It has been concluded:

1. that the contraction of the *Vorticella* stalks is due to the blocking of the negative excess charges of the contractile protein by Ca^{++};

2. that this change is reversed in the relaxation phase by means of ATP, which indicates that relaxation is the thermodynamically involuntary, and contraction the voluntary part of the movement cycle;

3. that the use of ATP is connected with the splitting of ATP;

4. that the mechanisms of vorticellar and muscular contraction differ.

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INTRACELLULAR PROTEIN AND NUCLEIC ACID TURNOVER IN RESTING YEAST CELLS*

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INTRODUCTION

In exponentially growing yeast, protein and nucleic acid are stable end products[1,2]. However, in resting yeast cells the situation appears to be quite different. Glucose utilization in resting cells leads to a loss of induced enzymes[3] and of an alphaglucoside permease[4]. Under such conditions, induced enzyme-synthesizing capacity is retained

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longer than one would expect, judging on the basis of the rapid disappearance of the free amino acid pool[5]. Finally, in resting yeast cells, there is a mechanism for internally replenishing the free amino acid and nucleotide pools[6].

Although indicative, the experiments so far reported cannot be considered as providing conclusive evidence for protein and nucleic acid turnover. They leave in doubt the origin of the nitrogen and carbon of the replenished pools, and do not delineate the extent to which the phenomenon represents cell lysis and reutilization of degradation products by intact cells, rather than intracellular degradation and resynthesis[7]. It was the purpose of the work reported here to analyze this phenomenon by means of isotopically labeled cells. The results support previous suggestions[5] that both intracellular protein and nucleic acid turnover occur in non-growing yeast cells.

MATERIALS AND METHODS

Organism used and condition of growth

Saccharomyces cerevisiae strain LK2G12 was used in this experiment. The conditions of growth have been described previously[1]. In all cases, cells from exponentially growing cultures were employed as starting material.

Conditions of incubation

The cells were centrifuged, washed twice with cold water and resuspended to a final density of 2.84 mg dry weight per ml in either $M/15$ phosphate buffer pH 4.5 or in a nitrogen- and carbohydrate-free buffer prepared by adding the following to one liter of water: KH_2PO_4, 8g; $CaCl_2$, 0.3g; $MgSO_4$, 0.5g; trace elements[8], 1.0 ml; and vitamin mixture[8], 1.0 ml. The pH was adjusted to 4.5. The cell suspensions were shaken at 30°C in Erlenmeyer flasks on a rotary shaker.

Fractionation of cells

The cell suspensions were centrifuged, washed and fractionated as previously described[1]. When radioautographs for identification of labeled components were desired, the proteins were hydrolyzed by refluxing them for 20 h with 6*N* HCl, following which the HCl was removed by repeatedly evaporating the samples to dryness. The amino acids were separated by two-dimensional chromatography employing a *tert.*-butanol–ethyl acetate–water (4:4:2) and *tert.*-butanol–methyl alcohol–water (4:5:1) solvent systems. Free purines from the pool and nucleic acid fractions were liberated by autoclaving the fractions with 1 *N* HCl for 2 hours, neutralizing the hydrolyzates with NH_3, and precipitating the purines with silver nitrate. The precipitates were washed; the free purines were extracted with hot 1 *N* HCl, and subsequently separated on Dowex-50 columns.

Glycine was reisolated from the pools by the following procedure. The amino acid components of the pools were first separated on Dowex-50 columns by the STEIN AND MOORE procedure[9]. The glycine fractions, slightly contaminated with alanine, were combined and repeatedly evaporated to remove the excess HCl. The glycine was separated from alanine by descending phenol–water chromatography. The glycine spot was eluted and its concentration measured by the MOORE AND STEIN ninhydrin reaction[10]. Aliquots of the eluates were employed to measure its radioactivity.

MATERIALS

Uniformly ^{14}C-labeled adenine, guanine and amino acids were isolated from *E. coli* grown on uniformly labeled sucrose (14 mc/mmole). 3-^{14}C-phenylalanine (2.1 mc/mmole) was obtained from the Commissariat à l'Energie Atomique France and 2-^{14}C-glycine (1.21 mc/mmole) from the Tracer Laboratory. $^{15}NH_4NO_3$(32 atom % ^{15}N) was obtained from Eastman Organic Chemicals. Since this strain of *E. coli* did not utilize nitrate as a source of N, cells were grown on $^{15}NH_4NO_3$ as a sole source of N. The adenine and guanine were isolated from the nucleic acid fractions, and crystallized as the HCl and H_2SO_4 salts respectively. These were both found to be spectrally pure and were used as such. ^{15}N analyses were conducted by the Service de Chimie-Physique, à l'Energie Atomique France.

The viability of single cells was measured by the method of DE FONBRUNE[11] with the assistance of Mr. ROBERT WRIGHT at the Department of Genetics, Madison, Wisconsin.

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EXPERIMENTAL RESULTS

1. *Dilution of the free amino acid pool*

The cold TCA-soluble pool of yeast contains approximately 10% of the N of the cell, primarily in the form of amino acids which serve as precursors of protein synthesis[6]. During N-starvation in resting cells, each of the amino acid components of this pool is incorporated into protein in a parallel manner. If one restricts the supply of energy during this starvation period, one can observe an internal replenishment of this pool from the endogenous N-reserves[5]. Therefore, although the net result of N-starvation is a depletion from the free amino acid pool into the protein fraction, the processes may represent a balance between internal replenishment and protein synthesis. In order to test this possibility, cells with preferentially labeled pools were prepared.

When cell densities of 2 mg dry wt./ml are employed, exogenous amino acids are rapidly incorporated both into the pool and into cell proteins[12]. However, with higher densities (12.4 mg dry wt./ml) the immediate assimilation of 2-^{14}C-glycine is primarily directed to the free amino acid pool (Fig. 1). When such cells are washed and diluted into fresh buffer containing glucose, the radioactivity of the free amino acid pool is incorporated into protein and nucleic acids (Fig. 2). Although in growing yeast 43% of the radioactivity from 2-^{14}C-glycine under the conditions described above is incorporated into nucleic acid purines[1], the nucleic acid fraction contains only 15% of

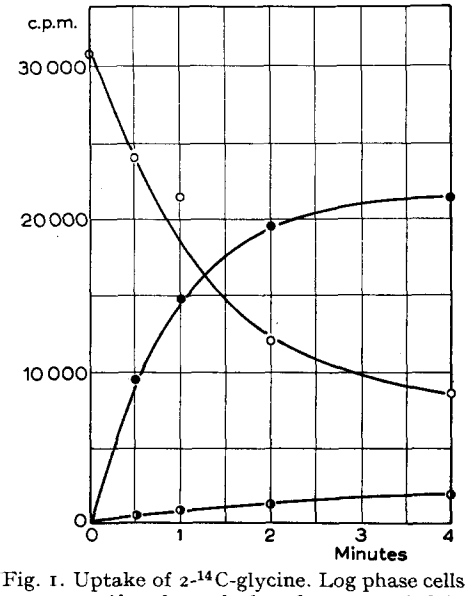

Fig. 1. Uptake of 2-^{14}C-glycine. Log phase cells were centrifuged, washed and resuspended in nitrogen- and carbohydrate-free medium to a density of 12.4 mg dry wt./ml. After thermal equilibration 110 μg 2-^{14}C-glycine (1.21 mc/mmole) were added. At intervals 2.0 ml samples were removed to pre-chilled tubes, centrifuged, washed twice with cold water and extracted with cold 5% TCA. Aliquots from the original supernatant were retained for analysis. ○ supernate. ● FAApool. ◑ Protein.

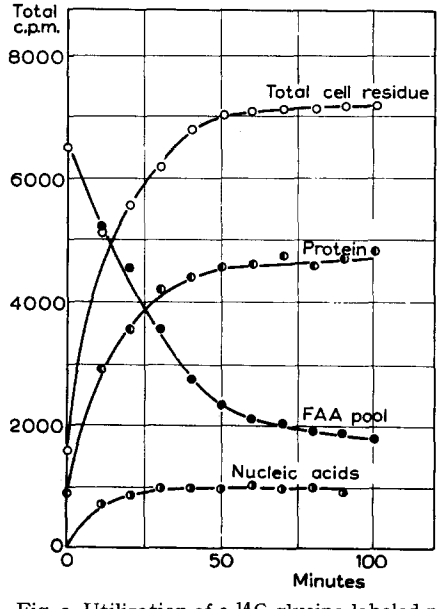

Fig. 2. Utilization of 2-^{14}C-glycine-labeled pools during N-starvation. Cells were incubated in the presence of 2-^{14}C-glycine for 2 minutes (see Fig. 1 for details), centrifuged, washed and diluted into warm N-free medium to a density of 2.84 mg dry wt./ml. The suspension was aerated at 30°; at intervals samples were withdrawn and fractionated for radioactivity.

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the radioactivity. Analysis of the nucleic acid fraction shows that only adenine and guanine are radioactive. Hydrolysis of the protein fraction from this experiment reveals that only glycine and, to a lesser extent, serine contain radioactivity.

If the internal replenishment mechanisms were operative during N-starvation, the result would be a dilution of the specific activity of the labeled amino acid in the preferentially labeled pools described above. An analysis from 2-^{14}C-glycine-labeled cells (Fig. 3) shows such a dilution indicating that active protein synthesis in resting yeast cells is accompanied by internal pool replenishment.

2. Carbon source of the replenished amino acids

There are at least two sources of N for internal replenishment of the cold TCA-soluble fraction: (a) protein and (b) nucleic acids. In the latter case N could be donated from nucleic acids and carbon from the carbohydrate reserves of the cell. If the proteins served as a source of N for amino acid replenishment, this should be demonstrable in cells grown in the presence of ^{14}C amino acids.

The distribution of radioactivity of cells during exponential growth in 3-^{14}C-phenylalanine is shown in Table I. The majority of the isotope is found in the protein fraction, with little radioactivity in either the nucleic acid or polysaccharide fractions. When these cells are incubated in the absence of an exogenous source of nitrogen and energy, a dramatic increase in the radioactivity of the cold TCA-soluble fraction occurs. The radioactivity released (3.4% of the total) is in close agreement with the release of N to this fraction (4% of the total). Data of Table I indicate that the radioactivity must arise from the TCA-precipitable (protein) fraction. During the starvation period, the increase in radioactivity of the cold TCA-soluble fraction greatly exceeds that of the alcohol-soluble fraction, while the radioactivity of the hot TCA-soluble (nucleic acid) fraction remains constant. The decrease in the alcohol-soluble fraction may be a result of a utilization of endogenous carbohydrate reserves. For the following reasons we shall consider this release of radioactivity from the TCA-precipitable fraction as protein breakdown: (1) the release of radioactivity is a general

TABLE I

DISTRIBUTION OF RADIOACTIVE CARBON AMONG THE CHEMICAL FRACTIONS OF CELLS GROWN IN 3-^{14}C-PHENYLALANINE

Flasks containing 100 ml of synthetic medium and 2 μmole of 3-^{14}C-phenylalanine (2.1 mc/mmole) were inoculated with yeast. After the cells had reached an optical density of 1.1 mg dry wt./ml the cells were resuspended in 500 ml medium and incubated for 4 h to diminish the radioactivity of the free amino acid pool. The cells were again centrifuged, washed, and divided. One fraction was resuspended in N and carbohydrate-free buffer to a density of 2.84 mg dry wt./ml and aerated at 30° C for 300 min on a platform shaker. Both cell suspensions were then chemically fractionated[1] and measured for radioactivity.

Fraction	*Radioactivity of*	
	Control culture c.p.m.	*Starved culture c.p.m.*
Cold TCA-soluble	364	1250
Alcohol-soluble	175	105
Hot TCA-soluble	506	520
TCA-precipitable	26,700	25,100
Medium	—	210
Total	27,745	27,185

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TABLE II

REPLENISHMENT RATES OF FREE AMINO ACID POOL COMPONENTS FROM LABELED PROTEIN

See Table I for details. After 5 h incubation, the rates of internal replenishment for each amino acid (k_2) was calculated from the following equation:

$$k_2 = \frac{\text{increase in free amino acid radioactivity}}{\text{total radioactivity x hours of incubation}}$$

Growth supplements		*Radioactivity*		
*^{14}C amino acid**	*^{12}C competitors***	*total c.p.m.*	*released c.p.m.*	*k_2 10^{-3}*
2-^{14}C-glycine	serine, adenine	$10.5 \cdot 10^6$	$3.15 \cdot 10^5$	6.0
Arginine***	—	$6.9 \cdot 10^4$	$2.2 \cdot 10^3$	6.4
Proline***	—	$6.55 \cdot 10^4$	$1.6 \cdot 10^3$	5.0
Threonine***	isoleucine	$6.67 \cdot 10^4$	$2.51 \cdot 10^3$	7.6
Aspartic acid***	lysine, homoserine	$4.0 \cdot 10^4$	$1.60 \cdot 10^3$	8.0
			Average	6.6

* 5 μmoles/100 ml. ** 10 μmoles/100 ml. *** totally ^{14}C-labeled.

phenomenon in cells grown in a variety of ^{14}C amino acids (see Table II); (2) the radioactivity of this fraction is not released by repeated reprecipitation of the proteins in the presence of excess unlabeled phenylalanine; (3) an exchange incorporation

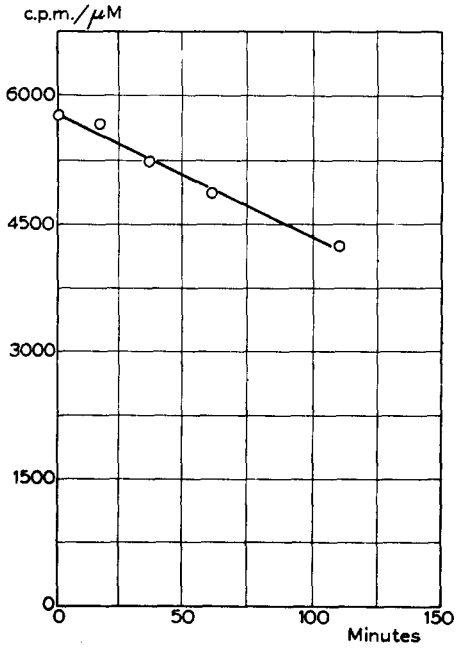

Fig. 3. Dilution of the 2-^{14}C-glycine-labeled pools during N-starvation. Cells were preferentially labeled with 2-^{14}C-glycine for 2 min (see Fig. 1 for details) and then incubated in N-free medium with 3 % glucose. At intervals, aliquots were removed, glycine was isolated from the free amino acid pools, and its specific activity determined.

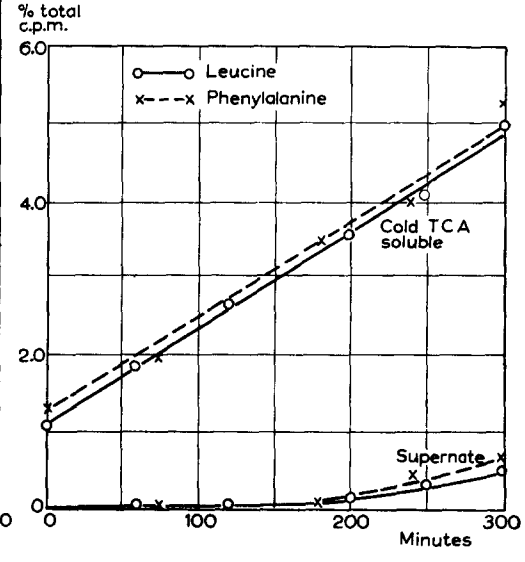

Fig. 4. Kinetics of replenishment of the free amino acid pool. (See Table I for details.) Cells were grown in the presence of either 5 μmoles of 2-^{14}C-phenylalanine or 5 μmoles of totally ^{14}C-labeled leucine (4.1 mc/mole). The % of the total radioactivity present in either the cold TCA-soluble fraction or in the supernatant is plotted against the time of incubation.

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system, of the type reported by GALE AND FOLKES[13], is not observed when the above labeled cells or extracts are incubated in excess unlabeled phenylalanine.

Fig. 4 shows the kinetics of internal replenishment from protein breakdown in cells grown in labeled phenylalanine and in leucine. In both cases most of the isotope released appears in the cold TCA-soluble fraction at a linear rate, while traces of radioactivity are released to the medium only after 200 minutes. The rate of accumulation in the cold TCA fraction (0.76%/h) is clearly a minimal estimate of protein breakdown, since the limited energy supply from endogenous reserves permits some reincorporation of isotope into proteins from the free amino acid pool.

One would expect general protein breakdown to release each amino acid in proportion to its distribution in the protein fraction. To test this hypothesis, cells were grown in the presence of various ^{14}C-labeled amino acids and unlabeled isotopic competitors to restrict secondary metabolism of the added labeled precursor. The increase in the cold TCA-soluble fraction after 5 h starvation (Table II) indicates that the amino acids are each released proportionally, thus supporting the conclusion that protein breakdown is involved. From the data of Fig. 2, the average rate of protein breakdown (k_2) is approximately $6.6 \cdot 10^{-3}$ in resting cells.

3. *Nucleic acid degradation*

In the growing cell, both protein and nucleic acid represent stable end products[1,2].

The release of nucleotides to the cold TCA-soluble fraction during N-starvation[6] or phosphorus-starvation[14,15] indicates the instability of nucleic acid in resting yeast. This breakdown is evident in cells in which the nucleic acid fraction is preferentially labeled with ^{14}C adenine (Fig. 5). When such cells are incubated under conditions which permit protein degradation, isotope is released into the cold TCA fraction and to a lesser extent into the medium at rates which are linear with respect to time. The rate of breakdown of nucleic acid (0.15%)/h) again represents a minimal figure, since cells under these conditions are capable of incorporating nucleotides into the nucleic acid fraction.

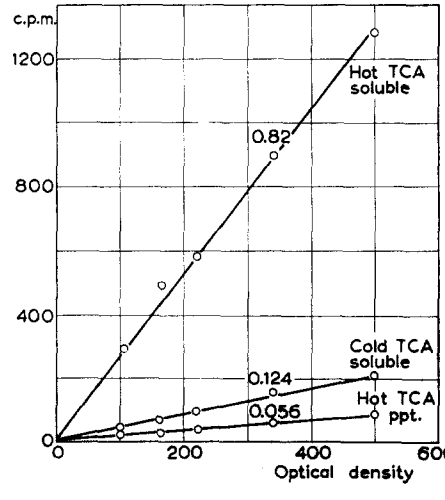

Fig. 5. Distribution of radioactivity in various cell fractions from ^{14}C adenine. Cells were grown in 25 ml of medium containing 0.53 mg of totally ^{14}C-labeled adenine. At intervals 5 ml aliquots were removed and fractionated for radioactivity.

Examination of the purines from acid hydrolysates of the cold TCA fraction from the experiment illustrated in Fig. 5 showed that the radioactivity resided in adenine, guanine, xanthine and hypoxanthine. The production of the latter two from adenine and guanine is not too surprising inasmuch as yeasts are known to contain purine deaminases[16]. The results, however, indicated the possibility that the amino group of adenine and guanine may be used for amino acid and subsequently protein synthesis. This conclusion is further supported by the fact that cells which had been subjected to a prolonged N-starvation could derive their N requirements for α-glucosidase

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synthesis from NH_3, adenine or guanine, but not from uracil, thymine, or xanthine (Fig. 7).

In order to measure the N flow from nucleic acids to proteins, cells grown on totally ^{15}N-labeled adenine and guanine were subjected to N-starvation in the presence of exogenous glucose (Table III). The hot TCA-soluble fraction contains both the highest specific activity and the greatest quantity of ^{15}N. A small, but significant, quantity of ^{15}N is found in the hot TCA-precipitable fraction. During N-starvation, although the cold TCA-soluble fraction decreases 55%, the % excess ^{15}N in this fraction actually increases 130% at the expense of the hot TCA-soluble fraction. The % excess ^{15}N in this latter fraction decreases 16.5% while its total N content increases 19%. The hot TCA-precipitable fraction (protein) increases its original ^{15}N content by 30%. Although the nucleic acid fraction actually increases during N-starvation, based on the increased N and ^{15}N content of the protein fraction and assuming that the nucleic acid purines contain 32% excess ^{15}N, approximately 1% of the N for protein synthesis was derived from nucleic acid purines.

TABLE III

NUCLEIC ACID TURNOVER DURING NITROGEN STARVATION

To a flask containing 1200 ml synthetic medium was added 34 mg ^{15}N adenine (32% excess), 44 mg ^{15}N guanine (32% excess) and yeast to a final O.D. of 0.0025. The flask was aerated at 30°C until it had reached an O.D. of 0.920. The cells were then centrifuged, washed and divided into 2 groups. The first group (A) was immediately frozen and the second (B) resuspended in 300 ml of N-free medium containing 3% glucose. Suspension B was aerated for 320 min at 30° C, centrifuged, and washed. Cells A and B were extracted twice with cold 5% TCA, centrifuged, and the residue extracted 3 times with boiling 5% TCA. Aliquots from each were removed and analyzed for N by the Kjeldahl procedure. The remainder of the sample was oxidized to NH_3 which was quantitatively distilled into H_2SO_4. The resulting $(NH_4)_2SO_4$ was crystallized and measured for ^{15}N. The % ^{15}N of the background was 0.363 ± 0.005

Sample	*N μg*	*^{15}N %*	*Excess ^{15}N %*	*Excess ^{15}N μg*
A Cold TCA-soluble	6,500	4.73 ± 0.2	4.37	284
Hot TCA-soluble	7,700	18.3 ± 0.5	17.94	1385
Hot TCA-precipitable	23,009	1.14 ± 0.05	0.78	185
Total	38,000			1854
B Cold TCA-soluble	2,900	10.3 ± 0.5	9.94	288
Hot TCA-soluble	8,350	15.4 ± 0.5	15.04	1255
Hot TCA-precipitable	26,000	1.29 ± 0.05	0.93	242
Total	37,250			1785

4. *Energy requirements of protein and nucleic acid breakdown*

A net accumulation of protein and nucleic acid precursors in the cold TCA-soluble fraction is favored by restricting the energy supply. The known mechanisms of protein or nucleic acid degradation involve the hydrolysis of peptide or nucleotide bonds by proteolytic enzymes, ribonucleases or desoxyribonucleases. Although the physiological function of those enzymes remains to be demonstrated *in vivo, in vitro* they are exergonic processes. It is therefore suprising that the breakdown of protein (Table IV) and nucleic acid (Table V) in resting yeast cells is suppressed by agents which inhibit energy-yielding reactions.

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The results reported here and elsewhere[17,18] indicate that the mechanism of nucleic acid and protein breakdown *in vivo* is not a direct hydrolysis, but is more complicated than is indicated by direct hydrolysis. An energy requirement for degradation might be expected if the reaction involved a complex acceptor other than water, as in the case of glycogen breakdown[19], or if energy were required to release the degradation system from an inactive state, as in the case of yeast catalase[20].

5. *Cellular integrity and viability during N-starvation*

At least two alternative mechanisms could lead to the observed pool replenishments from protein and nucleic acid: (a) intracellular turnover or (b) death and lysis of a fraction of the population with the internal accumulation of breakdown products by the remaining viable cells. In the case of hypothesis (b), one would expect the ap-

TABLE IV

INHIBITION OF PROTEIN BREAKDOWN IN LEUCINE-LABELED CELLS*

	Hours of incubation	*Free amino acid pool c.p.m.*	*Amino acid released c.p.m.*	*% Inhibition of release*
Zero time control	0	900		—
Incubated control	1	1360	460	—
	2	2060	1160	—
	4	3050	2150	—
$10^{-4}M$ dinitrophenol	1	1200	300	35
	2	1530	630	45
	4	2000	1100	48
$4 \cdot 10^{-4}M$ dinitrophenol	1	960	50	89
	2	1000	100	91
	4	1080	180	92

1. Total radioactivity in protein fraction: 72,500 c.p.m./10 ml.

* ^{14}C-leucine-labeled cells were prepared as described in experiment of Fig. 4. The suspension was aerated at 30°C in N- and carbohydrate-free medium and at intervals, 10 ml aliquots were removed and their free amino acid pools extracted.

TABLE V

EFFECT OF ENERGY INHIBITORS ON NUCLEIC ACID BREAKDOWN

Cells were grown in 100 ml of media containing 2.3 μmoles of totally ^{14}C-labeled guanine (7.3 mc/mmole) and subsequently treated as described in Fig. 4. The cells were aerated at 30°C for 300 min in N- and carbohydrate-free medium containing inhibitors, and fractionated for radioactivity. The figures represent the % radioactivity present in the supernatant (S) + cold TCA-soluble fraction (P).

Incubation conditions	*Incubation time minutes*	*S + P**	*Inhibition %*
Control	0	2.20	—
Control	300	5.01	0
$2 \cdot 10^{-4}M$ azide	300	2.12	100
$2 \cdot 10^{-4}M$ DNP	300	2.24	98
$2 \cdot 10^{-2}M$ arsenate**	300	2.15	100

* Per cent increase in radioactivity from 0 min control compared to 300 min control.

** In $M/15$ phthalate buffer pH 4.5.

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pearance of labeled components in the pool fraction to be preceded by the appearance of protein, nucleic acid, and soluble precursors in the medium, from cells which have lysed or lost their osmotic barriers. From Fig. 4 it is clear that products from labeled protein appear in the medium in detectable levels only after 200 min N-starvation. In the case of nucleic acid, although there appears to be no delay in the release of labeled purines to the medium (Fig. 6), its appearance does not precede the accumulation in the pool. The release of protein to the medium was further examined by measuring the appearance of α-glucosidase from fully induced cells during N-starvation. The kinetics of α-glucosidase release (Fig. 8) are in agreement with isotope experiments and indicate that approximately 0.5% of the cell components are released after 300 minutes.

The cell walls and particulate fractions of yeast contain approximately half of the

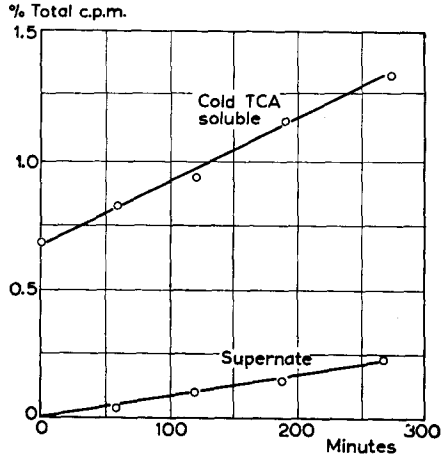

Fig. 6. Kinetics of replenishment of the nucleotide pool. Cells were grown in 100 ml of media containing 2 μmoles of totally ^{14}C-labeled adenine (8 mc/mmole) and subsequently treated as described in Table I.

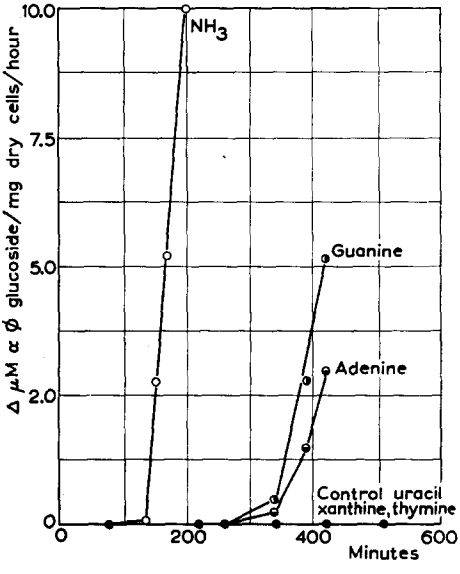

Fig. 7,

Fig. 7. Induced α-glucosidase synthesis in N-starved cells. Log phase cells were washed, suspended N-free buffer containing 4% glucose, and N-starved as previously described[5] for 12 h. The cells were then centrifuged, washed and resuspended in N-free medium containing 3% maltose. To various flasks were added NH_3, purines or pyrimidines at a final concentration of 10 μmoles/ml. At intervals aliquots were removed and assayed for α-glucosidase by the α-phenylglucoside assay[4].

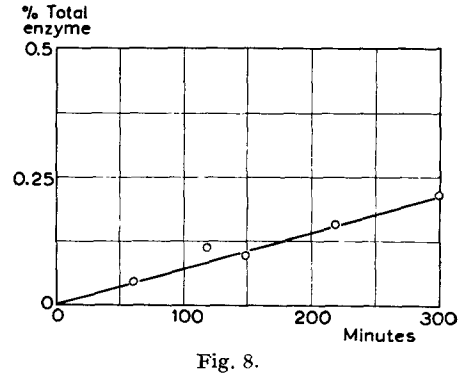

Fig. 8.

Fig. 8. Kinetics of α-glucosidase appearance in the medium. Maltose-grown cells were suspended in an N- and carbohydrate-free medium and aerated at 30°C. At intervals aliquots were removed, centrifuged and the supernatants assayed for α-glucosidase by the rate of hydrolysis of *p*-nitrophenyl-α-glucoside at pH 6.8. The % of the total α-glucosidase appearing in the medium is plottep against the time of aeration.

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total cell amino acid content. When the osmotic barrier of yeast is disrupted, either by mechanical means, TCA, boiling or autolysis, the outer structure of the cell microscopically appears intact. Therefore, if the entire pool replenishment is attributed to the internal accumulation of breakdown products in the viable cells, then approximately 10% of the yeast structures isolated after 5 hours N-starvation would be non-viable. However, single cell isolates from a culture which had been subjected to N-starvation for 5 h showed only 2 out of 124 cells (1.6%) to be non-viable, and even these two were microscopically indistinguishable from their viable relatives.

It therefore seems obvious that the precursors in the pool fraction arise entirely or almost entirely from intracellular turnover. The accumulation of cellular materials in the medium at the rate of approximately 0.1%/h may, on the other hand, arise from the lysis of a small fraction of the population.

DISCUSSION

In recent years, systems have been described in yeast[5] and mammalian cells[17,18] leading to internal replenishment of amino acid pools in resting cells. In yeast, and probably in *E. coli*[21], it has been possible to ascribe this phenomenon to intracellular events.

The augmentation of the free amino acid pool in yeast is seen when a restriction is placed upon the energy supply to minimize the incorporation[5] or when yeast cells are incubated with acridine orange[22]. From the dilution (Fig. 3) and ^{15}N (Table III) experiments, it seems clear that internal replenishment accompanies protein synthesis in resting cells when the energy supply is not limited. However, since internal replenishment is also an energy-requiring reaction in this and other systems[17,18] quantitative determinations of its magnitude permit only minimal estimates. If the dilution of the glycine pool during severe N-starvation is representative of the behavior of the free amino acid pool in general, then approximately 1% replenishment of the total cell N/h would be expected. This value is in close agreement with the data on isotope release under conditions of limited energy supply.

Experiments with cells, grown on various isotopically labeled precursors, show clearly that the carbon of the replenished amino acid pool is derived from proteins and the carbon of the nucleotide pool is derived from nucleic acids. Judging from the net increase in pool contents and from the failure to demonstrate exchange reactions, one cannot escape the conclusion that the processes involve protein and nucleic acid degradation. Since these resting systems are capable of both protein and nucleic acid synthesis, intracellular turnover must occur.

An interesting interrelationship was observed when ^{15}N purine-grown cells were subjected to N-starvation. The net N-flow from the pool to the nucleic acid and protein fractions was paralleled by a dilution of the specific activity of the ^{15}N of the nucleic acid fraction and an increase in the pool and protein ^{15}N-specific activity. Such N redistributions are understandable since yeasts are known to carry out the following reactions:

$$\text{adenine} + H_2O \xrightarrow{\text{adenase}} NH_3 + \text{hypoxanthine}$$

$$\text{guanine} + H_2O \xrightarrow{\text{guanase}} NH_3 + \text{xanthine}$$

When cells were grown on ^{14}C-purines, radioactive xanthine and hypoxanthine

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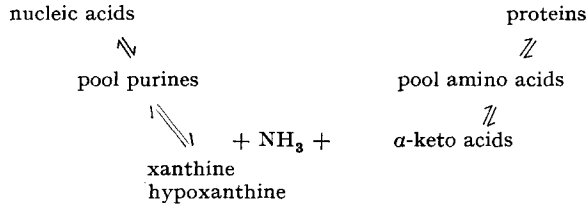
appeared in the replenished nucleotide pools. Furthermore, purines can support both the growth of yeast[16] and the synthesis of α-glucosidase in N-starved cells (Fig. 7). These results suggest the following N-interrelationship in yeast:



Nucleic acids, therefore, can serve to a limited extent as a source of N and as an energy reservoir[23] for protein synthesis in yeast. Although such flow does not quantitatively influence the replenishment of the free amino acid pool, the breakdown of nucleic acids furnishes a rich supply of the nucleotides that are utilized for the nucleic acid synthesis which accompanies protein synthesis[6]. PARDEE[24] has observed a similar slow nucleic acid breakdown and resynthesis of new nucleic acids when pyrimidine-less mutants of *E. coli* are incubated in the absence of pyrimidines.

In the absence of exogenous energy, the rates of protein and nucleic acid breakdown in resting cells can therefore be estimated in the absence of exogenous energy, by the amount of isotopes released to the pool fractions. Using this criterion, the average breakdown rates are constant over a 5 h period and total 0.0066 h^{-1} for protein and 0.0015 h^{-1} for nucleic acid. Higher values would be obtained if only a fraction of the protein or nucleic acids were subject to degradation. Employing cells subjected to prolonged N-starvation and using their ability to synthesize induced enzymes as a criterion of N-availability, HALVORSON, FRY AND SCHWEMMIN[5] concluded that proteins and probably nucleic acids are heterogeneous in their stability towards degradation. A similar process is found in the breakdown of the alcohol-soluble protein of *E. coli*[25] and the different metabolic decay rates of antibodies[25]. It is interesting to speculate that the loss of permeases and induced enzymes[4] during N-starvation represents degradation of such labile systems. A direct measurement of decreasing turnover rates during prolonged N-starvation is difficult, however, since loss of cellular integrity and viability is magnified under such conditions.

The present experiments do not confirm either of two theories concerning turnover, *i.e.*, the "wear and tear" hypothesis of partial replacement or the theory of complete degradation coupled with *de novo* synthesis. Nevertheless, the author is inclined to favor the latter. Experiments employing ^{35}S-labeled cells failed to detect exchange of amino residues in β-galactosidase in exponentially growing cells of *E. coli*[7]. However, it is doubtful that turnover *per se* of *E. coli* proteins occurs under these conditions. Also, on the basis of experiments employing amino acid antagonists and auxotrophic mutants, SPIEGELMAN, HALVORSON AND BEN-ISHAI[6] concluded that *de novo* synthesis was involved, at least for the induced enzyme, in resting yeast cells. Although exchange incorporation systems have been described for staphylococci[13], experiments designed to reveal their presence in yeast have been uniformly negative.

The concept of intracellular turnover has been subject to considerable debate in recent years. The data presented here and elsewhere[1] indicate that such turnover is characteristic of resting cells, but is absent or present to a much lesser extent in

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growing cells. It is difficult to believe that such relationships are not generally distributed in nature, since the development of a system for intracellular turnover would be of obvious selective advantage to a population faced with altering its enzymic constitution under conditions of limited nitrogen supply.

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SUMMARY

In the absence of exogenous energy and nitrogen, resting yeast cells are capable of replenishing both their nucleotide and free amino acid pools. This replenishment phenomenon is the result of intracellular protein and nucleic acid breakdown rather than of cell lysis. Isotopically labeled cells showed turnover rates of $6.6 \cdot 10^{-3}$ h^{-1} and $1.5 \cdot 10^{-3}$ h^{-1} for protein and nucleic acid respectively. Protein and nucleic acid degradation, as well as their synthesis, are energy-requiring reactions.

When exogenous energy is available, the degradation products are reutilized for protein and nucleic acid synthesis. Employing ^{15}N-purine-grown cells, it was found that the amino groups of nucleic acid purines can serve as nitrogen reservoirs for limited protein synthesis.

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