THE EFFECTS OF CERTAIN NUTRITIONAL CONDITIONS ON THE FORMATION OF PURINES AND OF RIBONUCLEIC ACID IN BAKER'S YEAST*

by

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Extensive alterations of purine and nucleic acid metabolism have been produced in bacteria whose growth was inhibited by antibiotics or in consequence of experimental mutations[1-4]. In view of the importance of these observations for the elucidation of the biosynthesis of purine compounds, it seemed useful to explore further possibilities of influencing the formation of purine compounds and nucleic acids in microorganisms. The experiments reported in this paper were carried out on bakers' yeast subjected to certain variations of nutritional conditions which could be expected to involve purine and protein metabolism.

One of the approaches to the problem was suggested by the discovery of SMITH AND SCHLENK[5] who found that the addition of methionine to cultures of bakers' yeast caused accumulation of large amounts of thiomethyladenosine in the cells. It is plausible to assume that the formation of this compound involves the intermediary condensation of methionine with ATP, in analogy to the reaction observed by CANTONI[6] in liver extracts.

In NEUBERG'S[7] phrasing, the accumulation of thiomethyladenosine can be considered as an intracellular trapping of adenine groups by methionine. The study of the effect of this trapping on the balance of purine compounds of the living yeast cell is the main topic of this investigation. The experiments had to be carried out in the presence of nutrients added to the media besides methionine. Owing to the capability of the yeast cells to form methionine from ammonium sulfate and glucose, correlations between the metabolism of added methionine and of that of purine compounds are partially masked under conditions favoring cell growth. Most of the experiments with methionine were therefore carried out on yeast cells incubated in deficient media. A brief description of some metabolic effects of these nutritional deficiencies is given in the first section of the experimental part which precedes the discussion of the effects of methionine on the purine metabolism of the yeast cells.

The attempts to depress cell growth led to experiments in which ethionine was added to the media. Thioethyladenosine was isolated in crystalline form from the

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bakers' yeast incubated under these conditions[8]. This observation is in agreement with the independent findings of SCHLENK AND TILLOTSON[9] who reported in 1954 the isolation of the substance from yeast incubated in the presence of ethionine. While the latter paper renders unnecessary the detailed publication of our procedure of the isolation and identification of thioethyladenosine, it may be mentioned that SCHLENK's evidence regarding its structure was supplemented by our observation that thioethyladenosine yielded ethyltrimethylammonium iodide during the Zeisel-Fanto procedure[10] when the reaction product was distilled into a receiver containing a methanolic solution of trimethylamine. The quarternary iodide was identified by paper chromatography with crystalline tetramethylammonium iodide and ethyltrimethylammonium iodide as reference substances. The effects of ethionine on the purine balance of bakers' yeast will be described together with those of methionine.

MATERIAL AND METHODS

Bakers' yeast (Fleischmann, Standard Brands, Inc., New York, N.Y.) was purchased on the day of each experiment from the wholesale distributor. Usually, the washed yeast cells were used directly for metabolic experiments in the form of 1 % (moist weight of the yeast before washing) suspensions in the experimental nutrients. In some instances, small inocula of the purchased yeast were grown in the laboratory in the synthetic medium of SCHULTZ AND ATKIN[11] prior to the metabolic experiments.

Media. Three types of media were used as indicated in the text: (1) the growth-promoting synthetic medium (containing ammonium sulfate as nitrogen source) of SCHULTZ AND ATKIN[7] referred to as medium G; (2) the medium described by JEENER AND BRACHET[12] which contains only ammonium sulfate, magnesium sulfate, potassium chloride, glucose and a citrate buffer (medium D_p deficient of phosphate); (3) a medium identical with D_p except for the fact that the sulfates were replaced by the corresponding chlorides (medium $D_{p,s}$ deficient of phosphate and sulfate).

Racemates of methionine and ethionine were used in the majority of the experiments, since no essential qualitative or quantitative differences were found in comparative experiments with the L-isomers and the racemic mixtures.

*The sum of the acid-soluble and the RNA-purines** was determined on aliquots of alkali digests of the centrifuged and washed yeast cells. It was found in many experiments that living bakers' yeast released only negligible amounts of UV-absorbing material into the medium. Some rare exceptions from this rule in experiments of excessively long duration will be mentioned in the text. The centrifuged cells obtained from 75 ml of the yeast suspension were incubated overnight with 10 ml of *N* aqueous sodium hydroxide at 37° in a shaker. The digest was freed from protein degradation products and from DNA by dropwise addition of 5 *N* sulfuric acid to a pH of approximately 2. An aliquot of the supernatant solution was hydrolyzed in 0.5 *N* sulfuric acid for 2 hours in a boiling water bath, and the purines were precipitated by the addition of 1 ml of an aqueous *N* solution of silver nitrate per 20 ml of hydrolysate. The washed purine-silver complexes were decomposed with *N* hydrochloric acid according to KERR AND SERAIDARIAN[13] and the resulting purine solutions were analyzed spectrophotometrically.

*The RNA purines*** were determined in a similar manner on yeast cells from which the acid-soluble purines had been extracted. For this purpose, the yeast cells were dehydrated by standing

* In the interest of brevity, the sum of the acid-soluble and RNA-purines is sometimes referred to in the text as the fraction of "total" purines.

** Essentially similar results were obtained in experiments in which the DNA-purines were included in the determinations. In these experiments the alkali-digestion of the yeast cells and the subsequent precipitation of the DNA of the digests (see below) prior to the acid hydrolysis was omitted. Instead, the acid hydrolysis was carried out directly on the suspensions on the washed yeast cells. Since in yeast, the DNA fraction amounts to less than 5 % of the RNA fraction, the results of total nucleic acid determinations mainly reflect the behavior of RNA.

Experimentally, however, the technique described in the text is preferable because the final solutions of the purines are practically colorless, whereas the direct hydrolysis of the yeast cells invariably yields light brownish solutions of the decomposed silver-purine complexes. Furthermore, the interpretation of the results is obviously simplified by eliminating the DNA-fraction from the analyses.

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overnight in at least 40 volumes of 95% alcohol. The suspension was centrifuged, the residue was washed several times with ether and dried in air at room temperature. The dried cells were extracted three times at room temperature with 0.25 *N* hydrochloric acid and twice with 0.05 *N* sulfuric acid, and the residue obtained after the final centrifugation was incubated with alkali and analyzed for purines as described before.

The adenine and guanine values were calculated from the extinctions of the purines at 240 and 262 mμ according to the binary equations of LORING *et al.*[14]. In many experiments, the guanine values obtained from absorption data were checked by determinations according to HITCHINGS[15]. This was found advisable since experimental errors in one of the absorption data used in the binary equations would have affected both the adenine and guanine figures in opposite directions. For this reason, the independent determination of the sum of adenine and guanine from the extinctions at the intersection points of both absorption curves is useless as a control of the accuracy of the data used in the binary equation although such determinations have been recommended for this purpose in the literature.

Orthophosphate was determined according to FISKE AND SUBBAROW[16]. For the determination of the inorganic phosphate of the cells, a suitable aliquot of the yeast suspension (usually 75 to 200 ml) was centrifuged and washed free of phosphate. The cells were suspended in 5 ml of water, and the suspension was heated for 10 minutes in a water bath of 80°. The volume was adjusted to 25 ml and the extracted cells were removed by centrifugation. 5 ml of the supernatant fluid were mixed with 1 ml of *N* hydrochloric acid and 1 ml of a 2% solution of barium chloride for the purpose of removing metaphosphate and small amounts of extracted nucleic acids. An aliquot of the supernatant solution obtained by centrifugation was used for the determination of the inorganic phosphate. The application of this procedure was found necessary since the extraction of inorganic phosphate from bakers' yeast by trichloroacetic acid is incomplete.

Determination of easily hydrolyzable (20'-) phosphorus. Owing to the high metaphosphate content of bakers' yeast and the incomplete extraction of this phosphorus fraction at acid reaction, the hydrolysis with *N* sulfuric acid was carried out separately on the acid extract obtained in the procedure for the nucleic acid determinations (see above), and on the residue of this extract. Since control experiments with ribonucleic acid showed that 30% of the RNA phosphorus were transformed to inorganic phosphate under these conditions the values obtained for the acid-insoluble fraction were corrected on the basis of the results of RNA determinations* on the analyzed yeast samples. In view of the relatively very large amounts of inorganic polyphosphates accumulating in bakers' yeast during the incubation in phosphate-containing media[18,19] this correction amounts to less than 5% of the direct values obtained for the 20'-P under such conditions. In the phosphate-starved yeast samples referred to in Table IV, lines 2 and 3, however, the inorganic phosphorus resulting from the hydrolysis of the ribonucleic acid accounted for as much as 50% of the uncorrected values of the 20'-phosphorus, and the figures of the corrected values must be considered as approximations. This criticism applies even to a higher degree to the phosphorus analyses of fresh and P-starved samples of pressed bakers' yeast whose contents of easily hydrolyzable phosphorus compounds, except of RNA, are extremely small. The values referred to in this discussion are nevertheless significant when correlated with other results of the corresponding experiments.

Total sulfur was determined gravimetrically as barium sulfate after ashing the yeast by fusion with sodium carbonate and sodium nitrate.

EXPERIMENTAL

1. *Nitrogen assimilation and formation of purine compounds in starved yeast*

A. Starvation period. Table I (lines 3, 4 and 5) shows the results of determinations of dry weight, total nitrogen, non-protein nitrogen, total sulfur, of the sum of the total acid-soluble and of the RNA-purines in yeast cells which had been aerated in the phosphate-free medium D_p during 16 hours, and which had subsequently been aerated for three hours in the medium D_p supplemented with 0.05 *M* potassium diphosphate. The purine values are essentially in agreement with earlier observations by JEENER AND BRACHET[12] who found that no net increase of the total purines

* The amounts of RNA were calculated from the extinctions at 260 mμ of the hydrolyzed alkali digests of the acid-insoluble fraction of the yeast cells on the basis of an average value of E(P) = 10,800[17].

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TABLE I

NITROGEN ASSIMILATION AND FORMATION OF PURINE COMPOUNDS IN STARVED BAKERS' YEAST

Line	*Incubation time (hours)*	*Medium*	*Dry weight grams per 1000 ml**	*Total N*	*NPN*	*Protein N*	*Sulfur*	*Acid-soluble RNA purine-N*	*RNA Purine-N*	*Number of cells per 1000 ml**
				*Milliatoms per 1000 ml of yeast suspension**						
1	0	G	8.0	31	—	—	—	3.4	—	—
2	3	G	14.9 (86)	52 (68)	—	—	—	5.7 (68)	—	—
3	0	—	2.9	16.8	2.50	14.30	0.266	1.70	1.42	$25 \cdot 10^9$
4	16	D_p	8.6 (197)	35.0 (112)	7.15 (187)	27.85 (94.5)	0.735 (180)	1.36 (—20)	0.95 (—33)	$68 \cdot 10^9$ (173)
5	3	D_p + phosphate	10.4 (21)	51.0 (46)	—	—	—	4.96 (265)	2.88 (188)	Not counted
6	0	—	2.6	13.6	—	—	—	1.85	Not det.	Not det.
7	16	D_{ps}	7.2 (177)	16.5 (21.4)	—	—	—	1.55 (—16.3)	1.15	Not det.
8	3	D_{ps} + phosphate	7.2 (0)	18.0 (8.1)	—	—	—	2.15 (38.9)	1.62 (41)	Not det.
9	3	D_{ps} + phosphate + sulfate	7.7 (7.2)	28.9 (75)	—	—	—	4.12 (166)	3.17 (175)	Not det.
10§§	0	—	2.5	15.5	2.1	13.4				
11	16	D_{ps}	6.9 (177)	22.4 (44.5)	5.3 (153)	17.1 (27.6)				
12	3	D_{ps} + phosphate	6.7 (—3)	27.7 (23.6)	9.4 (77.5)	18.3 (7.0)				
13	3	D_{ps} + ethionine (6.2 m*M*) + phosphate	6.1 (—11.5)	27.7 (23.6)	10.7 (102)	17.0 (—0.6)				
14	3	D_{ps} + methionine (6.6 m*M*) + phosphate	6.9 (0)	41.0 (83.0)	12.6 (138)	28.4 (66.0)				

* The figures in parenthesis are the differences between the values before and after incubation as expressed in per cent of the initial values.
** For definitions of media see page 136.
*** The figures of line 4 are the initial values for the incubation period referred to in line 5.
§ The figures of line 7 are the initial values for each of the incubation periods referred to in lines 8 and 9.
§§ The figures of line 10 are the initial values for each of the incubation periods referred to in lines 11, 12, 13, and 14.

occurred during the P-starvation period (lines 3 and 4). In our experiments, considerable decreases of the concentrations of ribonucleic acid purines were regularly observed. The absence of any net *de novo* synthesis of purines contrasts with the very considerable assimilation of nitrogen during this period. The amounts of total and non-protein nitrogen, and those of the total sulfur in the yeast cells per ml of the suspension increased by proportions of 112, 187 and 180% respectively, while the dry weight of the cells increased by 197%*. On the basis of data not included in the table, it can be stated that the excess of the increase of the cell weight over the formation of new protein was mainly accounted for by accumulation of polysaccharides while only negligible amounts of lipides were synthezised under these conditions.

The protein formation in P-starved yeast must be interpreted as actual cell growth since the number of cells per ml at the end of the starvation period showed an increment of 173% (line 4). This interpretation was also supported by the morphological aspect of the cells which showed frequent buddings at the end of the incubation in the phosphate-free medium in contrast to the initial absence of budding. When not only phosphorus sources, but also sulfur sources were omitted from the medium, (line 6 and 7), the assimilation of nitrogen was much smaller than it was in the presence of sulfate. Nevertheless, the weight of the cells showed an increase of 177% which was accounted for mainly by accumulation of polysaccharides.

Limit of growth and protein synthesis of P-starved yeast. 75 to 80% of the maximal amount of nitrogen assimilated by phosphate-starved yeast were taken up during one incubation period of 16 hours; during a subsequent 16 hour period in fresh medium D_p, a further increase of protein nitrogen of about 20% was observed, whereas a third extension of the phosphorus starvation resulted in only negligible increases of the nitrogen assimilation.

B. *Period of incubation of the starved yeast in phosphate- and sulfate-containing media.* The data of line 5 show the nitrogen assimilation of phosphate-starved yeast cells after their transfer to fresh medium containing orthophosphate. It can be seen that after a 3-hour incubation, the total purines and the nucleic acid purines increased by 265 and 188%, respectively, whereas the total nitrogen increased only by 46%.

The results reported in lines 8 and 9 show that not only the presence of phosphate, but also that of sulfate in the medium is necessary for extensive *de novo*-formation of purines and nucleic acids. When yeast which had been starved of both sulfate and phosphate was transferred to fresh medium supplemented only by the addition of phosphate, purine and nucleic acid formation was resumed at very slow rates (line 8). When, however, both phosphate and sulfate were added, the rates of purine and nucleic acid formation were approximately 4 times larger than those observed in a medium supplemented only with phosphate** (line 9).

The results of the experiments just described partially reestablish the conclusion

* Determinations of certain enzyme activities before and after incubation of commercial bakers' yeast in medium D_p were carried out on the acetone-dried yeast samples by M. Liss. The pyrophosphatase activity per 1000 ml of yeast suspension increased by 100%, the phosphatase activity by approximately 1000% during the period of P-starvation. The observations on phosphatase agree with similar findings obtained on P-starved *Torulopis utilis* by N. Rautanen and A. Kylä-Siurota, *Acta Chim. Scand.*, 8 (1954) 106.

** The purine formation in yeast incubated in medium D_p under the conditions described in the text continued for at least 9 hours, that in yeast incubated in medium D_{ps} for 6 hours, without apparent cell damage. After these intervals the cells invariably agglutinated, and a large part of their purine compounds appeared in the media.

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reached some years ago by JEENER AND BRACHET[12] who interpreted the increased basophily shown by phosphate-starved yeast cells after incubation in a phosphate-containing medium as a consequence of nucleic acid accumulation. This explanation had been abandoned when it was found that the accumulation of inorganic polyphosphate was mainly responsible for the basophily of the cells[18, 19].

2. Effects of methionine and ethionine on the formation of purine compounds in bakers' yeast

The influence of methionine and ethionine on the purine metabolism of bakers' yeast was studied under a variety of nutritional conditions. Owing to reasons set forth in the introduction, the clearest results were obtained with yeast which had been starved of phosphate and sulfate, and which was subsequently incubated in a phosphate-containing nutrient solution to which methionine or ethionine had been added as exclusive source of sulfur. Only these experiments will be reported in some detail.

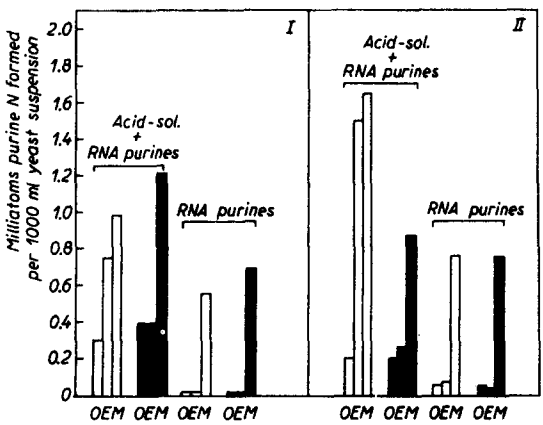

Fig. 1. Effect of methionine and ethionine on the formation of adenine and guanine groups in bakers' yeast. Yeast cells starved in medium D_{ps}. The bars represent the increment of purine groups during a subsequent 3-hour incubation in medium D_{ps} supplemented with phosphate. Abbreviations: O – No other additions, E – Ethionine added, M – Methionine added. White bars: adenine groups. Black bars: guanine groups. Experiment I: Concentration of either amino acid: 0.13 mM; Experiment II: 6.5 mM. Yeast suspension 1% (moist weight) in each experiment. All values calculated per 1000 ml of the yeast suspension prior to starvation period. All figures of experiments I and II respectively are comparable.

It might be mentioned, however, that the effects of these amino acids were essentially similar when the yeast cells were incubated in a growth-promoting synthetic medium, or when they had been starved only of phosphate, but not of sulfate prior to their transfer to the media containing methionine or ethionine.

Fig. 1 shows the results of two representative experiments in which the concentrations of adenine and guanine groups of the yeast cells were determined before and after incubation with methionine and ethionine respectively. The sum of the acid soluble and RNA purines and the amounts of the RNA purines were determined separately. All bars in the figures represent the increments of the purine fractions during the incubation periods, expressed in milliatoms of purine nitrogen per liter of yeast suspension. Paper chromatograms showed that adenine and guanine were the only purine groups present in the analyzed fractions. The increments of the adenine groups are represented by white bars, those of the guanine groups by black bars.

The first experiment (I) was carried out with 0.13 mM, the second, with 6.5 mM concentrations of methionine and ethionine respectively. It can be seen (white bars) that in both concentrations, ethionine and methionine caused strong increases of the formation of the "total" adenine groups in the yeast cells as compared to the behavior

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of the control samples incubated without a sulfur source. The effects of methionine and ethionine, however, differed essentially with regard to the distribution of the newly formed adenine groups in the acid-soluble and the RNA fraction: in the presence of methionine, both the synthesis of acid-soluble as well that of RNA-adenine was strongly stimulated; in the presence of ethionine, the newly formed adenine groups were almost entirely accounted for by the increases of acid-soluble adenine compounds, whereas the synthesis of RNA was not significantly influenced.

Furthermore, the two amino acids differed essentially with regard to their effects on the biosynthesis of guanine groups. The striking stimulation of adenine formation observed in the presence of methionine was always accompanied by a very considerable stimulation of the biosynthesis of guanine and pyrimidine groups. On the other hand, the addition of ethionine to the medium resulted exclusively in strong increases of the rate of adenine formation, whereas the biosynthesis of guanine (and that of the pyrimidines) was either not significantly influenced (Figs. 1 and 3) or even considerably depressed (Fig. 2).

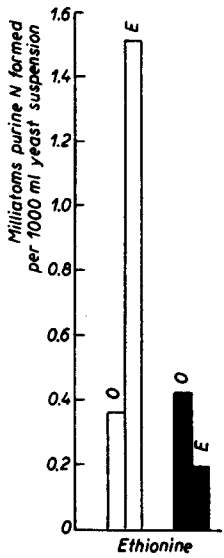

Fig. 2. Inhibitory effect of ethionine (1.3 mM) on the formation of guanine groups in bakers' yeast (conditions as indicated in legend to Fig. 1).

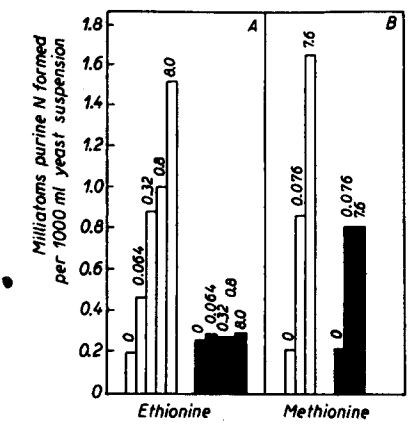

Fig. 3. Effect of different concentrations of ethionine (A) and methionine (B) respectively on the purine formation (sum of acid soluble and RNA purines) by two samples of bakers' yeast. The concentrations in mM of either amino acid is indicated on each bar. Other conditions as indicated in legend to Fig. 1.

Fig. 3A shows the effects of different ethionine concentrations on the formation of "total purines" by equal aliquots of the same yeast sample. Fig. 3B represents an analogous experiment with methionine. While ethionine had a marked stimulating effect on adenine formation already at 0.064 mM concentration, this effect increased strongly with increasing concentrations up to 8 mM. In agreement with the experiments of Fig. 1, no significant stimulation on guanine formation was observed at any of the concentrations used.

In the experiment with methionine, it is interesting to note that the strong stimulation of guanine formation observed at 0.076 mM concentration did not

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further increase when the methionine concentration was raised to 7.6 m*M*. On the other hand, the stimulation of adenine formation whose extent at a 0.076 m*M* concentration of methionine was very similar to the stimulation of the guanine formation increased to about twice this value when the methionine concentration was raised to 7.6 m*M*. This very likely means, that at low concentrations of methionine the utilization of adenine groups for nucleic acid formation predominates over the formation of thiomethyladenosine.

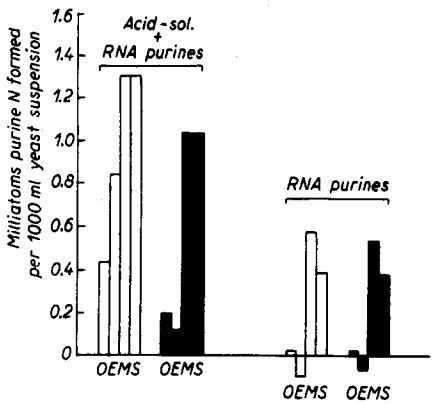

Fig. 4. Influence of small concentrations (0.13 m*M*) of ethionine, methionine, and sulfate on purine and RNA formation in bakers' yeast. Symbols: O, E, M as indicated in Fig. 1. S – sulfate-containing medium. General conditions of incubation as indicated in Fig. 1.

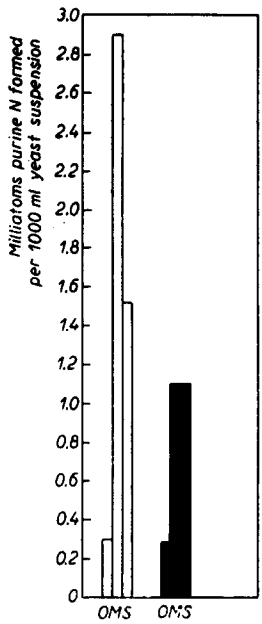

Fig. 5. Influence of high concentrations of methionine (6.5 m*M*) and sulfate (15.4 m*M*) on purine (sum of acid-soluble and RNA purines) formation in bakers' yeast. Symbols and conditions as indicated in Fig. 4.

This interpretation is supported by a comparison of the effects of low (0.13 m*M*) and high (6.5 m*M*) concentrations of methionine on purine formation with those of low (0.15 m*M*) and high (27 m*M*) sulfate concentrations (Figs. 4 and 5). Each of both sulfur sources produced very similar stimulations of the biosynthesis of guanine in all respective experiments. While the quantitative similarity between methionine and sulfate effects holds true for the adenine biosynthesis at low concentrations of methionine and sulfate, the stimulating effect of methionine in high concentrations was always considerably larger than that of sulfate in high concentrations.

The question of metabolic competition of ethionine with sulfate and methionine, respectively, was studied in experiments represented in Fig. 6. The experiments were so far limited to relatively high concentrations of ethionine, sulfate and methionine. It can be seen that the addition of sulfate (15.4 m*M*) to an ethionine-containing medium altered only very little the characteristic pattern of adenine-, guanine- and RNA-formation observed with 6.1 m*M* ethionine alone, whereas in mixtures of ethionine and methionine, the stimulation of guanine was almost as strong as that observed in the presence of methionine as the only sulfur compound. The adenine formation in mixtures of methionine and ethionine was even considerably larger than that observed with either amino acid.

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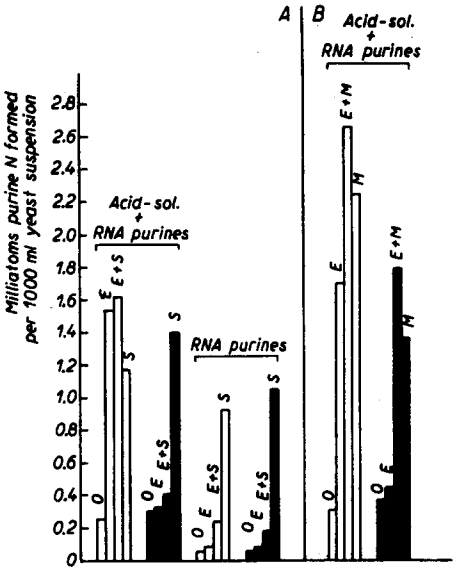

Fig. 6. Influence of mixtures of ethionine (6.1 mM) and sulfate (15.4 mM) (A) and of ethionine (2.3 mM) and methionine (5.3 mM) on purine formation in bakers' yeast. (For symbols and conditions see Fig. 1 and Fig. 4.)

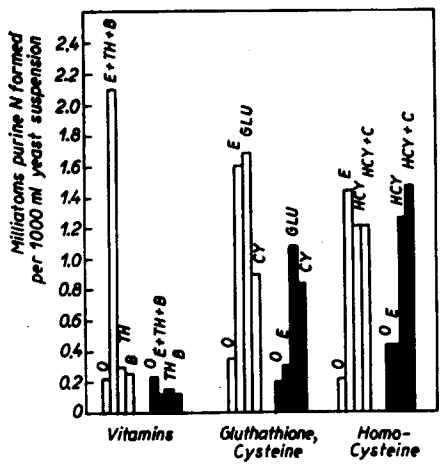

Fig. 7. Influence of some sulfur-containing vitamins (thiamine, 0.25 mM and biotin 0.008 mM) and amino acids (glutathione 3.2 mM, cysteine 6.4 mM, homocysteine 5.4 mM) on purine (sum of acid-soluble and RNA-purines) formation in bakers' yeast. Symbols: Th – Thiamine, B – Biotin, Glu – Glutathione, Cy – Cysteine, HCY – Homocysteine, C – Choline chloride (6.5 mM). Other symbols and conditions as indicated in Fig. 1.

The influences of some other sulfur sources on purine biosynthesis in yeast are shown in Fig. 7. The right section of the figure shows the results of experiments in which the influence of ethionine on the formation of purines was compared with that of glutathione, cysteine and homocysteine. The influence of these amino acids resembles in principle that of sulfate; the stimulation of purine, particularly of adenine, formation observed with glutathione was considerably stronger than that observed with cysteine.

The left section of Fig. 7 demonstrates the fact that neither thiamine nor biotin, alone or in the presence of ethionine, had significant effects on purine formation under the conditions studied.

The influence of some non-sulfur-containing metabolites on the formation of purines in sulfur-starved yeast was examined in experiments whose results are shown in Fig. 8.

Formate, glycine and choline were practically without any influence, whereas homoserine which might conceivably be a reaction product of the formation of thioalkyl adenosines had a measurable stimulating effect on the formation of both guanine and adenine.

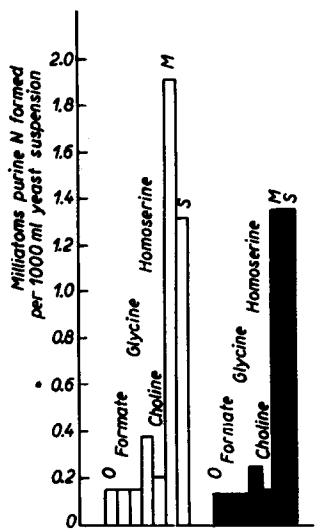

Fig. 8. Influence of some non-sulfur-containing metabolites on the formation of purines (sum of acid-soluble and RNA purines) of bakers' yeast. Symbols and conditions as in Fig. 1. Concentrations: Organic additions: 6.4 mM, ammonium sulfate: 15.4 mM.

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The extent of these effects, however, were small as compared to those observed with the active sulfur compounds.

3. *Insignificance of methionine as a donor of one-carbon-groups for purine formation in bakers' yeast*

The striking stimulation of purine formation in yeast by methionine raised the question of its participation in the intermediary mechanism of the synthesis of the purine ring, perhaps by furnishing one-carbon-groups required for ring closure. Such a possible role of methionine appeared unlikely on the basis of observations of SCHLENK AND SMITH[20] who found that radioactive carbon of $^{14}CH_3$-labelled methionine was incorporated only into the methyl group, but not into the adenine moiety of thiomethyladenosine. Since the studies of these authors were limited to the analyses of the adenine groups of this substance, we extended the examination to the total purines of the yeast samples incubated with methionine. The results of Table II show that the specific radioactivity of the newly formed purines was less than 2% of that of the added methionine regardless of whether the incubation with methionine was carried out in the presence or absence of inorganic sulfate. These results render a role of methionine as a one-carbon transmitter highly improbable even though the possibility of a continuous exchange of ureido carbons with unlabelled one-carbon sources by the action of transformylases cannot be entirely excluded.

TABLE II

INCORPORATION OF ^{14}C INTO PURINES OF YEAST DURING INCUBATION WITH $^{14}CH_3$-METHIONINE

Conditions		*Counts per μM of*		*Milliatoms of purine N per 1000 ml yeast suspension*	
Starved	*Incubated*	*Added methionine*	*Purine formed*	*Initial*	*Incubated with methionine*
Medium D_p 16 hours	D_p + 0.1 M phosphate + 0.008 M methionine 3 hours	95,000	950	1.02	4.05
Medium D_{ps} 16 hours	D_{ps} + 0.1 M phosphate + 0.008 M methionine 3 hours	95,000	1,120	1.55	3.18

4. *Extents of polyphosphate formation in bakers' yeast under various nutritional conditions; independence of polyphosphate formation from nucleic acid formation*

When washed bakers' yeast which had been cultured in the synthetic, growth-promoting medium G was transferred to fresh medium G from which any phosphorus source had been omitted, it continued to synthesize for several hours purine and pyrimidine compounds at rates very similar to those in the presence of phosphate (Fig. 9, left curves). The results of Table III (last column) show that the increase of the nucleic acid fraction parallels the behavior of the total purines. Since all the phosphorus required for the nucleic acid formation had to be supplied by intracellular phosphorus compounds, the behavior of the inorganic and the 20'-phosphorus fractions was also studied. The results of Table III show that neither the initial concentrations of inorganic phosphorus and of the acid-soluble 20'-P fraction, nor their changes during the experiment were large enough to account for the increases of the

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nucleic acid fraction. The acid-insoluble, easily hydrolyzable phosphorus fraction, however, underwent decreases whose extent was similar to that of the increments of the nucleic acid phosphorus. Since the easily hydrolyzable phosphorus fraction in bakers' yeast consists largely of inorganic polyphosphates, the results suggest the assumption that the acid-insoluble polyphosphate fraction can be utilized by the yeast cells for nucleic acid biosynthesis as readily as inorganic phosphate in the medium.

This interpretation is further supported by the fact that commercial pressed bakers' yeast, which contains only very small amounts of polyphosphates, forms only negligible amounts of purines and nucleic acids, when incubated in a medium in the absence of a phosphate source, although it is capable of synthesizing these compounds rapidly in the presence of orthophosphate in the medium (Fig. 9, right curves).

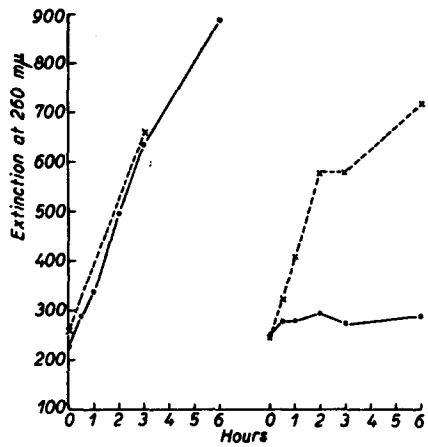

Fig. 9. Purine formation in bakers' yeastin P-free (continuous lines) and P-containing media (broken lines). Left curves: commercial bakers' yeast which had been grown in medium G for 16 hours, was incubated respectively in medium G from which phosphate was omitted and in medium G containing phosphate (8 m*M*). Right curves: Commercial bakers' yeast was incubated directly in these media. Extinctions determined in hydrolysate of yeast cells adjusted to the volume of the incubated yeast suspension. Yeast transferred to fresh media after 180 minutes.

The conclusion that acid-insoluble intracellular polyphosphates can be rapidly utilized for nucleic acid synthesis, possibly after intermediary conversion to orthophosphate is in agreement with a similar assumption advanced by JUNI *et al*[21]. as an explanation for the behavior of inorganic polyphosphates and nucleic acids during the incubation of yeast in a medium containing labelled orthophosphate.

On the other hand, the accumulation of large amounts of polyphosphates by phosphate-starved yeast appears to be independent of nucleic acid biosynthesis since it occurred even under conditions under which the formation of nucleic acids, at least that of ribonucleic acids, is entirely suppressed, namely in the absence of

TABLE III

NUCLEIC ACID-P AND 20'-P DURING INCUBATION OF GROWING YEAST IN P-FREE MEDIUM

Line	*Conditions of yeast*	*Inorganic P*	*Acid-soluble + 20'-P*	*Corrected** acid-insoluble 20'-P*	*RNA-P*
		Mg per 1000 ml of yeast suspension			
1	Initial*	6.6	11.7	34.5	9.6
2	Incubated 3 hours in P-free medium***	9.35	10.0	12.2**	34.1
3	Incubated 3 hours in P-free medium*** with 6.5 m*M* methionine	8.0	11.0	13.1**	35.6

* Harvested after 24 hours growth in medium G.

** See section on MATERIALS AND METHODS for comments on the determination of the 20'-P values.

*** Medium G except that phosphate was omitted.

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sulfur sources from the media (Table IV, E; Figs. 1 and 4), or in the presence of ethionine as the only sulfur-containing component of the medium.

TABLE IV

POLYPHOSPHATE CONTENTS OF BAKERS' YEAST UNDER VARIOUS NUTRITIONAL CONDITIONS

1	2	3	4
Designation of sample	*Conditions of yeast*	*Not incubated*	*Incubated for 3 hours in presence of 0.05 M monopotassium phosphate (media indicated in Column 2)*
		20'-P (per cent of dry weight)	
A	Grown in medium G, 16 hours	0.61	Not measured
B	Grown in medium G in presence of 0.05 *M* phosphate, 16 hours	1.0	1.4
C	Purchased pressed bakers' yeast (Not incubated)	0.01	2.7
D	Sample C, starved of phosphate (medium D_p), 16 hours	<0.01	6.7
E	Sample C, starved of phosphate and sulfate (medium D_{ps}), 16 hours	<0.01	4.7

In view of the accumulation of large concentrations of polyphosphates in phosphate-starved yeast cells after a short subsequent incubation in a phosphate-containing medium, it was of interest to determine the quantities of polyphosphates in bakers' yeast under a variety of nutritional conditions. The results of such experiments are reported in Table IV. The data show that freshly purchased yeast which contains only extremely small amounts of polyphosphates, rapidly forms very considerable quantities of these compounds under all conditions of incubation in orthophosphate-containing nutrient solutions The actual polyphosphate concentrations accumulating ranged between 0.61 g of polyphosphate phosphorus per 100 g of dried yeast cells in yeast cultured from small (0.1 g of fresh yeast per 1000 ml of medium) inocula in the complete growth-promoting medium G (line A, column 3) and 6.7% of polyphosphate phosphorus, found when 25 g of phosphate-starved yeast was incubated for 3 hours in medium D_p supplemented with 0.05*M* of orthophosphate (line D, column 4). It will be noted that the extent of polyphosphate formation observed during the incubation of large (10 g per 1000 ml of medium) inocula of freshly purchased yeast in phosphate-supplemented medium D_p was between the concentrations observed under optimal growth conditions (small inocula, medium G; line A, column 3). On the one hand, and under conditions at which the incubation of large (10 g per 1000 ml of medium) inocula in phosphate-supplemented medium D_p had been preceded by a period of phosphate-starvation, on the other hand.

5. *Effect of methionine and ethionine on the extent of protein formation on bakers' yeast*

The interpretation of the different effects of methionine and ethionine on nucleic acid formation required information regarding the behavior of protein biosynthesis in presence of either of these amino acids. The representative results of protein nitrogen determinations reported in lines 10 to 14 of Table I strongly suggest a correlation between the respective effects of these amino acids on both processes.

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A comparison between the values of protein nitrogen before and after a three hour incubation with ethionine in the presence of phosphate (lines 11 and 13) shows that no formation of protein occurred under these conditions, while in the absence of any sulfur source from the phosphate-containing medium, only a 7% increase of the protein nitrogen was observed (line 12). On the other hand, the addition of methionine to the medium (line 14) resulted in a 66% increase of the protein nitrogen within 3 hours.

The influence of sulfate on protein formation was not tested in the series of experiments reported in lines 10 to 14, but on the basis of the data of line 9 there can be no doubt as to the vigorous stimulation of protein formation by this ion.

DISCUSSION

The observations reported in the first section of the experimental part have a bearing on the problem of a metabolic connection between ribonucleic acid biosynthesis and protein biosynthesis in as much as they show that the normal interdependence between both processes may be disengaged to a very considerable extent in the living yeast cell under certain nutritional conditions. In phosphate-free media, yeast cells are capable of doubling their amounts of protein without any simultaneous formation of purines or nucleic acids; when, subsequently, phosphate is made available to the cells their purine and nucleic acid formation (expressed as percent of the initial amounts) is resumed at rates which are several times faster than that of their simultaneous protein synthesis.

The essential role of the availability of utilizable sulfur compounds—either during the period of P-starvation or during the subsequent incubation in the presence of phosphate—for the resumption of nucleic acid synthesis after supplementing the medium with phosphate strongly suggests an essential correlation between nucleic acid and protein formation in the sense that the latter controls the former. This assumption is further supported by the failure of the yeast cells to resume nucleic acid formation when the medium contains ethionine as exclusive sulfur compound. Under these conditions no protein formation was observed.

The assumption of a controlling influence of protein formation on nucleic acid biosynthesis provides a plausible explanation for the fact that the ratio between the rates of nucleic acid formation and protein formation in phosphate-starved yeast cells after their transfer to a phosphate-containing medium is about three times that between nucleic acid concentration and protein concentration in the phosphate-starved yeast cells. The large amount of protein formed during the period of phosphate starvation without nucleic acid formation appears as the principal factor responsible for the excessive nucleic acid biosynthesis once phosphate is made again available to the cells.

Regarding the nature of this influence, two alternatives seem possible. The relative depletion of intracellular ribonucleoprotein concentration caused by the continuing cell growth in the absence of phosphate might be compensated after transfer to a phosphate-containing medium by simultaneous and perhaps interdependent formation of ribonucleic acid and of the specific protein moieties of ribonucleoproteins which will be designated as aporibonucleoprotein.

On the other hand, it might also be possible that both nucleic acid and protein

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biosynthesis must not necessarily occur simultaneously, but that considerable amounts of aporibonucleoproteins might have been formed in the absence of phosphate without any *de novo* formation of ribonucleic acid. As soon as phosphate becomes available the accumulation of proteins would stimulate the formation of ribonucleic acids and the aporibonucleoproteins would combine with them to ribonucleoproteins. The extent of protein formation in the absence of phosphate (see section 1) might be limited by the final concentrations of the hypothetical aporibonucleoproteins in the water-soluble phase of the cytoplasm. Their subsequent combination with newly formed ribonucleic acids would result in their transformation to insoluble particulate ribonucleoproteins, and thereby in a disturbance of the equilibrium conditions of the soluble phase favorable to the resumption of protein synthesis.

The dependence of the overall process of ribonucleic acid formation on protein biosynthesis raises the question as to which one of the several partial reactions involved in ribonucleic acid formation is controlled by *de novo* formation of proteins. The observations reported in the second section of the experimental part exclude in all probability the biosynthesis of adenine groups from this consideration since extensive increases of the amounts of adenine groups occurred in the presence of ethionine without any formation of protein or of ribonucleic acid. These increases resulted almost certainly from the continuous disturbance of the equilibrium between adenine nucleotides and their precursors produced by the trapping of adenine groups in the form of thioethyladenosine. The failure of ethionine, on the other hand, to produce stimulation of guanine and pyrimidine formation (in contrast to methionine) can be explained by the fact that no opportunity for equilibrium disturbances is provided since neither ribonucleic acid nor other trapping compounds analogous to thioethyladenosine are formed. An alternative explanation for the behavior of the guanine and pyrimidine formation in the presence of ethionine would be the assumption that biosynthesis of these groups might require a cofactor which could be formed from methionine or some other sulfur compounds but not from ethionine. This possibility has not been excluded so far, but it is rather implausible in view of the simultaneous inhibition of guanine and pyrimidine formation.

The striking net increases of the adenine groups in the presence of ethionine implicitly[22,23] exclude the assumption that lack of ribose formation or ribose phosphorylation could have been the cause for the inhibition of ribonucleic acid biosynthesis by ethionine or by the lack of protein formation or by the lack of a utilizable sulfur source.

Thus, it appears that probably the only step of ribonucleic acid biosynthesis which cannot be accomplished by the yeast cells in the absence of protein formation is the polymerization of nucleotide units.

Concerning the inhibition of ribonucleic acid formation in the presence of ethionine, it remains to be seen whether or not there exists a direct effect of ethionine on the process of nucleotide polymerization. It would be of interest to test this possibility on the polynucleotide phosphorylase discovered by GRUNBERG-MANAGO AND OCHOA[24] in *Azotobacter vinelandii*.

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SUMMARY

1. Bakers' yeast, when incubated in the absence of phosphate, more than doubles its content of protein nitrogen and that of its organic non-protein nitrogen without any net increase of its content of purine compounds. On the other hand, when phosphate-starved yeast cells are transferred to a phosphate-containing medium, purine and nucleic acid biosynthesis is resumed at rates which are approximately four times larger than those of the simultaneous protein and amino acid biosynthesis. (The rates were measured as the per cent increments of the initial concentrations of the respective cell constituents during a given time of incubation.)

2. By the use of ethionine as a reagent for the intracellular trapping of adenine groups it has been demonstrated that yeast cells are capable of intense *de novo* synthesis of adenine groups without any simultaneous formation of RNA.

3. Methionine has no significant role as a donor of one-carbon groups for purine biosynthesis in yeast.

4. Acid-insoluble intracellular polyphosphates are utilized for nucleic acid biosynthesis in yeast as efficiently as orthophosphate present in the nutrient solutions. On the other hand, large accumulations of inorganic polyphosphates occur in yeast cells under conditions of complete inhibition of RNA formation.

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