3. Adrenalectomy reduced or abolished seasonal changes in heart, but not those in brain. Changes in heart were unaffected by removal of the adrenal medulla; concentrations of catechol compounds in kidney were reduced.

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The Relationship of Fermentation to Cell Structure in Yeast

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From a biochemical point of view, the sequence of reactions in fermentation and glycolysis is well understood, and many of the individual enzymes have been purified and extensively characterized. However, from a cytological point of view, the relationship of fermentation to cellular structure is far from clear. Certain experiments suggest that the glycolysis reactions are located in the periphery of the cell (Rothstein, 1954a). For example, in the red blood cell, the uptake of ^{32}P-labelled phosphate apparently proceeds in part via esterification into glycolytic intermediates in the stroma (Gourley, 1952; Prankerd & Altman, 1954). In muscle, radioautographic studies indicate that ^{32}P-labelled phosphate is esterified in the periphery of the cell

(Causey & Harris, 1951). In yeast cells, results of detailed studies of sugar uptake have also pointed in the same direction (Rothstein, 1954b).

Studies with certain cell-free preparations of yeast suggest a relationship between glycolytic processes and cellular structure. There are basically two types of yeast preparation: the structureless, soluble fermenting system, and the dried-yeast preparations, in which much of the enzyme activity is associated with insoluble cellular residues. In the soluble fermenting systems (press-juice, Lebedev maceration juice, extracts from frozen and thawed cells, and yeast treated by sonic oscillation) certain characteristic properties are found which were described by Harden (1932). Such preparations show a dependence on orthophosphate and possess a characteristic sequence of production of carbon dioxide. In the absence of phosphate, the course of

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fermentation of yeast juice with glucose shows a slight preliminary acceleration, due to free phosphate in the juice, after which there is a steady rate of production of carbon dioxide, which continues for a considerable period. In the presence of phosphate, the rate rapidly rises to a value several times that of the normal rate, then gradually returns to the steady rate obtained with the extract in the absence of phosphate. The extracts can ferment hexose diphosphate at a rate equal to that for glucose. In dried-yeast preparations, however, the fermentation is much more like that of the intact cell, with no Harden–Young sequence, less dependence on orthophosphate and lower rates of fermentation of hexose diphosphate (Nilsson & Alm, 1941; Meyerhof & Wilson, 1949). Meyerhof (1945) has demonstrated that the determining factor in the behaviour of the dried preparation is the preservation of apyrase activity. Its inhibition or destruction results in the appearance of the typical Harden–Young sequence and phosphate-dependence.

The similarity between the fermentation pattern of the dried-yeast preparations and that of intact cells suggested that the dehydration–rehydration process used in the preparation of the dried yeast constituted a minimal disturbance of a cellular structure which carried the glycolytic enzymes. On this premise, the fermentation properties of several dried-yeast preparations were determined. This paper describes the detailed study of one type of preparation whose fermentation and respiration, phosphate uptake and permeability possessed interesting properties. Evidence is presented that the glycolysis reactions are indeed associated with a cellular structure.

METHODS

Yeast preparations

Baker's yeast (Standard Brands Inc., Buffalo, N.Y., U.S.A.) was thoroughly washed, spread in a thin layer (about ¼ in. thick) and slowly dried at 22°. In drying, it passes through a crumbly stage, when it can be broken into small granules by hand-kneading. After 24 hr., the yeast material was in the form of a granular dry powder which was lyophilized to a constant dry weight (21 % of the wet wt.). The lyophilized yeast was then ground to a fine powder in a Wiley laboratory rotatory mill until it would pass a 50-mesh sieve. The dry preparation was extracted with 10 vol. of acetone and air-dried. It was then treated and washed in a variety of procedures described below. Plating of the preparation on Sabouraud medium revealed no viable cells capable of forming colonies.

Analytical methods

Orthophosphate. This was determined by the method of Fiske & Subbarow (1925) modified by the addition of 15 %

ethanol before colour development (Meyerhof & Oesper, 1947).

Total phosphate. This was estimated as orthophosphate after hydrolysis of organic phosphates with H_2SO_4.

Potassium. This was estimated by means of the flame photometer (Beckman attachment with a model DU Beckman spectrophotometer).

Glucose. This was determined by the method of Nelson (1944).

Glycogen. This was estimated by the method of Scott & Melvin (1953).

Protein. A volume (4 ml.) of biuret reagent (300 ml. containing 1 g. of $CuSO_4$ and 75 g. of carbonate-free NaOH) was added to 2 ml. of supernatant, then diluted to a total of 10 ml. The colour was read in a Lumetron colorimeter (Photovolt Corp., New York, U.S.A.) with a $525 \mu\mu$ filter and calibrated against egg albumin.

Ethanol. This was identified by the formation of a derivative with p-nitrobenzoyl chloride (Schriner & Fuson, 1940), which was used in the determination of a mixed melting point after microdistillation as described by Gettler, Niederl & Benedetti-Pichler (1932). The estimation of ethanol was carried out by the chromate method (Westerfield, Stotz & Berg, 1942).

Hexose diphosphate. This was estimated as the fructose moiety by the method of Roe, Epstein & Goldstein (1949).

Pyruvate. This was estimated by the method suggested by Umbreit, Burris & Stauffer (1949).

Carbon dioxide and oxygen. These were estimated by the standard Warburg techniques.

Paper chromatography of phosphate compounds. These were separated two-dimensionally on Schleicher and Schuell 589 orange ribbon paper. The solvents used were trichloroacetic acid–acetone at 4° (Burrows, Grylls & Harrison, 1952) and butyric acid–NaOH at 25° (Wade & Morgan, 1955). The presence of the various radioactive compounds on the chromatogram was detected by radioautography with Kodak no-screen X-ray film. The method of Berg (1958) was used for the chemical determination of the position of esters on the chromatogram. The radioactive spots were identified by cochromatography with known pure phosphate compounds.

Measurement of radioactivity. The activity of ^{32}P in the supernatant was determined with 1 ml. of solution by using an end-window Geiger–Müller counter.

Buffer solutions. The buffer used throughout all the experiments was an equimolar mixture of succinic and tartaric acids adjusted to the required pH with pure triethylamine. The final concentration of each acid in the buffer solution was 0·01 M.

Treatment of the dried yeast. The dried yeast, unless it is stated otherwise, was directly suspended in water to produce a final concentration of between 10 and 20 mg./ml. For any one chemical determination, 5 ml. of suspension was removed at the appropriate time and centrifuged. 2 ml. of the supernatant was removed for analysis of the medium. It was appropriately diluted according to the analytical method. In some experiments involving changes in the phosphate content of the medium, precipitation of protein proved to be necessary. A volume (2 ml.) of the supernatant was treated with 5 ml. of trichloroacetic acid (final concentration 10 %), and centrifuged and the protein-free supernatant appropriately diluted. This treatment was unnecessary in experiments with washed yeast.

The residue from the above centrifuging, after washing and resuspension in a suitable volume of water, was used for the chemical analysis of the dried yeast. When the orthophosphate content of the dried yeast was determined, the residue was directly extracted twice with 5 ml. of 10% trichloroacetic acid and the extract made up to a suitable volume for analysis. For total phosphate and potassium, 1 ml. of the dried-yeast suspension was heated under reflux with 0·4 ml. of 10N-H_2SO_4 for 15–17 hr. on a sand bath. The temperature of the sand bath was adjusted so that there was gentle refluxing and little liquid lost. The acid-treated material was then boiled with 30% H_2O_2, which was added slowly a drop at a time until the solution was clear (usually, about 1 ml. was added). The solution was analysed as described above. It was necessary for an analysis of the glycogen content to extract 1 ml. of the suspension with 10 ml. of 10% trichloroacetic acid. The residue was then digested with 8 ml. of 30% KOH and the resulting solution analysed. A larger quantity of dried yeast was used for the examination of the phosphate com-pounds within the preparation. The dried-yeast suspension (35 ml.; 100 mg./ml.) was spun down after treatment with ^{32}P, and quickly washed with water. The residue was extracted three times with 10 ml. of 10% trichloroacetic acid at 5°. The barium-insoluble, ethanol-insoluble phosphates (Umbreit et al. 1949) were chromatographed after treatment with Dowex 50 in the sodium form. About 40μl. of the resulting solution (usually 8–12 ml.) was applied to the chromatogram.

RESULTS

Respiration and fermentation of dried yeast

The gas exchanges of the dried yeast with or without substrate (glucose or hexose diphosphate) are shown in Fig. 1. With no substrate no anaerobic production of CO_2 could be measured, whereas in air a definite rate of respiration was observed, with an R.Q. of 1·0 (equal O_2 consumption and CO_2 production). With hexose diphosphate as a substrate, a similar result was obtained. Anaerobic production of CO_2 was essentially zero, whereas respiration proceeded at a rate considerably above endogenous. Again the rates of consumption of O_2 and of production of CO_2 were similar. With glucose as a substrate the pattern was quite different. The rate of respiration of glucose was similar to that of hexose diphosphate but, in contrast, the rates of anaerobic and of aerobic CO_2 production were very high, about five times that of O_2 consumption.

The rate of anaerobic CO_2 production and O_2 consumption by washed dried yeast in the presence of various substrates are given in Table 1. Only in the presence of sugars, glucose, mannose and fructose was there a high rate of anaerobic CO_2
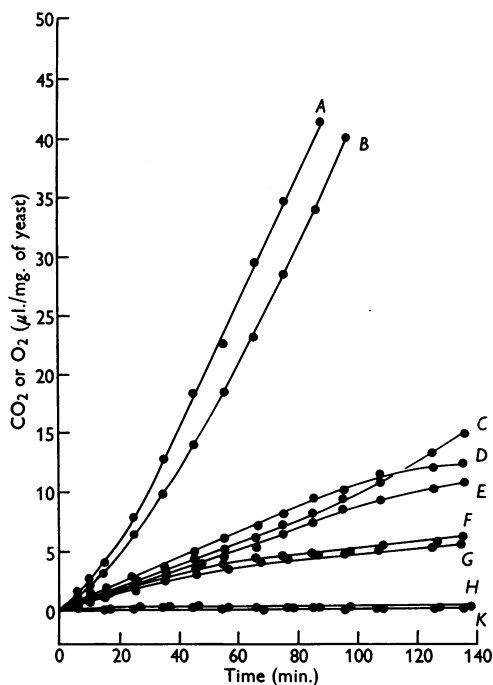

Fig. 1. Gas exchanges of washed dried yeast with no substrate, with glucose and with hexose diphosphate. Each Warburg flask contained 5 mg. of dried yeast (equivalent to 24 mg. of live yeast) in 2 ml. of buffer, pH 5·5. A volume (0·5 ml.) of this buffer, containing substrate, was tipped from the side arm at zero time to give a final substrate concentration of 0·1 M. In anaerobic experiments, the flasks were flushed with N_2; in aerobic experiments, with air. Temperature, 25°. A, Glucose anaerobic CO_2; B, glucose aerobic CO_2; C, glucose O_2; D, hexose diphosphate O_2; E, hexose diphosphate aerobic CO_2; F, endogenous O_2; G, endogenous aerobic CO_2; H, hexose diphosphate anaerobic CO_2; K, endogenous anaerobic CO_2.

Table 1. *Anaerobic production of carbon dioxide and consumption of oxygen by washed dried yeast in the presence of various substrates*

Results represent averages of three to five determinations. Flasks contained 20 mg. of dried yeast in 2 ml. of buffer, pH 5·5–6·0. A volume (0·5 ml.) of buffer containing substrate was tipped in at zero time to give a final substrate concentration of 0·1 M. Temperature, 25°. In anaerobic experiments the flasks were flushed with N_2, in aerobic experiments with air.

Substrate	Anaerobic production of CO_2 (μl./mg./hr.)	Consumption of O_2 (μl./mg./hr.)
None	0·2	2·4
Glucose	46·0	8·2
Mannose	37·0	6·2
Fructose	40·0	6·4
Galactose	0·2	8·1
Hexose diphosphate	0·2	8·0
Glucose 1-phosphate	0·2	4·8
Glucose 6-phosphate	0·2	3·5
Glycerophosphate	0·2	2·2
Pyruvate	0·2	10·0

production, whereas in the presence of galactose, sugar phosphates, pyruvate and endogenous stores, although the rate of respiration was about the same as that for the sugars, there was only a very low rate of anaerobic CO_2 production.

The glucose disappearance from 0·01M-solution in the presence of 10 mg. dry wt. of dried yeast/ml. in air or in N_2 was about 90% in 2 hr. The gas exchanges could account for 80% of the glucose removed in air and 70% in N_2. Thus most of the glucose which disappears can be accounted for as CO_2 production or O_2 consumption. The end products of glucose breakdown by the dried-yeast preparation were primarily CO_2 and ethanol. The ethanol was not only measured analytically by the chromate method (Westerfield et al. 1942) but was identified by the formation of a derivative and the determination of mixed melting points. On the other hand, little glycogen was formed.

Even though hexose diphosphate, at the same concentration as glucose, gave little or no anaerobic CO_2, it disappeared to the extent of 50% in 2 hr. The hexose diphosphate that is removed does not appear as pyruvate. In fact, when the dried yeast is suspended with 0·01M-pyruvate, 70% disappears within 2 hr. Both substrates disappear at the same rate in air and in N_2, and the loss is not nearly accounted for by the quantity of O_2 consumed.

Effect of washing on metabolism

The experiments of Table 1 were carried out with the dried-yeast residue remaining after washing once with 100 vol. of water. If the yeast was not washed, the pattern of metabolism was the same, with one exception. The unwashed yeast possessed the capacity to produce CO_2 from pyruvate under anaerobic conditions owing to the presence of a soluble carboxylase, as reported previously (Barron, Ardac & Hearon, 1950). However, the rates of metabolism of the washed residues were only half of those before washing. The reduced rates are attributable in large part to the loss of electrolytes, particularly K^+ ions, shown to be necessary by Meyerhof & Kaplan (1951). The K^+ ion-dependence could also be demonstrated by dialysis, or by treating the suspension of dried yeast with a Na^+ cation-exchange resin. The depressed rates with washing, dialysis or resin-treatment could be largely but not completely restored by the addition of 0·1M-K^+ ions. Mg^{2+} ions also had a slight stimulating action in restoring the rate of fermentation of the washed yeast. Other additions had no pronounced effect on the rate of fermentation. These included adenosine triphosphate (ATP), adenylic acid, diphosphopyridine nucleotide (DPN), boiled-yeast juice, Ca^{2+} ions, Na^+ ions and inorganic phosphate. In this respect

the slowly dried yeast differs from the quickly dried yeast of Meyerhof & Kaplan (1951), the fermentation of which is increased by ATP, DPN and boiled-yeast juice.

The dependence of the dried yeast on K^+ ions is also related to its dependence on pH, as shown in Fig. 2. For comparative purposes, data on live yeast are also included (see also Rothstein & Demis, 1953). Fermentation by the dried yeast was less tolerant of low pH than that by live yeast. Furthermore, the fermentation by dried yeast was stimulated by K^+ ions in the range pH 3·5–6·0, whereas that by live yeast was stimulated at values below pH 3·5. The rate of fermentation by the dried yeast was somewhat lower than that of live yeast. For example, in Fig. 2, the ratio of rates (dried to live) was 0·35 without K^+ ions and 0·55 with K^+ ions (at pH 5·0). The respiration of glucose was reduced to a much greater degree than was the fermentation. The maximal ratio of rates (dried to live) was only 0·15.

The ability of the fermentation of dried yeast to proceed rapidly is associated with the integrity of an insoluble structure. Treatment with a homogenizer, blendor or ultrasonic oscillator solubilizes the enzymes, but results in a marked depression in the rate of fermentation as well as a marked change in the pattern of metabolism. For example, hexose diphosphate can be fermented.

Permeability of dried yeast to phosphates

The dehydration–rehydration process used in the preparation of the dried yeast alters the cells in a variety of ways. They can no longer divide. Not
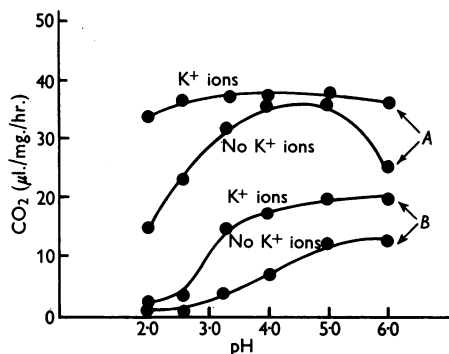
Fig. 2. Effect of potassium on the fermentation of dried and live yeast at different pH values. Each Warburg flask contained 5 mg. of dried yeast (equivalent to 24 mg. of live yeast) or 10 mg. of live yeast in 2 ml. of buffer at the appropriate pH. A volume (0·5 ml.) of this buffer, containing glucose and K^+ ions, was tipped from the side arm at zero time to give a final concentration of 0·1M-glucose and 0·1M-K^+ ions. The flasks were flushed with N_2. Temperature, 25°. A, Live yeast; B, dried yeast.

only is the pattern of metabolism and the dependence on extracellular electrolytes altered but drastic changes in permeability were also observed. Washing with water (4 × 100 ml./g. of dried yeast) removed as much as 25 % of the dry weight, much of it accounted for by the soluble constituents of the cytoplasm such as potassium, phosphates, soluble proteins, organic acids and amino acids. For example, with potassium, 80 % was extracted, reducing the level from 0·14 mole/kg. of original cell material to 0·3 mole/kg. The release of anions measured in terms of buffer capacity in the range pH 4–8 was about the same as that for K^+ ion, 0·13 mole/kg. A large part of the buffer release was due to phosphate compounds and to organic acids such as succinic and acetic acids. As indicated previously, carboxylase activity was readily washed out of the dried yeast. Additional protein to the extent of about 3 % of the original yeast material (on a wet-weight basis) was also solubilized.

The extraction of phosphate materials was studied in some detail (Table 2). For example, four successive washes of 100 ml./g. of dried yeast removed 20 m-moles of orthophosphate/kg. of original cells, essentially all of the inorganic phosphate of the cell. Additional extractions with trichloroacetic acid removed almost no additional phosphate. However, the orthophosphate washed out rather slowly. The duration of the washing period in this experiment was 30 min., and a ratio of wash to solid of 100 to 1, yet the first wash contained only 50–60 % of the phosphate. Nor was the slow release due to a slow liberation of phosphate from organic esters by phosphatases, because phosphate was released at almost the same rate at 0° and 25° (Table 2). Furthermore, no phosphatase activity could be detected by adding phosphate esters to the preparation. Presumably, the outward diffusion of phosphate was impeded by the presence of cellular structures, perhaps precipitated by the dehydration procedure.

During the fermentation of glucose the behaviour of the dried yeast toward phosphates was quite different from that in the absence of glucose (Fig. 3). The release of phosphate esters was blocked and the inorganic phosphate was rapidly absorbed rather than released. If ^{32}P-labelled orthophosphate was used in the experiment, no changes in the specific activity of the phosphate in the supernatant occurred, indicating that there was no phosphate exchange between the medium and the residue. Furthermore, the absorbed phosphate was found to be in the form of phosphate esters rather than inorganic phosphate, as shown by analysis of a trichloroacetic acid extract of the dried yeast. By the use of ^{32}P-labelled orthophosphate and two-dimensional paper chromatography,

many of the trichloroacetic acid-extractable esters formed during the process of phosphate absorption were identified. Included were ATP, the sugar phosphates (glucose 1-phosphate, glucose 6-phosphate, fructose 6-phosphate and especially hexose diphosphate), as well as a variety of incompletely identified substances, mainly nucleotides and triose phosphates (identification based on published R_F values [Burrows et al. 1952; Wade & Morgan, 1955]).

The amounts of phosphate esters formed are small compared with the amount of glucose consumed. For example, in Fig. 3, the total phosphate absorbed in 2 hr. was 10 m-moles/kg. of original live yeast. The amount of glucose absorbed in the same period was 600 m-moles/kg. Thus the phosphate uptake is less than 2 % of the sugar uptake. The phosphate esters already present in the dried-yeast residue, and those newly formed by esterification of absorbed phosphate, must be capable of

Table 2. *Cumulative release of inorganic phosphate by dried yeast with washing*

Volume of washing solution was 100 ml. for 1 g. of dry yeast. Water was used for washes 1–4 and trichloroacetic acid (10 %) for washes 5 and 6. Washing time was 30 min. All values are in m-moles/kg. of yeast, based on original wet weight, and are the sum of amounts in the wash together with that in each preceding wash.

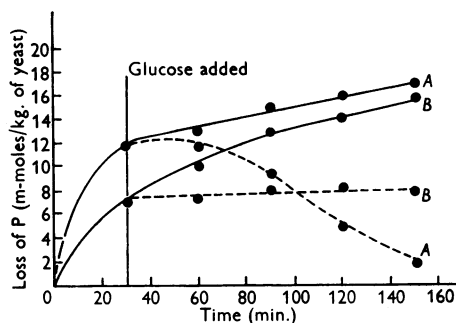| Wash	Temperature	
	0°	25°
1	10·4	11·9
2	14·0	16·9
3	17·0	19·0
4	18·4	19·5
5	19·8	20·8
6	20·0	21·0

Fig. 3. Gain or loss of inorganic or ester phosphate by dried yeast with or without glucose. The dried-yeast concentration was 16·7 mg./ml. (equivalent to 80 mg. of live yeast/ml.); 0·05 M-glucose when added, and 0·02 M-triethylamine–tartaric acid–succinic acid buffer, pH 5·0. Temperature, 25°. *A*, Orthophosphate; *B*, ester phosphate. Broken line, glucose in the medium; continuous line, glucose-free medium.

turning over rapidly during the fermentation of glucose, accounting for the lack of dependence on the orthophosphate concentration of the medium.

All of the ^{32}P-labelled ester phosphates formed during the absorption of orthophosphate were trapped within the yeast residue, none being detectable in the medium during the course of the experiment. The internal labelled esters did not exchange with unlabelled phosphate esters added to the medium. Thus the fermenting structure is impermeable to phosphate esters, a property which accounts for its inability to ferment sugar phosphates, even though it can utilize glucose, producing alcohol and CO_2, with sugar phosphates as intermediates.

A distribution study with glucose 1-phosphate and glucose 6-phosphate indicated that 87 % of the original cell volume is freely permeable to these substances (compared to 11 % for live yeast). Thus the sugar phosphate-impermeable space, in which the fermentative enzymes are located, must occupy 13 % or less of the original cell. This value is, of course, based on the assumption that the volume occupied by the various cellular structures after rehydration is equal to the original volume.

DISCUSSION

Although in the dried yeast the integrity of the cell as a whole is destroyed so that cell division cannot occur, and soluble constituents of the cytoplasm leak out, the insoluble residues retain many of the properties of the intact cell in so far as fermentation is concerned. In both the live and dried yeast the sugars, glucose, fructose and mannose are fermented rapidly; but other substrates such as endogenous stores (Stier & Stannard, 1936; Spiegelman & Nozawa, 1945), galactose—unless cells are adapted (Spiegelman, Reiner & Cohnberg, 1947), sugar phosphates—unless first split by cellular phosphatases (Rothstein & Meier, 1949), and pyruvate (Barron et al. 1950) do not produce carbon dioxide under anaerobic conditions.

Another similarity between the dried-yeast residues and live yeast is the behaviour toward inorganic phosphate and phosphate esters. Both absorb, inorganic phosphate without exchange (Goodman & Rothstein, 1957), with the formation of ester phosphates. Both the fermenting residue of dried yeast and the live yeast cell are impermeable to inflow or outflow of sugar phosphates (Rothstein & Meier, 1949).

The enzymes responsible for fermentation are readily solubilized by homogenization, blending, grinding and similar procedures. They remain in an insoluble residue in the dried-yeast preparation either because they were originally present in the cells as a structural element which has been preserved, or because they have been precipitated as an artifact in the course of the dehydration process. The latter possibility seems unlikely on several grounds. First, dehydration at room temperature is a relatively mild treatment. In Nature, it is not uncommon for certain cells such as those of the Protozoa, spores, slime moulds and seeds to withstand drying, which emphasizes that certain structures can withstand drying without loss of function and that the treatment need not be drastic. Presumably, little chemical alteration is involved, although physical alteration and changes in solubility may certainly occur. These physical changes may be the major factor responsible for any differences between the properties of the intact cell and the preparation produced by drying. Nevertheless, the treatment should be mild enough to allow part of the organization of the cell to remain intact. Secondly, it seems highly unlikely that a series of at least nine soluble enzymes, cofactors and intermediates should be precipitated together in a highly organized system capable of carrying out fermentation at rates approaching that of the intact cell, with no dependence on added orthophosphate, with a rapid turnover of the phosphorylated intermediates and an impermeability to phosphate esters and to pyruvate. Thirdly, Lampen* (personal communication) has found that, with certain yeast samples but not with others, broken-cell preparations obtained by grinding the cells with glass beads showed all the glycolytic activity in the particulate fraction. On more extensive grinding of the preparations, or by the use of higher temperatures or partial autolysis, completely soluble extracts were obtained.

There is little evidence of the exact nature of the structural entity. The glycolytic enzymes could be present in solution within a permeability barrier or within a gel-like structure. The fact that it is unaffected by the dehydration and by treatment with acetone suggests that the idea of a membrane enclosing a solution is less probable than some kind of matrix.

Many recent studies indicate that respiratory activities of cells are associated with mitochondria. In the present studies certain differences between respiration and fermentation suggest that the fermentative structure is distinct from the mitochondria. The dried-yeast preparation will respire glucose, but at a much lower rate, relatively speaking, than it will ferment. Also, the Pasteur effect is weak and there is a high rate of aerobic fermentation. Several substrates, such as hexose diphosphate and pyruvate, that have only a very low rate of production of carbon dioxide under anaerobic conditions, are respired at the same rate

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as glucose. Yet neither of these can enter the fermentative structure. The respiration must proceed in a location different from that of the fermentation. The metabolic pathway involved in the respiratory activity of the dried yeast is of some interest. If the glucose or hexose diphosphate is converted into pyruvate via the Embden–Meyerhof scheme, followed by pyruvate oxidation via the Krebs cycle, then it must be postulated that there are two distinct locations of the glycolytic enzymes in the cell. On the other hand, the respiration may proceed via the phosphogluconate shunt and the pentose cycle. No choice between the two alternatives can be made at present. The rate of respiration of pyruvate is as fast as that of glucose and hexose diphosphate, so the Embden–Meyerhof pathway cannot be excluded. On the other hand, the shunt pathway is known to account for 13–30 % of the respiratory activity of the live yeast cell (Beevers & Gibbs, 1954; Blumenthal, Lewis & Weinhouse, 1954; Wang, Gregg, Forbusch, Christenson & Cheldelin, 1956) and the rate of respiration of the dried yeast is only 10–15 % of that of the original intact yeast. The shunt pathway could therefore account for the respiration.

Although the loss of hexose diphosphate might be accounted for by breakdown through at least part of the Embden–Meyerhof pathway, there is no evidence that the full course of fermentation is completed. The breakdown of hexose diphosphate, by unwashed dried yeast (where carboxylase activity is present) remains unaltered in rate under aerobic or anaerobic conditions and is neither associated with an anaerobic production of carbon dioxide nor is it associated with an accumulation of pyruvate. In fact, pyruvate is rapidly removed under anaerobic conditions with little production of carbon dioxide. Although this is not direct evidence, it does seem likely that enzymes associated with the insoluble residue are those responsible for at least the major part, if not all, of the fermentation observed in the intact cell.

The fermentative activity of the living yeast cell is apparently located in the periphery of the cell. The evidence is based on a variety of data which are reviewed in detail elsewhere (Rothstein, 1954 *a*, *b*, 1955). Briefly, the fermentative activities are directly influenced by extracellular concentrations of H^+ and K^+ ions, rather than by intracellular concentrations of the same ions. Not only are the rates of fermentation affected, but the end products as well. Other evidence is concerned with the close coupling of fermentative reactions with the absorption of phosphate. In cells other than yeast, evidence of a different nature has been cited for the peripheral location of glycolytic activity. Of immediate interest is a recent paper of Shaw & Stadie (1957) on muscle, which indicates two locations for sugar metabolism: a peripheral one concerned with glycolysis and an internal one concerned with other pathways. One presumes that the structural entity described above is also peripherally located in the intact yeast cell.

SUMMARY

1. Baker's yeast was slowly dried, lyophilized and extracted with acetone. The yeast thus treated was unable to divide, and many of its permeability barriers were largely destroyed so that a large proportion of the soluble constituents of the cytoplasm could be washed out.

2. After washing, a residue remained which could ferment glucose to carbon dioxide and ethanol at a rate about half of that of the intact cell. Mannose and fructose could also be fermented, but sugar phosphates, triose phosphates, pyruvate and endogenous stores of carbohydrate produced no carbon dioxide under anaerobic conditions. Respiration of glucose proceeded at a much lower rate. Hexose diphosphate, galactose, pyruvate and endogenous carbohydrates were also respired.

3. The metabolism of the dried-yeast preparations was dependent on electrolytes, particularly K^+ and H^+ ions.

4. The dried yeast, during fermentation of glucose, absorbed inorganic phosphate without exchange, forming the expected phosphorylated intermediates of the glycolysis system. However, the latter, once formed, could not escape from the cell residues.

5. From the properties of the dried-yeast residues, it was concluded that the enzymes for fermentation exist in the cell within an organized structure distinct from the mitochondria.

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Respiratory Enzyme Studies in *Tetrahymena pyriformis*

4. STABILIZATION OF ELECTRON-TRANSPORT COMPONENTS*

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Early attempts to demonstrate an active succinoxidase system in cell-free homogenates of the ciliated protozoan *Tetrahymena pyriformis* were unsuccessful when preparations were made in a glass homogenizer or Waring Blendor with the use of sea sand, Ballotini, alumina or different grades of quartz. Such homogenates were either completely inactive or consumed very little extra oxygen in the presence of added succinate. However, the passage of whole cells through a Logeman homogenizer yielded preparations with quite active electron-transport systems as reflected by succinoxidase, succinic cytochrome *c* reductase, reduced diphosphopyridine nucleotide oxidase, reduced diphosphopyridine nucleotide cytochrome *c* reductase and diaphorase activities (Eichel, 1954, 1956a, c). On the other hand, kinetic studies of enzymes in these homogenates are seriously handicapped by the extraordinary instability of the electron-transport systems present in the particles, and purification of the enzymes noted above is rendered exceedingly difficult. Although the marked lability of the electron-transport chains argued for seeking other methods of cell disruption

which might give more stable and perhaps standardized enzyme preparations, it was felt that the reasonably high initial activities of these homogenates, and the great ease with which they could be prepared without the use of 'contaminating' grinding materials, spoke for a search for means of stabilizing them to facilitate certain types of enzymic studies. In addition, an effective stabilizing agent or group of agents might be expected to yield information about the nature of instability of the particulate electron-transport systems. In part 3 of this series (Eichel, 1956b), as part of a study of some properties of the reduced diphosphopyridine nucleotide oxidase system, it was reported that the latter was stabilized for brief periods by suspending and homogenizing whole cells in alkaline phosphate buffer. This procedure, however, resulted in a 50–60 % loss of the initial activity.

This paper deals with the use of two types of agents which have been found to be effective stabilizers of the entire electron-transport systems encompassing the span of either succinate or reduced diphosphopyridine nucleotide to oxygen, as well as of glutamic and β-hydroxybutyric dehydrogenases. One is a relatively thermostable diffusate obtained from commercial preparations of

* Part 3: Eichel (1956b).