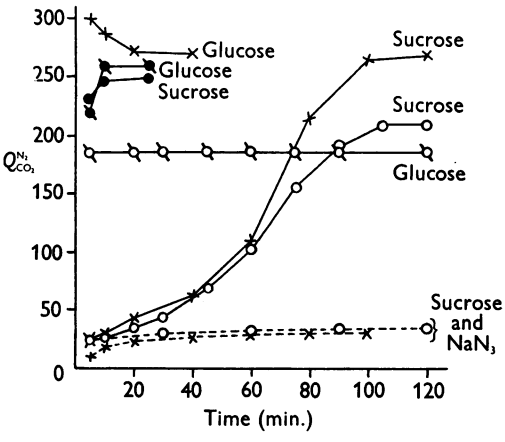

Fig. 1. Fermentation of glucose and sucrose by washed suspensions of *S. fragilis*. Warburg manometers contained in a total vol. of 3·5 ml.: 0·057 M-phosphate buffer, pH 5·0, 0·084 M-sugar, 2·5 mg. dry wt. of cells and, where added, 3×10^{-4} M-NaN_3. Gas phase: N_2. ○—○ and ◔—◔, grown in basal medium + 2% glucose; ●—● and ◕—◕, grown in basal medium + 2% sucrose; ×—×, grown in synthetic medium + 10% glucose.

ability to ferment sucrose is partially adaptive in character. The adaptive enzymes involved in galactose and lactose fermentation are not readily synthesized anaerobically in suspensions prepared from cells grown in basal medium plus 2% glucose, since they do not appear within 6 hr., even when amino acids and small amounts of glucose are added (cf. Spiegelman, 1946; Monod, 1947; Pollock & Wainwright, 1948).

S. fragilis will grow to a limited extent on basal medium alone, and washed suspensions prepared from cells grown in this way exhibit quite a different

Table 1. *Adaptive fermentation of galactose, lactose and raffinose by* S. fragilis

(*S. fragilis* grown on basal medium plus 2% sugar for 24 hr. at 25°. Warburg manometers contained, in a volume of 3·5 ml.: 0·057 M-phosphate buffer pH 5·0, 0·084 M-sugar, 2·5 mg. dry wt. of yeast. Gas phase: N_2. Activities as μl. CO_2/mg. dry wt. of cells/hr.)

Additions to basal medium	Substrate for fermentation				
	Glucose	Sucrose	Galactose	Lactose	Raffinose
Glucose	233	240	(3)*	(3)	(3)
Sucrose	256	245	(2)	(1)	20
Galactose	154	197	233	240	10
Lactose	182	185	173	230	11
Raffinose	177	163	0	0	164

* The figures in brackets are probably not significantly different from zero.

pattern of sugar fermentation from that described above. Glucose and sucrose are fermented at similar rates immediately, but the rates increase to 3 or more times their initial values over a period of 2–3 hr. Galactose, lactose and raffinose are fermented very slowly at first and the rates increase for 5–6 hr. until, for lactose and raffinose, they approximate to that for glucose. The increases in rate do not occur in the presence of 3×10^{-4} M-sodium azide, except in the case of glucose and sucrose, but even here the final rate is considerably less than that reached in the absence of azide (Fig. 2). Occasionally the increase in rate of glucose fermentation is also prevented by azide. The increase in rate of sucrose fermentation observed with cells grown in basal medium plus 2 % glucose is

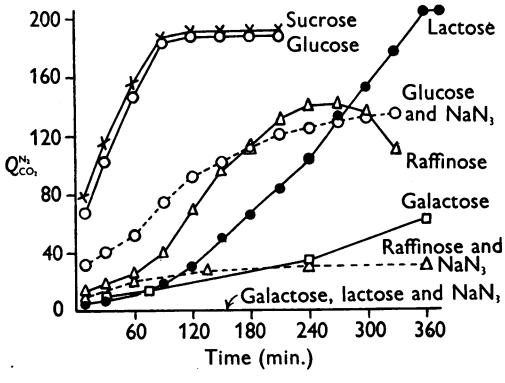

Fig. 2. Fermentation of sugars by washed suspensions of *S. fragilis* grown in basal medium alone. Conditions as for Fig. 1.

also prevented by 3×10^{-4} M-azide (Fig. 1). This effect of azide is similar to that described by Spiegelman (1947) for galactose and maltose fermentation by *S. cerevisiae*. Lactose fermentation by *S. fragilis* is inhibited to the extent of 30 % by 3×10^{-4} M-azide and it cannot be definitely concluded that adaptation is inhibited by azide in this case.

The increase in the rate of fermentation of glucose shown by cells which have been grown on basal medium alone suggests that part of the increase in the rate of galactose, lactose and raffinose fermentation might be due to a general rise in the activity of that part of the glycolysis system that is common to all sugars. This possibility is supported by the results of the following experiment. Suspensions of *S. fragilis*, grown in basal medium and showing a low initial rate of fermentation of glucose ($Q^{N_3}_{CO_2} = 43$ in 0·1 M-phosphate, pH 5·0), were incubated in 0·15 M-glucose–0·1 M-phosphate, pH 5·0, until the fermentation rate was at a maximum ($Q^{N_3}_{CO_2} = 189$). The cells were recovered, washed in water and the rates of fermentation of galactose and lactose measured under the same conditions. The $Q^{N_3}_{CO_2}$ for galactose had risen from 9 to 27 and for lactose from 3 to 35.

It is clear from these experiments that the composition of the growth medium affects not only the enzymic composition of the cells, but also the subsequent ability of the cells to acquire new activities. Following growth for 24 hr. in basal medium plus 2 % glucose, the cells are unable to ferment galactose, lactose and raffinose and are unable to adapt to ferment these sugars within 6 hr. The ability to ferment sucrose is initially low, but adaptation can occur rapidly. If growth takes place in basal medium alone, then the resulting cells ferment galactose, lactose and raffinose at a very low initial rate but can now adapt with varying degrees of rapidity, while fermentation of sucrose proceeds at a rate equal to that of glucose.

Fermentation and hydrolysis of sucrose

The plot of fermentation rate against time for sucrose fermentation by *S. fragilis* grown in basal medium plus 2 % glucose gives the typical S-shaped curve described by other workers for the formation of adaptive enzymes (e.g. Spiegelman, 1946), and the effect of azide is consistent with the view that adaptive enzyme formation is involved. Table 2 (*a*) shows that the increase in rate of sucrose fermentation is in fact accompanied by an increase in invertase activity, and that this increase does not occur in presence of azide (Table 2*b*). During the adaptation there is an increase in cell substance, as determined turbidimetrically, and also an increase in cell numbers which is of borderline significance (Table 3). Similar increases also occur when glucose replaces sucrose, though in this case very little invertase is formed and therefore the changes in cell substance may be unrelated to invertase formation. If it is assumed that the new invertase is formed only in the new cell substance, then this would have an invertase content of 44 units/mg. dry wt., a value which is by no means impossible since values as high as 150 have been observed for this yeast (see Table 9).

Attempts were made by various means to obtain preparations from unadapted cells having activities approaching those of adapted cells, but in no case was this successful (Table 4).

Rate of formation of invertase by cell suspensions. This is shown in Fig. 3. The curve is of the typical S-shape characteristic of many adaptive-enzyme systems. The 'growth rate' constant for invertase formation (see Table 7), calculated from the linear portion of the ln (invertase units) plot (Fig. 3), is 2·03, which is very similar to the value of 2·87 for maltase formation calculated from the figures given by Halvorson & Spiegelman (1952).

Effect of pH *on invertase formation by cell suspensions.* A washed-cell suspension of density 8·0 mg. dry wt./ml. was prepared from a 22 hr. culture in basal medium plus 10% glucose, and samples were incubated with sucrose in citrate-phosphate buffers of the appropriate pH for 1·5 hr. The cells were recovered, washed twice with distilled water and suspended in water at a density of

Table 2. *Formation of invertase during adaptation to sucrose by washed-cell suspensions of* S. fragilis

(Fermentation rates measured as in Table 1. Adaptations carried out by incubating 2 vol. of yeast suspension (10 mg. dry wt. cells/ml.) with 3 vol. of 0·2 M-K_2HPO_4-citric acid buffer pH 4·5 and 2 vol. of 10% (w/v) sucrose in air at 25°, without shaking, for the times indicated below. The cells were then recovered, washed twice with distilled water and resuspended in distilled water. Invertase content of the cells was estimated by measuring the increase in reducing sugars when the cell suspension was incubated with sucrose at pH 4·5 using iodoacetate as inhibitor of fermentation. Invertase units/mg. dry wt. = μmole sucrose hydrolysed/mg. dry wt. yeast/hr.)

(*a*) *Correlation between invertase content and rate of sucrose fermentation*

(Organism grown in basal medium + 2% glucose for 19 hr.)

Period of adaptation (min.)	Q_{CO_2} (glucose)	Q_{CO_2} (sucrose)	Invertase content (units/mg. dry wt.)
0	225	36	1·4
30	249	194	4·1
90	252	302	8·4

(*b*) *Inhibition of invertase formation by* 10^{-3} M-NaN_3

(Organism grown in basal medium + 10% glucose for 20 hr. Period of adaptation 1·5 hr.)

Sugar present in adaptation system	Invertase content (units/mg. dry wt.) Intact cells	Smashed cells	Extract
None	0·12	0·12	0·18
Glucose	0·47	—	—
Sucrose	3·51	3·40	3·51
Sucrose + 10^{-3} M-NaN_3	0·23	0·18	0·18
Initial invertase	0·18	0·18	0·18

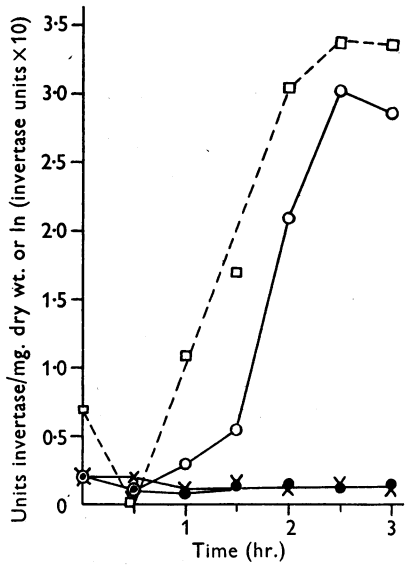

Fig. 3. Invertase formation by washed-cell suspensions of *S. fragilis*. Cell suspensions incubated with citrate-phosphate buffer, pH 4·5, ●—●; buffer plus sucrose, ○—○; buffer plus glucose ×—× as described in Table 2. □—□, ln (invertase units).

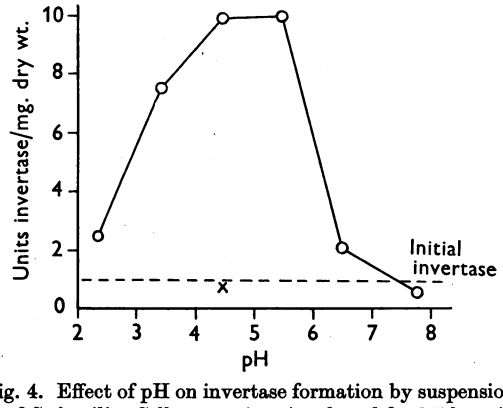

Fig. 4. Effect of pH on invertase formation by suspensions of *S. fragilis*. Cell suspensions incubated for 1·5 hr. with sucrose in citrate-phosphate buffer of the appropriate pH as described in Table 2. Invertase estimated in presence of 0·01 M-iodoacetate. × = Invertase content when incubated with glucose at pH 4·5.

Table 3. *Changes in dry matter and in total cell numbers during adaptation to sucrose in washed cell suspensions*

(Adaptations and invertase estimations carried out as in Table 2; period of adaptation 2·5 hr.; invertase content expressed as units/mg. dry wt. cells. Dry matter estimated turbidimetrically. Each cell count is the mean of nine separate determinations.)

Sugar present in adaptation system	Invertase content Initial	Final	Dry matter (mg./ml.) Initial	Final	Cell numbers × 10^{-5} Initial	Final
None	0·50	0·53	1·61	1·67	145 ± 20	146 ± 20
Glucose	0·50	0·70	1·57	2·06	143 ± 20	159 ± 20
Sucrose	0·50	7·0	1·60	1·88	137 ± 20	164 ± 20

Table 4. *Effect of various treatments on invertase activities of unadapted and adapted* S. fragilis

(The yeast was grown in basal medium + (*a*) 10% glucose for 24 hr., (*b*) 2% glucose for 42 hr., (*c*) 2% glucose for 16 hr. for unadapted yeast or 1% raffinose for adapted yeast. In (*a*) the adapted cells were obtained by incubating the unadapted cells with sucrose and buffer for 2 hr. as described in Table 2. For the invertase estimations CTAB was omitted from the normal test system. Treatments were carried out as follows: (1) *CTAB*—0·25 mg. CTAB/mg. dry wt. yeast was added to the yeast suspension and the invertase activity measured; (2) *Mickle*—10 ml. suspension (116 mg. dry wt. yeast) was shaken with glass beads in the Mickle disintegrator for 30 min. and invertase estimated in the resulting preparation; (3) *acetone*—10 ml. suspension (116 mg. dry wt. yeast) was added rapidly to 80 ml. acetone at 0° and allowed to stand at 0° for 20 min., cells were recovered, washed with acetone and ether and dried *in vacuo*; (4) *alumina*—yeast equivalent to 232 mg. dry wt. ground with alumina as described by McIlwain (1948) and 0·2 M-K_2HPO_4-citric acid buffer, pH 4·5, added to give a total vol. of 20 ml.; invertase estimated on the resulting mixture.)

	Treatment given to suspension				
Kind of cells	None	CTAB	Mickle	Acetone	Alumina
(*a*) Unadapted	0·40	0·50	0·56	0·38	—
Adapted	4·55	4·80	4·69	5·10	—
(*b*) Adapted	18·7	18·3	18·2	14·7	15·7
(*c*) Adapted	—	108·2	—	—	—
Unadapted	0·09	0·28	0·28	—	—
Unadapted plus boiled extract of adapted cells	—	0·22	0·28	—	—

Table 5. *Effect of acetate on sucrose fermentation and on adaptation to sucrose by cell suspensions*

(Fermentation rates were measured on the adapted cells as in Table 1. The yeast was grown in basal medium + 10% glucose for 18 hr. For (*a*) adaptation was carried out in sucrose + 0·2 M-phosphate buffer, pH 5·0; for (*b*) adaptation was carried out in N_2 in Warburg manometers containing 0·084 M-sucrose, 0·2 M-buffer (phosphate or acetate) of the appropriate pH, and 2·1 mg. dry wt. of yeast in a total volume of 3·5 ml. The values for $Q_{CO_2}^{N_2}$ at pH 6·0 have been corrected for retention of CO_2.)

		(*a*) $Q_{CO_2}^{N_2}$		(*b*) $Q_{CO_2}^{N_2}$ (sucrose)	
pH	Buffer	Glucose	Sucrose	Initial	After 2·5 hr. adaptation
6·0	Phosphate	286	217	7	232
	Acetate	300	260	6	101
5·5	Phosphate	300	242	7	228
	Acetate	294	240	6	39
5·0	Phosphate	295	255	12	234
	Acetate	100	88	4	20
4·5	Phosphate	268	250	—	—
	Acetate	40	48	4	4

Table 6. *Inhibition of adaptation to sucrose by acetate*

(The yeast was grown in basal medium + 10% glucose for 17 hr. Adaptation was carried out in N_2 in Warburg manometers containing 2·5 mg. dry wt. of cells, 0·084 M-sucrose, 0·086 M-phosphate buffer pH 5·5, potassium acetate buffer, pH 5·5, to give the acetate concentrations indicated in the table, and sufficient 0·2 M-KCl to give 0·143 M-K^+ in all manometers.)

	Acetate concentration (M)					
	0	0·011	0·023	0·034	0·046	0·057
$Q_{CO_2}^{N_2}$ (glucose), initial	153	154	157	164	167	161
$Q_{CO_2}^{N_2}$ (sucrose), initial	8	8	8	8	5	5
$Q_{CO_2}^{N_2}$ (sucrose), after 2 hr.	132	140	69	42	27	19

4·0 mg. dry wt./ml. Invertase was determined using iodoacetate as inhibitor of fermentation. The result is given in Fig. 4. Maximum invertase is formed at pH 4·5–5·5. The same result was obtained if the rate of increase of $Q_{CO_2}^{N_2}$ (sucrose) was used as the measure of adaptation.

Effect of acetate on sucrose fermentation. In the course of the experiments just described, sodium acetate buffers were used to extend the pH range beyond that of the KH_2PO_4-Na_2HPO_4 system. It was found that for values of pH below 5·5 the rate of fermentation of glucose or sucrose was markedly

different in the two buffer systems. The rate rapidly diminished in acetate buffer below pH 5·5. Development of the ability to ferment sucrose is also impaired in acetate buffers when compared with the corresponding phosphate buffers (Table 5). Citrate-phosphate buffers give the same results as phosphate buffers. Failure to adapt in acetate buffer at pH 5·5 is due neither to lack of phosphate nor of potassium ions, since the inhibition still occurs in mixed acetate-phosphate buffer at this pH. At pH 5·5 the fermentation rates for glucose and sucrose are the same in either buffer. If the concentrations of phosphate and potassium ions and the ionic strength are held constant then, at pH 5·5, the

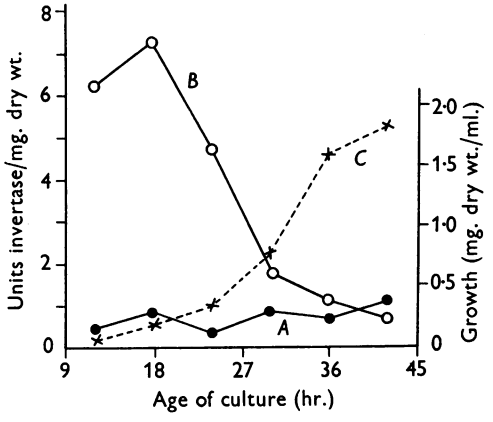

Fig. 5. Effect of 'age of culture' on ability of washed suspensions of *S. fragilis* to form invertase. The yeast was grown in basal medium + 10% glucose and adaptation was carried out at pH 4·5 for 2 hr. as described in Table 2. Curve A = invertase content of cells/mg. dry wt. before adaptation; curve B = same after adaptation; curve C = growth of cells in mg. dry wt./ml. culture.

ability to adapt is inversely related to the acetate concentration (Table 6). Adaptation does occur in acetate buffers at, or above, pH 6·0, and it would seem possible that the lack of adaptation is due to inhibition by undissociated acetic acid.

Effect of time of growth on ability to adapt. The extent to which adaptive formation of invertase occurs in cell suspensions incubated with sucrose and buffer varies with the time for which the cells have been grown (Fig. 5). Adaptation takes place most readily in young cells growing at their maximum rate and falls off as the growth rate decreases until, towards the end of growth, the cells cease to respond to sucrose. These observations are in agreement with those of Hegarty (1939) on adaptation to galactose by *Streptococcus lactis* and the opposite of those of De Ley & Vandamme (1951) for invertase formation by a bottom yeast.

Effect of time of growth on invertase content of cells grown in basal medium plus various sugars. (*a*) 2% Glucose. Occasional batches of cells which had been grown for longer than 24 hr. on this medium were found to ferment sucrose at the maximum rate without an initial period of adaptation. An investigation of the variation of invertase content of the cells with time of growth gave the results shown in Fig. 6. The invertase content remains low throughout the early, rapid phase of growth, and only starts to increase when growth slows down after about 18 hr. It increases very rapidly about the time that growth ceases. The values for the total intracellular invertase present in each Roux bottle are also shown in Fig. 6, and it will be seen that this increases continuously throughout the growth period.

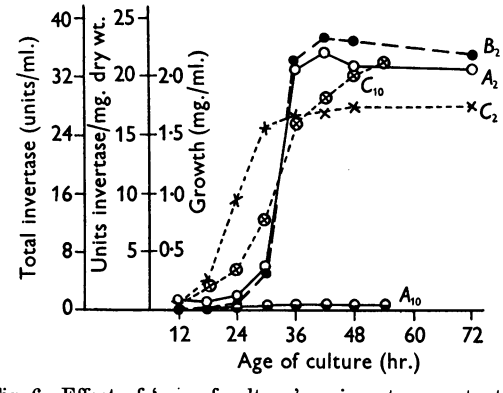

Fig. 6. Effect of 'age of culture' on invertase content of *S. fragilis* and on total intracellular invertase formed during growth. The yeast was grown in basal medium + (*a*) 2% glucose, or (*b*) 10% glucose, the inoculum being taken from a 24 hr. culture in basal medium + 2% glucose. Total intracellular invertase content of culture/ml. = (invertase/mg. dry wt. cells) × (dry wt. cells/ml. culture). Curves A_2 and A_{10} = invertase content of cells/mg. dry wt.; curve B_2 = total intracellular invertase/ml. culture; curves C_2 and C_{10} = growth of cells/ml. culture. Subscripts indicate the glucose content of the growth medium.

(*b*) 10% Glucose. With 10% glucose in the growth medium the invertase content of the cells remains low throughout the growth period of 54 hr. (Fig. 6, curves A_{10} and C_{10}).

(*c*) 2% Sucrose. The results with 2% sucrose in the growth medium are shown in Fig. 7. It was expected that the invertase content of the cells would reach a high level in the early stages of growth and would remain high, possibly falling after growth had ceased. The observed behaviour is quite different. The enzyme content is certainly high in very young cells, but during the period of maximum growth rate it rapidly falls, to rise again towards the end of growth. The total amount of intracellular invertase present in the culture is seen to increase throughout the growth period. The

variation in invertase content per unit mass of cells is therefore due to a difference between the rate of invertase synthesis and the rate of cell growth. This is more clearly seen in Table 7, where the 'growth rates' for cell substance and invertase are compared for cultures grown on basal medium plus 2 % glucose or 2 % sucrose. In the very early stages of growth on sucrose, following inoculation with 24 hr. cells grown on 2 % glucose, the cells of the inoculum probably become adapted in the same way as do washed-cell suspensions incubated with buffered sucrose solutions. Hence the high invertase content of the very young cells. During the logarithmic growth phase, and for some time after, cell growth proceeds faster than invertase synthesis, the latter in fact decreasing. In the later stages the cell growth rate falls but the 'growth rate' for invertase increases again up to the point where growth ceases, when it drops to zero. When the growth medium contains 2 % glucose the 'growth rate' for invertase does not show the fall in the middle periods which is found in the sucrose medium under the conditions given in Table 7*b*.

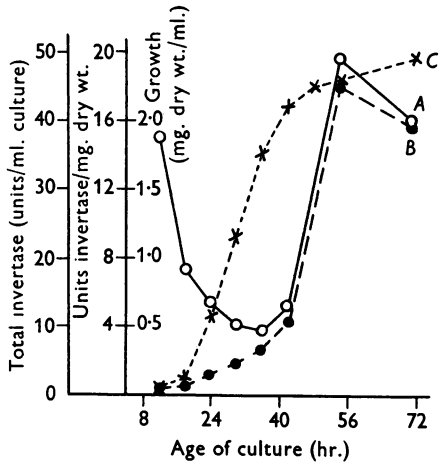

Fig. 7. Effect of 'age of culture' on invertase content of *S. fragilis* and on total intracellular invertase formed during growth on basal medium + 2 % sucrose. The inoculum was as for Fig. 6. Curve *A* = invertase content of cells/mg. dry wt.; curve *B* = total intracellular invertase/ml. culture; curve *C* = growth of cells in mg. dry wt./ml. culture.

(*d*) 1 % Raffinose. The failure of cells growing in basal medium plus 10 % glucose to synthesize invertase might be interpreted as an inhibition of synthesis by glucose. The other growth experiments give no clear evidence in support of this. However, evidence which strongly suggests that glucose may be inhibitory of invertase formation was obtained from an examination of the invertase content of cells growing in basal medium plus 1 % raffinose. The results are shown in Fig. 8. In this case the invertase content of the cells increases rapidly to reach a peak value at the end of the logarithmic growth phase which is almost 20 times higher than the maximum value found for cells growing in 2 % sucrose medium. In the later stages of growth the invertase content falls somewhat, but is still about 10 times higher than the value for cells grown in 2 % sucrose medium. The total intracellular invertase content of the culture increases more or less in parallel with cell growth. This great increase in invertase is not to be ascribed specifically to an

Table 7. *'Growth rates' for cell substance and invertase for* S. fragilis *growing on (a) glucose, (b) and (c) sucrose*

(The yeast was grown in basal medium plus (*a*) 2 % glucose, (*b*) and (*c*) 2 % sucrose. In (*a*) and (*b*) the media were inoculated from a 24 hr. culture in basal medium plus 2 % glucose; in (*c*) the inoculum was from a 24 hr. culture in basal medium plus 10 % glucose. Invertase was determined in presence of CTAB.)

$$\text{Growth rate (cell substance)} = \frac{1}{t_2 - t_1} \ln \frac{\text{(mg. dry wt. cells/ml. culture) at } t_2}{\text{(mg. dry wt. cells/ml. culture) at } t_1},$$

$$\text{'Growth rate' (invertase)} = \frac{1}{t_2 - t_1} \ln \frac{\text{(total intracellular invertase/ml. culture) at } t_2}{\text{(total intracellular invertase/ml. culture) at } t_1}.$$

Period of growth (hr.)	'Growth rate' (*a*) Cell substance	(*a*) Invertase	(*b*) Cell substance	(*b*) Invertase	(*c*) Cell substance	(*c*) Invertase
0–12	0·24	0·19	0·20	0·39	0·19	0·24
12–18	0·22	0·185	0·25	0·12	0·21	0·12
18–24	0·24	0·34	0·20	0·15	0·26	0·29
24–30	0·085	0·26	0·12	0·08	0·13	0·33
30–36	0·01	0·31	0·065	0·05	0·06	0·39
36–42	0·0	0·015	0·03	0·09	0·004	0·08
42–48	0·007	0·0	0·01	0·14	0·006	0·02
48–54	0·004	0·0	0·004	0·10	0·0	0·003

effect of raffinose, since growth in the basal medium alone also yields cells with a high invertase content. Thus, *S. fragilis* harvested after 18 hr. growth in basal medium alone has an invertase content of 132 units/mg. dry wt., compared with 7·3 for cells grown in basal medium plus 2 % sucrose and 138 for cells grown in basal medium plus 1 % raffinose.

Effect of the inoculum on the course of invertase formation. It is conceivable that the invertase content of cells growing in a sucrose medium may be affected by the initial invertase content of the inoculum. In the experiments of Fig. 7 and Table 7 the inoculum was taken from a 24 hr. culture on 2 % glucose-basal medium and the cells had an invertase

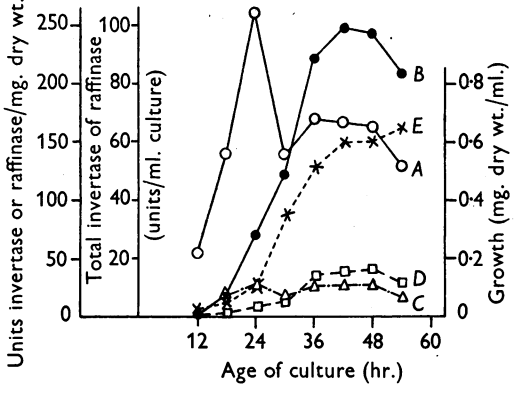

Fig. 8. Effect of age of culture on invertase and raffinase content of *S. fragilis* and on total intracellular invertase and raffinase formed during growth on basal medium + 1 % raffinose. The inoculum was as for Fig. 6. Curve *A* = invertase content of cells/mg. dry wt.; curve *B* = total intracellular invertase/ml. culture; curve *C* = raffinase content of cells/mg. dry wt.; curve *D* = total intracellular raffinase/ml. culture; curve *E* = growth of cells in mg. dry wt./ml. culture.

content of 1·4 units/mg. dry wt. The experiment was repeated using an inoculum taken from a 24 hr. culture in basal medium plus 10 % glucose, in which the cells had an invertase content of 0·28 unit/mg. dry wt. Such cells are able to form invertase when incubated in phosphate buffer plus sucrose (see Fig. 5). The results obtained are given in Fig. 9 and Table 7*c*. The initial rise in invertase content is now absent and the 'growth rate' for invertase increases throughout the growth period. In fact, the behaviour is similar to that found when growth takes place on glucose instead of sucrose. When the inoculum is grown in basal medium plus 2 % sucrose (invertase content of inoculum 7·0 units/mg. dry wt. cells) the variation of invertase content with time of incubation follows a pattern similar to that obtained when the inoculum is taken from a 2 % glucose culture (Fig. 10). It is clear from these experiments that the changes in invertase content during growth on 2 % sucrose medium are markedly influenced by the history of the inoculum used. It is by no means impossible that similar effects occur with other enzymes.

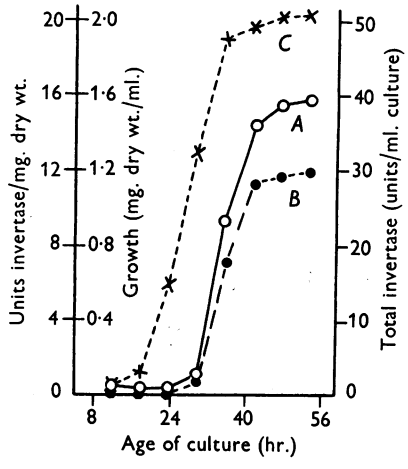

Fig. 9. As for Fig. 7, but inoculum taken from a 24 hr. culture in basal medium + 10 % glucose. Curve *A* = invertase content of cells/mg. dry wt.; curve *B* = total intracellular invertase/ml. culture; curve *C* = growth of cells in mg. dry wt./ml. culture.

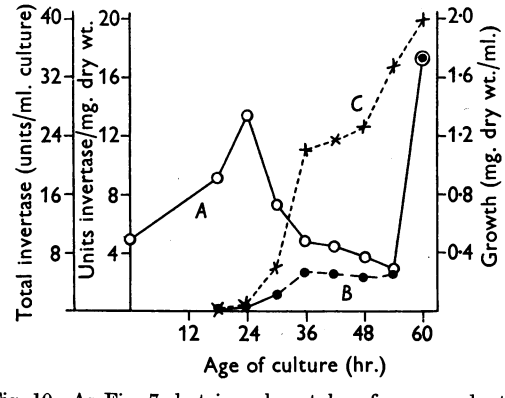

Fig. 10. As Fig. 7, but inoculum taken from an adapted culture grown for 15 hr. in basal medium + 2 % sucrose. Curve *A* = invertase content of cells/mg. dry wt.; curve *B* = total intracellular invertase/ml. culture; curve *C* = growth of cells in mg. dry wt./ml. culture.

Stimulation of invertase formation in washed suspensions of S. fragilis *by sugars other than sucrose.* The ability of a number of sugars to substitute for sucrose in stimulating the formation of invertase in washed cell suspensions was investigated using the test system described in the Methods section. With a sugar concentration of 2·86 % there was no significant stimulation by glucose, raffinose, lactose, galactose or melibiose, although fructose appeared to support some synthesis. The failure of raffinose to

stimulate is noteworthy, since raffinose is generally believed to be hydrolysed by invertase, and is in fact hydrolysed by cell suspensions of *S. fragilis* at about one-tenth the rate of sucrose. An investigation of the influence of sugar concentration on invertase formation under these conditions shows that the stimulation is not specific for sucrose. Fig. 11 shows the relationship between sugar concentration and extent of adaptation for sucrose, glucose, fructose and raffinose. Low concentrations of glucose and

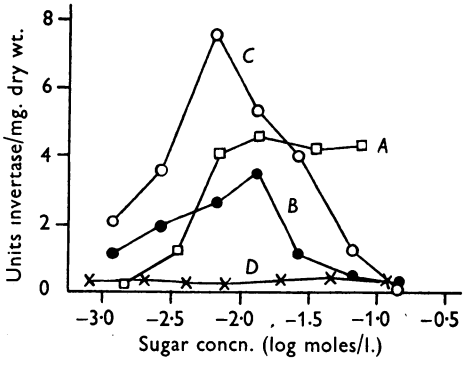

Fig. 11. Effect of sugar concentration on stimulation of invertase formation by washed-cell suspensions of *S. fragilis*. The yeast was grown in basal medium + 10% glucose for 18 hr. Adaptation was carried out at pH 4·5 for 3 hr. as described in Table 2. Curve *A* = sucrose; curve *B* = glucose; curve *C* = fructose; curve *D* = raffinose.

fructose are as effective as sucrose in stimulating invertase formation, but high concentrations are strongly inhibitory. Willstätter *et al.* (1925) showed that the slow addition of glucose was as effective as that of sucrose in stimulating invertase formation in a brewing yeast.

Fermentation and hydrolysis of raffinose

Quantitative estimation of the carbon dioxide liberated when raffinose was fermented to completion by washed-cell suspensions of *S. fragilis* in phosphate buffer at pH 4·5 and in the presence of 3×10^{-4} M-azide, gave the value 1·83 moles carbon dioxide/mole raffinose fermented. This suggests that the products of raffinose fermentation by this yeast are ethanol, carbon dioxide, fructose and melibiose. Melibiose cannot be metabolized by *S. fragilis*, and it has been shown by paper chromatography (Diding, unpublished results) that melibiose accumulates in the medium during growth of the yeast on basal medium plus 1% raffinose, and also during the fermentation of raffinose by washed suspensions of the organism. No sugars other than raffinose, fructose and melibiose could be detected.

Fermentation of raffinose by washed-cell suspensions. It has been shown (Fig. 2) that washed-cell suspensions of *S. fragilis* grown in basal medium alone slowly acquire the ability to ferment raffinose and that this response does not occur in the presence of 3×10^{-4} M-azide. On the other hand, fermentation of raffinose by cells grown in its presence is unaffected by this concentration of azide (Fig. 12). These observations would suggest that the increase in fermentation rate is due to an adaptive increase in some part of the raffinose fermenting system, although the situation is obviously complicated by the fact that the rate of fermentation of glucose also increases. However, in an experiment designed to examine the effect of adaptation to raffinose in cell suspensions on the subsequent ability to ferment

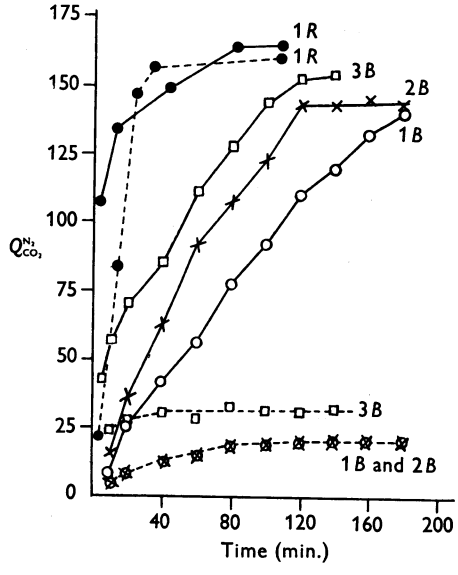

Fig. 12. Fermentation of raffinose, glucose and fructose by washed-cell suspensions of *S. fragilis* grown in basal medium alone or + 2% raffinose. Warburg-manometer contents as in Fig. 1. $NaN_3 = 10^{-3}$ M. Full lines = no NaN_3; broken lines = + NaN_3. *B* = cells grown in basal medium alone; *R* = cells grown in basal medium + 2% raffinose. Curve 1 = raffinose fermentation; curve 2 = glucose fermentation; curve 3 = fructose fermentation.

glucose, galactose and lactose, it was found that the rate of fermentation of raffinose by the adapted cells fell to about one-fourth to one-fifth when they were washed twice in distilled water. Glucose fermentation was not affected. Washing in 0·067 M-potassium dihydrogen phosphate, with or without 0·01 M-cysteine, had no protective effect. Removal of the cells by centrifugation and resuspension in buffer without washing also produced the drop in activity (Table 8). The washed cells gradually regained their activity when incubated with raffinose for 2–3 hr. That removal of an exogenous raffinase is not the explanation of these effects was shown by incu-

Table 8. *Effect of washing on fermentation of raffinose and glucose*

(The yeast was grown in basal medium alone for 48 hr. For fermentation rates Warburg manometers contained in a total vol. of 3·5 ml.: 0·086 M-phosphate buffer, pH 5·0, 0·084 M-sugar, 2·5 mg. dry wt. cells; gas phase, N_2. Adaptation to raffinose carried out as described for sucrose in Table 2.)

Treatment of suspension	$Q_{CO_2}^{N_2}$ Expt. I Raffinose	Expt. II Raffinose	Expt. II Glucose	Expt. III Raffinose	Expt. IV Raffinose	Expt. IV Supernatant fluid from adaptation system	Expt. IV Fructose	Expt. IV Glucose
(*a*) Initial suspension	16	5	—	15	15	—	45	49
(*b*) Incubated with raffinose for 3·5 hr.	182	164	166	192	140	—	140	148
(*c*) Cells from (*b*) recovered and:								
(i) resuspended in buffer without washing	—	—	—	71	—	—	—	—
(ii) washed twice with distilled water	33	48	146	—	51	101	130	139
(iii) washed twice with 0·07 M-KH_2PO_4	—	50	155	66	—	—	—	—
(iv) washed twice with 0·07 M-KH_2PO_4 + 0·01 M-cysteine	—	—	—	64	—	—	—	—

Table 9. *Invertase and raffinase content of* S. fragilis *grown in various media*

(Invertase and raffinase contents given as units/mg. dry wt. of cells.)

	Additions to basal medium: None	2% Raffinose		2% Galactose	2% Lactose	2% Glucose		10% Glucose	2% Fructose		10% Fructose		2% Sucrose	
Growth time (hr.)	16	24	48	16	16	16	42	16	16	48	16	48	24	48
(*a*) Invertase	157·7	138·0	123·8	33·5	29·6	0·7	21·2	0·54	0·56	31·8	0·24	0·83	0·48	23·2
(*b*) Raffinase	15·6	16·2	13·6	4·1	4·7	—	1·5	0·07	0·09	4·05	0·04	0·12	0·05	2·9
Ratio *a*/*b*	10·1	8·5	9·1	8·2	6·3	—	14·1	7·7	6·2	7·9	6·0	6·9	9·6	8·0

bating the cells with raffinose until adaptation was complete and then testing the ability of the cell-free suspension fluid to hydrolyse raffinose. There was no hydrolysis in 16 hr. The loss in activity was very much less if the adapted cells, washed twice with distilled water, were replaced in the fluid in which the adaptation was originally carried out, instead of in fresh raffinose solution. Moreover, the twice-washed cells could ferment fructose at an almost undiminished rate (Table 8, Expt. IV). If the maximum rate of carbon dioxide production from raffinose is only attained when an appreciable amount of fructose has accumulated in the medium (which may be a slow process), then the reduction in the rate of carbon dioxide production from raffinose brought about by washing the adapted cells could be due to the removal of fructose. Against this explanation, however, is the fact that the cells show no loss of activity on washing if they have been grown in basal medium plus 2% raffinose. For example, a sample of such a culture to which extra raffinose was added had a $Q_{CO_2}^{N_2} = 181$, while for the cells washed twice in distilled water $Q_{CO_2}^{N_2} = 164$. This difference in behaviour between cells adapted to raffinose in suspension and those adapted during growth could be easily explained if the latter cells had a much higher raffinase content than the former. This would allow a sufficient concentration of fructose to be built up much more quickly. However, direct measurement of the raffinase content of *S. fragilis* grown in basal medium, either alone or with the addition of 2% raffinose, shows no appreciable difference between the two, and in both cases it is much higher than is necessary to account for the observed value of $Q_{CO_2}^{N_2}$ (raffinose) for adapted cells (Table 9).

Raffinase content of cells grown under various conditions. A comparison of the raffinase and in-

Table 10. *Raffinase formation during adaptation to sucrose by washed-cell suspensions of* S. fragilis

(Experimental conditions as for Fig. 12, Expt. 1. Results given as invertase or raffinase units/mg. dry wt. of cells.)

	Initial enzyme	Sugar added to adaptation system			
		None	Sucrose	Glucose	Raffinose
(*a*) Invertase	0·54	0·44	7·85	0·68	0·63
(*b*) Raffinase	0·08	0·07	0·93	0·06	0·10
Ratio *a*/*b*	6·8	6·3	8·4	10·1	6·3

vertase contents of *S. fragilis* grown in various media is given in Table 9. It will be seen that there is only a twofold variation in the ratio (invertase content)/(raffinase content) for a 200-fold variation in the raffinase content. Fig. 8 shows that the invertase and raffinase activities vary in a similar manner during growth of the yeast on basal medium plus 1 % raffinose, the ratio of the two activities ranging from 5·8 to 9·8.

Raffinase content of cells following adaptation to sucrose. If raffinose and sucrose are hydrolysed by different enzymes then the two enzymes might be expected to respond differently when unadapted cells are induced to synthesize one or other enzyme in washed-cell suspensions. Table 10 shows that when unadapted *S. fragilis* is induced to form invertase by incubating in a sucrose-buffer mixture, raffinase is also formed. The difference in the ratios of the two activities before and after adaptation is probably not significant.

DISCUSSION

It is well known that the formation of many enzymes is suppressed when glucose is present in the growth medium (see review by Gale, 1943). Invertase formation by *S. fragilis* provides yet another example of this phenomenon. Not only is invertase formation in growing cultures suppressed, but its formation by washed cell suspensions, where cell multiplication is probably unimportant, is likewise prevented if a sufficiently high concentration of glucose is present. Fructose, so far as its effect has been examined, behaves similarly to glucose. If the enzymic activities exhibited by cells grown in basal medium alone are assumed to represent the levels attainable in the absence of any inhibition, then galactose, lactose and sucrose also inhibit the formation of invertase and raffinase. It is difficult to differentiate between the possibilities that the reduction in enzyme content is due to a true inhibition of the invertase and raffinase synthesizing systems by the sugars, or to an inability of these systems to compete with other systems for the available raw materials (cf. Monod, 1947; Spiegelman, 1946; Mandelstam, 1952; Mandelstam & Yudkin, 1952). The fact that sucrose is also apparently inhibitory is perhaps significant in this connexion. Hestrin & Lindegren (1952) have shown that the formation of maltase and of α-methylglucosidase is depressed when glucose is present in the growth medium along with maltose or α-methylglucoside, and they point out that 'the formation of glucosidases in a medium in which glucose is replaced by a glucoside may result simply from the absence of glucose and is not necessarily an outcome of an adaptive response to the glucoside as such'. They also suggest that in a system which has synthesized a glucosidase beyond the level necessary to keep the fermentation system maximally employed, the rising glucose concentration might prevent excessive formation of the glucosidase. No convincing evidence was presented in support of these ideas. The experiments described in this paper, however, do lend considerable support to these suggestions, at least for the enzyme invertase. When the effect of glucose is removed by growing the yeast in basal medium alone, the invertase content of the cells rises to about 10 times that found in cells grown in the presence of 2 % sucrose. Nevertheless, the invertase content of the sucrose-grown cells is still more than sufficient to account for the observed rates of fermentation of glucose and sucrose. The very high invertase content of cells grown in raffinose medium can also be readily explained if the assumption is made that raffinase and invertase are the same enzyme (see below). Raffinose is hydrolysed at only one-tenth the rate of sucrose, so that with raffinose as substrate the amount of enzyme needed to allow hexose to accumulate at the same rate as with sucrose as substrate would have to be about 20 times as great (raffinose hydrolysis yields only one fermentable hexose; sucrose yields two). If therefore accumulation of free hexose is in fact responsible for limiting the amount of invertase synthesized, the limit would not be reached with raffinose as substrate until 20 times as much invertase had been formed as with sucrose. Other factors probably come into play before this limit is reached and, as a result, cells grown in raffinose medium respond as if there was no hexose present and have the same invertase content as cells grown in basal medium alone.

It is a reasonable assumption that, in those cases of enzyme formation in which the inducing substrate is also the source of energy for enzyme synthesis, the

rate of formation of the enzyme will be a direct function of the initial amount of enzyme within the cells. On this basis failure of *S. fragilis* cells grown in glucose medium to adapt to ferment galactose, lactose and raffinose could be due to complete suppression, by glucose, of the formation of one or more of the enzymes required for the adapted state. Growth in the basal medium alone would allow a small synthesis of these enzymes, sufficient for adaptation to occur when the cells are incubated with the inducing substrate. It would appear from the effect of fructose on invertase formation in cell suspensions, and also from the invertase contents of cells grown with fructose, galactose or lactose as carbon sources, that inhibition of enzyme formation is not a specific property of glucose. Fructose would seem to be equally as effective as glucose. The reduced invertase content of cells grown in basal medium plus galactose or lactose, as compared with basal medium alone, could however be a competition effect.

With regard to the hydrolysis of raffinose by *S. fragilis*, the results presented in this paper show that despite a 200-fold variation in the ability of this yeast to hydrolyse sucrose and raffinose, the ratio of the two activities shows only a twofold variation. The values obtained for this ratio, 6·3–14·2, are not very different from those obtained by Adams *et al.* (1943), 4·11–8·59, for various preparations of purified invertase from brewer's and baker's yeasts. The observations do not support the view that sucrose and raffinose are hydrolysed by different enzymes in *S. fragilis*, but rather the reverse, that the same enzyme is involved in both cases.

It has been shown that *S. fragilis* cells grown in basal medium plus 10% glucose develop increased invertase and raffinase activities when incubated with sucrose at pH 4·5 for 2–3 hr., and at the same time the rate of sucrose fermentation rises to that for glucose. Despite the fact that the unadapted cells possess raffinase activity, they do not exhibit an increased ability to ferment raffinose when exposed to this sugar for as long as 6 hr., nor is there any change in their raffinase content. Failure to adapt in the presence of raffinose could be due to inability of this sugar to induce enzyme formation (cf. Monod, Cohen-Bazire & Cohn, 1951), but this seems unlikely. A more probable explanation would be that it is due to the much lower activity of the unadapted cells towards raffinose than towards sucrose. Invertase formation in the presence of sucrose shows a lag of about 30 min., and if the length of the lag is a function of the activity of the enzyme initially present in the cells, then a reduction in activity to one-tenth, as is the case when raffinose is substituted for sucrose, might be expected to lengthen the lag considerably. Even at the end of the lag period, the rate at which energy can be made available from raffinose is still only one-tenth of the rate from sucrose (one-twentieth if both hexoses from sucrose are completely fermented), which again would lengthen the adaptation time. The combination of these two effects may well result in no observable adaptation to raffinose within a period of 6 hr.

The raffinase activity of the yeast grown in basal medium alone is as high as that of yeast grown in the presence of raffinose. This would indicate that raffinase is not an adaptive enzyme in *S. fragilis*. Nevertheless, the manometric evidence shows that a period of adaptation is necessary before carbon dioxide is evolved from raffinose at the maximum rate and that azide prevents the attainment of this maximum rate. It would seem therefore that fermentation of raffinose by *S. fragilis* involves the formation of an adaptive enzyme, but that the enzyme concerned is not the one responsible for the hydrolysis of raffinose. The rate of fermentation of glucose and of fructose by cells grown in basal medium alone is also very low initially and increases slowly during contact with the substrate, the increase being prevented by azide. This would suggest that increases in some of the enzymes of the glycolytic system may be a factor in glucose fermentation by such cells and, consequently, in raffinose fermentation also. Fowler (1951) has suggested that the enzyme system responsible for anaerobic utilization of glucose by *Escherichia coli* can behave as an adaptive system.

SUMMARY

1. The ability of washed cell suspensions of *Saccharomyces fragilis* to ferment glucose, sucrose, galactose, lactose and raffinose following growth in a basal medium, either unsupplemented or supplemented with one of these sugars, has been studied.

2. Ability to ferment galactose, lactose or raffinose is an adaptive property in *S. fragilis*.

3. Following growth in basal medium plus 10% glucose, washed-cell suspensions of *S. fragilis* do not acquire the ability to ferment galactose, lactose or raffinose when incubated with these sugars in phosphate buffer, pH 5·0, for 6 hr. The initial ability of such cell suspensions to ferment sucrose is very low, but when incubated with sucrose and phosphate buffer for 2 hr. the fermentation rate increases to that for glucose fermentation. This increase is prevented by 3×10^{-4} M-sodium azide.

4. The increase in the rate of sucrose fermentation is accompanied by an increase in invertase activity, sufficient to account for the increase in fermentation rate. The increase in invertase activity is most rapid with very young cells and at pH 4·5–5·0, and does not occur in presence of sodium azide.

5. Following growth in unsupplemented basal medium, washed-cell suspensions of *S. fragilis* can acquire the ability to ferment raffinose, lactose and galactose, the rate of adaptation decreasing in that order. Adaptation does not occur in presence of sodium azide. The initial rate of glucose and fructose fermentation by these suspensions is very low and maximum rate is not reached for 2 hr., the increase in rate being prevented by sodium azide.

6. Failure to ferment raffinose following growth in unsupplemented basal medium is not due to lack of hydrolytic activity towards raffinose, but appears to be due to a deficiency in the glycolytic system.

7. Evidence is presented which suggests that glucose and fructose strongly inhibit invertase synthesis by *S. fragilis*.

I wish to thank Dr Nils Diding and Mr V. W. Leonard for their help with some of the experimental work, and the former for a gift of melibiose. My thanks are also due to Dr E. F. Gale for his continual encouragement and advice.

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Fibrinolysins of Human Plasma

A COMPARISON OF FIBRINOLYTIC PLASMA FROM NORMAL SUBJECTS AND FROM CADAVER BLOOD WITH PLASMIN PREPARED BY ACTIVATION WITH CHLOROFORM

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(*Received* 25 *March* 1953)

This paper is concerned with the nature of the fibrinolytic activity which develops in human blood in a variety of circumstances. The centre of interest is the spontaneous fibrinolysis produced in the normal subject by physical or emotional disturbances such as severe exercise or fear or by injection of adrenaline (Macfarlane, 1937; Macfarlane & Pilling, 1946; Biggs, Macfarlane & Pilling, 1947). Fibrinolytic activity can also be produced *in vitro* by treating plasma with chloroform or certain bacterial filtrates, and this has been shown to be due to the conversion of a zymogen ('plasminogen') present in normal plasma to its active, proteolytic form ('plasmin') (Christensen, 1945, 1946; Christensen & MacLeod, 1945). Normal plasma and serum were found to contain a powerful inhibitor to plasmin. Intense fibrinolytic activity sometimes occurs in blood taken post mortem, particularly in cases of sudden or violent death (Mole, 1948).