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ENERGY METABOLISM OF *SACCHAROMYCES CEREVISIAE* DISCREPANCY BETWEEN ATP BALANCE AND KNOWN METABOLIC FUNCTIONS*

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SUMMARY

The contribution of metabolic pathways to the catabolism of glucose, galactose and ethanol by *Saccharomyces cerevisiae* in aerobiosis has been studied. The results suggest that:

1. Of the total ATP formed in catabolism yeast obtain as much as 60 % from ethylic fermentation during logarithmic growth on glucose. However, about 80 % of ATP is formed in oxidation of galactose. Oxidation seems to be the only important catabolic pathway of ethanol.

2. The ratios between growth yield and ATP formed in catabolism were approx. 9, 7 and 3 g dry yeast/mol ATP in glucose, galactose and ethanol cultures, respectively.

3. The balance between ATP produced in catabolism of substrates and the requirements of ATP for the biosynthesis of cellular material indicates that as much as 60 % of ATP is spent in functions other than net biosynthesis.

4. The rate of ATP expenditure in non net-biosynthetic functions during growth was approx. 20 mmol/g dry yeast per h.

5. In conditions in which no growth occurred but cell viability was maintained, that is, in the absence of exogenous carbon and nitrogen source, the ATP production rate was approx. 1 mmol ATP/g dry yeast per h.

6. These results indicate that the ATP required for maintaining the yeast alive, what would be considered maintenance energy "sensu stricto", is only a minor proportion of the ATP spent in non net-biosynthetic functions during growth. The identification of the processes related to growth which spend more energy than that required for net biosynthesis could lead to important insights in cell biology.

INTRODUCTION

It is well known that, due to catabolite repression of certain enzymes of the

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respiratory chain and citric acid cycle, the energy metabolism of *Saccharomyces cerevisiae* depends not only on the oxygen availability but also on the carbon source present in the medium. The activities of some enzymes during growth on glucose, galactose and ethanol, as well as the potential capacity of yeast to respire and ferment have been previously reported [1–4]. However, scarce information exists on the quantitative contribution of fermentative and oxidative pathways to the ATP formation during growth on these substrates. To provide information on the actual effect caused in the energy metabolism of yeast by the catabolite repression produced by these sugars, accumulation of fermentation products in the culture media, and fermentation and respiration rates of yeast actively growing on the three substrates have been measured.

A balance between ATP production and consumption by the cells has to be established in order to obtain a complete picture of their energy metabolism. An approach to this problem has been attempted in this work by comparing the ATP produced in catabolism with the theoretical requirements for the biosynthesis of cellular components [5]. The ATP spent in non net-biosynthetic functions has been calculated in growing and starving yeast. The results indicate that the proportion of ATP spent in non-biochemically defined functions is very high during growth. Since the fate of this relevant amount of ATP is actually unknown, a detailed study of its functions is required for the understanding of the energy metabolism of this organism. The possible functions fulfilled by this ATP expenditure are discussed.

MATERIALS AND METHODS

Reagents

Enzymes and nucleotides were from Boehringer Mannheim GmbH (Mannheim, G.F.R.), the yeast extract from Difco Laboratories (Detroit, Mich., U.S.A.). All other reagents were of analytical grade.

Microorganisms

The strain of *Saccharomyces cerevisiae* used, S-13-Gal, was isolated for its ability to grow on a synthetic medium with galactose as only carbon and energy source, from the haploid strain 1724–14A (originally provided by Dr. Hawthorne, Seattle). The strain was maintained in agar glucose slants.

Growth conditions

Yeasts were grown aerobically in a rotatory shaker at 30° C in a synthetic medium with the following composition per l: 3.4 g $(NH_4)_2HPO_4$, 1.7 ml of 85 % H_3PO_4, 0.2 g KH_2PO_4, 0.25 g $MgSO_4+7\ H_2O$, 0.25 g NaCl, 1.8 mg $ZnSO_4$, 1 mg $Fe(NH_4)_2(SO_4)_2+6\ H_2O$, 100 μg $CuSO_4$, 20 μg biotin, 500 μg calcium D-pantotenate, 10 mg inositol, 4 mg thiamin chlorhydrate, and 1 mg pyridoxine. The medium was adjusted to pH 6.0 with 5 M KOH. 2 % Glucose, 2 % galactose or 1 % ethanol were respectively used as only carbon and energy sources.

Inocula were prepared by transferring the micro-organisms from an agar slant into a medium with 0.3 % yeast extract Difco and 2 % glucose. An adequate quantity of this culture was used to inoculate the main culture. The amount of inocula used was always less than 1 % of the growth yield.

Starvation conditions

Cultures at the middle of exponential growth were filtered in vacuum through Millipore filters. The cells obtained were washed by resuspension in 2 vols. of a medium without carbon and nitrogen source in which $(NH_4)_2HPO_4$ was substituted for K_2HPO_4. After being filtered again, yeasts were resuspended in a volume of the same medium and maintained in a rotatory shaker at 30 °C.

Analytical procedures

Yeast growth was measured by determining dry weight at different stages of growth.

Oxygen consumption was measured by the direct method, i.e. alkali in the center well [6]. Aliquots of growing cultures or starving suspensions were poured into Warburg vessels placed in a Warburg respirometer at 30 °C and the increments in the manometers followed for 1 h. In the case of growing cultures the values were corrected for yeast growth in the vessel during the experiments with the formula

$$\Delta_0 = \Delta_t \frac{\mu t}{e^{\mu t} - 1}$$

where Δ_0 is the corrected value, Δ_t the increment in the manometer, μ, the specific growth rate, and t, the time elapsed in the experiment.

Glucose, galactose, ethanol and their products of catabolism were estimated in aliquots of the cultures' media filtered through Millipore filters 0.47 μm pore size by conventional enzymatic methods [7]. In all cases, internal standards were used.

The viability of starving yeast suspension was measured by placing aliquots on a solid medium composed of 1.5 % agar, 2 % glucose and 0.3 % yeast extract. After incubation at 30 °C for 48 h, colonies were counted.

RESULTS

Contribution of different metabolic pathways to catabolism of substrates during logarithmic growth

During growth, cells use up the substrates to meet two major requirements: energy (i.e. ATP), and metabolic intermediates to form cellular material. Yeast can obtain the energy from oxidative and fermentative pathways. Ethanol and glycerol were the major products of fermentation of glucose and galactose (Table I), while none of the assayed fermentation products accumulated in ethanol cultures at significant concentrations. The rate of oxygen consumption was almost the same in yeast growing on ethanol and galactose, and three times lower in yeast growing on glucose (Fig. 1). Since the rate of oxygen consumption was constant during logarithmic growth on the three substrates, the expenditure of oxygen in a given interval could be calculated from the growth equation and the rate of oxygen consumption as shown in the legend of Table II. The biosynthesis of unsaturated fatty acids and sterols requires molecular oxygen as a substrate [9]; nevertheless, no important amount would be spent for this purpose since the lipid content of yeast is very low [10]. To our knowledge, no other major pathway consumes molecular oxygen in yeast. Therefore, most of the oxygen is probably used up in the respiratory chain and the amount of oxidized substrate in the respiratory chain can be tentatively calculated as is shown in Table II.

TABLE I

PRODUCTS ACCUMULATED IN THE CULTURE MEDIUM OF *S. CEREVISIAE* DURING LOGARITHMIC GROWTH ON GLUCOSE, GALACTOSE OR ETHANOL

Measurements were done as indicated in Methods. The values are the differences between the concentration at the beginning of experiments (see Fig. 1) and that at different steps during logarithmic growth. Mean values and standard deviation of four experiments are shown.

Substrate in the culture	Substrate consumed (mmol/l)	Growth yield (g dry yeast)	Ethanol (mmol/l)	Glycerol (mmol/l)	Acetaldehyde (mmol/l)	Acetate (mmol/l)	Pyruvate (mmol/l)
Glucose	55± 4	1.1±0.04	77± 6	12.2 ±0.9	≦ 0.3	0.47±0.05	0.33±0.02
	76± 4	1.7±0.04	108± 5	17.8± 1.4	≦ 0.3	0.58±0.07	0.50±0.02
	104± 1	2.6±0.01	151± 8	22.2 ±1.7	0.6 ±0.07	1.02±0.1	0.40±0.14
Galactose	18± 3	0.6±0.02	15± 0.1	1.0± 0.2	0.14±0.01	0.87±0.06	0.10±0.01
	32± 3	1.5±0.02	34± 0.6	1.96±0.2	0.29±0.03	1.79±0.05	0.14±0.03
	56± 1	2.4±0.01	63± 4.9	2.70±0.1	0.90±0.1	1.76±0.2	0.04±0.006
	92± 3	4.2±0.09	104±10	3.86±0.7	0.63±0.15	1.29±0.4	0.04±0.01
Ethanol	44±10	0.9±0.1	–	0.19±0.07	0.67±0.08	0.40±0.15	≦ 0.02
	109±16	2.3±0.2	–	0.16±0.07	0.19±0.01	0.10±0.03	≦ 0.02
	141±15	3.1±0.4	–	0.23±0.07	≦ 0.02	0.13±0.03	≦ 0.02

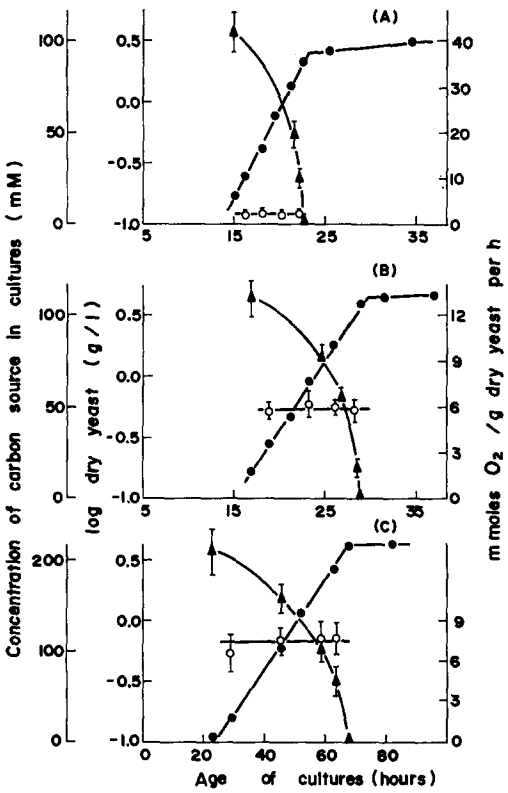

Fig. 1. Growth rate, carbon source utilization, and oxygen consumption of *S. cerevisiae* growing (A), on glucose, (B), galactose and (C), ethanol. Measurements were done as indicated in Methods. ●—●, growth, ▲—▲, carbon source utilization, ○—○, oxygen consumption. The mean values and standard deviation of four or more experiments are shown.

In the experimental conditions used in this work, with a sole carbon source in the culture media, all cellular material formed during growth, except for mineral material, nitrogen and vitamins, is supplied by the carbon source. Calculations of the proportion of carbon sources assimilated are shown in Table III. Assimilated and oxidized substrates, and fermentation products accumulated in the media, account for more than 90 % of the total substrates consumed (Table IV). This indicates that no other major pathways occur under our experimental conditions. The hexose monophosphate pathway, a very important metabolic pathway responsible for the supply of the reducing power in almost all types of cells, has been found in a previous work to account for only 2.5 % of the total glucose metabolized [10].

About 40 % of the utilized ethanol was assimilated as carbon skeletons, while in the case of glucose and galactose only 13 % and 26 %, respectively, was used for this purpose (Table IV). Among the substrates studied as nutritional repressors, differences became apparent in the proportion of substrates which were metabolized in oxidative pathways. While about 50 % of ethanol and 15 % of galactose was oxidized, in the case of glucose the oxidation reached only 3 %. The higher proportion of glyceric fermentation in glucose with respect to galactose cultures supports pre-

TABLE II

AMOUNT OF CARBON SOURCE OXIDIZED DURING LOGARITHMIC GROWTH

B_0, amount of yeast at the initiation of the experiment; μ, specific growth rate: $\mu = {}^e\lg 2$/generation time; t, time spent in consumption of 100 mM substrate (Fig. 1); Q_{O_2}, rate of oxygen consumption. Mean values and standard deviation of data of Fig. 1 are shown; O_2, oxygen consumed/100 mmol substrate utilized. Calculated by the formula: $O_2 = Q_{O_2} \cdot B_0 \cdot 1/\mu\ (e^{\mu t} - 1)$ [8]; oxidized substrate has been calculated by substitution of O_2 values in the equations:

$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$

for sugars, and

$2\ CH_3\text{-}CH_2OH + 6\ O_2 \rightarrow 4\ CO_2 + 6\ H_2O$

for ethanol (see text).

Carbon source in the culture	B_0 (g dry yeast/l)	μ (h^{-1})	t (h)	Q_{O_2} (mmol O_2/g per h)	O_2 (mmol/100 mmol substrate consumed)	Oxidized substrate (mmol/100 mmol substrate consumed)
Glucose	0.17	0.34	8.3	2.4±0.2	19±1	3.2±0.2
Galactose	0.17	0.26	11.8	6.0±0.3	88±4	14.8±0.7
Ethanol	0.11	0.087	33.5	7.5±0.4	166±9	55 ±3.0

TABLE III

CARBON SOURCE ASSIMILATED DURING GROWTH

Carbon source in the medium	Substrate consumed[a] (g/g dry yeast)	Yeast organic matter formed[b] (g/g dry yeast)	Carbon content (g)		Assimilated carbon[e] (%)
			of the consumed substrate[c]	of the yeast organic matter formed[d]	
Glucose	8.1	0.91	3.24	0.43	13
Galactose	4.2	0.91	1.66	0.43	26
Ethanol	2.2	0.91	1.12	0.43	39

[a] Amount of substrate consumed per g dry yeast formed. Calculated from Table I. Mean values are shown.

[b] Amount of organic material per g dry yeast. Ash content of yeast is 9 % of dry weight [11].

[c] Calculated by the formula: (a) · C/mass, where C/mass is the ratio between carbon content of 1 mol of substrate and the molecular weight of substrate: 72/180 = 0.40 for sugars and 24/46 = 0.52 for ethanol.

[d] Calculated by the formula: (b) · C'/mass'. For the calculation of C'/mass' the following assumptions have been made: (i) Average composition of yeast carbohydrate: $n(C_6H_{10}O_5)$; C/mass = 72/162 = 0.44. (ii) Average composition of yeast proteins: $n(C_4H_7NO)$; C/mass = 48/85 = 0.56. (iii) Average composition of yeast lipids: $n(C_{18}H_{32}O_2)$; C/mass = 216/280 = 0.77. (iv) Average composition of the nucleotides: $n(C_{10}N_5P_1O_6H_{11})$; C/mass = 120/328 = 0.37. (v) Organic chemical composition of yeast: 37 % carbohydrates; 43 % proteins; 5 % lipids; 7 % nucleotides [10] C'/mass' = $[(37\times0.44)+(43\times0.56)+(5\times0.77)+(7\times0.37)]/100 = 0.47$.

[e] Calculated by the formula: (d) × 100/(c).

TABLE IV

BALANCE OF GLUCOSE, GALACTOSE AND ETHANOL UTILIZATION DURING LOGARITHMIC GROWTH

Proportion of oxidized and assimilated substrates have been taken from data of Tables II and III, respectively. Fermented substrates have been calculated from the data of Table I, taking into account that each mol of sugar produces 2 mol of fermentation products.

Functions	Substrate (%) utilized		
	Glucose	Galactose	Ethanol
Assimilated substrate	13	26	39
Ethanol production	71	53	–
Glycerol production	11	2.4	$\leqq$ 0.5
Acetaldehyde production	$\leqq$ 0.5	0.5	$\leqq$ 1
Acetate production	0.4	2	$\leqq$ 0.5
Pyruvate production	0.3	0.2	$<$ 0.01
Substrate oxidation	3.2	14	55

vious findings on the role of glycerol in maintaining NAD^+/NADH ratio in yeast [10]. In glucose cultures where repression of respiratory chain is high [2], and therefore reoxidation of NADH in this pathway is limited, glyceric fermentation was 10 % of the metabolized substrate while in galactose, with a lower repression effect [2], glyceric fermentation was only 2 %.

Production of ATP in the catabolism of substrates

Production of ATP in fermentation can be easily calculated, since each mol of sugar transformed to ethanol, acetate, acetaldehyde or pyruvate produces a net gain of 2 mol of ATP, and an expenditure of 2 mol of ATP takes place in the transformation of 1 mol of sugar to glycerol. Somewhat more doubtful is the amount of ATP produced in respiration, since different phosphorylation efficiencies of the yeast respiratory chain have been reported [12–15]. However, later results strongly support the assumption that when catabolite repression occurs, only two phosphorylation sites are operative [16] and that the other site (site I) is induced when a complete development of the respiratory chain is favored [16, 17].

In the calculations of ATP production shown in Table V, P/O has been assumed to equal 2 and 3 since, due to the quantitative differences in the catabolite repression effect produced by the substrates studied, differences between phosphorylation efficiencies of the respiratory chain most probably occur in our experimental conditions. With both assumptions, it seems that while in galactose cultures the greater proportion of ATP was produced in oxidation, fermentation was responsible for more than half of the ATP production in glucose cultures. The total amount of ATP was different on each carbon source.

Balance of the production and utilization of ATP

Stouthamer has calculated the amount of ATP required for the formation of microbial cells growing under various conditions, taking into account the amount of ATP spent in biosynthesis and polymerization of macromolecules from a given

TABLE V

CALCULATED NET PRODUCTION OF ATP IN CATABOLISM OF SUBSTRATES

Data from Table IV have been used. 2 mol of ATP are gained in the transformation of 1 mol of sugar to ethanol, acetaldehyde, acetate and pyruvate. 2 mol of ATP are spent in the transformation of 1 mol of sugar to glycerol. P/O equal 3 and 2 (in brackets) in the respiratory chain have been assumed. With P/O = 2, 28 mmol of ATP would be produced in the oxidation of 1 mol of sugars and 11 in the oxidation of 1 mol of ethanol.' With P/O = 3, 38 and 16 respectively would be produced.

Substrate in the culture	ATP production (mmol ATP formed/100 mmol utilized substrate) in the formation of						
	Ethanol	Glycerol	Acetaldehyde	Acetate	Pyruvate	Oxidation	Total
Glucose	142	−22	2	0.8	0.6	122 (90)	245 (213)
Galactose	106	− 5	1	4.0	0.4	532 (390)	638 (500)
Ethanol	–	–	–	–	–	880 (605)	880 (605)

substrate, and the ATP required for turnover of mRNA and transport processes [5]. According to this calculation, yeast growing on glucose or galactose as sole carbon and energy source would yield approx. 29 g of dry matter per mol of ATP spent in biosynthesis and approx. 10 g when yeast is growing on ethanol. A comparison of Stouthamer's data with the Y_{ATP} values (ratio between growth yield and ATP produced) found in our experimental conditions indicates that, whether 2 or 3 phosphorylation sites are assumed, much lower amounts of cell material than the theoretically possible are actually synthetized from the three studied substrates (Table VI). It seems that of the overall ATP produced in catabolism, only about 30 % is used in biosynthesis of cellular components, the remainder being spent in functions other than net-biosynthesis (Table VI).

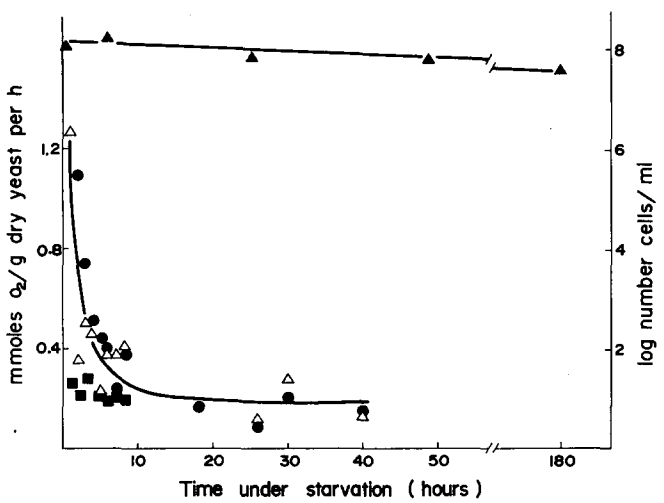

Fig. 2. Oxygen consumption and viability of starving yeast. Yeast was harvested at the middle of exponential growth. Starvation was achieved as indicated in Methods. At the indicated times respiration of starving yeast grown on glucose (△—△), galactose (●—●), and ethanol (■—■) was measured. The viability of starving yeast grown on glucose was measured (▲—▲). Each point is the mean value of four experiments.

TABLE VI

BALANCE BETWEEN THE CALCULATED PRODUCTION OF ATP IN THE CATABOLISM OF SUBSTRATES AND THE THEORETICAL ATP EXPENDITURE DURING GROWTH

Values in brackets are those obtained assuming P/O = 2 in respiratory chain. The other ones, assuming P/O = 3.

Substrate in the culture	Growth yield[a] (g/100 mmol utilized substrate)	ATP[b] (mmol/100 mmol utilized substrate)	Y_{ATP}[c] (g dry yeast/ mol ATP)	Maximal Y_{ATP}[d] (g dry yeast/ mol ATP)	ATP spent in biosynthesis[e] (%)	ATP spent in non-defined functions[f] (%)
Glucose	2.19	245 (213)	8.9 (10.2)	29	31 (35)	69 (65)
Galactose	4.24	638 (500)	6.6 (8.4)	29	23 (29)	77 (70)
Ethanol	2.07	880 (605)	2.3 (3.4)	10	23 (34)	77 (66)

[a] Data from Table I.
[b] Total ATP produced in catabolism of substrates. From Table V.
[c] Dry weight of yeast formed per mol of ATP produced in catabolism: (a)/(b).
[d] Theoretical amount of dry weight of yeast which could be formed per mol of ATP used in biosynthesis. From Stouthamer [5].
[e] Calculated by the formula: $(Y_{ATP}/\text{Maximal } Y_{ATP}) \times 100$.
[f] Calculated by the subtraction: 100 − (e).

TABLE VII

CALCULATED ATP SPENT IN NON-DEFINED FUNCTIONS DURING LOGARITHMIC GROWTH AND STARVATION

Values in brackets are those obtained assuming P/O = 2 in respiratory chain. The other ones assuming P/O = 3.

Substrate in the culture	Total Q_{ATP} during growth[a] (mmol/g dry yeast per h)	Q_{ATP} spent in non-defined functions (mmol/g dry yeast per h)	
		Growth[b]	Starvation[c]
Glucose	30 (26)	21 (17)	≈ 1.2
Galactose	46 (36)	35 (25)	≈ 1.2
Ethanol	43 (30)	33 (23)	≈ 1.2

[a] Rate of ATP production during logarithmic growth calculated by the formula: $ATP = Q_{ATP} \cdot B_0 \cdot 1/\mu\ (e^{\mu t}-1)$, where ATP is the total ATP produced in catabolism of substrates during a given interval of the logarithmic growth (Table V), Q_{ATP} the rate of ATP production in catabolism during logarithmic growth, B_0 the amount of dry yeast at the initiation of the interval (Table II), μ the specific growth rate (Table II), t = time elapsed in the interval (Table II).

[b] Calculated by the formula: (Q_{ATP}/% ATP spent in non-defined functions) × 100 (see Table VI).

[c] Calculated from data of Fig. 2 assuming 6 mol of ATP formed per mol of oxygen consumed, according to the equation: $2\ NADH_2 + O_2 + 6\ ADP + 6\ Pi \rightarrow 2\ NAD^+ + 2\ H_2O + 6\ ATP$

Rate of ATP production in endogenous metabolism during starvation

A comparison between ATP produced in endogenous metabolism during starvation, in which no net biosynthesis occurs, with ATP spent in non-defined functions during growth would give valuable information on energy metabolism of yeast. Values of respiration rate of yeast deprived of carbon and nitrogen source are shown in Fig. 2. After 5 h of starvation yeast respiration reached a value which remained almost constant for at least 40 h. Endogenous fermentation was not detected. The correlation between oxygen consumption and ATP gain can be established by a reasoning analogous to that reported above. The ATP production rate, once oxygen consumption became constant, reached a value of approx. 1.2 mmol ATP/g dry yeast per h (Table VII). Similar values were calculated from continuous culture data extrapolated to zero dilution rate [18, 19]. This value is more than 20 times lower than the ATP spent in non-defined functions during growth (Table VII). The viability of yeast under starvation was very good for at least 180 h (Fig. 2).

DISCUSSION

The results clearly indicate the effects of the differences among glucose, galactose and ethanol as nutritional repressors on the energy metabolism of *S. cerevisiae*. In the presence of an excess of glucose in aerobic cultures, yeast obtained most of its energy from fermentation (Table V). However, although fermentation accounted for more than 50 % of the metabolized galactose, it seems that the main proportion of ATP formed in the metabolism of this substrate occurred at the respiratory chain (Table V). In ethanol, oxidation seems to be the only important catabolic pathway (Table V). The ratio between growth yield and ATP gained in catabolism, Y_{ATP}, was different with the three substrates (Table VI). This is in agreement with the view of

Stouthamer and Bettenhaussen on the variability of Y_{ATP} of microorganisms depending on the culture conditions [20].

A comparison of the Y_{ATP} values with the theoretical amount of cellular material which would be formed if ATP were exclusively used to fulfill biosynthetic requirements indicates that only a small amount of the overall ATP produced by the cell is apparently spent in functions directly related with biosynthesis and that more than 60 %, 20 or more mmol/g per h, are spent in functions which cannot be biochemically defined at present (Tables VI and VII). A similar value has been recently reported by Hempfling and Mainzer in *Escherichia coli* growing anaerobically on glucose [21]. This ATP expenditure has been related with the energy needed for the turnover of cell components and the preservation of the right ionic composition [20, 22]. However, its quantitative significance which seems to be general in microorganisms [22, 23], strongly demands a detailed study of its role. The drastic decrease in the ATP needed for viability of starving yeast with respect to growing yeast (approx. 1 mmol ATP/g per h during starvation vs. 20 or more during growth) (Table VII) suggests that the energy required just to keep the cell alive, what would be considered maintenance energy "sensu stricto", is actually very small and that the largest part of the ATP dedicated to non-biosynthetic reactions during growth is spent in functions that depend on growth or active metabolism. A similar conclusion has been reached by Stouthamer and Bettenhaussen from experiments with *Aerobacter aerogenes*. It seems that this microorganism does not use the largest part of the maintenance energy, defined as the energy expended independently of growth, for true maintenance processes but for purposes unknown at present [24].

Biological functions which, in principle, could consume energy in yeast and are not directly related with net biosynthesis, can be tentatively listed as follows: (a) transport reactions for preservation of the right ionic strength, pH, and intracellular gradient concentration. Also, transport of macromolecules such as RNA and proteins from a cellular compartment to another might be ATP dependent; (b) futile cycles, in which hydrolysis of ATP gratuitously occurs, cannot be excluded in principle. It seems possible that in some cases, ATP is produced at higher rates than can be used with profit and is then wasted in futile cycles. As pointed out by Senez [25], resting cells metabolizing an energy-yielding substrate could be the extreme case of such situation. The existence of a futile cycle, consisting in the rapid turnover of trehalose, has been reported by Avigad in resting cells with high external glucose [26]. (c) A certain amount of ATP may be spent in redox reactions for the preservation of the reduced state of certain groups of proteins needed for some biological activities. (d) Turnover of macromolecules and cellular structures can also contribute to ATP utilization. In the last few years, important progress has been made on the knowledge of enzymes related with protein turnover [27]; however we are still far from understanding the functions and controls of this phenomenon. (e) Processing of certain macromolecules such as those of mRNA, which recently were shown to be far more complex in eucaryotes than could be expected from the early information on procaryotes [28]. (f) Assembly of macromolecules to form cellular structures. Even a tentative evaluation of this possibility seems very difficult at present. (g) ATP consuming reactions specifically related to cell cycle may also occur. (h) Possibly even some additional process related to growth as yet unidentified. In this connection, it should be kept in mind that some biosynthetic processes could turn out to spend more ATP than is actually acknowledged.

An estimation of these functions under growth and starvation conditions would probably give very valuable information on the utilization of the ATP fraction which is not yet accounted for. Only functions occurring at levels significantly higher during growth than during starvation, would be good candidates as important ATP consumers. Among the functions listed above, some would be operative in every metabolic condition during cell life, while others would obviously be dependent on growth or active metabolism, e.g. cell division and futile cycles. Perhaps functions related with the preservation of internal environment such as pH and ionic strength would also decrease under starvation.

The observations reported in this paper indicate that yeast spends an important amount of energy in growth-accompanying processes, which is well above the energy required for mere survival. Since the same seems to occur in bacteria [24], a general principle may be involved in this phenomenon. The realization that as yet unrecognized processes related to growth spend more energy than that required for net biosynthesis and mere survival should stimulate the search to identify the nature of the major component(s) of these processes. This search could lead to important insights in cell biology.

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