Sequential Induction of Maltose-Permease and Maltase Systems in *Saccharomyces cerevisiae*

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The induction of maltose utilization in many strains of *Saccharomyces cerevisiae* is well known. The process in resting cells involves the synthesis of maltase at the expense of the free amino acid pool (Halvorson & Spiegelman, 1952, 1953*a, b*), which may be replenished by protein turnover (Halvorson, Fry & Schwemmin, 1955; Halvorson, 1958, 1960). Various lines of evidence (Sutton & Lampen, 1962; de la Fuente & Sols, 1962) suggest that a specific inducible permease is also necessary for maltose fermentation. Such a system, distinct from the maltotriose permease described by Harris & Thompson (1960*b*), has been found in one strain by Harris & Thompson (1961). This system differs from the constitutive α -glucoside permease, described in another strain by Robertson & Halvorson (1957), not only by virtue of its inducible character, but also because of differences in competitive effects between maltose and methyl α -glucoside. The observations of Burger, Hejmová & Kleinzeller (1959) and Terui, Okada & Oshima (1959) also suggest that the transport of methyl α -glucoside is not necessarily related to that of maltose. Maltose permease constitutes an 'active-transport' system (cf. Cirillo, 1961) and the synthesis of protein is apparently involved in its induction (Harris & Thompson, 1961; de la Fuente & Sols, 1962). The present paper describes a more detailed examination of the course of the inductive processes leading to the utilization of maltose by several strains of *S. cerevisiae*.

MATERIALS AND METHODS

Organisms. *S. cerevisiae* (National Collection of Yeast Cultures nos. 239, 240 and 366) were maintained at 2–5° in a liquid medium containing malt extract, yeast extract, glucose and peptone (Wickerham, 1951).

Culture media. In addition to Wickerham's (1951) medium, two synthetic media having glucose as sole carbon source were employed. These were the 'full rich medium' of Kilkenny & Hinshelwood (1951) and that described by Harris & Thompson (1960*a*). The principal differences between these media are: glucose concentration [2% and 0.5% (w/v) respectively], CaCl_2 content (250 and 17.5 mg./l. respectively) and pH (4.8 and 6.7 respectively). The higher pH value of Harris & Thompson's (1960*a*) medium is achieved by replacing the KH_2PO_4 of the 'full rich medium' by a mixture of KH_2PO_4 and Na_2HPO_4 .

Cells lacking the ability to metabolize maltose. Strains 239 and 366 were subcultured (1 loopful/100 ml. of fresh medium) five times in 'full rich medium' and then maintained in this medium at 2–5°. Strain 240, after repeated subculture in this medium, was subcultured several times in the medium of Harris & Thompson (1960*a*) and a sample streaked on plates containing 'full rich medium' and 2% agar. After growth at 25° for several days two types of colonies were apparent. One was faintly yellow and exhibited small pseudomycelia typical of the original strain. The second type, presumed to be a mutant and subsequently referred to as 240M, appeared white and shiny and formed only small groups of cells. Both types of colonies were isolated and subsequently grown and maintained in 'full rich medium'.

Suitable cultures of resting cells were obtained by inoculating fresh medium (0.5–2 ml. of inoculum/100 ml.) from the appropriate stock culture and growing for 20–48 hr. All cultures were grown at 25° with gentle shaking in 200 ml.-capacity flat screw-capped bottles or Roux flasks. Cells were harvested by centrifuging and washed three times with water at 0–5°.

Induction of maltose-utilizing systems. Washed cells were suspended (1–3 mg. dry wt./ml.) in one of the following media: A, 'full rich medium' without glucose, pH 4.8; B, 0.66M- KH_2PO_4 and 0.02% acid-hydrolysed casein (Oxoid Ltd.), pH 4.6; C, 0.66M- KH_2PO_4 only, together with various concentrations of either commercial maltose (British Drug Houses Ltd.) or the pure sugar. The suspensions were shaken aerobically at 25° and samples withdrawn at suitable intervals for assay of maltase and of maltose permease. Respiration of the cells under identical conditions was observed manometrically in a conventional Warburg apparatus (Umbreit, Burris & Stauffer, 1957).

Maltase activity. Washed cells (approx. 100 mg.) were collected on a glass sinter (porosity 4), pressed as dry as possible and treated with 0.03 ml. of ethyl acetate. At this stage, for the maltase to be completely available to the substrate, all cells must readily take up methylene blue (Jørgensen, 1948) and all aggregations of cells must be dispersed. The suspension, diluted to 3–4 ml. with water, was then sufficient for three enzyme reactions together with appropriate blank and dry-weight determinations.

The reaction mixture, consisting of 0.5 ml. of the ethyl acetate-treated suspension, 1 ml. of Na_2HPO_4 -citric acid buffer, pH 6.5 (McIlvaine, 1921), and 1 ml. of 0.5% maltose (British Drug Houses Ltd.), was incubated with shaking for 2–20 min. at 30° and the reaction terminated by heating at 100° for 5 min. After centrifuging, the supernatant solutions, combined with washings, were passed individually through short columns (2 cm. $\times$ 1 cm.) of acid-washed alumina (chromatographic grade, E. Merck) and the effluents (5 ml.) separately collected. Portions were then

used for the determination of glucose by the modified Barfoed's procedure of Cook & Phillips (1957) or for measurement of reducing power with alkaline ferricyanide (Hagedorn & Jensen, 1923) as described by Cooper & Pollock (1957). To counteract departures from Beer's law of the final coloured solutions in the former method, samples containing 0.15–0.3 mg. were used and a reference curve was constructed for each series of determinations by adding known amounts of glucose to the blank.

Maltase activity is expressed as the initial rate of glucose production ($\mu\text{moles/min./mg. dry wt. of yeast}$) obtained by plotting the observed rates against time and extrapolating to zero time.

Maltose permease. [^{14}C]Maltose (The Radiochemical Centre, Amersham, Bucks.) was used at a specific activity of 0.02–0.045 $\mu\text{C}/\mu\text{mole}$ and its uptake at 30° was measured as described by Harris & Thompson (1961). Some experiments with strain 240 M were performed at pH 4.6 in 0.66 M KH_2PO_4 instead of at pH 8.5 as described by Harris & Thompson (1961), and in certain of these [^{14}C]maltose was included in the inducing medium, when its specific activity was 0.001 $\mu\text{C}/\mu\text{mole}$. Corrections for adsorption of maltose on the cell walls were made by observing the uptake by uninduced cells kept at 2° for 10 min. in the appropriate radioactive medium.

The dry samples were burnt to $^{14}\text{CO}_2$, the radioactivity of which was measured in a gas-counting Geiger tube. Corrections were made for background counts, coincidence where necessary, and tube efficiency. The last-named was determined by combustion of a standard polymer reference sample (The Radiochemical Centre) (0.428 μmc gave 479 counts/min.). Standard errors were not greater than 2%.

Radioautographs. Cells were extracted with water at 100° or with 80% (v/v) ethanol at room temperature. Chromatograms were run and radioautographs prepared as described by Harris & Thompson (1961).

Identification of [^{14}C]trehalose. Radioautographs of cell extracts prepared from strain 239 after incubation with [^{14}C]maltose at pH 8.5 (Harris & Thompson, 1961) revealed a spot having R_{maltose} 0.78. Chromatograms prepared from extracts of larger samples of yeast incubated with unlabelled maltose under precisely the same conditions revealed, on development with a modified periodate-benzidine reagent (cf. Cifonelli & Smith, 1954), a prominent spot with R_{maltose} 0.78 running in the same position as authentic trehalose. This substance failed to give any visible colour with diphenylamine–aniline–phosphoric acid reagent (Harris & MacWilliam, 1954). A duplicate zone was eluted with 5 ml. of warm Na_2HPO_4 –citric acid buffer solution (McIlvaine, 1921), and 5 mg. of crude yeast maltase was added. This preparation hydrolyses maltose and maltotriose but not trehalose (Phillips, 1959). The mixture was incubated at 37° for 62 hr. under toluene, then passed successively through short columns of alumina, Amberlite IR-120 (H^+ form) and Amberlite IR-4B (OH^- form). The effluent was evaporated in a vacuum and again chromatographed on paper, when a single spot corresponding to authentic trehalose was revealed with the periodate-benzidine reagent.

The original radioactive spot was then treated in a precisely similar manner except that only a marker strip from the final chromatogram was developed. The area corresponding to trehalose in the undeveloped portion was

eluted with a little water and the solution made approx. 8N with respect to H_2SO_4 . After 16 hr. in a sealed tube at 100° the acid solution was neutralized with $\text{Ba}(\text{OH})_2$ and, after centrifuging, the supernatant solution was desalted by passage through Amberlite resins, evaporated in a vacuum and again chromatographed, with [^{14}C]glucose as marker. A radioautograph prepared from this chromatogram indicated glucose as the major radioactive hydrolysis product.

RESULTS

Cultures of uninduced cells

Cells of *S. cerevisiae*, strains 239 and 366, lacking the ability to ferment maltose, were readily obtained by growth in 'full rich medium'. Cells of strain 240, however, after fifteen successive subcultures in this medium still showed an immediate respiratory response to maltose and contained 0.066 unit of maltase, a value typical for cells grown in the presence of maltose. Nevertheless, as reported by Harris & Thompson (1960a), serial subculture in the medium described by these authors led to uninduced cells. This apparent paradox was resolved by plating cultures previously grown in Harris & Thompson's (1960a) medium, when two types of colonies, faintly yellow and white, appeared. When previous growth was in 'full rich medium', only yellow colonies were visible. After isolation, the yellow colonies showed an immediate respiratory response to maltose whereas the white colonies did not. Growth of the white colonies was markedly faster than that of the yellow in synthetic media. The white colonies should presumably be regarded as a mutant (240M) derived from strain 240, and Harris & Thompson's (1960a) medium is particularly favourable for its selection. The parent strain would appear to be constitutive for maltose fermentation. Uninduced cells of all three of the above strains possessed neither maltase nor maltose permease detectable under the conditions described above.

Respiratory studies during induction

The characteristic patterns of carbon dioxide evolution during induction of each strain in media A, B and C may be summarized as follows (see Table 1).

Strain 239. Over the range of concentrations employed only this strain showed any variation in the induction time with change in the maltose content (2–4%, w/v) of the medium. Thus with 2% maltose an induction period of 3.75 hr. was required before evolution of carbon dioxide was maximal, whereas with a 4% solution of the sugar 2.25 hr. sufficed. With a 4% solution of glucose-free maltose, however, the time was increased to

about 2.75 hr. These times were independent of the other variables, including nitrogen, in the inducing media.

Strain 240M. The time of induction was invariably about 3.5 hr. to maximal evolution of carbon dioxide regardless of maltose concentration. In the absence of an exogenous nitrogen source (medium *C*), however, no appreciable evolution of carbon dioxide was observed even after 5 hr. in the presence of maltose. The addition of casein hydrolysate to the yeast suspension in medium *C* at any

time less than 3 hr. after the addition of the maltose gave rise to a pattern of carbon dioxide evolution indistinguishable from that observed in medium *B*. When the amino acid mixture was added after a minimum of 3 hr. of preincubation with maltose the respiratory response was almost immediate (Fig. 1).

Strain 366. The induction period was comparatively brief, i.e. about 1.5 hr. to maximal carbon dioxide output, and was independent of variables in the medium.

The final metabolic quotients achieved by all three strains were principally dependent on the nitrogen content of the inducing media. Rates of oxygen uptake generally increased only slightly during induction, but a respiratory quotient of approximately unity was observed throughout the induction of strain 240M in medium *B*.

Induction of maltase

Maltase activities determined during the course of induction are recorded, together with the relevant metabolic quotients, in Table 1.

Increments in rates of carbon dioxide evolution closely reflect changes observed in the maltase content of the cells. Between two and six molecules of carbon dioxide should be produced under aerobic conditions from each molecule of glucose made available by the enzyme. The results all lie within this range except in the later stages of induction in strain 366 and a single result after 1.5 hr. of induction of strain 239. Here the enzyme activities are higher than expected from the observed metabolic quotients.

Fig. 1. Carbon dioxide evolution during induction of maltose utilization in *Saccharomyces cerevisiae* (strain 240M). Samples (2.5 ml.) of a suspension of cells (1.5 mg. dry wt./ml.) in 0.66M-KH₂PO₄ were added to 0.5 ml. of 18% (w/v) maltose in the main compartments of Warburg flasks. Then 0.5 ml. of 0.12% casein hydrolysate in 0.66M-KH₂PO₄ was added from the side arm after 0.4 hr. (●) or 4.75 hr. (▲) (↑).

Table 1. Induction of maltase in *Saccharomyces cerevisiae*

Experimental conditions are given in the text. Maltase activity is expressed as the initial rate of glucose production (μmoles/mg. dry wt. of cells/min.). Metabolic quotients, $q_{CO_2}^0$, are expressed as μmoles of CO₂/mg. dry wt. of cells/min. In medium *A* corrections were made for minor increases in dry weight during the experiments.

Strain no.	Inducing medium	Concn. of maltose (% w/v)	Time of induction (hr.)	10 ³ × Maltase content	10 ³ $q_{CO_2}^0$
239	A	2	2	0.0	2.0
			4	2.6	12.6
			1.5	3.1	4.8
	C	4	4	6.6	16.2
			1.5	—	2.2
			4	—	6.3
240M	B	3	3.2	0.0	1.5
			3.5	1.4	4.8
			4	2.7	10.4
	C	3	4	0.0	0.7
366	B	3	0.75	0.6	2.2
			1.0	1.4	4.5
			1.25	3.2	5.6
			2	—	6.1
			1.0	—	3.7
	C	3	1.25	—	4.1
			2	3.1	4.8

Table 2. *Maltose transport in Saccharomyces cerevisiae (strain 366)*

The yeast samples were suspended (approx. 2 mg. dry wt./ml.) in 12.5 mM-tris buffer solution, adjusted to pH 8.5 with 0.2N-HCl, containing maltose (0.5 μ mole/ml.) of specific activity 0.022 μ C/ μ mole. Samples were removed at intervals, washed once with tris buffer solution at 0–5°, dried and assayed. Corrections were made for adsorption on cell walls (see the text).

Yeast sample	Time in radioactive medium (hr.)	Uptake of [14 C]maltose (counts/min./mg. of yeast)
Grown in Wickerham's (1951) medium	0.4	52
	1.0	103
	1.5	176
Uninduced	1.0	17
	1.5	30
Induced (1.25 hr. in medium B)	0.5	11
	1.0	36
Induced (2.5 hr. in medium B)	0.5	73
	1.0	117
Induced (1.6 hr. in medium C)	1.0	38
	1.5	35

Induction of maltose permease

Direct evidence for active transport of maltose in strains 239 and 366 was not forthcoming because, after incubation with [14 C]maltose at pH 8.5 (Harris & Thompson, 1961), radioactive materials other than maltose were evident in radioautographs prepared from cell extracts. The radioautographs prepared from strain 239 revealed a prominent trehalose spot (see the Materials and Methods section), several slower-moving unidentified compounds and only a trace of maltose. Radioautographs originating from extracts of strain 366 exhibited maltose as the most intense spot, a less-intense region corresponding to the position of trehalose (R_{maltose} 0.78) and, in some samples, a trace of a slow-running component.

Uptake measurements during induction of strain 239 indicated that radioactivity only started to enter the cell in significant amounts at about the time the sugar began to be utilized, strongly suggesting the induction of a specific transport mechanism. In similar experiments with strain 366 no evidence was obtained for induction of a transport mechanism up to the time of maximum rate of carbon dioxide evolution (Table 2). Uptake of labelling similar to that observed in cells grown in Wickerham's (1951) medium was only achieved after 2.5 hr. of induction in medium B, an hour or more after the rate of carbon dioxide output was maximal.

The presence of a maltose permease in strain 240M has already been demonstrated (Harris & Thompson, 1961). The manometric studies (Fig. 1) suggested that, in the absence of exogenous nitrogen, maltose was not metabolized. Induction of maltose permease under these conditions was conceivable, however, and two series of experiments were performed to test this possibility. In the first,

Table 3. *Induction of maltose permease in Saccharomyces cerevisiae (strain 240M)*

The inducing medium was 0.66M-KH₂PO₄ containing 3% (w/v) of maltose. Samples were removed at intervals, washed twice with water at 0–5° and resuspended (approx. 2 mg. dry wt./ml.) in 0.66M-KH₂PO₄ containing maltose (0.5 μ mole/ml.) of specific activity 0.045 μ C/ μ mole. Samples were taken again at intervals, and the cells were washed twice with water at 0–5°, dried and assayed. Uptake of [14 C]maltose (column 3) was corrected for adsorption on cell walls, and the weight of maltose (column 4) was further corrected approximately for insoluble radioactive materials (see the text).

Induction time (hr.)	Time in radioactive medium (hr.)	Uptake of [14 C]maltose (counts/min./mg. of yeast)	Total uptake of maltose (μ g./mg. of yeast)
0	0.5	129	0.42
	1.0	184	0.60
	1.5	266	0.86
3	0.5	3809	12.4
	1.0	5698	18.5
3.5	0.5	5379	17.5
	1.0	7773	25.4

[14 C]maltose was included in the inducing medium [medium C plus 3% (w/v) of maltose] at low specific activity and samples of yeast were removed at intervals for assay. Radioactivity started to enter the cell in appreciable amounts after about 2.75 hr. in the inducing medium and had reached a maximum after 4.5 hr. Radioautographs prepared from cell extracts obtained during these experiments showed maltose as the only radioactive material. In the second series, cells were transferred from the inducing medium to 0.66M-potassium dihydrogen phosphate containing maltose (0.5 μ mole/ml.) of higher specific activity and sampled at intervals (Table 3).

In these experiments radioautographs derived from the cell extracts showed a minor slow-running spot in addition to maltose. In parallel experiments only 5–10 % of the total radioactivity of the cell was found in an insoluble form. These results indicate that, in strain 240M, maltose permease is induced in the absence of exogenous nitrogen. No fermentation of maltose is concurrently induced (Fig. 1), nor is any maltase detectable under these conditions (Table 1).

DISCUSSION

Cells of *S. cerevisiae* strains in which maltose utilization is inducible normally fail to ferment maltose when previously grown in the absence of that sugar. Strains 239 and 366 employed in the present studies fell into this category, but strain 240 behaved as if the ability to ferment maltose was constitutive when grown in 'full rich medium'. Growth of strain 240 at higher pH was necessary for the production of uninduced cells. This process apparently involves the selection of a mutant (240M) but this finding does not materially alter any of the conclusions of Harris & Thompson (1960*a*, 1961) derived before this was apparent.

Maltose permease could not be unequivocally demonstrated in strains 239 and 366 owing to the appearance of trehalose in the radioautographs obtained after incubation with [^{14}C]maltose at pH 8.5. Avigad (1960) has shown that most of the radioactivity fixed by the cells on exposure to [^{14}C]maltose at pH 6.8 resides in trehalose. Further, Trevelyan (1958) indicated that trehalose formation from glucose is favoured by increasing the pH. However, although glucose is still completely metabolized at pH 8.5 (Sussman, Spiegelman & Reiner, 1947), maltose is not fermented at pH values higher than 8.1 (Spiegelman, Reiner & Sussman, 1947). Therefore, since the disaccharide still penetrates the cell under alkaline conditions (Harris & Thompson, 1961), the enzyme inhibited must be maltase. To explain the passage of radioactivity from maltose to trehalose at pH 8.5, observed in strains 239 and 366, it might be supposed that the maltase in these particular strains is still active at this high extracellular pH. Alternatively, some other pathway, not involving maltase, might supply suitable units for trehalose synthesis from maltose. Such possibilities are maltose phosphorylase, which exists in certain *Neisseria* species (Fitting & Doudoroff, 1952; Selinger & Schramm, 1961) and in beer lactobacilli (Wood & Rainbow, 1961), or a transglucosidase such as amylomaltase, which is well characterized in *Escherichia coli* (cf. Wiesmeyer & Cohn, 1960) and has been postulated to be present in baker's yeast (Blair & Pigman, 1954).

The course of induction revealed by the manometric studies is, in general, much as expected from the results of previous investigators using different strains. Thus it has been shown (Spiegelman, Reiner & Cohnberg, 1947; Suomalainen, Axelsson & Oura, 1956; Halvorson, 1960; de la Fuente & Sols, 1962) that small amounts of fermentable hexoses stimulate the inductive process, presumably by supplying the energy necessary for enzyme synthesis. The maltose used as inducer in the present work contains approximately 0.3 % of glucose, giving 0.15–0.5 μmole of hexose/mg. of yeast as the inducer concentration varied. This probably explains the effect of varying the inducer concentration on strain 239, especially in view of its behaviour towards a purified sample of the inducer. Within this range of glucose concentration no effect on strains 240M and 366 was noticed, whereas the strain studied by de la Fuente & Sols (1962) was markedly stimulated by as little as 0.1 μmole of glucose/mg. of yeast. The presence of assimilable exogenous nitrogen has long been known to stimulate induction (cf. Spiegelman & Dunn, 1947). The enzyme-synthesizing ability of uninduced cells depends, in the first instance, on the amount of the free amino acid pool. This can be replenished readily by exogenous ammonia or amino acids (Halvorson *et al.* 1955). Thus, in the current experiments on stationary cells, exogenous nitrogen determines the final amount of enzyme synthesized but does not affect the induction period.

During induction of the maltose-utilizing systems in strains 239 and 240M it is apparent that sequential induction of the transport and hydrolysing systems takes place. The induction of the permease is probably a prerequisite for the induction of the maltase system. This is brought out especially clearly in the results for strain 240M. Here the addition of exogenous amino acids causes synthesis of maltase with very slight lag once the permease has been formed, but has no such immediate effect before the transport system is present (Fig. 1). This is reminiscent of the induction of β -galactosidase in *E. coli*. The work of Herzenberg (1959) suggests that the role of the permease is to provide a suitable intracellular concentration of the inducer when the external concentration is low. At sufficiently high external concentrations of inducer, lack of permease in a cryptic strain did not retard β -galactosidase synthesis, presumably because diffusion of the inducer was adequate. Such considerations may explain the behaviour of strain 366 where induction of the maltase system apparently took place before the transport system was synthesized.

The induction of maltose permease involves protein synthesis at the expense of free amino acids (Harris & Thompson, 1961; de la Fuente & Sols, 1962) in a manner analogous to the induction of

maltase (Halvorson & Spiegelman, 1952, 1953*a, b*). The complete chronological separation of the induction of the transport and hydrolysing systems here described for strain 240 M by withholding the amino acids necessary to form the latter system therefore suggests a number of possibilities.

The first is that, fortuitously, the free amino acid pool and available labile protein are sufficient to enable synthesis of the permease, but are then so depleted as to render any further induction impossible. Prolonged nitrogen starvation, however, appears to be necessary to eliminate protein turnover (Halvorson *et al.* 1955; Strange, 1961). Conceivably, the mechanism for protein turnover is defective in this particular strain. The second possibility is that the pools of amino acids for synthesis of maltose permease and maltase are metabolically distinct. Separate pools have been detected in *Candida utilis* (Cowie & McClure, 1959). Thirdly, the possibility exists that the mechanisms involved in the two syntheses are different. It is reasonable to suppose that permease synthesis takes place at the cytoplasmic membrane, and protein formation at such sites may differ, at least in detail, from those operative in other cell organelles (cf. Hendler, 1962; Hunter & Godson, 1961). Finally, it is possible that the repressors for maltose-permease and maltase synthesis, either at genetic or template level (cf. Jacob & Monod, 1961; Hauge, MacQuillan, Cline & Halvorson, 1961), may be chemically distinct and have differing affinities, modified by additional amino acids, for the inducer.

Mandelstam (1961) has shown that, in stationary cells of *E. coli*, protein turnover is sufficient to account for the induced synthesis of β -galactosidase at a differential rate similar to that in growing cells, and Halvorson (1960) suggests that a similar situation exists during enzyme induction in stationary *S. cerevisiae*. It is clear, however, that the products of protein turnover, if present in the system here studied (strain 240 M), are not directly available for maltase synthesis. Therefore, since the availability of amino acids limits the induction of maltase, one may perhaps visualize preferential synthesis of the maltase system in these circumstances. The system described, therefore, presents the dual possibility of studying on the one hand the formation of maltose permease, and on the other hand, by adding amino acids, the synthesis of the maltase system.

SUMMARY

1. The induction of maltose-permease and maltase systems has been studied in stationary cells of three strains of *Saccharomyces cerevisiae*.

2. In strain 240 M maltose permease is induced 2.75–3.5 hr. after adding the inducer. This syn-

thesis is independent of the presence of an external nitrogen source. In the absence of exogenous nitrogen no detectable maltase is formed, but if nitrogen is present the maltase system is induced 3.2–3.5 hr. after the addition of the inducer.

3. If uninduced cells of strain 240 M are exposed to maltose for 3.5 hr. or more in the absence of nitrogen and if amino acids are then added, the maltase system is synthesized with little further lag over a period of about 20 min. Thus separate induction of transport and hydrolysing systems is possible in this strain.

4. By contrast, in strains 366 and 239, induction of the maltase systems appears to be independent of a supply of exogenous nitrogen. Indeed, in strain 366 it appears also to be independent of pre-induction of the transport system.

5. Strain 239 possesses a marked ability to transfer ^{14}C from [^{14}C]maltose to [^{14}C]trehalose at an external pH of 8.5 where yeast maltases are usually inactive. Strain 366 also exhibits this ability to a much smaller degree.

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The Metabolism of Acetaldehyde by the Brain *in vivo*

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The concentrations of certain metabolites in the rat brain are profoundly affected by the physiological state of the animal (Richter & Dawson, 1948; Richter & Crossland, 1949; Dawson & Richter, 1950). In particular, stress (induced by gentle tumbling) is accompanied by an increase in the concentrations of lactate and phosphocreatine, and a decrease in that of acetylcholine. It was decided to extend this work and to ascertain whether these biochemical effects of stress on the central nervous system were modified by the ingestion of ethanol. However, it soon became clear that the effects of ethanol in the absence of stress were sufficiently well-defined to merit investigation, and the results are presented below.

EXPERIMENTAL

Treatment of animals. Albino rats weighing 140–160 g. were used. Any animal that objected to normal handling was rejected. The general procedure was to administer the ethanol, and after various known times to kill the animal,

remove the brain and carry out the chemical determinations.

Ethanol was administered in aqueous solution by stomach tube. The dose used was 3 ml. of 30% (v/v) ethanol/150 g. rat, unless otherwise stated.

The animals were killed by immersion in liquid O₂ for 2–3 min. The head was sawn off and the skull was exposed. By careful saw-cuts the skull could be removed without damaging the brain, which could then be levered out, generally, in one piece. The excision took 5–6 min., and during this time the head and instruments were kept cold by frequent immersion in liquid O₂. The freshly excised frozen brain was weighed and homogenized at 0° immediately.

Blood samples were taken under ether anaesthesia. The blood was collected in citrated syringes from the inferior vena cava, care being taken not to include any air bubbles, and transferred immediately to stoppered tubes each containing 5 ml. of ice-cold 10% (w/v) trichloroacetic acid, well mixed and weighed. If the brain of the same animal was required it was killed by immersion in liquid O₂ immediately after the blood sample was taken, and the brain removed as described above.

In some experiments the aldehyde-dehydrogenase inhibitor tetraethylthiuram disulphide was administered 16 hr. before the ethanol. The inhibitor was given as an aqueous suspension by stomach tube (4 ml./150 g. rat). The suspension was made up by mixing 300 mg. each of

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