Aerobic Fermentation and the Depletion of the Amino Acid Pool in Yeast Cells

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ABSTRACT The amino acid pool of yeast cells, *Saccharomyces cerevisiae*, incubated with galactose remains at a constant level for 100 minutes. This is 30 minutes beyond the time at which the oxidative phase of the induced-enzyme formation begins. Washed yeast cells, the pools of which have been depleted 60 per cent by incubation with glucose, do not replenish their pools as do washed cells incubated without a substrate. These facts indicate that the induced enzymes are formed at least partially from pool-replenishing amino acids. The time of onset of pool depletion is the time at which the aerobic fermentation phase of induced-enzyme formation begins for cells incubated with galactose. With 0.1 per cent galactose the respiratory phase begins at 100 minutes but no aerobic fermentation nor pool depletion occurs. The rates of respiration and aerobic fermentation are constant for four glucose concentrations from 0.1 to 1.0 per cent. The amount of aerobic fermentation is proportional to the initial concentration of glucose. Amino acid pool depletion occurs for all concentrations but depletion ceases and is followed by pool replenishment after aerobic fermentation is complete. Ultraviolet radiations, which delay the appearance of the respiratory phase of induced-enzyme formation, completely eliminate both the appearance of aerobic fermentation and pool depletion. The results indicate an intimate association between aerobic fermentation and amino acid pool depletion.

Previous work by Swenson and Dott (1) has shown that in the absence of an exogenous energy source, amino acids leak slowly from yeast cells and that accompanying this leakage there is a slow increase in the free amino acid pool level of the cells. The addition of glucose greatly increases the metabolic rate, resulting in the leakage of fewer amino acids and in the depletion of the amino acid pool by about 50 per cent after 2 hours. Pool depletion during glucose metabolism was interpreted to be the result of protein synthesis. If glucose is removed from the suspending medium after the pool level has been

stabilized, the pool level increases by an internal replenishment process which is thought to involve the degradation of certain labile proteins to amino acids (1, 3). Presumably, the slow increase in the amino acid pool for freshly harvested cells without glucose is the result of the same internal pool replenishment process. Ultraviolet radiations have been shown to modify these pool and leakage relationships (1). With high dosages the pool level falls to zero as amino acids rapidly leak from the cell; if glucose is added after the cells are irradiated, both the rate and extent of leakage are greatly increased.

It was thought to be of importance to explore the pool and leakage relationships of cells in the presence of galactose, a sugar which induces the formation of an enzyme complex necessary for the metabolism of the sugar. Evidence has been accumulated which indicates that induced enzymes in yeast are formed from the free amino acid pool (4). Enzymatic induction for "galactozymase" formation in yeast may be followed manometrically by measuring oxygen consumption. First, there is a lag period of 70 minutes, characterized by a low endogenous rate of respiration; following this period the rate of oxygen consumption gradually increases until a maximum rate is attained.

Sheffner and Lindegren (6) have shown that in addition to the oxidative phase of galactose utilization, there occurs in yeast a second phase, one of aerobic fermentation characterized by evolution of large volumes of carbon dioxide. This phase was shown by them to begin 80 minutes after the onset of the oxidative phase. Aerobic fermentation of glucose is well known in yeast and is defined as the process of formation of carbon dioxide and ethyl alcohol in the presence of molecular oxygen. The term was first used by Meyerhof (7) who gave reasons for assuming that the RQ for glucose-metabolizing yeast is 1.0 and concluded that the carbon dioxide produced in excess of oxygen consumed is an index of the fermentation process taking place concurrently with respiration.

Evidence is presented in this paper that in yeast the depletion of the amino acid pool in the presence of glucose and galactose is dependent upon aerobic fermentation activity.

METHODS

A strain of bakers' yeast, *Saccharomyces cerevisiae*, originally isolated at the University of Toronto was employed in this work. The organisms were maintained on agar slants containing 1.0 per cent yeast extract, 2.0 per cent glucose, and 2.2 per cent agar. Twenty-four hours prior to an experimental run the yeast were inoculated from a slant by means of a sterile needle into a flask containing 125 ml of sterile liquid medium. The composition of the medium was an aqueous solution of 2.0 per cent glucose and 1.0 per cent yeast extract. The inoculated flasks were placed in an incubator shaker, the temperature of which was maintained at 30°C. After

incubation for 24 hours, the cells were washed four times with distilled water and suspended in distilled water at a concentration of 2.1×10^8 cells per ml. These were the conditions employed for the experiments on which the present work is based (1).

For the pool and leakage experiments, 10 ml of cell suspension were pipetted into as many 150 ml Pyrex beakers as samples were to be taken in a given experiment. To the cell suspension was added 0.5 ml of an appropriate concentration of glucose or galactose or 0.5 ml of distilled water for cells to which no exogenous substrate was to be added. The sugar concentrations listed in the text and on the graphs are the final concentrations in the cell suspension. The beakers were covered with aluminum foil and placed in the incubator shaker at 30°C. At intervals beakers were removed from the shaker and 2.5 ml samples of the suspension were drawn off for analysis of the levels of the amino acid pools and, in certain instances, of the amino acids which leaked out of the cells. The zero-time samples were taken from 10 ml cell suspensions to which 0.5 ml of water had been added. The withdrawn samples were centrifuged and the supernatants containing the leakage amino acids were decanted and saved for analysis. The cells were washed once with distilled water and resuspended in 2.5 ml of distilled water. The cells were then heated in a boiling water bath for 10 minutes, a process which coagulates the cell proteins and destroys the cell membranes. The cell residues were packed by centrifugation and the supernatants containing the pool amino acids (8) were decanted and saved for analysis.

The level of amino acids in the leakage and pool samples was determined using the ninhydrin method described by Moore and Stein (9) for the analysis of amino acids in effluents from an ion exchange column. The use of this method for pool and leakage level determinations has been discussed by Swenson and Dott (1). Aliquots of 0.1 ml of pool or leakage samples were pipetted into test tubes and 1.0 ml of ninhydrin solution was added to each. The tube contents were then heated for 20 minutes in a boiling water bath to develop the characteristic purple color. After cooling and diluting with 5.0 ml of *n*-propyl alcohol, the absorbance of the solutions was measured at 570 mμ using a Bausch and Lomb spectronic 20 spectrophotometer. The results are expressed as micromoles of leucine per 4.6 mg dry weight of cells.

Respiration and aerobic fermentation were followed using the Warburg manometric technique. Large 100 ml flasks of cylindrical shape were used.[1] The diameter of these vessels is 55 mm, the same diameter as for the 150 ml beakers used for the pool leakage experiments. This provided the same surface to volume ratio for all cell suspensions in experiments in which pool changes were to be correlated with aerobic fermentation. Sugar solutions (0.5 ml) of appropriate concentrations to give the desired final concentrations were in the vessel sidearms from which they could be mixed with the cell suspensions at the desired time. Respiratory changes were followed using 0.4 ml of 40 per cent KOH to absorb the carbon dioxide. Aerobic fermentation was followed in vessels in which no KOH was present. An RQ of 1 was assumed for respiration of carbohydrate (7, 10) and the carbon dioxide in excess of respiratory oxygen was calculated from the positive pressure changes registered on the manometers for which no KOH was present in the vessels.

[1] Supplied by the American Instrument Company, Silver Springs, Maryland. Catalogue No. 5-210.

Ultraviolet irradiation was carried out using two 14 watt General Electric germicidal lamps. Twenty ml of cell suspension were irradiated in a crystallizing dish 80 mm in diameter and 40 mm deep. The depth of the cell suspension was 4 mm and the cells were stirred during irradiation with a magnetic stirrer. The lamps were mounted 61 mm above the level of the suspension. These lamps put out 95 per cent of their energy in the 2537 A region. The intensity of the radiations at the center of the dish was 59 ergs/mm²/sec. as measured with a Hanovia ultraviolet meter. Following irradiation the cells were handled only in dim yellow light to prevent photoreactivation.

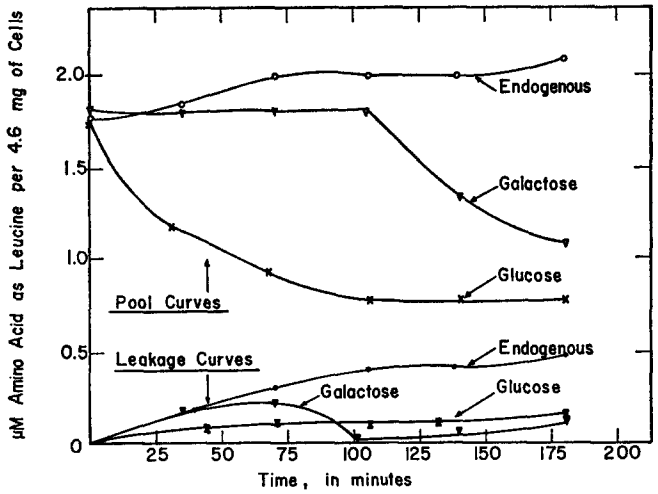

FIGURE 1. Amino acid leakage and pool curves for yeast cells incubated with no substrate (endogenous), glucose, and galactose. Substrate concentrations are 1.0 per cent.

RESULTS

Fig. 1 shows the leakage and pool patterns for yeast incubated under three different conditions: (*a*) with no added substrate (endogenous conditions); (*b*) with glucose; and (*c*) with galactose. The endogenous cells slowly leak amino acids and a pool replenishment process is at work, indicated by an increase in the amino acid pool. The pool of the glucose cells is depleted rapidly after addition of the substrate and at about 2 hours reaches a stabilized level of about 50 per cent of the zero-time value. The patterns are quite different for the galactose cells. Leakage of amino acids occurs at approximately the same rate as for the endogenous control for about 70 minutes, after which time leakage ceases and the amino acids which have leaked out are drawn back into the cells. The level of the pool remains constant for 100 minutes after which it is depleted rapidly.

The constancy of the level of the amino acid pool for 100 minutes in the galactose cells is of interest because the rate of oxygen consumption during

the 70 minute induction period is approximately equal to that of the endogenous cells. Either the pool replenishment process characteristic of endogenous cells is not operative or, if replenishment is occurring, an amino acid utilization process is taking place, probably for the production of the induced enzyme. It was postulated that the latter possibility is correct. A test of this hypothesis is made possible by using cells, the pools of which are depleted while in contact with glucose. Normally, pool replenishment occurs after washing the pool-depleted cells and resuspending them in water (1, 3). If

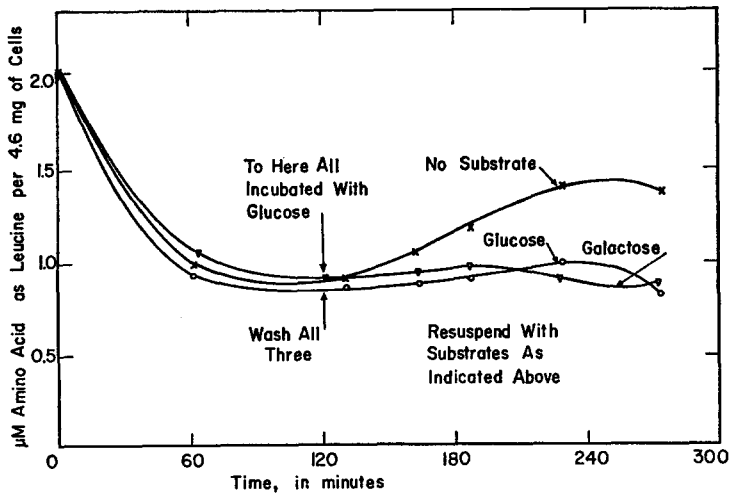

FIGURE 2. The influence of galactose, glucose, and endogenous conditions on the amino acid pool levels of cells previously depleted by incubation with glucose. Substrate concentrations are 1.0 per cent. See text for details.

the amino acids from labile proteins are utilized for synthesis of the induced enzyme, addition of galactose should prevent pool replenishment. Fig. 2 shows the results of such an experiment in which cells were incubated with glucose for 2 hours followed by washing and by suspension of the cells in water with no substrate or with glucose or galactose. Pool replenishment occurs when no substrate is added but not when either glucose or galactose is present. Manometric experiments showed that the galactose cells formed the enzyme according to schedule.

A second point of interest in connection with Fig. 1 is that amino acid pool depletion does not occur until some 30 minutes after enzymatic activity, as measured by oxygen consumption, has appeared. Thus, cell respiration of itself does not appear to cause pool depletion. The work of Sheffner and Lindegren (6) on aerobic fermentation by yeast forming induced enzymes in response to galactose was thought to provide a clue to the understanding of the pool depletion problem. Aerobic fermentation is characterized by the

production of large quantities of carbon dioxide and ethyl alcohol. Sheffner and Lindegren (6) showed that the fermentation phase in their strain of bakers' yeast commences at 170 minutes, a time which is 80 minutes after the onset of oxygen consumption. Fig. 3 shows that aerobic fermentation in our strain begins at approximately 100 minutes. Fig. 3 also shows that when

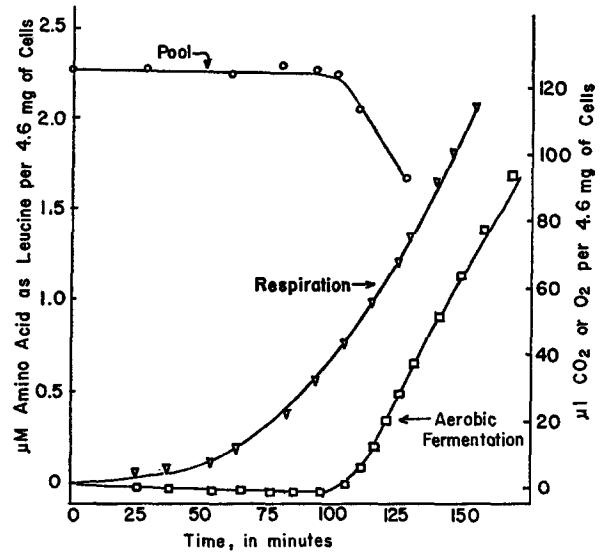

FIGURE 3. Graph showing the time relationships among respiration, aerobic fermentation, and the amino acid pool level of galactose-incubated cells. Galactose concentration 1.0 per cent.

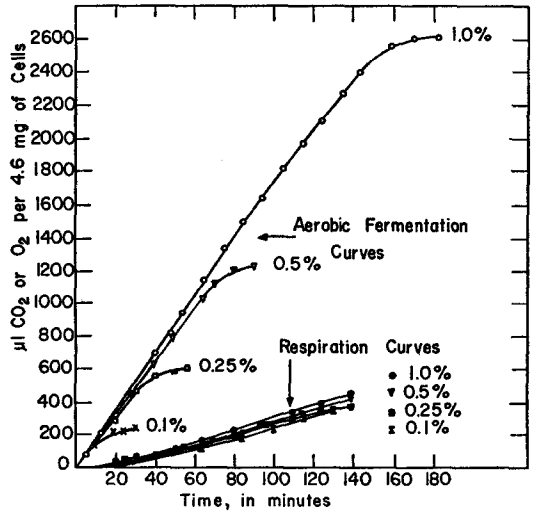

FIGURE 4. The influence of various concentrations of glucose on respiration and aerobic fermentation.

aerobic fermentation, respiration, and pool levels are followed concurrently for galactose-incubated cells, the fall of the amino acid pool is closely correlated in time with the onset of fermentation.

The above results led to the prediction that the pool level would not fall if aerobic fermentation were prevented. To test the hypothesis that pool depletion is dependent upon aerobic fermentation, an experiment was designed using yeast cells metabolizing glucose. It had been observed for cells incubated with 1.0 per cent glucose that aerobic fermentation ceased after 2½ hours

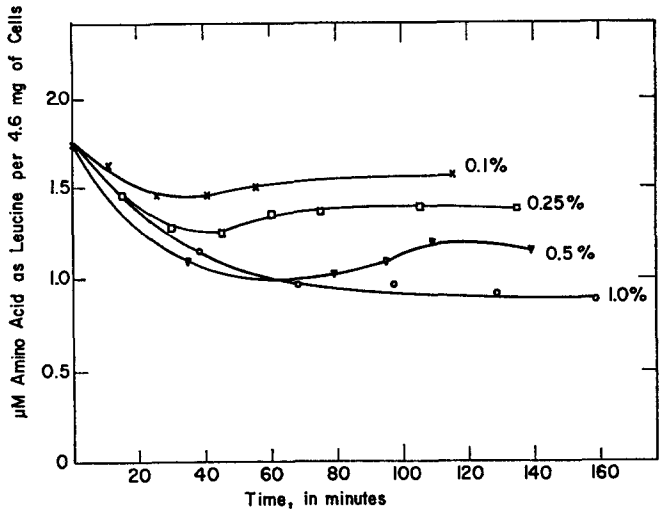

FIGURE 5. The influence of various concentrations of glucose on amino acid pool depletion.

but that the respiration continued at a constant rate. By lowering the initial concentration of glucose it was found that the total amount of aerobic fermentation could be reduced. Fig. 4 shows the aerobic fermentation and respiration curves for four initial concentrations of glucose between 0.1 and 1.0 per cent. The rate of aerobic fermentation is the same for all concentrations and the amount of fermentation is proportional to the initial concentration. The rate of respiration is influenced only slightly by the concentration of glucose. The aerobic fermentation takes place at a rate approximately six times that of respiration.

In Fig. 5 are plotted the pool responses of cells incubated with each of the four concentrations of glucose. Pool depletion occurs in the usual manner for the 1.0 per cent cells, reaching a stabilized level and remaining there. The 0.5 per cent cells exhibit pool depletion until 70 minutes, the time at which aerobic fermentation is about to terminate; after the cessation of aerobic fermentation, pool replenishment occurs. The situation is much the same for

the 0.25 per cent cells; aerobic fermentation and pool depletion cease at 30 minutes and pool replenishment follows. The pool of the 0.1 per cent cells is depleted slightly and the amount of carbon dioxide from aerobic fermentation is also quite small.

A similar set of experiments using three initial concentrations of substrate was carried out for galactose-utilizing cells. The respiration and aerobic

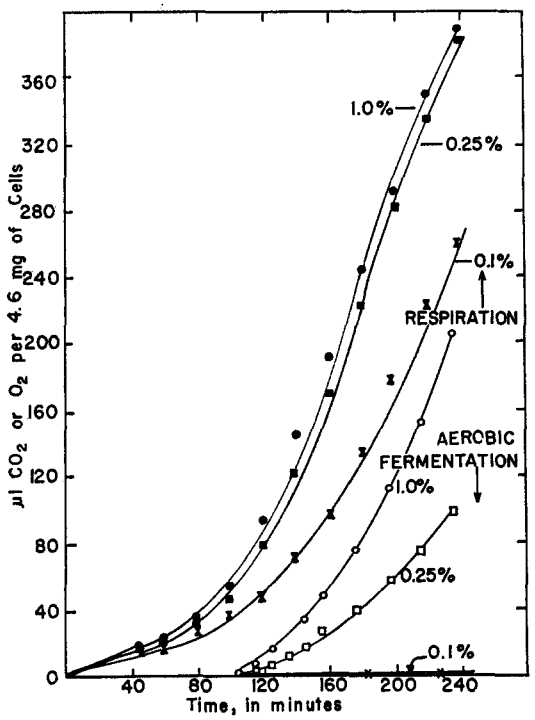

FIGURE 6. The influence of various concentrations of galactose on respiration and aerobic fermentation.

fermentation curves are presented in Fig. 6. The respiration curves for 1.0 and 0.25 per cent galactose are identical but with 0.1 per cent galactose the transition to rapid oxygen consumption is delayed by about 20 minutes. The aerobic fermentation rate for 1.0 per cent galactose is approximately equal to that of respiration. At a concentration of 0.25 per cent the rate of aerobic fermentation is somewhat reduced and for 0.1 per cent galactose no aerobic fermentation is seen. Fig. 7 shows that pool depletion is most extensive for the 1.0 per cent cells; depletion is considerable, although delayed, for 0.25 per cent. Pool depletion does not occur with 0.1 per cent galactose, the concentration for which no aerobic fermentation is seen.

Swenson and Giese (11) have shown that ultraviolet radiations inhibit the

formation of the enzymes induced by the presence of galactose. High dosages prevent the appearance of any enzyme activity; lower dosages delay the formation of the enzyme but when activity does appear, the rate of utilization of galactose as measured by oxygen consumption is equal to that of the con-

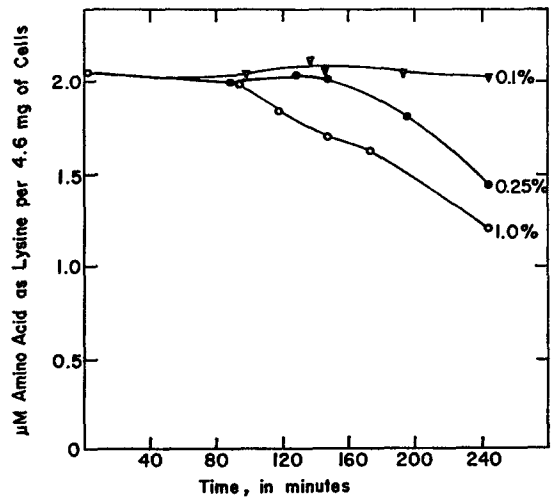

FIGURE 7. The influence of various concentrations of galactose on amino acid pool depletion.

TABLE I

THE INFLUENCE OF ULTRAVIOLET RADIATIONS ON RESPIRATION, AEROBIC FERMENTATION, AND FINAL POOL LEVELS OF GALACTOSE-INCUBATED CELLS

	Respiration	Aerobic fermentation	Per cent of Original pool	Length of time that normal O_2 consumption took place
	µl/4.6 mg cells/hr.	*µl/4.6 mg cells/hr.*		*min.*
Irradiated	170	6	94	115
Non-irradiated	179	190	52	175

The initial concentration of galactose was 1.0 per cent. Irradiation time was 4 minutes. The total time of incubation was 240 minutes. The formation of the induced enzymes for the irradiated cells was delayed 60 minutes as indicated by the onset of respiratory activity.

trol. It was thought that there might be a differential effect of the radiations on respiration and aerobic fermentation in such cells. If so, this would provide another opportunity to test the hypothesis that pool depletion is dependent upon aerobic fermentation. For this experiment the cells were irradiated for 4 minutes; this corresponds to a dosage which has little effect on the amino acid pool of endogenous cells. The results are seen in Table I. The increase in oxygen consumption, signifying the appearance of the enzyme, is delayed

by 110 minutes for the irradiated cells but the final respiration rates of the two samples are almost identical. On the other hand, aerobic fermentation is seen for the control cells but does not occur in the irradiated cells. Significantly, pool depletion occurs in the control but not in the irradiated cells.

DISCUSSION

Leakage of amino acids from endogenous yeast cells has been interpreted to be the result of the absence of an energy source required for the uptake of amino acids from the suspending medium (1). In this light the leakage of amino acids from the galactose cells during the 70 minute induction period is understandable. At 70 minutes the respiration rate increases above the low endogenous rate and as a result net leakage ceases and the external amino acids are moved to the cell interior.

The absence of a net change in the amino acid pool level during and beyond the 70 minute induction period is at first surprising, considering the fact that during this period protein synthesis is thought to be taking place. It may be, however, that for synthesis of the induced enzymes sufficient amino acids are provided by the breakdown of labile proteins during the normal replenishment process whether it be in freshly harvested cells or in cells, the pools of which have been depleted by the metabolism of glucose. Three enzymes are formed during the induction period and the activities of these enzymes are essentially zero prior to the addition of galactose to the cells (12). It is evident from Fig. 2 that approximately 0.5 micromole of amino acids is added to the pool of 1 ml of washed, pool-depleted cells during a 2 hour period by the process of replenishment. Each milliliter contains 2.1×10^8 cells which means 2.4×10^{-15} moles or 1.44×10^9 molecules of amino acids per cell. If it is assumed that each enzyme has 1000 amino acid residues, there is a sufficient number of amino acids available to form 1.44×10^6 molecules of protein. The total nitrogen of yeast is given as 8.94 per cent of the dry weight of the yeast (13). Multiplying this value by 6.45 gives approximately 58 gm of protein per 100 gm of dry weight. Each of the enzymes of the glycolytic scheme constitutes greater than 0.1 per cent of the total cell protein; the figure given for hexokinase is 0.3 per cent (14). If protein is assumed to make up 58 per cent of the dry weight of the cell and the dry weight of a single cell is 2.2×10^{-11} gm, there are 3.8×10^{-14} gm of hexokinase in a yeast cell. The molecular weight of hexokinase is 9.6×10^4 (15). There are, therefore, approximately 2.38×10^5 hexokinase molecules in the cell.

It is evident from the foregoing assumptions and calculations that there are sufficient amino acids provided by the replenishment process after pool depletion to synthesize the three induced enzymes of the galactozymase complex if each of them is present in the amount of 0.3 per cent of the total

protein of the cell. Pool replenishment in freshly harvested endogenous cells provides 50 per cent fewer amino acids during a 2 hour period but the number would be sufficient to synthesize the three enzymes.

There may be more replenishment of amino acids occurring than is evident from net changes. It is also possible that the presence of the inducer, galactose, could cause other proteins to be degraded in addition to those normally contributing to replenishment. Spiegelman and Dunn (16) for example, showed that during the course of "adaptation" to galactose, the glucozymase activity of the cells decreased as the galactozymase activity appeared. Likewise, from the data in this paper it cannot be judged whether or not the induced enzyme is formed entirely of amino acids from degraded proteins because these amino acids might well enter the pool where they are indistinguishable from others of the same type. Thus, the results of the present experiments are not in conflict with the strong evidence that free amino acids are the precursors of induced enzymes (4,5). Because of the difficulty in labeling the amino acid pool without labeling the cellular protein, it has not been possible to obtain unqualified evidence that induced enzymes are formed solely from the amino acids in the pool at the time of addition of the inductor (4). Halvorson, who has found significant turnover rates for protein in resting yeast cells (3) assigns to the replenishment process a prominent role in the preferential synthesis of induced enzymes (4). By preferential synthesis is meant the formation of specific enzymes without an increase in the total protein of the cell.

Aldous *et al.* (17) were the first to make concurrent measurements in yeast cells of oxygen consumption and carbon dioxide production using different concentrations of glucose. They found that with concentrations of 0.1 per cent glucose and below, no aerobic fermentation occurred. On the other hand, between 0.1 and 1.0 per cent they found a slight drop in the respiratory rate and an almost twofold increase in the rate of total carbon dioxide production. Stickland (10) also worked with bakers' yeast and found similar results. The respiratory rate was found by him to be constant for glucose concentrations between 0.1 and 10.0 per cent. Aerobic fermentation was negligible below 0.1 per cent and above this concentration became quite significant, although for 1.0 per cent glucose the carbon dioxide produced by fermentation was only about 20 per cent of the oxygen consumed by respiration. The results of Aldous *et al.* (17) and of Stickland (10) differ from those of the present report in which the rate of aerobic fermentation is seen to be five times that of respiration and independent of the concentration of glucose. In neither of these earlier papers are there presented time-course curves of aerobic fermentation and the duration of this process for any concentration of glucose is not given.

Eaton and Klein (18) investigated the degradation of glucose under aerobic

conditions. Using glucose in a final concentration of 0.12 per cent, they found a burst of carbon dioxide production lasting for 30 minutes after addition of glucose before a significant amount of oxygen uptake had occurred. This is in agreement with our findings. When they employed glucose labeled with C^{14} in the 3 and 4 positions, 80 to 90 per cent of the labeling appeared in the carbon dioxide evolved during the 30 minute aerobic fermentation period. Ethanol formation accompanied the carbon dioxide production and later, as ethanol disappeared, acetate accumulated. Analysis revealed that virtually all of the glucose is first fermented *via* the glycolytic pathway.

Any discussion of aerobic fermentation raises the question of the mechanism of the Pasteur effect. The Pasteur effect occurs when yeast or other cells are transferred from an anaerobic to an aerobic environment. The two chief features, aside from respiratory activity, are a reduction in the rate of glucose utilization and a reduction of fermentation activity. The Pasteur effect has been extensively studied and a number of theories have been advanced to explain the facts. The subject has been reviewed recently by van Eys (19) who noted that the various explanations fall into three categories: (*a*) inhibition of glycolysis by oxygen; (*b*) the action of a metabolic intermediate generated during aerobic conditions acting as an inhibitor in the glycolytic scheme; and (*c*) competition between the processes of glycolysis and respiration for a common intermediate. As van Eys points out, there is general agreement among workers in the field that no theory is completely satisfactory.

If, as has been shown by Eaton and Klein (18), fermentation and respiration have a common pathway to pyruvate, aerobic fermentation would seem to be the result of some rate-limiting step in the tricarboxylic acid cycle or electron transport system. Holzer *et al.* (20) cite evidence that the rate-limiting step is at the pyruvate level and that the balance between respiration and fermentation is governed by the intracellular concentration of pyruvate. The steady-state concentration of pyruvate was found by them to be higher under anaerobic than under aerobic conditions. Holzer *et al.* also found that the pyruvate concentration in the cell was lowered considerably in aerobic cells poisoned with iodoacetic acid, conditions which allow normal respiration but completely eliminate fermentation. As a consequence of a rate-limiting step, possibly pyruvic oxidase, pyruvate accumulates and is decarboxylated to produce carbon dioxide and acetaldehyde which acts as the hydrogen and electron acceptor for reduced DPN. Because some of the glucose is oxidized *via* the tricarboxylic acid cycle, a certain amount of oxygen consumption occurs together with an equal amount of carbon dioxide production, in conformity with an expected RQ of 1.0 for carbohydrate. The carbon dioxide in excess of oxygen consumption is the carbon dioxide of a fermentation process occurring in the presence of oxygen and it is a measure of the extent of the process called aerobic fermentation.

In accordance with this outline of events it is to be expected that if the enzyme of the postulated rate-limiting step were saturated at concentrations of 0.1 per cent glucose and above, the respiration rate would be little affected by increasing the concentration of glucose. Because the rate of aerobic fermentation is also independent of the concentration of glucose, there must be an additional rate-limiting step in the glycolytic system above the pyruvate level. This step may be the one catalyzed by the enzyme triose phosphate dehydrogenase. Lynen (21) and Johnson (22) proposed independently that this step, and hence the rate at which glucose is metabolized, is influenced by the intracellular concentration of inorganic orthophosphate. Some of the evidence dealing with the regulation of glucose metabolism by inorganic phosphate and by other metabolites has been discussed recently by Holzer (23, 24), Lynen *et al.* (25), and by van Eys (19).

The concept of rate-limiting steps in the metabolism of hexoses *via* the glycolytic scheme and the tricarboxylic acid cycle can be used to analyze the results for the galactose cells. Sheffner and McClary (26) have concluded that the fermentation phase of the adaptation of yeast to galactose is the result of the accumulation of metabolic intermediates and saturation of the respiratory system. The induced enzymes in the galactose system of our strain of yeast are formed or become functional at about 70 minutes as indicated by the increase in oxygen consumption over the endogenous rate. More and more enzymes are formed and become functional until eventually galactose molecules are processed in such numbers per unit time that the rate-limiting step below pyruvate becomes saturated. At this time the respiration rate is at its maximum. Any additional induced enzymes that are formed will increase the rate of processing of additional galactose molecules but the result will be fermentation and not an increase in respiration. Consider first the situation for the 1.0 per cent cells for which the final respiration rate is the same but for which the final aerobic fermentation rate is much lower than for the 1.0 per cent glucose cells. Glucose and galactose share a common pathway between glucose-6-phosphate and pyruvate; it, therefore, appears that the rate of fermentation of galactose is limited by the activity of one of the newly formed enzymes. The situation for galactose must be more complex than this, however, as can be seen by the differences in respiration and fermentation rates for the lower concentrations of substrate. Perhaps the concentration of the inducing substrate influences the amounts of new enzymes which are synthesized. Aerobic fermentation occurs earlier in this strain of bakers' yeast than for the one used by Sheffner and Lindegren (6). On one important point, however, there is agreement between their results and ours; aerobic fermentation occurs with 1.0 per cent galactose but not with 0.1 per cent galactose.

Aerobic fermentation and pool depletion begin immediately upon addition of glucose to a yeast suspension and when aerobic fermentation ceases for the

0.5, 0.25, and 0.1 per cent concentrations, pool replenishment begins. Replenishment is never complete, even with washed cells. Pool replenishment has been interpreted as the result of the degradation of labile proteins in the absence of an energy source (3, 1). Now it appears that aerobic fermentation of a carbohydrate is necessary because the cells still have ethyl alcohol, the end product of fermentation, as an energy source after aerobic fermentation has ceased. Halvorson *et al.* (2) found that ethyl alcohol does not promote pool depletion but that pool replenishment occurs as has been shown for endogenous cells. A puzzling inconsistency, however, is that pool replenishment has never been observed with 1.0 per cent glucose over a 6 hour period although aerobic fermentation ceases after 2½ hours (1). The crucial test of the hypothesis that pool depletion occurs only in cells carrying on aerobic fermentation is seen with the 0.1 per cent galactose cells where neither aerobic fermentation nor pool depletion occurs. Pool depletion for the 1.0 per cent galactose cells begins at the onset of fermentation but in each experimental run the 0.25 per cent pool delayed falling for 40 to 50 minutes after aerobic fermentation. The rate of fall, once it has begun, is roughly the same as for the 1.0 per cent cells.

Additional evidence that aerobic fermentation and pool depletion are linked is provided by the experiment in which ultraviolet radiations are shown to completely inhibit pool depletion and the aerobic fermentation phase of galactozymase formation but are shown only to delay the oxidative phase. Ultraviolet radiations inhibit a number of energy-requiring processes in yeast including colony formation, induced enzyme formation (1), and uptake of phosphate (27). Giese and Swanson (28) demonstrated that ultraviolet radiations prevent storage of carbohydrate in yeast. This inhibition may have been associated with a reduction of aerobic fermentation: experiments were cited in which alcohol production was reduced in some cases to 50 per cent of the non-irradiated controls. It is significant that Sheffner and McClary (26) in their study of the galactose system did find a close relationship between aerobic fermentation and carbohydrate storage. The amount of glycogen stored by yeast cells decreased slightly during the induction period and increased only moderately during the oxidative period. However, shortly after the onset of aerobic fermentation there began a rapid accumulation of glycogen, resulting in a doubling of these reserves in a 2 hour period.

What is the significance of the association between aerobic fermentation and cellular synthesis? In the case of the glucose-metabolizing yeast cells described in the present paper it is possible that the energy from aerobic fermentation promotes the synthesis of proteins from the amino acid pool. For each oxygen molecule consumed in respiration about 6 ATP molecules are gained; for each carbon dioxide molecule produced in fermentation the cell nets 1 ATP molecule. Thus, if the fermentation rate is six times that of

respiration, the cells are approximately doubling the amount of energy per unit time obtained by respiration alone, while aerobic fermentation is in progress. For the galactose cells, however, much less extra energy is made available. For the 1.0 per cent cells the aerobic fermentation rate is approximately equal to that of respiration. This would increase the rate of production of ATP by 16 per cent; the increase would be even less for the 0.25 per cent cells. It is of passing interest that Warburg's theory of the origin of cancer cells involves metabolic changes (29) whereby the respiratory mechanism is injured and the cells subsequently switch to a fermentation system for gaining energy. Warburg cautions that although aerobic fermentation is a property of all growing cancer cells, it is the capacity to ferment vigorously under nitrogen that definitely characterizes cancer cells.

Division of yeast cells is also associated with aerobic fermentation. Holzer *et al.* (20) have shown that ammonium sulfate added to glucose-metabolizing cells will increase the rate of aerobic fermentation during the course of the promotion of cell division. For the first hour after the addition of ammonium ions, during which time the aerobic fermentation rate is markedly increased, the respiratory rate is not altered. These results show once again that aerobic fermentation and cellular synthesis go hand in hand. A notable exception to his rule is described in this paper; it is the case of induced-enzyme formation by yeast cells in the presence of galactose. Nevertheless, the close time relationships that exist between aerobic fermentation and amino acid pool depletion, together with other experimental evidence from the literature, indicate that aerobic fermentation must be considered seriously in the regulation of synthetic activities of the yeast cell.

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