CHAPTER I

INHIBITORY EFFECT OF GLUCOSE AND CYCLIC ADENOSINE 3' -5' MONOPHOSPHATE ON THE SYNTHESIS OF INDUCIBLE N-ACETYL-GLUCOSAMINE CATABOLIC ENZYMES IN YEAST

SUMMARY

A number of catabolic enzymes are induced in the yeast *Candida albicans* and *Saccharomyces cerevisiae* 3059 when the cells are grown in the presence of an amino sugar, N-acetyl-glucosamine. Glucose can block the utilization of this amino sugar in *Saccharomyces cerevisiae* - a facultative aerobe but not in *Candida albicans* - an obligatory aerobe. Furthermore, glucose was found to repress the synthesis of the inducible enzymes of N-acetyl-glucosamine catabolic pathway in *Saccharomyces cerevisiae* but not in *Candida albicans*. Addition of cAMP to *Saccharomyces cerevisiae* cells maintained in glucose medium can not bring about their release from catabolite repression. On the contrary, in the presence of cAMP, the synthesis of inducible enzymes of N-acetyl-glucosamine pathway was inhibited in both the yeasts. This seems to indicate that cAMP can readily penetrate into the cells. In addition, the total protein synthesis was inhibited in both the yeasts by cAMP, suggesting that protein synthesis in yeast is under cAMP control.

INTRODUCTION

Carbon catabolite repression (138) is a regulatory mechanism of considerable importance in prokaryotic as well as eukaryotic organisms. Whereas the important aspects of the molecular mechanism of catabolite repression in prokaryotes are fairly well understood (139), much less is known about the analogous processes in eukaryotes. It is known that glucose can also repress the synthesis of various rat liver enzymes, such as, tyrosine aminotransferase (TAT), phosphoenolpyruvate carboxykinase (PEPCK), and serine dehydratase. These enzymes are induced in rat liver under conditions of glucose deprivation, when glucose or a source of glucose was not given to the animal. On refeeding these rats with glucose, the levels of these enzymes were reduced drastically showing that they are subject to glucose repression (140).

Saccharomyces cerevisiae, a simple eukaryote, appears to be particularly suited for studies on catabolite repression. In this organism the levels of a great number of enzymes are dependent on the carbon source utilized during growth (141). In yeast, the addition of glucose or metabolically related sugars to cells adapted to a sugar lacking medium causes a time dependent disappearance of the activity of some enzymes. Among the gluconeogenetic enzymes, fructose biphosphatase (142), cytoplasmic malate dehydrogenase (143)

and PEPCK (144) have been reported to have catabolite inactivation. In *Saccharomyces cerevisiae* a wide variety of enzymes and other proteins are known to be subject to catabolite repression. In the cell wall and its inner surface there is internal invertase, plasma membrane transport system for α- methyl glucoside and galactose, and gluconeogenetic enzymes (145). In our previous studies we also demonstrated that, like other inducible systems present in bacteria and yeast, glucose could repress the induction of enzymes involved in N-acetyl-glucosamine (GlcNAc) metabolism in *Saccharomyces cerevisiae*. However, we also found that these enzymes are not sensitive to catabolite repression in *Candida albicans*, a pathogenic strain of yeast (146)

In prokaryotes it has been shown that catabolite repression can be relieved by cAMP. However, in eukaryotes the relationship between cAMP and catabolite repression is not absolutely clear and the role of cAMP in relation to catabolite repression, so far as the molecular mechanism is concerned, remains to be worked out in detail. In eukaryotes the effect of cAMP appears to be to exert a primarily selective effect on the synthesis of enzymes. One of the rat liver enzymes, TAT, has been studied in detail in this respect and its activity appears to be regulated by cAMP. It was found that the concentration of cAMP rises prior to any increase in TAT in cultured hepatic cells (147). In the same system, it was shown that dibutyryl cAMP (Bt_2 cAMP).

an analogue of cAMP, caused a rapid and marked rise in PEPCK activity which depended on the presence of the chemical in the media (148). Studies on serine dehydratase (SDH) of liver showed that amino acids can induce SDH in liver and the effect is mediated via an increase in cAMP concentration. Bt_2 cAMP was also shown to be able to partially reverse the glucose effect on the induction of SDH (149). Apart from the important liver enzymes, studies with various other enzymes, such as, ornithine decarboxylase (150) and gonadal hormones (150, 151) indicate that cAMP influences the activities of these enzymes by its action on protein synthesis, since the effect could be blocked by protein synthesis inhibitors.

It has also been found that cAMP or Bt_2 cAMP is capable of causing suppression of enzyme induction. This gave an important dimension to the problem of metabolic regulation by cAMP. The earliest reports came from studies on glucokinase (152), the rise in the activity of which could be blocked by Bt_2 cAMP (153). Later, it was reported that glucose 6-phosphate dehydrogenase induction, produced by feeding rats a high carbohydrate diet and induction of fatty acid synthetase by refeeding fasted normal rats with high carbohydrate diet, could be prevented by Bt_2 cAMP (154, 155). These results indicated that cAMP could exert both a positive and a negative effect on specific protein synthesis.

The role of cAMP in intracellular regulation in yeast, in general and *Saccharomyces cerevisiae* in particular, is not clear, especially with respect to its significance and in relevance to catabolite repression.

The content of cAMP in wild type cells of *Saccharomyces fragilis* (156) was found to be higher on lactate than on glucose. In *Saccharomyces cerevisiae* it has been shown that cells grown on glucose have a cAMP concentration one-sixth of that in cells grown on less repressing galactose. Coincident with these, there were changes in the levels of two catabolite sensitive enzymes - L-glucosidase and succinate dehydrogenase (157). In *Schizosaccharomyces pombe* which is an obligatory aerobe, specific correlation was shown between the decrease in glucose concentration and increase in the levels of cAMP (158).

Studies on *Saccharomyces cerevisiae* with the δ -amino laevulinate dehydrogenase system have shown that a rapid release from catabolite repression occurs when cells were transferred from glucose to a derepression medium (145). It was also shown that cAMP could bring about release from repression even in the presence of glucose with similar kinetics. There was also an increase in the intracellular levels of cAMP when glucose was removed from the medium to initiate normal release in absence of cAMP. Also other derepressible activities in cytosol and mitochondria could

be made to respond to cAMP in a similar manner. Conversely, the intracellular cAMP levels were shown to decrease three-fold when glucose was added to ethanol grown (derepressed) yeast cells (145).

Further studies have been conducted on the cAMP levels and derepression by using various mutants. A mutant of *Saccharomyces carlsbergensis*, which is partially resistant to catabolite repression for several enzymes, still exhibited wild type levels of the nucleotide on either glucose or galactose (159). Whether providing exogenous cAMP can overcome glucose repression of several catabolite repressible activities or not is not clear. Results which do not show a correlation between cAMP levels and glucose repression, have led to the proposal that in fungi an elevated cAMP level may stimulate the mobilization of endogenous carbohydrate reserves (glycogen and trehalose) and their subsequent glycolysis (160). Studies on mutants of *Saccharomyces cerevisiae* show that mutants which are highly permeable to cAMP, show endogenous levels of cAMP about two orders of magnitude above the endogenous cAMP concentrations of normal yeast cells, even when grown on carbon source other than glucose. But the cells did not prevent glucose repression of glucokinase synthesis, even in the presence of considerably high concentrations (5 mM) of exogenous cAMP (161). We have shown that cAMP, which is believed to mediate glucose repre-

ssion in yeast (162), inhibits protein synthesis in *Candida albicans* (163).

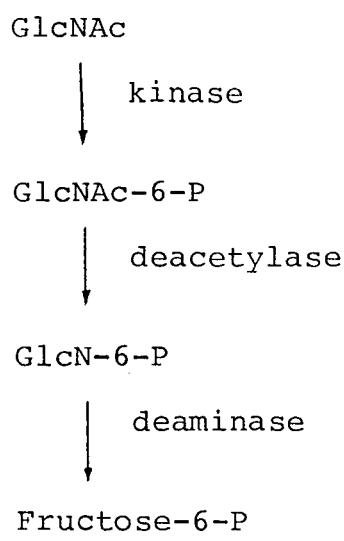
In our present study we have found that, unlike many other catabolite sensitive systems, the inducible GlcNAc catabolic pathway in *Saccharomycopsis fibuligera* could not be rescued from catabolite repression by cAMP. On the contrary, it appears that there was a cAMP mediated inhibition of protein synthesis not only in *Candida albicans*, an obligatory aerobe (where "glucose effect" i.e. catabolite repression is not operative), but also in *Saccharomycopsis fibuligera*, facultative aerobe (where catabolite repression is operative).

RESULTS

Effect of Glucose on N-acetyl-glucosamine Utilization by Yeast :

As shown in Fig. 4 and Fig. 5 both *Saccharomycopsis fibuligera* 3059 and *Candida albicans* utilize N-acetyl-glucosamine (GlcNAc) as a carbon source. Normally only pathogenic yeast e.g. *Candida albicans* can utilize GlcNAc as carbon source, whereas, other non-pathogenic yeasts e.g. *Saccharomycopsis fibuligera* can not utilize this amino sugar. However, this particular strain i.e. *Saccharomycopsis fibuligera* 3059 becomes pathogenic under certain conditions and it can also utilize GlcNAc. Like glucose, GlcNAc is taken up readily

as the cells start to multiply after 2-3 hrs lag. The following steps are involved in GlcNAc catabolic pathway (164, 165).



N-acetyl-glucosamine kinase (EC 2.7.1.59) is absent in glucose grown cells but is synthesized in presence of GlcNAc in *Candida albicans*. However, this enzyme could not be detected in *Saccharomyces cerevisiae* or any other yeast, tested so far in our laboratory (127). A high affinity uptake system (permease) for GlcNAc is induced in both the yeasts and GlcNAc enters the cells as phosphorylated derivative. Furthermore, glucosamine-6-phosphate deaminase (EC 5.3.1.10) is present in glucose grown cells of both the yeasts, but its level increases several-fold when cells are transferred to GlcNAc medium (166).

In order to show that the repression caused by D-glucose resulted from the phenomena of catabolite

FIGURE 4 : EFFECT OF GLUCOSE ON THE UTILISATION OF N-ACETYLGLUCOSAMINE BY *Pseudomonas aeruginosa*.

Cells collected in their exponential phase of growth were resuspended in 0.3 KH_2PO_4 containing 0.3 Glc NAc. In a separate culture glucose was added at a concentration of 1 at zero time of growth. GlcNAc and glucose concentrations were assayed in the supernatant after removing, the cells as described in 'Materials and Methods'.

o——o control

●——● with glucose

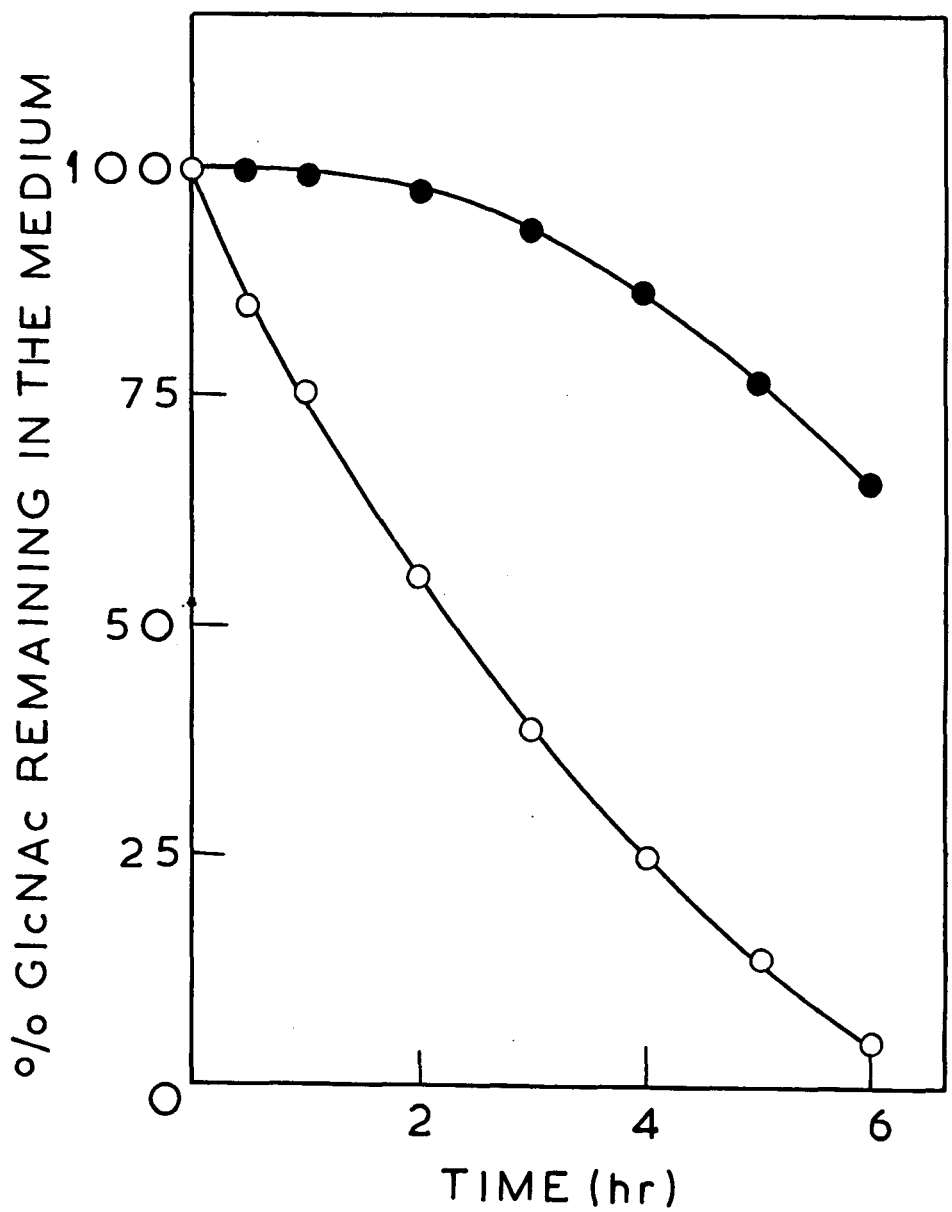
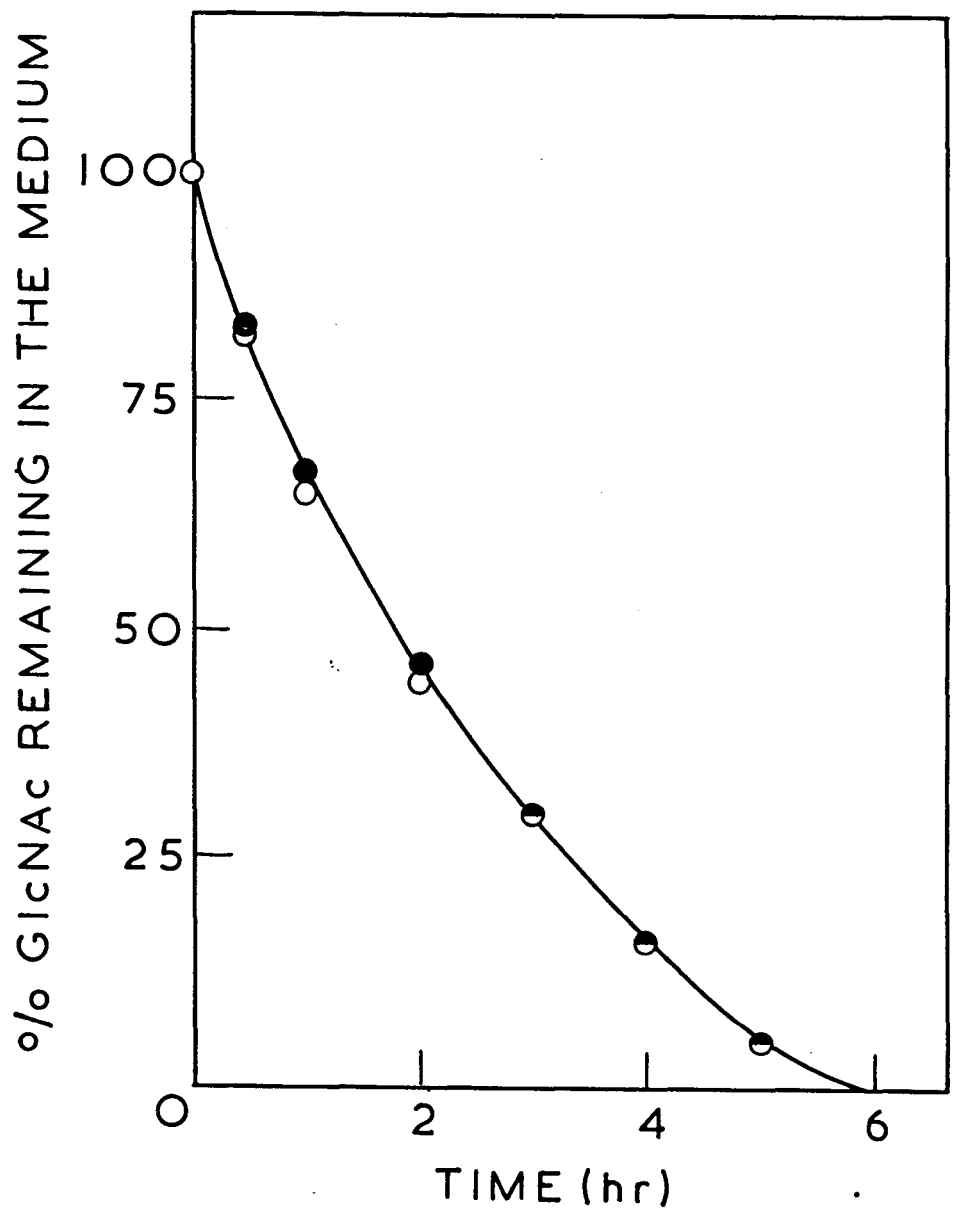

% GlcNAc REMAINING IN THE MEDIUM
100
75
50
25
0
2
4
6
TIME (hr)

FIGURE 5 : EFFECT OF GLUCOSE ON THE UTILIZATION OF N-ACETYL-GLUCOSE AMINE IN [illegible].

Cells from the exponential phase of growth were collected and resuspended in 0.3% KH_2PO_4 containing 0.5% GlcNAc. GlcNAc and glucose concentrations were determined in the supernatant after removing the cells as described in 'Materials and Methods'.

o——o control

●——● with glucose.


% GlcNAc REMAINING IN THE MEDIUM
100
75
50
25
0
2
4
6
TIME (hr)

repression or inhibition, utilization of GlcNAc was compared in the presence and absence of glucose. When glucose was added along with the inducer (GlcNAc), the utilisation of GlcNAc was severely affected in *Saccharomyces cerevisiae* (Fig. 4) but, on the contrary, in *Candida albicans*, GlcNAc utilization remained almost unaffected by glucose (Fig. 5). Similarly, glucose had an inhibitory effect on the synthesis of inducible GlcNAc catabolic enzymes in *Saccharomyces cerevisiae* but not in *Candida albicans* (146). Inhibition in the utilisation of GlcNAc in *Saccharomyces cerevisiae* by glucose could be due to the inhibition of the transport of GlcNAc into the cells. If this were the case, the catabolite enzymes would probably be switched off due to an apparent lack of GlcNAc. However, Fig. 6 shows that this was not the case. When uptake of GlcNAc was studied in 3 hrs induced cells in presence of a high concentration of glucose (100 times higher than that of GlcNAc), there was no significant inhibitory effect on uptake. Furthermore, gluclose does not inhibit the specific binding of GlcNAc to its partially purified carrier (167). Therefore, the results obtained in this investigation show that inducible enzymes of *Saccharomyces cerevisiae* are sensitive to catabolite repression, whereas it is absent in *Candida albicans*.

Effect on cAMP on Inducible Enzyme Synthesis :

Cyclic AMP is taken up by both *Saccharomyces cerevisiae* (Fig.7) and *Candida albicans* (Fig.8) readily and the rates are comparable. Cells ((8-10) x 10^6 cells/ml) exposed to labelled cAMP (total concentration = 0.1 mM) are able to accumulate the nucleotide at an initial (no more than 5 min) linear rate of 0.6 nmol/min per mg cell protein.

Cyclic AMP has been shown to rescue bacteria as well as yeast from catabolite repression. This is particularly so in the case of facultative aerobic yeasts e.g. *Saccharomyces cerevisiae* (162). But under our experimental conditions, cAMP failed to release inducible GlcNAc catabolic enzymes from catabolite repression in yeast. The concentration of the cyclic nucleotide required to produce inhibition is critical. Maximum inhibition of GlcNAc permease induction by cAMP (35-40% inhibition) was observed at a concentration of 3 mM in both *Saccharomyces cerevisiae* and *Candida albicans* (Table II). Furthermore, at concentrations higher than 3 mM, cAMP had no significant effect on permease induction. This is in agreement with the published observations (29) that at much higher concentrations cAMP inhibits the activity of cAMP dependent protein kinase. In the rest of the experiments, this concentration was used to study the cAMP mediated inhibition.

FIGURE 6 : EFFECT OF HIGH CONCENTRATION OF GLUCOSE (5 mM) ON INDUCIBLE N-ACETYL-GLUCOSAMINE UPTAKE.

GlcNAc uptake was assayed in 3 hrs induced cells as described in 'Materials and Methods,' except that glucose was added along with the radioactive GlcNAc (0.05 mM) wherever indicated.

o——o uninduced cells

●——● induced cells

△——△ induced cells plus glucose.

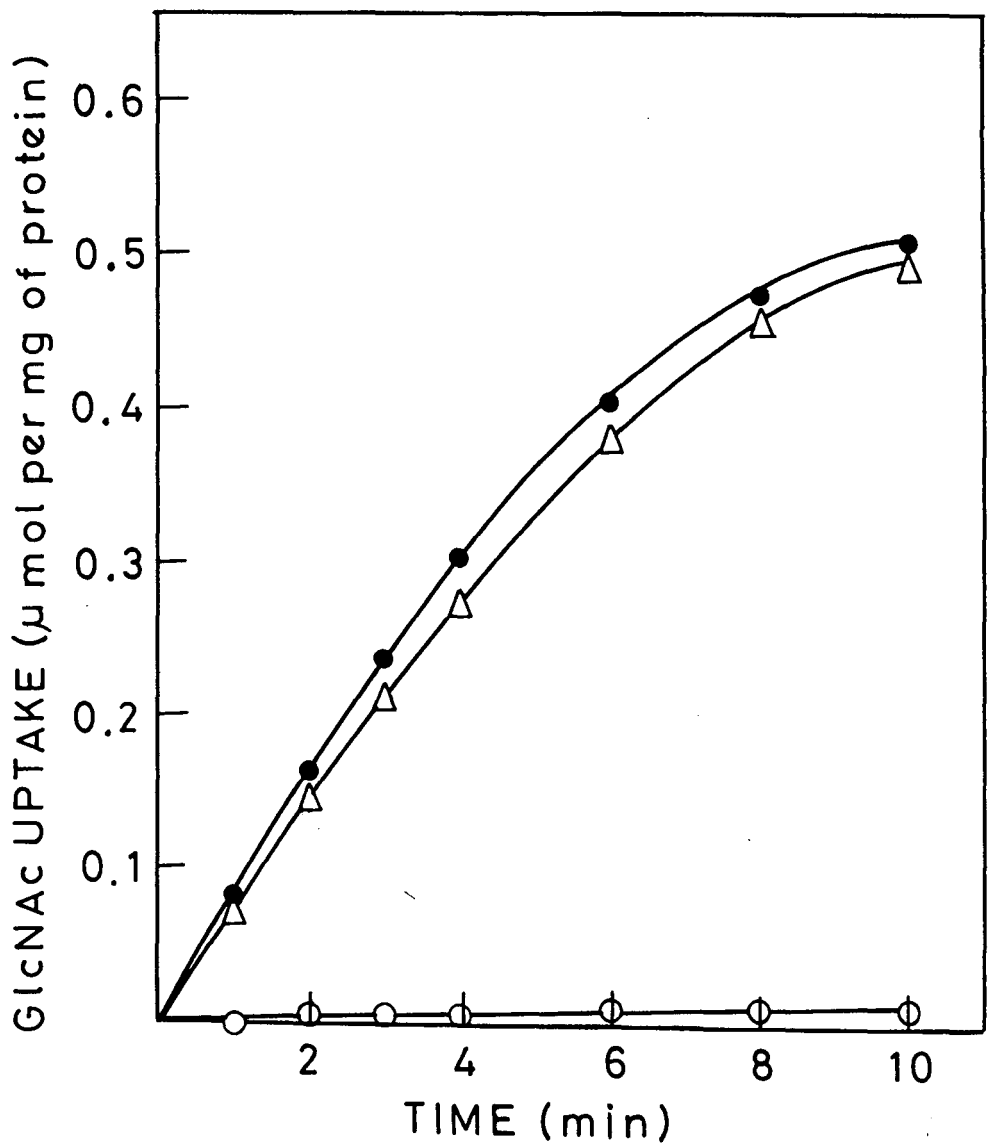

GlcNAc UPTAKE (μ mol per mg of protein)
0.6
0.5
0.4
0.3
0.2
0.1
2
4
6
8
10
TIME (min)

FIGURE 7 : UPTAKE OF (^{3}H) cAMP IN *Saccharomyces cerevisiae*.

Cells harvested from midlog phase were resuspended in water at density of (8-10)x10^6 cells/ml. cyclic (^{3}H)AMP (2.5 μCi/ml) was added to give a final concentration of 0.1 mM. At indicated times 0.5 ml aliquots were immediately filtered on GF/C filters and washed with cold water, dried and radioactivity determined by scintillation counting. A sample taken immediately after adding the labelled cAMP was used to give the 'blank' value which was subtracted from the other cellular radioactivity estimations.

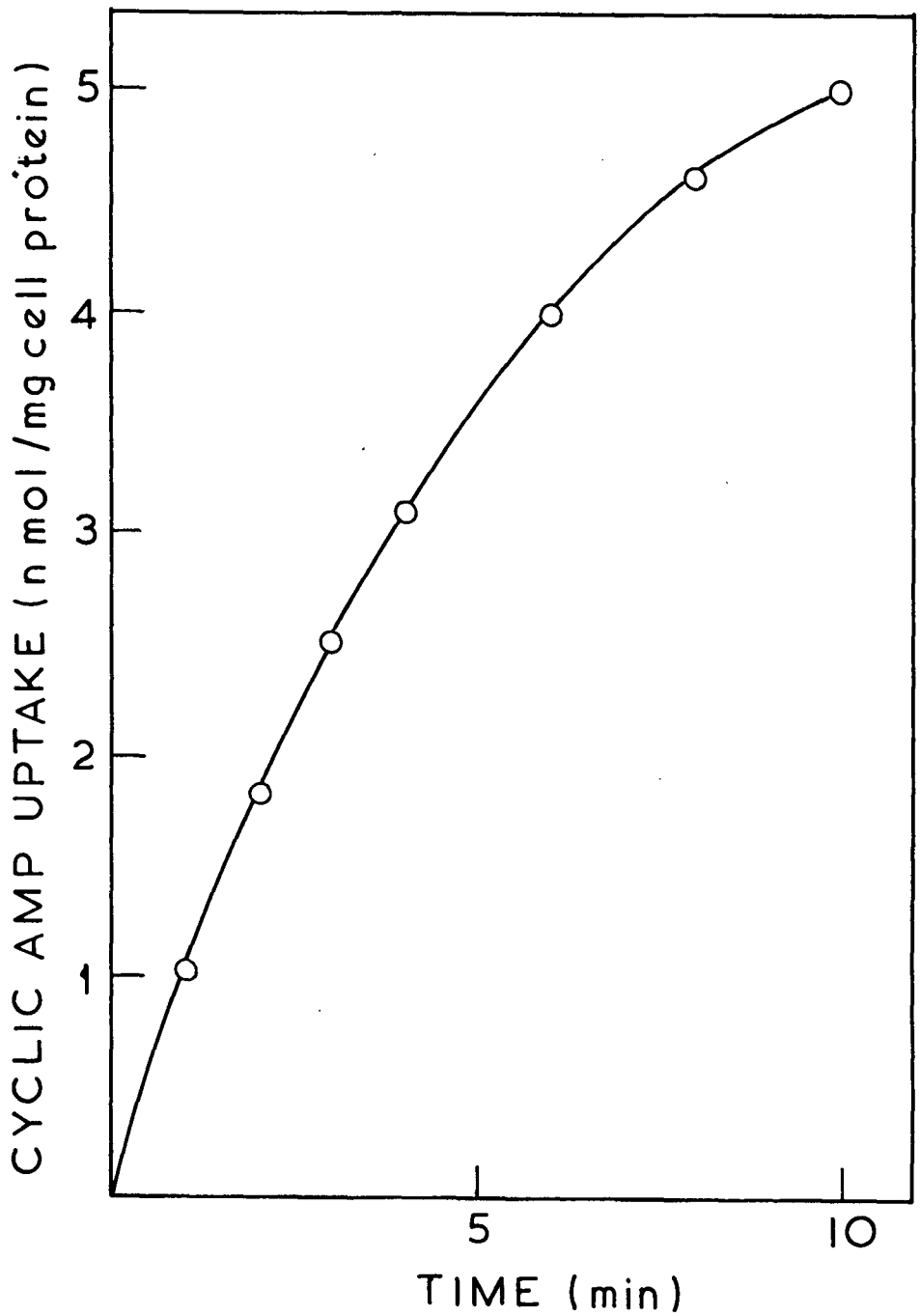

CYCLIC AMP UPTAKE (n mol /mg cell protein)
1
2
3
4
5
5
10
TIME (min)

FIGURE 8 : UPTAKE OF (^{3}H) cAMP IN *Candida albicans*.

Cells harvested from midlog phase were resuspended in water at a density of (8-10)x10^6 cells/ml. Cyclic (^{3}H) AMP (2.5 uCi/ml) was added to give a final concentration of 0.1 mM. At indicated times 0.5 ml aliquots were immediately filered on GF/C filters and washed with cold water, dried and radioactivity determined by scintillation counting. A sample taken immediately after adding the labelled cAMP was used to give the 'blank' value which was subtracted from the other cellular radioactivity estimations.

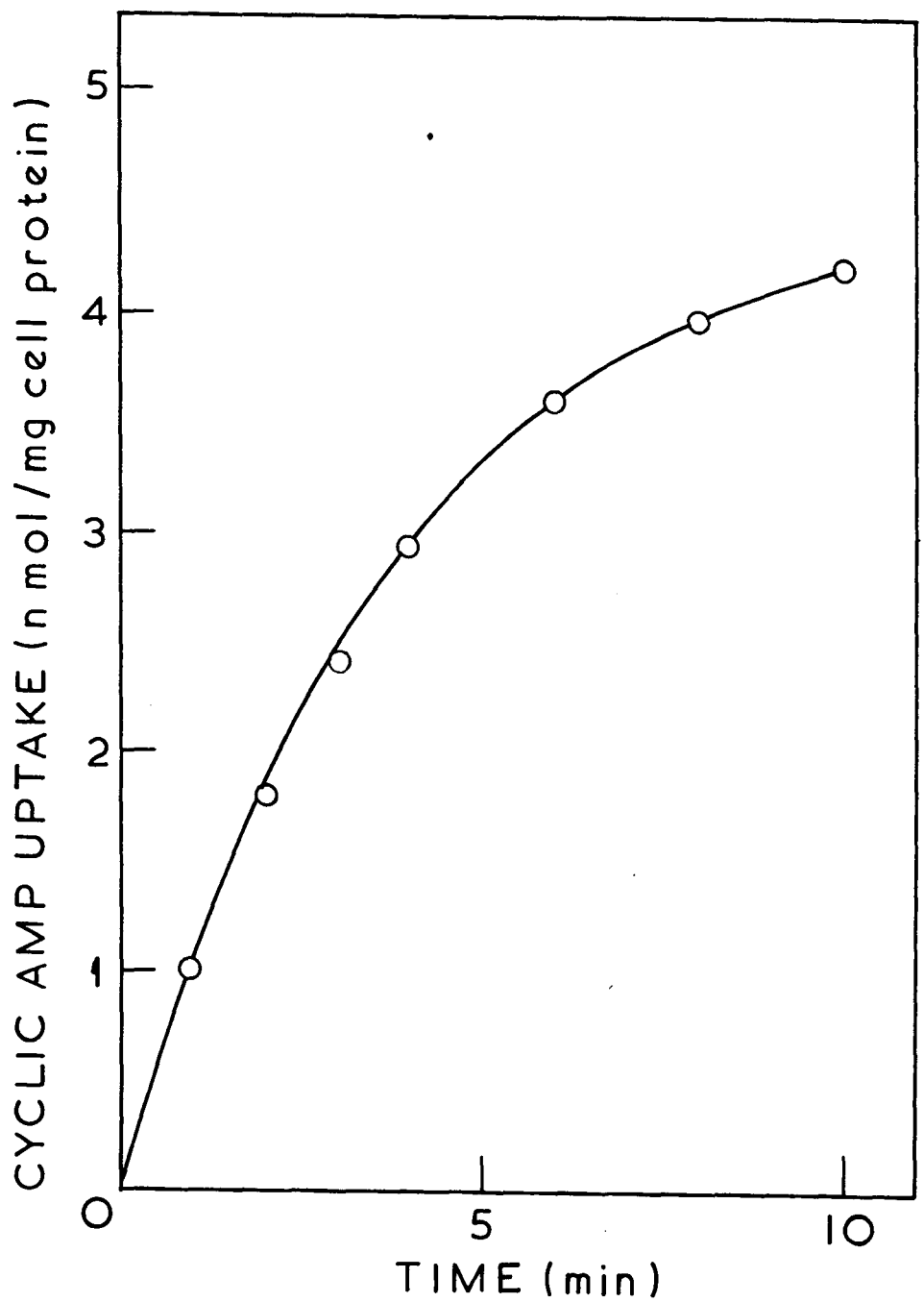

CYCLIC AMP UPTAKE (n mol / mg cell protein)
5
4
3
2
1
0
5
10
TIME (min)

TABLE II : Cells were harvested from the exponential phase, washed and resuspended in induction medium containing 0.5% GlcNAc and 0.3% KH_2PO_4. In separate cultures cyclic AMP at various concentrations were added at the beginning of induction. Cells after 4 h of induction were collected and the uptake of N-acetyl-glucosamine (0.05 mM) was determined as described in 'Materials and Methods'.

TABLE II

EFFECT OF CYCLIC AMP ON THE INDUCTION OF THE N-ACETYL-GLUCOSAMINE UPTAKE SYSTEM IN YEAST

Concentration of cyclic AMP (mM)	*Candida albicans*		*Saccharomyces cerevisiae*	
	GlcNAc uptake (μmol/5 min per mg protein)	% of control	GlcNAc uptake (μmol/5 min per mg protein)	% of control
0	0.390	100	0.381	100
0.2	0.385	99	0.390	102
0.5	0.371	95	0.341	90
1.0	0.315	81	0.285	75
3.0	0.249	64	0.223	59
6.0	0.294	75	0.250	66
10.0	0.310	80	0.265	70

FIGURE 9 : EFFECT OF cAMP ON THE INDUCTION OF N-ACETYL-GLUCOSAMINE PERMEASE IN [illegible].

Cells collected in their exponential phase of growth were suspended in absence (○) or presence (●) of cAMP (3 mM). $(8\text{-}10)\times10^6$ cells/ml were suspended in water and preincubated for 5 min at 30°C. N-(^{3}H) acetyl-glucosamine (1 µCi/ml) was then added to give a final concentrations of 0.05 mM. Cells were collected on GF/C filters and radioactivity determined as explained in 'Materials and Methods'.

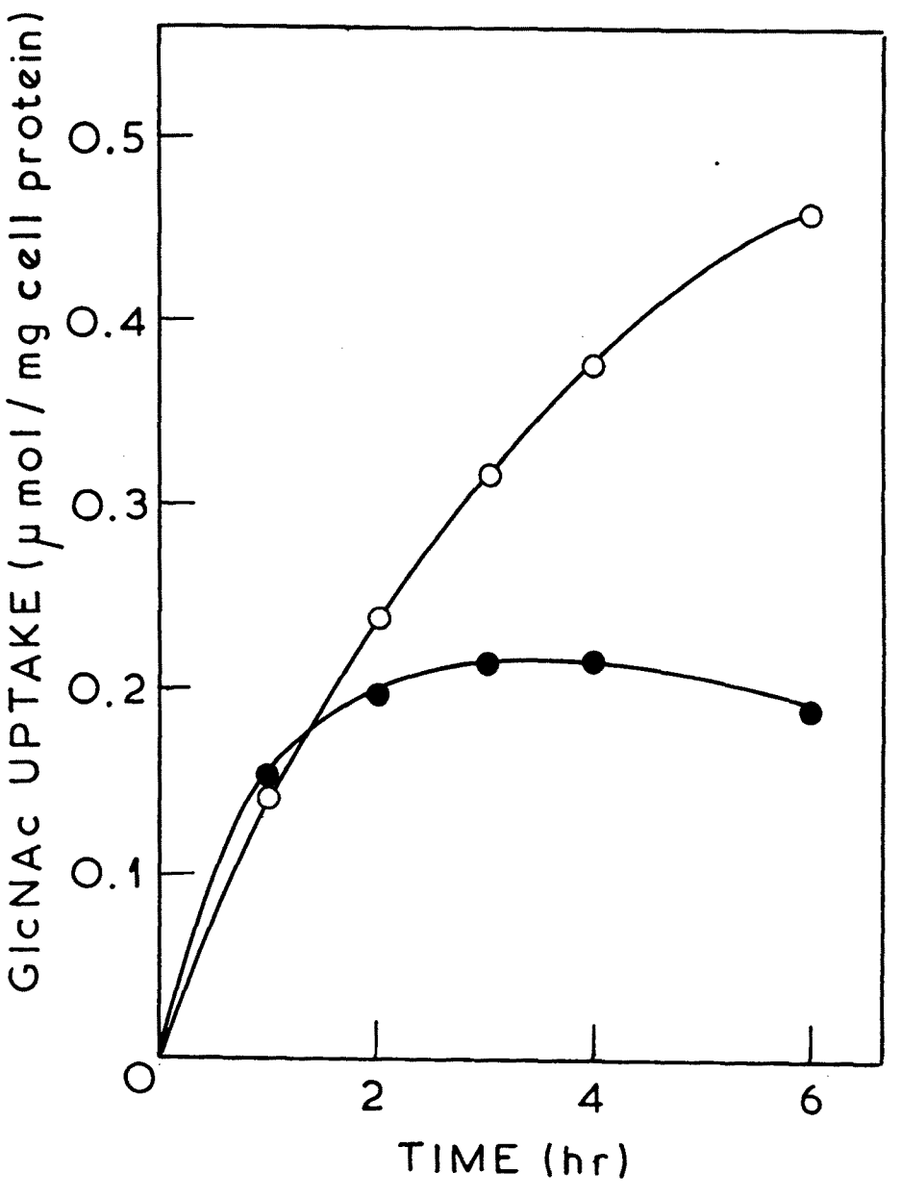

GlcNAc UPTAKE (μ mol / mg cell protein)
0.5
0.4
0.3
0.2
0.1
0
2
4
6
TIME (hr)

FIGURE 10 : EFFECT OF cAMP ON THE INDUCTION OF N-ACETYL-GLUCOSAMINE PERMEASE IN [illegible].

Cells collected in their exponential phase of growth were suspended in absence (○) and presence (●) of cAMP (3 mM). (8-10)x10^6 cells/ml were suspended in water and preincubated for 5 min at 30°C. N-(^{3}H) acetyl-glucosamine (1 µCi/ml) was then added to give a final concentration of 0.05 mM. Cells were than collected on GF/C filters and radioactivity was determined as explained in "Materials and Methods".

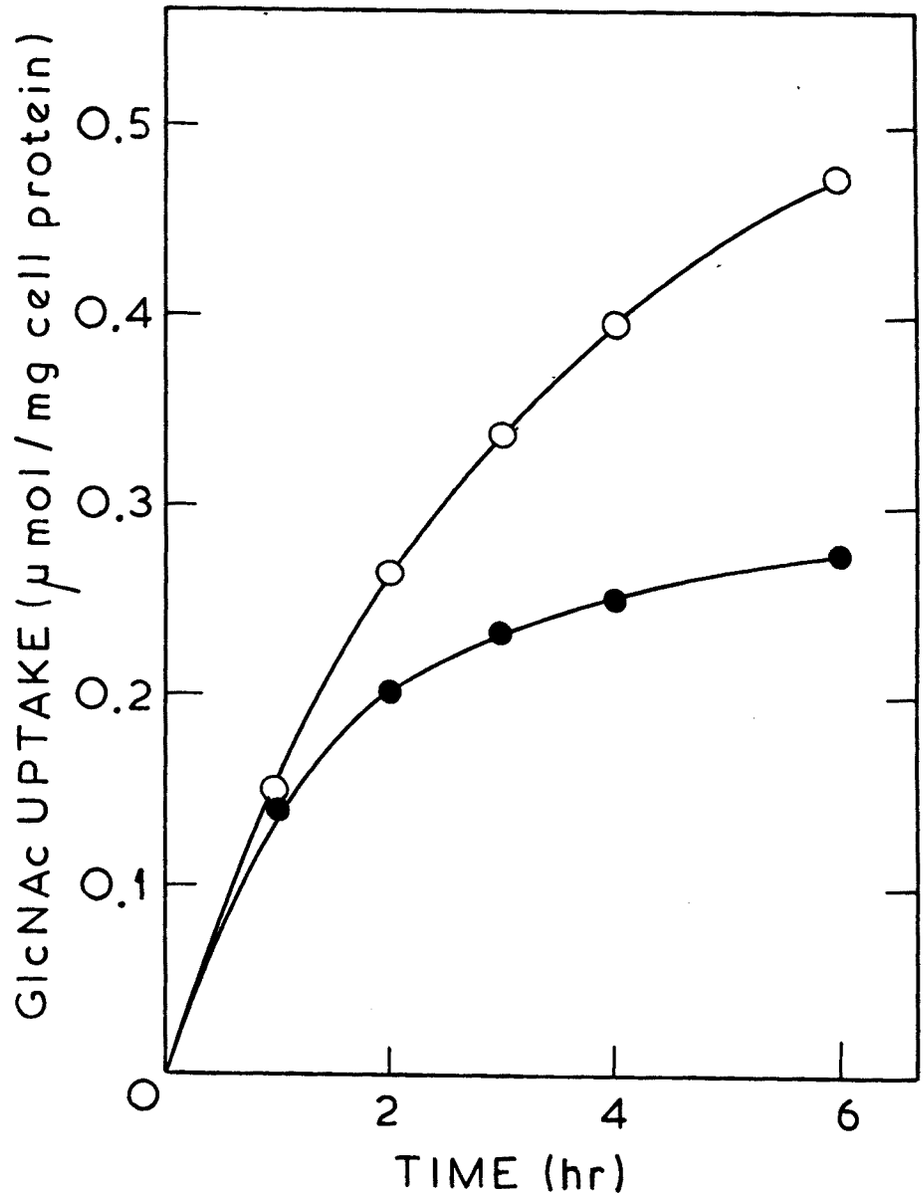

GlcNAc UPTAKE (μ mol / mg cell protein)
0.5
0.4
0.3
0.2
0.1
0
2
4
6
TIME (hr)

FIGURE 11 : EFFECT OF cAMP ON THE INDUCTION OF GLUCOSAMINE- 6-PHOSPHATE DEAMINASE IN *Klebsiella pneumoniae*.

Cells from midlog phase were harvested and grown in the presence and absence of cAMP (3 mM). At indicated times cells were taken from the medium and the specific activity of glucosamine -6-phosphate (μmol of substrate deaminated per min per mg protein) was determined as in 'Materials and Methods'.

o- - - - - - - - o without cAMP

●———————● with cAMP

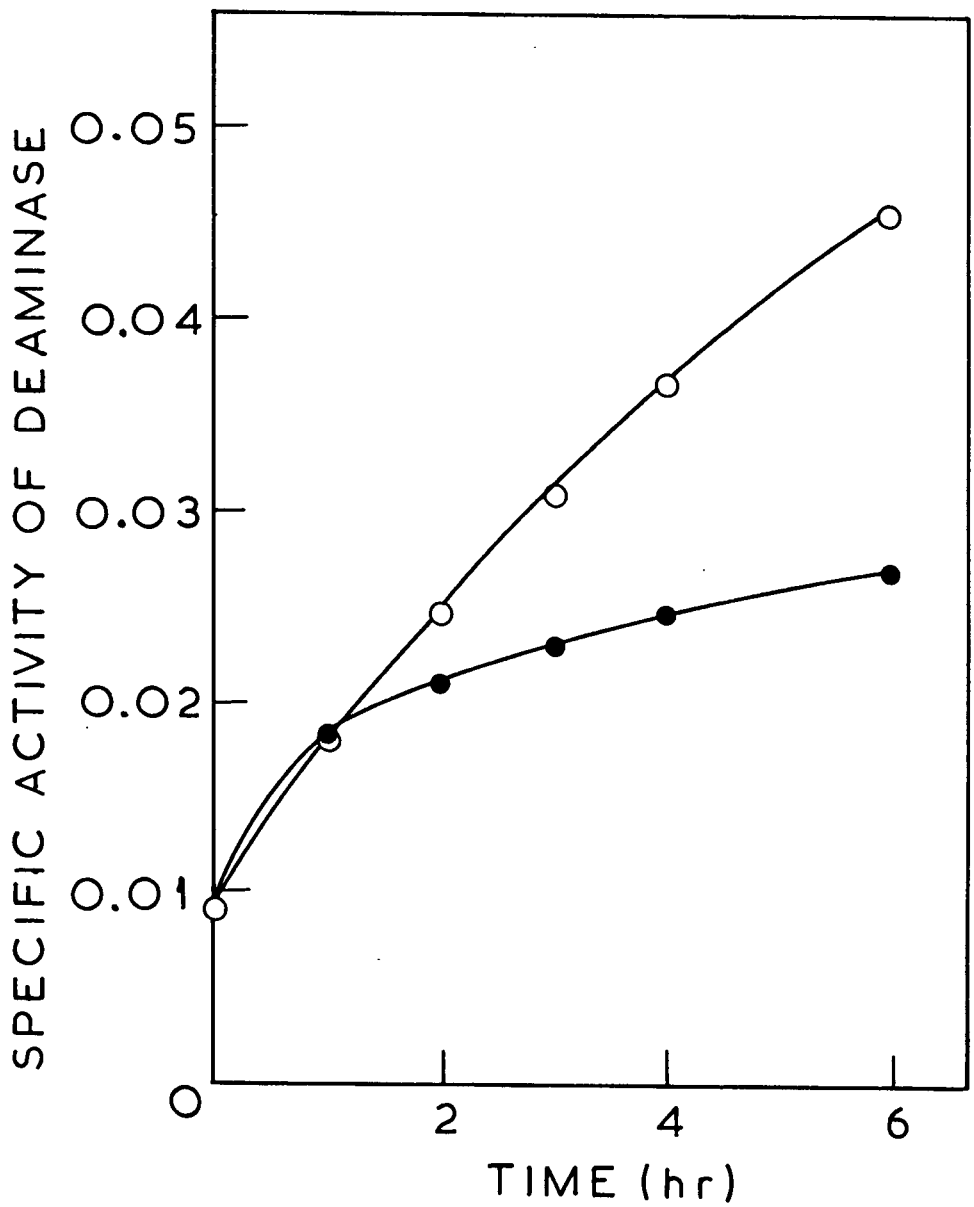

SPECIFIC ACTIVITY OF DEAMINASE
0.05
0.04
0.03
0.02
0.01
0
2
4
6
TIME (hr)

FIGURE 12 : EFFECT OF cAMP ON THE INDUCTION OF GLUCOSAMINE-6-PHOSPHATE DEAMINASE IN [illegible].

Cells from midlog phase were harvested and grown in the presence and absence of cAMP (3 mM). At indicated times cells were taken from the medium and the specific activity of glucosamine-6-phosphate (μmol of substrate deaminated per min per mg protein) was determined as in 'Materials and Methods'.

○——○ Without cAMP

●——● With cAMP.

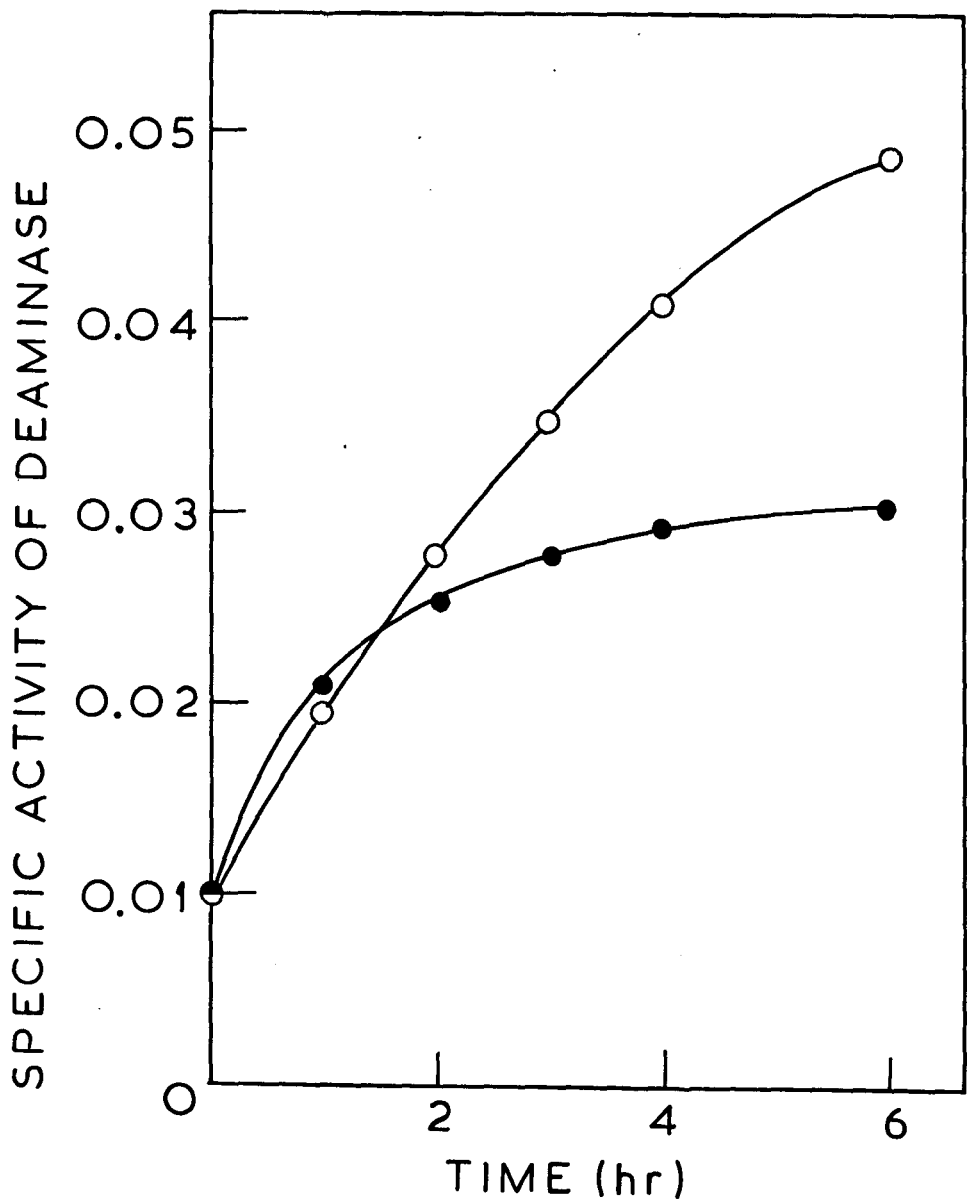

SPECIFIC ACTIVITY OF DEAMINASE
0.05
0.04
0.03
0.02
0.01
0
2
4
6
TIME (hr)

FIGURE 13 : EFFECT OF cAMP ON PULSE-LABELLED PROTEIN SYNTHESIS DURING INDUCTION IN *Saccharomyces cerevisiae*.

Cells harvested from the exponential phase were washed and resuspended in induction medium to give $(8\text{-}10)\times10^6$ cells/ml. At zero time, cells were divided into two portions: one sample (control) remained untreated and the other (experiemental) received 3mM cAMP. At different times, 1 ml suspension was taken out and then labelled with 2 μCi (^{3}H) leucine for 5 min. Radioactivity in each aliquot was determined as described in 'Materials and Methods'.

[illegible] without cAMP

[illegible] with cAMP (3 mM)

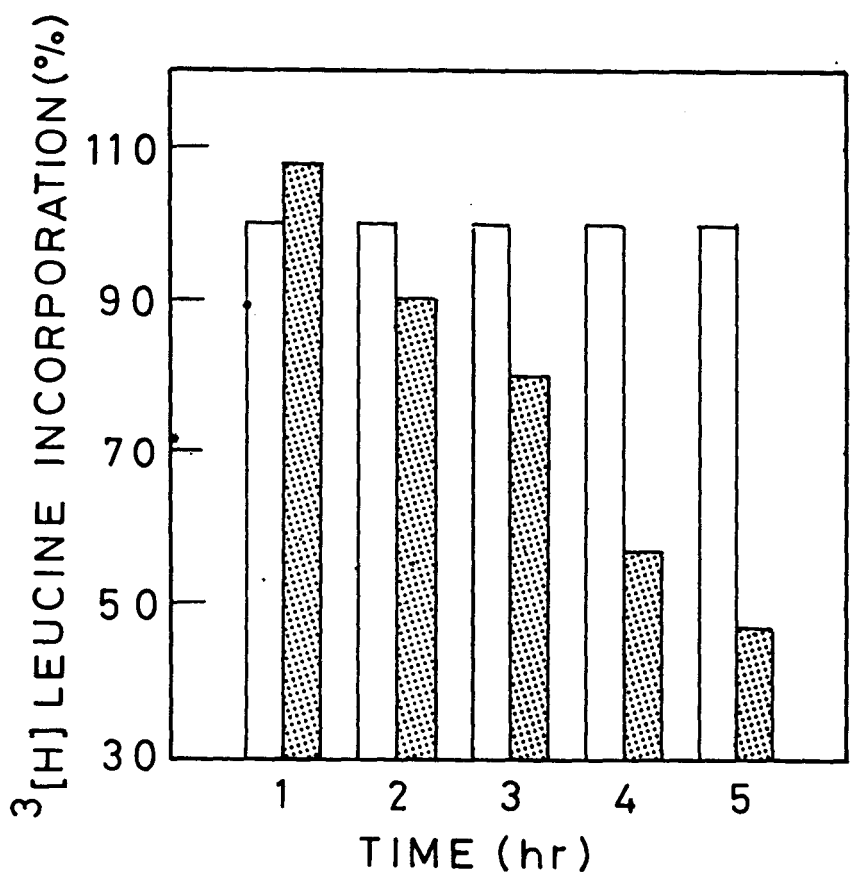

^{3}[H] LEUCINE INCORPORATION (%)
110
90
70
50
30
1
2
3
4
5
TIME (hr)

TABLE III : Cells from exponential phase of growth were collected, washed and resuspended in 0.3% KH_4PO_4 containing the indicated sugar. After incubating for 3 h, cell-free extracts were prepared and cyclic AMP concentration in this extract was determined as described in 'Materials and Methods'.

TABLE III

INTRACELLULAR CONCENTRATION OF
CYCLIC AMP UNDER VARIOUS CONDITIONS

Growth conditions	Cyclic AMP concentration (pmol per mg cell protein)	
	Candida albicans	*Saccharomyces cerevisiae*
Glucose grown midlog phase cells	32	29
Washed cells suspended in :		
GlcNAc (0.5%)	30	31
Glucose (0.5%)	31	27
Glucose (1%)	30	28

Fig. 9 and Fig. 10 show the effect of cAMP on the induction of GlcNAc permease in the two yeasts. Initially, cAMP was without any effect for about 1 hr, after which a marked inhibition of permease synthesis was observed. However, the reason for the delayed action of cAMP is not known.

Glucosamine-6-phosphate deaminase, which is a semi-constitutive enzyme present in both the yeasts (166), provides an ideal system for the present study. As shown, the inducible synthesis of deaminase was affected in the manner similar to GlcNAc permease in both *Saccharomyces cerevisiae* (catabolite repression positive) (Fig. 11) and *Candida albicans* (catabolite repression negative) (Fig.12). This could suggest that cAMP mediated inhibition is independent of glucose effect.

Effect of cAMP on Protein Synthesis :

To examine whether cAMP mediated inhibition is limited only to inducible enzyme synthesis or is of a general nature, the cAMP effect on the rate of protein synthesis during induction was studied. The results (Fig. 13) clearly suggest that the rate of cellular protein synthesis comes down to the same extent as that of inducible enzymes. The fact that cAMP inhibits protein synthesis in both the yeasts, but glucose inhibits inducible enzyme synthesis only in *Saccharomyces cerevisiae* and not in *Candida albicans* indicates that the two inhibitory mechanisms could be different. Moreover, the

level of intracellular cAMP remains unchanged under various conditions employed in the present study (Table III).

DISCUSSION

The involvement of glucose and cyclic nucleotides in catabolite repression seems to be a rather general phenomenon, but the details of the mutual relationship between these two signals remain obscure. A correlation has been shown between concentrations of glucose in the medium and intracellular cAMP levels in yeast (158), but it does not explain the details of the time course and specificity of this effect. Studies on mutants resistant to glucose repression have not shown any correlation between the levels of cAMP and one or more catabolite repressible enzymes (168). PEPCK showed high activity in *Saccharomyces cerevisiae* grown on gluconeogenic carbon sources e.g. ethanol. Addition of glucose to such cultures caused a rapid loss of PEPCK activity. The inactivation was shown to be an irreversible process since the regain of activity depended on *de novo* protein synthesis. Cycloheximide did not prevent but considerably retarded the inactivation. At 10 µg/ml, cycloheximide inhibited the incorporation of amino acids into protein without any effect on the rate of glucose fermentation (169). No correlation could be observed between the cAMP level in yeast cells and the sensitivity of invertase synthesis to glucose repression (170). Furthermore, it has also

been shown that catabolite repression of certain enzymes in *Saccharomyces cerevisiae* cannot be rescued by cAMP (171). A recent report (172) has shown that the addition of glucose to yeast cells, [illegible] X 2180, causes an immediate rise in the intracellular concentration of cAMP. This is in contrast to reports that cAMP concentration in glucose grown yeast cells is low (158, 159). In this strain, inactivation of fructose biphosphatase by glucose is initially reversible and this inactivation has been shown to be due to phosphorylation of the enzyme. Such increase in cAMP levels, on resuspending commercial Baker's yeast on glucose containing medium, has been seen and it is likely that glucose produces a transient elevation of cAMP followed by sustained decrease (172). In this study we have shown that the cAMP content of the glucose grown cells was same as that of the GlcNAc grown cells (Table III). Furthermore, we have also shown that cAMP cannot release the GlcNAc catabolic enzymes from catabolite repression in *Saccharomyces cerevisiae*.

The mechanism of action of cAMP, in relation to glucose, in control of enzyme synthesis has been studied with various enzymes. Different enzymes show different effects of cAMP. A number of such reports predict an effect on overall mRNA metabolism. The mechanism of control of the synthesis PEPCK appears to be at the level of transcription since administration of Bt_2 cAMP to glucose fed rats rapidly

increases the level of cytosolic PEPCK mRNA, and this effect could be blocked by simultaneous administration of cordycepin or actinomycin D (173).

Recent reports suggest that primarily the action of cAMP in various systems is at the level of protein synthesis. In mouse hepatoma cell line culture it was shown that in the presence of 1 mM Bt_2 cAMP, the synthesis and secretion of albumin, α-fetoprotein and transferrin were elevated above controls by 24 hrs. However, there seemed to be no effect on size distribution of total polyribosomes or albumin synthesizing polyribosomes. Moreover, results indicated that the protein synthesis initiation rate on albumin mRNA was also decreased. Hence, there appeared to be non-specific effects of Bt_2 cAMP on protein synthetic parameters (174). A very recent report shows that cAMP, at a concentration of 0.3 mM, elicits maximal induction of PEPCK synthesis in preincubated rat liver cell suspension, whereas at 1 mM concentration or higher there was a consistent inhibition of total protein synthesis, accompanied by a decrease in the relative rate of PEPCK synthesis (175). We have also shown in this study that protein synthesis is inhibited by cAMP in both *Saccharomyces cerevisiae* and *Candida albicans* irrespective of the presence or absence of glucose repression. Furthermore, cAMP also inhibited the induction of GlcNAc catabolic enzymes in both the strains of yeast and

its effect was not related to catabolite repression.

It has been shown that in [illegible] oocytes incubation with cAMP inhibits the incorporation of amino acids into proteins and delays onset of maturation (176). In *Saccharomyces cerevisiae* X2180 also, an increase in intracellular cAMP level caused by glucose, causes a concomitant inhibition of the transport of amino acids driven by membrane potential (172). A recent report has shown that caffeine, which inhibits RNA and protein synthesis in yeast, also causes a decrease in cAMP levels, which is stimulated by glucose in yeast. Glucose inactivation of fructose biphosphatase PEPCK and malate dehydrogenase is also retarded by caffeine. This effect is likely to be mediated via cAMP and there may be a link with protein synthesis (177). It has been shown in retic lysate that cAMP affects protein synthesis . It was suggested that a cAMP dependent protein kinase, in turn activates a cAMP independent protein kinase (the inhibitor) which inhibits protein synthesis (178). But there is no direct evidence showing the presence of such an enzyme. Our present studies indicate that in lower eukaryotes also, protein synthesis is under cAMP control.
