Changes in Dry Weight, Protein, Deoxyribonucleic Acid, Ribonucleic Acid and Reserve and Structural Carbohydrate during the Aerobic Growth Cycle of Yeast

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1. Changes in dry weight, DNA, RNA, protein and reserve and structural carbohydrate were measured during the aerobic growth of yeast on 0.9% glucose in an aerobic synthetic medium. 2. After glucose had been consumed and during the growth of yeast on ethanol and acetate, the rate of formation of DNA remained about the same but the rate of increase of dry weight was greatly diminished. 3. During the second stage of growth the ratios dry weight/DNA, protein/DNA, RNA/DNA and carbohydrate/DNA decreased to about 30% of the corresponding values during the first stage of growth. 4. A higher fraction of the dry weight of the yeast cells could be accounted for by the reserve carbohydrate content of the cells during the second stage of growth. 5. By the end of the first stage of growth an increase in the reserve carbohydrate content of the cells was observed. Part of this reserve carbohydrate was consumed by the cells in the beginning of the second stage of growth. The possibility of adaptation of cells at the expense of their reserves is discussed.

The metabolism of carbohydrate, protein and nucleic acids in yeast cells has been studied by many workers.

Thus in actively growing yeast the synthesis of polysaccharides was studied by Gilvarg (1952), Chung & Nickerson (1954) and Chen (1959*a,b*). In non-proliferating yeast the synthesis and degradation of carbohydrate in the presence of an exogenous source of carbon under anaerobic and aerobic conditions were examined by Fales & Baumberger (1948) and Fales (1951), and Fales (1960) described the effect of different degrees of aeration on the carbohydrate and fat content of the yeast. The endogenous metabolism of the reserve carbohydrate of anaerobically grown yeast under both aerobic and anaerobic conditions was examined by Chester (1963, 1964).

Protein and RNA turnover in growing and resting yeast cells was studied by Halvorsen (1958*a,b*), and Shortman & Fukuhara (1963) failed to demonstrate any change in the rate of RNA synthesis during the respiratory adaptation of non-proliferating yeast cells.

The role of RNA in the growth of bacteria and the changes in the rate of RNA synthesis accompanying the changes in the growth rate were studied in detail by Schaechter, Maaløe & Kjeldgaard (1958), Kjeldgaard, Maaløe & Schaechter (1958) and Neidhardt & Magasanik (1960).

In the present paper changes in DNA, dry weight, RNA, protein and carbohydrate contents during the aerobic growth of yeast are examined. The aim of the work is to determine any major changes in the anabolic processes concomitant with the changes of structure and enzyme activity (Polakis, Bartley & Meek, 1964, 1965; Polakis & Bartley, 1965).

METHODS

Maintenance and growth of yeast. *Saccharomyces cerevisiae* strain no. 77 of the National Collection of Yeast Cultures (Brewing Industry Research Foundation, Nutfield, Surrey) was used. It was maintained on agar slopes of the synthetic medium for growth described by Polakis & Bartley (1965). Cells were grown at 35° on 0.9% glucose in the liquid synthetic medium described by the same authors. These are referred to below as the standard conditions of growth.

Determination of dry weight. Cells were washed several times at 0° and dried overnight at 105°.

Determination of glucose in the growth medium. Glucose was determined by the glucose-oxidase method of Huggett & Nixon (1957).

Determination of cell constituents. At the times indicated, four or five samples were removed from the growth medium. The cells were spun down and washed twice with ice-cold water at 0°. The samples were used for the determination of protein, dry weight, carbohydrate and nucleic acids.

Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). Crystalline bovine plasma albumin (Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex) was used as standard.

Table 1. *Changes in dry weight, DNA, RNA, protein and carbohydrate contents of yeast cells during aerobic growth in synthetic medium on 0.9% glucose (50 μ moles/ml.)*

Methods of fractionation and determination are given in the Methods section. Reserve carbohydrate, trehalose+glycogen; structural carbohydrate, mannan+glucan.													
Time of growth (hr.)	Glucose left in medium (μ mole/ml.)	Content (μ g. in yeast cells present in 1 ml. of medium)										Recovery of total carbohydrate (%)	
		DNA	RNA	Protein	Dry wt.	Trehalose	Glycogen	Reserve carbohydrate	Total carbohydrate				
									Mannan	Glucan	Structural carbohydrate		
14	42.7	0.23	19.2	108.8	328	1.8	15.6	17.4	21.4	16	37.4	54.8	—
16	21.3	0.41	39.1	230.0	620	2.6	43.3	45.9	35.3	32.8	68.1	114.0	—
18	0.04	0.56	54.8	320.0	882	17.8	97.5	115.3	51.9	49.2	101.1	216.4	—
19½	0.02	0.66	67.2	352.0	976	28.5	62.4	90.9	78.0	58.8	136.8	227.7	—
27	—	2.75	85.0	412.0	1550	66.1	68.6	134.7	108.2	67.7	175.9	310.6	94.7
28	—	3.03	92.2	462.0	1660	88.5	75.9	164.4	122.9	72.2	195.1	359.5	95.9
30	—	3.99	100.0	480.4	1880	99.4	97.1	196.5	149.4	100.3	249.7	446.2	95.7
36	—	5.08	109.2	545.0	2200	63.1	113.8	176.9	153.3	102.9	256.2	433.1	—
40	—	5.20	108.2	546.0	2240	58.4	136.9	195.3	150.2	114.6	264.8	460.1	—

Methods of fractionation and determination are given in the Methods section. Reserve carbohydrate, trehalose+glycogen; structural carbohydrate, mannan+glucan.

Nucleic acids were fractionated by the method of Schmidt & Thannhauser (1945) as described by Hutchison & Munro (1961). DNA was estimated by the diphenylamine method of Burton (1956), all the quantities being scaled down to one-fourth. RNA was measured by the orcinol method, as described by Schneider (1957). Salmon-sperm DNA and yeast RNA were used as standards.

The reserve and structural carbohydrate was fractionated by the method of Trevelyan & Harrison (1956). The sum of acid-soluble glycogen and alkali-soluble glycogen is referred to as glycogen. Total carbohydrate and the amount of sugar in the different fractions were determined by the anthrone method, as described by Ashwell (1957). Samples and the anthrone reagent were chilled in an ice-water bath and mixed by vigorous shaking while immersed in the bath. The tubes were heated at 90° for 16 min. and then cooled, and the resulting green-coloured solutions were read at 625 m μ . Glucose and mannose were used as standards.

RESULTS

Yeast was grown aerobically in the synthetic medium containing glucose (0.9%) at 35°. At the times indicated, samples of cells were removed for determination of dry weight, DNA, RNA, protein and carbohydrate. The results of the experiments are given in Table 1.

Changes in DNA and dry weight. The changes in the amounts of DNA and dry weight of yeast cells in 1 ml. of growth medium during aerobic growth of the yeast are given in Fig. 1. In Fig. 1 the amounts of glucose left in the medium at different times are also presented. When glucose had been consumed there was an immediate fall in the rate of increase of

Fig. 1. Changes in DNA and dry weight of yeast cells during growth under standard conditions. $\circ$, dry weight (mg./l.); $\square$ DNA (μ g./l.). The glucose remaining in the medium is also shown (Δ).

dry weight, but the rate of DNA synthesis was not affected. This is clearly shown in Fig. 2, in which changes in dry weight are plotted against the changes in DNA. At the time glucose disappeared from the medium, the rate of increase of dry weight changed from about 1500 $\mu\text{g.}$ dry weight/ $\mu\text{g.}$ of DNA to about 300 $\mu\text{g.}$ dry weight/ $\mu\text{g.}$ of DNA.

Changes in protein. Relative changes in the ratios protein/DNA and protein/dry weight are shown in Fig. 3. The values for protein/DNA and protein/dry

weight at any time are expressed relative to the corresponding values after 14 hr. of growth, these being taken as unity. Initially, as cells were approaching the end of the growth period on glucose, there was a small increase in both ratios which during the second stage of growth fell below unity. Both increase and decrease were more pronounced for the protein/DNA ratio.

Fig. 2. Changes in the dry weight of yeast cells in relation to the change of DNA during growth under standard conditions. When glucose was consumed the slope of the line changed.

Fig. 3. Relative changes in the ratios protein/DNA and protein/dry weight during growth under standard conditions. The ratios are expressed relative to those after 14 hr. of growth, which were taken as unity. $\circ$, Protein/DNA; $\bullet$, protein/dry weight.

Fig. 4. Relative changes in the ratios RNA/protein, RNA/DNA and RNA/dry weight during growth under standard conditions. The ratios are expressed relative to those after 14 hr. of growth, which were taken as unity. $\blacktriangle$, RNA/protein; $\bullet$, RNA/dry weight; $\blacksquare$, RNA/DNA.

Fig. 5. Relative changes in the ratios carbohydrate/DNA during growth under standard conditions. The ratios are expressed relative to those after 14 hr. of growth, which were taken as unity. $\bullet$, Reserve carbohydrate/DNA; $\blacksquare$, structural carbohydrate/DNA; $\blacktriangle$, total carbohydrate/DNA.

Changes in RNA. Changes in the relative ratios RNA/DNA, RNA/dry weight and RNA/protein are presented in Fig. 4. Changes in RNA/DNA and RNA/dry weight followed the same pattern as the corresponding protein ratios. The RNA/protein ratio, which remained constant during growth on glucose, changed to another almost constant value 15% higher than the first one during the subsequent growth on ethanol and acetate, in spite of the decrease in the rate of growth of yeast (cf. Schaechter *et al.* 1958; Kjeldgaard *et al.* 1958; Neidhardt & Magasanik, 1960; Kjeldgaard, 1961; Neidhardt & Fraenkel, 1961).

Changes in carbohydrate. Changes in carbohydrate are shown in Figs. 5 and 6.

Initially there was a slow increase in the structural carbohydrate/DNA ratio (Fig. 5). In the second stage of growth the relative ratio fell to 0.3–0.4 (Fig. 5). The changes in structural carbohydrate/dry weight are presented in Fig. 6. The amount of structural carbohydrate varied very little in relation to dry weight. Reserve carbohydrate was the cell constituent subjected to the greatest changes during the growth cycle. Particularly noticeable was the increase in the amounts of reserve carbohydrate at the end of growth on glucose (Figs. 5 and 6). Both the relative ratios reserve carbohydrate/DNA and reserve carbohydrate/dry weight increased 2.5–3-fold. A part of this newly synthesized carbohydrate at the last stages of growth on glucose (16–18 hr.)

was consumed during the subsequent 1.5 hr. of growth (18–19.5 hr.) (Table 1). The ratio reserve carbohydrate/DNA fell initially sharply in the beginning of the second stage of growth and then continued decreasing very slowly. In spite of this, during the growth of yeast on acetate and ethanol a higher fraction of the dry weight was composed of carbohydrate than during growth on glucose (Fig. 6). Of the two major components of carbohydrate, trehalose showed the greatest increase on passing from the first to the second stage of growth.

DISCUSSION

Williamson & Scopes (1961) separated yeast into two fractions of different mean size and showed that, in spite of the wide differences in the mean content of large and small cells in RNA, protein and dry weight, the DNA content/cell was the same. If the DNA content/yeast cell is considered constant during growth of the yeast, then the results described in this section are consistent with a sharp decrease in the mean size of cells when glucose was consumed and growth continued on the degradation products of glucose. Electron microscopy confirmed the change in size. Dependency of cell mass on growth rate had also been observed by Schaechter *et al.* (1958). The bigger size of cells during growth at a higher rate in comparison with cells growing at a lower rate was ascribed by these authors to the need of an increase in the number of centres of protein synthesis (ribosomes) in the rapidly growing cells. The change in the size of the cells observed in this work resulted in an increase of the surface area for a given volume, thus possibly facilitating the uptake of different nutrients by the cells, including oxygen, as well as the excretion of the end products of metabolism.

The rate of increase in dry weight and DNA with time (Fig. 1) closely resembles the changes of the same constituents observed by Tustanoff & Bartley (1964) during anaerobic growth of yeast on glucose. The presence of glucose in the medium resulted in a high growth rate and the formation of giant cells, thus possibly decreasing the uptake of nutrients per given volume or mass of cells (assuming the density of cells to be constant). Any relation between this phenomenon and the repression by glucose of the respiratory and tricarboxylic acid-cycle enzymes cannot be assessed at present.

The increase in the carbohydrate content of the cells by the end of the first stage of growth could be attributed to an increased degree of aerobiosis as the glucose concentration in the medium was decreased to a low level. Fales (1960) studied the effect of the degree of aerobiosis on the carbohydrate content of yeast and showed that strong aeration

Fig. 6. Relative changes in the ratios carbohydrate/dry weight during growth under standard conditions. The ratios are expressed relative to those after 14 hr. of growth, which were taken as unity. ●, Reserve carbohydrate/dry weight; ■, structural carbohydrate/dry weight; ▲, total carbohydrate/dry weight.

resulted in an increase in the synthesis of cellular carbohydrate. Brandt (1941) observed a parallelism between the trehalose content of yeast cells and the degree of aerobiosis, and S. L. Chen & K. J. H. Lauer (quoted in Chen, 1959b) also observed great increases in trehalose content on changing from anaerobic to aerobic conditions. Considering the fact that the presence of glucose in the growth medium results in a semi-anaerobic pattern of metabolism by the yeast cells, the results described in the present paper are in agreement with the work of the authors mentioned.

The sudden breakdown of these reserves in the beginning of the second stage of growth could suggest that yeast cells adapt to a fully aerobic type of metabolism at the expense of their reserve carbohydrate. In fact, Chester (1963) showed synthesis of cytochrome *c* oxidase during the aerobic dissimilation of reserve carbohydrate of anaerobically grown yeast and inhibition of dissimilation by inhibitors of protein synthesis.

The increased carbohydrate/dry weight ratio in the second stage of growth was possibly due to the development of the enzymes of the glyoxylate pathway supplying oxaloacetate, and to a preferential allotment of energy to the formation of reserve carbohydrate rather than protein and lipids (Polakis & Bartley, 1965, 1966).

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