Effect of Biotin Deficiency on the Synthesis of Nucleic Acids and Protein by *Saccharomyces cerevisiae*

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(Received 28 June 1960)

SUMMARY

A strain of *Saccharomyces cerevisiae*, grown in a medium containing a suboptimal concentration (0.4×10^{-10} M) of biotin, was shown to contain less deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein but, during the early stages of growth, increased concentrations of acid-soluble ultraviolet (u.v.)-absorbing substances, as compared with the same organism grown in the presence of an optimal concentration (8.0×10^{-10} M) of biotin. The concentration of acid-soluble u.v.-absorbing substances in the biotin-deficient yeast was higher, irrespective of the nature of the extracting acid (0.2 N-perchloric acid, 5 % (w/v) and 10 % (w/v) trichloroacetic acid, or 5 % (v/v) *n*-butanol in M/15 KH_2PO_4). Raising the temperature of extraction from 3° to 21° or 30° had little or no effect on the amounts of these u.v.-absorbing substances extracted. Analyses of the nucleotides and nucleobases in the yeast RNA showed these to have a ratio of purine:pyrimidine bases of 1.00-1.15, with the exception of the RNA from 5-day cultures of biotin-deficient yeast which had a slightly but consistently higher ratio. The significance of these results is discussed in relation to the metabolic function of biotin.

INTRODUCTION

Biotin has for some time been recognized as a growth factor for micro-organisms, but, although the metabolic roles of many other vitamins and growth factors have been elucidated, no specific function for biotin in the metabolism of micro-organisms has as yet been unequivocally established. Two main lines of study have been pursued in an attempt to gain information on the role of biotin in microbial metabolism. Growth of certain biotin-requiring micro-organisms in media containing suboptimal concentrations of biotin has been shown to be accompanied by the appearance in the medium of biosynthetic intermediates, the further metabolism of which is inhibited under conditions of biotin deficiency. Accumulation of an aromatic amine, 5-amino-imidazole riboside (Chamberlain, Cutts & Rainbow, 1952; Chamberlain & Rainbow, 1954; Moat, Wilkins & Friedman, 1956) and of hypoxanthine (Chamberlain & Rainbow, 1954) by *Saccharomyces cerevisiae* is the result of an impairment in the ability of the biotin-deficient yeast to complete the synthesis of purines. The inability to synthesize adequate quantities of purine under these conditions is also thought to cause a derangement in the synthesis of pyridine nucleotides by *S. cerevisiae*, which is manifested in the accumulation of

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nicotinic acid and nicotinic acid adenine dinucleotide in the culture medium (Rose, 1960*a, b*), while the same metabolic disturbance is probably responsible for the subnormal contents of adenosine triphosphate (ATP) and coenzyme A in biotin-deficient *Piricularia oryzae* (Katsuki, 1959*a*) and *Bacillus macerans* (Katsuki, 1959*b*).

Several workers have searched for a more specific locus of biotin function and have been able to show that, in certain biotin-deficient micro-organisms, the activity of some enzymes is markedly depressed. For example, a deficiency of biotin affects adversely the decarboxylation of oxaloacetate in *Lactobacillus arabinosus* (Lardy, Potter & Elvehjem, 1947), oxalosuccinate carboxylation in *Escherichia coli* (Shive & Rogers, 1947) and succinate decarboxylation in *Propionibacterium pentosaceum* (Delwiche, 1950). Biotin has also been shown to be essential for the activity of certain enzymes involved in amino acid metabolism, including those responsible for the aspartate to α -ketoglutarate transformation (Rossi, Rossi & Rossi, 1957), and for the deamination of aspartic acid (Lichstein & Umbreit, 1947), of threonine (Lichstein & Christman, 1948) and of serine (Nadkarni & Sreenivasan, 1957). Hexokinase activity in *Saccharomyces cerevisiae* (Strauss & Moat, 1958) and the ability to convert ornithine and carbamyl phosphate into citrulline in *Streptococcus lactis* (Estes, Ravel & Shive, 1956) have also been reported to be decreased under conditions of biotin deficiency.

However, the function of biotin in these biotin-dependent enzyme reactions has not yet been established. The activity of some of the enzymes in cell-free preparations is known to be stimulated on adding biotin, but a coenzymic role for the vitamin has not been demonstrated directly. Indirect evidence, such as the demonstration of a correlation between the activity of enzyme preparations and the content of bound biotin, is taken by some workers (e.g. Lichstein, 1955) to indicate a possible coenzymic role. There is, however, no evidence to show that the bound forms of biotin which have been isolated (such as ϵ -N-biotinyl-L-lysine, 'biocytin') are coenzymic forms of the vitamin (Wright *et al.* 1952). With other biotin-dependent enzyme systems no stimulation of activity occurs on adding biotin to the cell-free preparations, and this has led some workers to ascribe an indirect role for biotin, probably in enzyme synthesis. Sund, Ravel & Shive (1958), for example, did not obtain any immediate increase in activity of a preparation of the ornithine→citrulline enzyme from biotin-deficient *Streptococcus lactis* on adding biotin, and concluded that biotin is probably concerned in synthesis of the enzyme. Further evidence to support the view that biotin is concerned only indirectly in the activity of this enzyme comes from more recent data (Ravel, Grona, Humphreys & Shive, 1959), which showed that purified preparations of the enzyme contained less biotin than did the original cell-free extract. Similarly, Chambers & Delwiche (1954), as a result of their studies on the function of biotin in *Propionibacterium pentosaceum*, suggested that the vitamin functions in the synthesis of the coenzyme or apoenzyme concerned in the carboxylation of succinate.

The results from both of these lines of study suggest, therefore, that biotin is concerned in protein synthesis, either via the synthesis of purines or in the formation of specific enzymes. The work reported in this paper was carried out in order to examine the effect of biotin deficiency on the synthesis of nucleic acids and total protein in *Saccharomyces cerevisiae*. The results show that, under these conditions, synthesis of both of these groups of substances was impaired.

METHODS

Organism. The strain of *Saccharomyces cerevisiae* (Fleischmann) used was obtained from the Division of Applied Biology, National Research Council of Canada, Ottawa, and was maintained on slopes of malt wort agar: 10 % (w/v) spray-dried malt extract ('Muntona', Munton & Fison Ltd., Stowmarket, Suffolk) + 2 % (w/v) agar. Cultures were stored at 3°.

Experimental cultures. The chemically defined medium of Rose & Nickerson (1956) was used. Portions of the medium (100 ml.), containing either an optimal (8.0×10^{-10} M) or a suboptimal (0.4×10^{-10} M) concentration of biotin, were dispensed into 350 ml. conical flasks, which were plugged and sterilized by autoclaving momentarily at 10 lb./sq.in. The medium was inoculated by the procedure described by Rose (1960*b*), and cultures were incubated statically at 25°. Growth was measured turbidimetrically by determining the optical density of a portion (6 ml.) of culture in the Hilger 'Spekker' absorptiometer (model H 760), using neutral green-grey H 508 filters and a water blank. Optical density measurements were related to dry weight of yeast by a calibration curve.

Nucleic acid estimations. Yeast grown in media containing either an optimal or a suboptimal concentration of biotin was washed three times with M/15 KH_2PO_4 , (pH 4.5), and triplicate 3 mg. portions of the crop were taken for nucleic acid estimations. The pellet of yeast was extracted twice, in 15 ml. tapered centrifuge tubes, with 2.0 ml. portions of 0.2 N-perchloric acid at room temperature to remove acid-soluble ultraviolet (u.v.)-absorbing substances. The extracts were pooled, neutralized with N-NaOH, and made up to 5.0 ml. with M/15 KH_2PO_4 ; the optical density of this extract was measured at 260 m μ , with the Unicam S.P. 500 quartz spectrophotometer, and the reading taken as a measure of the acid-soluble u.v.-absorbing substances in the yeast. The yeast pellet was then extracted twice with 8 ml. of a boiling mixture of 95 % (v/v) ethanol in water (8 vol.) + ether (1 vol.) for 2 min. to extract lipids, and the extracts rejected. The ribonucleic acid (RNA) in the residue was hydrolysed to acid-soluble nucleotides by suspending the material in 2.0 ml. N-NaOH for 1 hr. at room temperature (Schmidt & Thannhauser, 1945; Bonar & Duggan, 1955), after which perchloric acid (N) was added to a concentration of 0.2 N. The supernatant fluid containing the soluble RNA nucleotides was separated from the precipitate of deoxyribonucleic acid (DNA) and protein, which was then washed twice with 1.0 ml. portions of 0.2 N perchloric acid, and the washings combined with the RNA extract. The combined volume was neutralized with N-NaOH, made to 10.0 ml. with M/15 KH_2PO_4 , and the optical density at 260 m μ taken as a measure of the RNA content of the yeast.

The residue of DNA and protein was suspended in 2.0 ml. N-perchloric acid, and held at 90° for 15 min. This hydrolysed the DNA to acid-soluble nucleotides, which were removed in the supernatant fluid. Extracts were made up to 3.0 ml. with N-perchloric acid, and the optical density of the solution at 260 m μ taken as a measure of the DNA content of the yeast.

Protein estimations. Protein in the residue remaining after the nucleic acids had been extracted was determined by the conventional micro-Kjeldahl technique (Markham, 1942) with a mercuric oxide catalyst (Miller & Houghton, 1945). Protein contents are expressed as mg. Kjeldahl nitrogen/3 mg. dry weight yeast.

Analysis of ribonucleic acids. For the electrophoretic separation of ribonucleotides, the RNA extract from 40 mg. dry weight of yeast was adjusted to pH 4.0 by careful addition of 10.0 N-KOH, and potassium perchlorate removed by centrifugation (Davidson & Smellie, 1952). Portions (4.0 mg.) of a commercial preparation of yeast RNA (L. Light and Co. Ltd. Colnbrook, Buckinghamshire), which was used as a control, were dissolved in 1.0 ml. of 0.8 N-KOH, incubated at 37° for 18 hr., and the supernatant fluid removed by centrifugation after neutralization with 9.2 N-perchloric acid. The supernatant liquid was then adjusted to pH 4.0 with 10.0 N-KOH, and potassium perchlorate removed by centrifugation. Samples of the solutions (300–500 μ l.) were applied as a short band (1.5–2.0 cm.) about 15 cm. from the end of a strip of Whatman no. 3 MM paper (57 cm. $\times$ 10 cm.). The paper was soaked in 0.02 M-citrate buffer (pH 3.5), and a potential gradient of 21 V./cm. applied for 7 hr. After removal, the paper was dried with a hair dryer, examined under u.v. radiation (Hanovia 'Chromatolite'), and the positions of the nucleotide spots marked with a pencil. The areas of paper containing the spots were cut out, and the nucleotides eluted from the shredded paper by soaking in 0.01 N-HCl overnight at 37° in a stoppered test tube. The eluate was centrifuged to remove cellulose fibres. The optical density of the eluate was then measured at the appropriate wavelength (adenylic acid, 260 $m\mu$; guanylic acid, 260 $m\mu$ (Volkin & Carter, 1951); cytidylic acid, 278 $m\mu$; uridylic acid, 262 $m\mu$ (Ploeser & Loring, 1949)), and the molar concentrations of the nucleotides in the eluates calculated from the optical density measurements by using the appropriate millimolar extinction coefficients. Controls of adenylic acid, guanylic acid, cytidylic acid and uridylic acid were run with each electrophoretogram.

Ribonucleobases were obtained by perchloric acid oxidation of the nucleotides. The extract of RNA mononucleotides from 40 mg. dry weight yeast was acidified to pH 5 and evaporated to dryness on a boiling water bath. This was followed by the addition of 0.2 ml. of 12.0 N perchloric acid, after which the solution was heated on the boiling water bath for 70 min. On cooling, 0.2 ml. distilled water was added, and the perchloric acid neutralized by addition of 10.0 N-KOH. The supernatant liquid was then acidified with pure HCl to 2.0 N-HCl, heated in a boiling water bath for a further 5 min. and cooled, after which the precipitate of potassium perchlorate and carbon was removed by centrifugation. This precipitate was washed with 0.1 N-HCl and the washings combined with the original extract. This was then applied as a band (1.0–1.5 cm.) on Whatman no. 3 MM paper, and examined by descending chromatography, using a solvent of *isopropanol* + conc. HCl + water (68 + 16.4 + 15.6; Wyatt, 1951). The paper was irrigated for 40 hr. at room temperature, dried, and examined beneath u.v. radiation. The nucleobases appeared as discrete absorbing spots, the guanine spot being easily distinguished by its bluish tinge. The appropriate areas of paper were cut out, and the nucleobases eluted from the shredded paper by soaking overnight at 37° in 0.10 N-HCl. The optical densities of the eluates were measured at the appropriate wavelengths (adenine, 260 $m\mu$; guanine, 250 $m\mu$; cytosine, 275 $m\mu$; uracil, 260 $m\mu$; Wyatt, 1951), and the molar concentrations of nucleobases calculated by using the appropriate millimolar extinction coefficients. Controls of adenine, guanine, cytosine and uracil were run with each chromatogram.

RESULTS

Effect of biotin deficiency on the concentrations of nucleic acids, protein and acid-soluble u.v.-absorbing substances in yeast

Cultures of the yeast, grown in media containing either an optimal ($8.0 \times 10^{-10} \text{M}$) or a suboptimal ($0.4 \times 10^{-10} \text{M}$) concentration of biotin, were removed at intervals and, after growth had been measured, the yeast was washed and analysed for DNA, RNA, protein and acid-soluble u.v.-absorbing substances. The results are shown in Figs. 1 and 2. Under conditions of biotin deficiency, growth of the yeast was restricted and, after *c.* 120 hr. of incubation, the biotin-deficient yeast was coloured pink instead of the usual creamy-white (Chamberlain *et al.* 1952). This restriction in growth and change in colour of the yeast was accompanied by marked changes in

Fig. 1

Fig. 2

Figs. 1, 2. Effect of incubation time on growth (●—●, mg. dry wt./ml.) and on the concentrations of DNA (×—×), RNA (○—○), acid-soluble u.v.-absorbing substances (●—●) and Kjeldahl protein nitrogen (△—△, mg./3 mg. dry wt. yeast × 10) in yeast grown in media containing either an optimal ($8.0 \times 10^{-10} \text{M}$) (Fig. 1) or a suboptimal ($0.4 \times 10^{-10} \text{M}$) (Fig. 2) concentration of biotin. Analyses were conducted on triplicate 3.0 mg. portions of yeast. Concentrations of DNA, RNA and acid-soluble u.v.-absorbing substances are expressed as the optical densities at 260 mμ of extracts from the yeast made up to 3.0, 10.0 and 5.0 ml. respectively with $\text{m}/15 \text{KH}_2\text{PO}_4$.

the concentrations of DNA, RNA, protein and acid-soluble u.v.-absorbing substances. After an initial slight decrease, the concentrations of DNA in both types of yeast remained constant throughout the period of growth, although under conditions of biotin deficiency, the concentration was significantly lower than in the yeast (biotin-optimal) grown in medium containing an optimal concentration of biotin. The sequence of changes observed in the RNA content of the biotin-optimal yeast was similar to that previously reported by other workers (Di Carlo & Schultz, 1948). In the biotin-deficient yeast, however, the concentration of RNA was, by comparison, low and remained so during the observed period of growth. The

concentration of Kjeldahl protein-nitrogen in the biotin-optimal yeast was highest during the very early stages of the exponential phase of growth, but declined steadily as the culture aged. In the biotin-deficient yeast, the protein nitrogen content increased up to 160 hr., when it was approximately half of that in exponential phase biotin-optimal yeast, but, thereafter, gradually declined. The biotin-optimal yeast contained an appreciable amount of acid-soluble u.v.-absorbing substances during the early stages of the exponential phase of growth but on further incubation the concentration declined rapidly and, at the end of the exponential phase, had become extremely small. The biotin-deficient yeast contained significantly higher concentrations of these substances during the early stages of growth although, after c. 200 hr., this concentration too had decreased to a low value.

Concentration of acid-soluble u.v.-absorbing substances and stability of RNA in biotin-deficient yeast

The comparatively high concentration of acid-soluble u.v.-absorbing substances in the biotin-deficient yeast during the early stages of growth was obviously of interest in relation to the inability of the yeast to synthesize normal amounts of RNA under conditions of biotin deficiency. It was possible that these u.v.-absorbing substances arose as a result of the breakdown of RNA in the biotin-deficient yeast during extraction with perchloric acid; alternatively, they may have represented purine- and pyrimidine-containing substances that had failed to be polymerized into DNA and RNA. A study was therefore made of the effect of using various acid solutions, including 0.2 N and 1.0 N-perchloric acid, 5 % (w/v) and 10 % (w/v) trichloroacetic acid, and 5 % (v/v) *n*-butanol in M/15 KH_2PO_4 (pH 4.5), to extract these u.v.-absorbing substances from 120 hr. biotin-deficient yeast and from exponential phase (40 hr.) and stationary phase (120 hr.) biotin-optimal yeast. Triplicate 3 mg. portions of washed yeast were taken, and were extracted five or, when necessary, more times with 4.0 ml. portions of the extracting solution at 3° for 5 min. After centrifugation, the supernatant liquid was decanted, adjusted to pH 4.5, and made up to 5.0 ml. with M/15 KH_2PO_4 . The optical densities of these extracts were then measured at 260 $\text{m}\mu$, with a blank of the appropriate reagent. Removal of the u.v.-absorbing substances was usually complete in five extractions, although complete removal from biotin-deficient yeast with the 5 % aqueous butanol required seven separate extractions. After all the u.v.-absorbing substances had been extracted, the residue was defatted and the RNA estimated in the usual way.

Perchloric acid (0.2 N) extracted all the u.v.-absorbing substances fairly rapidly from biotin-deficient yeast and from biotin-optimal yeast. Consistently larger amounts of u.v.-absorbing substances were extracted from biotin-deficient yeast than from either exponentially growing or stationary phase yeast grown in presence of optimal biotin (Fig. 3). Higher concentrations of perchloric acid (e.g. N) are known to hydrolyse RNA, and this is used as a means of extracting RNA from tissues (Ogur & Rosen, 1950). However, it would seem from the data shown in Fig. 4 that the RNA in biotin-deficient yeast was hydrolysed more quickly by N-perchloric acid as compared with RNA in biotin-optimal yeast.

Many workers have recommended the use of trichloroacetic acid for the extraction

of u.v.-absorbing substances before estimation of nucleic acids in tissues (Davidson, Frazer & Hutchinson, 1951; Schmidt & Thannhauser, 1945; Schneider, 1945). Trichloroacetic acid at concentrations of 5% (w/v) or 10% (w/v) rapidly extracted the bulk of the acid-soluble u.v.-absorbing substances from the biotin-deficient yeast and from the biotin-optimal yeast. The amounts extracted from biotin-deficient yeast again exceeded those from non-deficient yeast (Fig. 5). Little difference was

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Figs. 3-6. Extraction of acid-soluble u.v.-absorbing substances from 3.0 mg. portions of biotin-optimal (40 and 120 hr.) and biotin-deficient (120 hr.) yeast with separate 4.0 ml. portions of 0.2N-HClO₄ (Fig. 3), 1.0N-HClO₄ (Fig. 4), 5% (w/v) trichloroacetic acid (Fig. 5) or 5% (v/v) *n*-butanol in *m*/15KH₂PO₄ (Fig. 6). Extracts were adjusted to pH 4.5, made up to 5.0 ml. with *m*/15 KH₂PO₄, and the optical densities measured at 260 mμ. RNA was extracted as described under Methods.

observed between the amounts extracted by 5% and 10% trichloroacetic acid, although a slightly decreased content of RNA in biotin-deficient yeast that had been extracted with 10% trichloroacetic acid suggested that some of the nucleic acid may have been hydrolysed during extraction.

Extraction of u.v.-absorbing substances by aqueous *n*-butanol was used by Mitchell & Moyle (1951) in their studies on the chemical anatomy of *Staphylococcus*

aureus (*Micrococcus pyogenes*). This reagent is far milder than either perchloric acid or trichloroacetic acid, and was used in an attempt to minimize possible hydrolytic breakdown of RNA during extraction. Concentrations of *n*-butanol up to 4 % (v/v) in *m*/15 KH_2PO_4 failed to extract detectable amounts of u.v.-absorbing substances from the yeast. But by using a concentration of 5 % (v/v) butanol in *m*/15 KH_2PO_4 , the u.v.-absorbing substances were extracted, although at least seven separate extractions were required for complete removal of these substances from biotin-deficient yeast (Fig. 6).

Effect of temperature. Further information about the stability of the RNA in biotin-deficient yeast during extraction of the acid-soluble u.v.-absorbing substances was obtained when these extractions were carried out at 21° or 30° instead of at 3°. The results obtained showed that elevation of the temperature had no significant effect on the amounts of u.v.-absorbing substances and of RNA extracted, as compared with the amounts extracted at the lower temperature. Extraction at these elevated temperatures did not affect the amounts of acid-soluble u.v.-absorbing substances extracted from biotin-optimal yeast. Spectrophotometric examination of the various extracts of acid-soluble u.v.-absorbing substances revealed that, in all instances, these showed maximum u.v. absorption at or very close to 260 *mμ*.

Effect of biotin deficiency on the nucleotide and nucleobase compositions of the yeast RNA

The nucleotide and nucleobase compositions were determined on the RNA extracted from 40 mg. dry wt. yeast. The yeast was extracted with 10 ml. portions of 5 % (w/v) trichloroacetic acid at 3° until the acid-soluble u.v.-absorbing substances had been completely removed; this required five to eight separate extrac-

Table 1. *Molar concentrations of adenylic, guanylic, cytidylic and uridylic acids (based on adenylic acid = 10) in a commercial yeast RNA and in extracts of RNA from Saccharomyces cerevisiae grown in media containing an optimal (8.0×10^{-10} M) or a suboptimal (0.4×10^{-10} M) concentration of biotin*

Source	Age of culture (hr.)	Molar concentration				Ratio: purine/pyrimidine
		Adenylic acid	Guanylic acid	Cytidylic acid	Uridylic acid	
Commercial yeast RNA	—	10.0	12.64	7.73	12.20	1.13
Biotin-optimal yeast	40	10.0	11.12	8.00	11.31	1.11
	96	10.0	10.6	8.20	11.60	1.03
Biotin-deficient yeast	120	10.0	11.90	7.40	9.76	1.28
	168	10.0	12.70	8.19	12.08	1.12

tions. The tissue was defatted by extracting twice with 10 ml. portions of a boiling mixture of 95 % (v/v) ethanol in water (3 vol.) + ether (1 vol.), and the residue treated with 2.0 ml. 0.3 *N*-KOH for 18 hr. at 37° (Davidson & Smellie, 1952) to hydrolyse polyribonucleotides to soluble mononucleotides. Shorter periods of incubation were tried and, although these were sufficient to allow for the hydrolysis of RNA to acid-soluble nucleotides as detected spectrophotometrically, nevertheless it was shown electrophoretically that incubation for 18 hr. at 37° was necessary

to obtain complete hydrolysis to the mononucleotides. The nucleotide and nucleobase compositions of the RNA extract were then determined as described under Methods.

The data in Table 1 show the molar concentrations (with adenylic acid expressed as 10) of RNA nucleotides in a sample of commercial yeast RNA and in the RNA from *Saccharomyces cerevisiae* grown in media containing either an optimal or a suboptimal concentration of biotin. These results show the molar ratio of purine to pyrimidine nucleotides ranged from 1.00 to 1.15 in the commercial yeast RNA

Table 2. *Molar concentrations of adenine, guanine, cytosine and uracil (based on adenine = 10) in a commercial yeast RNA and in extracts of RNA from Saccharomyces cerevisiae grown in media containing an optimal (8.0×10^{-10} M) or a suboptimal (0.4×10^{-10} M) concentration of biotin*

Source	Age of culture (hr.)	Molar concentration				Ratio: purine/pyrimidine
		Adenine	Guanine	Cytosine	Uracil	
Commercial yeast RNA	—	10.0	10.10	8.07	11.03	1.05
Biotin-optimal yeast	40	10.0	11.36	7.91	11.50	1.10
	96	10.0	11.14	8.50	11.40	1.04
Biotin-deficient yeast	120	10.0	11.60	7.56	10.10	1.23
	168	10.0	11.60	9.10	11.90	1.02

and in biotin-optimal yeast during the exponential and stationary phases of growth. In biotin-deficient yeast from 120 hr. cultures, the ratio was slightly but consistently higher, the average value obtained being *c.* 1.28; but in yeast from 7-day biotin-deficient cultures, the ratio had decreased to within the range 1.00–1.15. Closely similar results were obtained when the ratio of purine to pyrimidine bases in the yeast RNA was determined. As shown in Table 2, this ratio was in the range 1.00–1.10 in all of the samples of RNA studied, with the exception of that obtained from 120 hr. cultures of biotin-deficient yeast in which it averaged 1.23.

DISCUSSION

Since purine- and pyrimidine-containing nucleotides (e.g. ATP, DPN, coenzyme A) and polynucleotides (nucleic acids) are essential components of all living cells, it is to be expected that any metabolic stress which causes a derangement in the biosynthetic processes leading to the formation of purines or pyrimidines will result in the decreased synthesis of nucleotides and nucleic acids. Although the presence of diminished amounts of total purine in biotin-deficient micro-organisms has not been demonstrated directly, several workers have obtained evidence that biotin is concerned in the synthesis of these nitrogenous bases. The effects of this biotin-conditioned purine deficiency on the synthesis of certain nucleotides in biotin-requiring micro-organisms has already been reported. Thus, Katsuki (1959*a, b*) has shown that biotin-deficient *Piricularia oryzae* and *Bacillus macerans* contained diminished amounts of ATP, DNP and coenzyme A, and Rose (1960*b*) reported that the excretion of nicotinic acid and nicotinic acid adenine dinucleotide, two biosynthetic precursors of pyridine nucleotides which appear in the culture medium during growth of *Saccharomyces cerevisiae* under conditions of biotin deficiency, is suppressed on adding adenine to the medium. The results obtained in the present

investigation showed that biotin deficiency during growth of a strain of *S. cerevisiae* had a profound effect also on the synthesis of nucleic acids. It was somewhat surprising to discover that, during the early stages of growth, RNA in the biotin-deficient yeast contained an abnormally high content of purine, in view of the adverse effect of biotin deficiency on purine biosynthesis. There was further evidence of a difference between the RNA from 5-day biotin-deficient cultures and that from other cultures, in that the former was more readily hydrolysed by N-perchloric acid. This may indicate a certain instability in structure, a reflexion perhaps of the slightly abnormal base ratio. It is important to note, however, that the RNA extracted from the yeast was heterogeneous and consisted of a mixture of ribosomal, soluble and nuclear RNA. The slight difference in the overall base ratio of the mixture might then be caused by a more significant variation in the base ratio of one of these RNA fractions.

This diminution in the amounts of nucleic acids synthesized under conditions of biotin deficiency was accompanied, during the early stages of growth, by a significant increase in the concentration of intracellular acid-soluble u.v.-absorbing substances which, since they absorbed maximally at approximately 260 m μ , were taken to be purine- and pyrimidine-containing substances. The substances were detected initially in extracts made with the strong acids perchloric and trichloroacetic acids, and it was possible that they represented products from the acid degradation of RNA and DNA. When it was discovered, however, that these u.v.-absorbing substances were also extracted, albeit more slowly, with aqueous *n*-butanol at pH 4.5, a much less drastic reagent, then it was assumed that they were present in the yeast in the soluble state and that they did not represent artefacts of extraction. Some evidence to support this contention came from the discovery that the amounts of u.v.-absorbing substances extracted with acid did not increase significantly when the temperature of extraction was raised from 3° to 21° or 30°, as might have been expected had they arisen as the result of hydrolysis of RNA. No analyses of the composition of this acid-soluble fraction were made in the present study, so it is not known what type of purine- or pyrimidine-containing compounds were present. But at least two u.v.-absorbing purine precursors, 5-amino-imidazole riboside (Moat *et al.* 1956; Lones, Rainbow & Woodward, 1958) and hypoxanthine (Chamberlain & Rainbow, 1954), are known to be excreted by *Saccharomyces cerevisiae* growing under conditions of biotin deficiency, so that it is likely that these also accumulate in the cells. It is possible too, that ribonucleotides are present in this fraction since these are known to accumulate intracellularly under conditions of decreased RNA synthesis. This has been demonstrated, for example, with a strain of *Escherichia coli* following addition to the culture of the purine analogue 6-azauracil (Skoda & Sorm, 1958).

The role of RNA in protein synthesis is well known, and there is also some reason for believing that protein synthesis is essential for the synthesis of RNA. The results obtained in the present study showed that, under conditions of biotin deficiency, synthesis of total protein by *Saccharomyces cerevisiae* was markedly diminished, and it is possible that this was a result, at least in part, of the decrease in the amount of RNA synthesized and of the formation of possibly abnormal RNA. But for protein synthesis to take place, it is also essential to have in the cell an adequate reservoir or pool of amino acids as well as sufficient energy, in the

form of ATP, to activate these amino acids. It is probable, however, that neither of these requirements is met in biotin-deficient yeast, for, not only does biotin-deficient *S. cerevisiae* contain reduced amounts of ATP (Dr M. H. Briggs, personal communication), but it has also been found to contain decreased concentrations of water-soluble ninhydrin-positive substances (Mr A. L. S. Munro, unpublished observations) as compared with biotin-optimal yeast. It would appear, therefore, that there are several metabolic deficiencies contributing towards this overall reduction in protein synthesis.

The diminution in the amount of protein synthesized in biotin-deficient yeast must clearly affect the enzymic activities of the yeast. It is possible that the production of only certain enzymes is affected under conditions of biotin deficiency, which would explain why only a limited number of enzymic activities have been reported to be impaired in yeast grown under this metabolic stress. On the other hand, biotin deficiency may have a non-specific effect on protein synthesis, with the result that production of all of the enzymes in the cell is decreased to about the same extent. Then the deficiency might only be observed in those metabolic reactions for which the enzymes are present in rate-limiting concentrations.

The authors wish to thank Dr P. Mitchell (Department of Zoology, University of Edinburgh), and Dr J. K. Heyes (Department of Botany, University of Edinburgh) for valuable suggestions with regard to carrying out the nucleic acid analyses.

REFERENCES

- BONAR, R. A. & DUGGAN, E. L. (1955). The preparation of ribonucleoprotein from yeast. *J. biol. Chem.* **212**, 697.
- CARLO, F. J. DI & SCHULTZ, A. S. (1948). Yeast nucleic acid. I. Quantitative determination by spectrophotometry; ammonium and phosphate ions as factors in biosynthesis. *Arch. Biochem.* **17**, 293.
- CHAMBERLAIN, N. & RAINBOW, C. (1954). The formation of diazotizable amine and hypoxanthine by yeast: possible implications in the biosynthesis of purines. *J. gen. Microbiol.* **11**, 180.
- CHAMBERLAIN, N., CUTTS, N. S. & RAINBOW, C. (1952). The formation of pigment and arylamine by yeasts. *J. gen. Microbiol.* **7**, 54.
- CHAMBERS, E. A. & DELWICHE, E. A. (1954). Biotin and succinate decarboxylation. *J. Bact.* **68**, 131.
- DAVIDSON, J. N. & SMELLIE, R. M. S. (1952). Phosphorus compounds in the cell. 2. The separation by ionophoresis on paper of the constituent nucleotides of ribonucleic acid. *Biochem. J.* **52**, 594.
- DAVIDSON, J. N., FRAZER, S. C. & HUTCHINSON, W. C. (1951). Phosphorus compounds in the cell. 1. Protein-bound phosphorus fractions studied with the aid of radioactive phosphorus. *Biochem. J.* **49**, 313.
- DELWICHE, E. A. (1950). A biotin function in succinic acid decarboxylation by *Propionibacterium pentosaceum*. *J. Bact.* **59**, 439.
- ESTES, J. M., RAVEL, J. M. & SHIVE, W. (1956). A role of biotin in the interaction of ornithine and carbamyl phosphate. *J. Amer. chem. Soc.* **78**, 6410.
- KATSUKI, H. (1959a). Studies on the metabolic function of biotin. III. Accumulation of α -keto acids in biotin-deficient cultures of *Piricularia oryzae*. *J. Biochem., Tokyo*, **46**, 621.
- KATSUKI, H. (1959b). Studies on the metabolic function of biotin. IV. Function of biotin in α -keto acid oxidation. *J. Biochem., Tokyo*, **46**, 979.
- LARDY, H. A., POTTER, R. L. & ELVEHJEM, C. A. (1947). The role of biotin in bicarbonate utilization by bacteria. *J. biol. Chem.* **169**, 451.

- LICHSTEIN, H. C. (1955). The presence of bound biotin in purified preparations of oxal-acetic carboxylase. *J. biol. Chem.* **212**, 217.
- LICHSTEIN, H. C. & CHRISTMAN, J. R. (1948). The role of biotin and adenylic acid in amino acid deaminases. *J. biol. Chem.* **175**, 649.
- LICHSTEIN, H. C. & UMBREIT, W. W. (1947). Biotin activation of certain deaminases. *J. biol. Chem.* **170**, 423.
- LONES, D. P., RAINBOW, C. & WOODWARD, J. D. (1958). A diazotizable amine produced by yeast; its chemical nature and factors affecting its accumulation. *J. gen. Microbiol.* **19**, 46.
- MARKHAM, R. (1942). A steam distillation apparatus suitable for micro-Kjeldahl analysis. *Biochem. J.* **36**, 790.
- MILLER, L. & HOUGHTON, J. A. (1945). The micro-Kjeldahl determination of the nitrogen content of amino acids and proteins. *J. biol. Chem.* **159**, 373.
- MITCHELL, P. & MOYLE, J. (1951). The glycerophosphoprotein complex envelope of *Micrococcus pyogenes*. *J. gen. Microbiol.* **5**, 981.
- MOAT, A. G., WILKINS, C. N. & FRIEDMAN, H. (1956). A role for biotin in purine biosynthesis. *J. biol. Chem.* **223**, 985.
- NADKARNI, G. B. & SREENIVASAN, A. (1957). The metabolism of serine in biotin deficiency. *Proc. Ind. Acad. Sci.* **46**, B, 229.
- OGUR, M. & ROSEN, G. (1950). The nucleic acids of plant tissues. I. The extraction and estimation of deoxypentose nucleic acid and pentose nucleic acid. *Arch. Biochem.* **25**, 262.
- PLOESER, J. MCT. & LORING, H. S. (1949). The ultraviolet absorption spectra of the pyrimidine ribonucleosides and ribonucleotides. *J. biol. Chem.* **178**, 431.
- RAVEL, J. M., GRONA, M. L., HUMPHREYS, J. S. & SHIVE, W. (1959). Properties and biotin content of purified preparations of the ornithine-citrulline enzyme of *Streptococcus lactis*. *J. biol. Chem.* **234**, 1452.
- ROSE, A. H. (1960a). Excretion of nicotinic acid and nicotinic acid adenine dinucleotide by biotin-deficient yeast. *Nature, Lond.* **186**, 139.
- ROSE, A. H. (1960b). Excretion of nicotinic acid by biotin-deficient *Saccharomyces cerevisiae*. *J. gen. Microbiol.* **23**, 143.
- ROSE, A. H. & NICKERSON, W. J. (1956). Nicotinic acid secretion by biotin-dependent yeasts. *J. Bact.* **72**, 324.
- ROSSI, C. S., ROSSI, F. & ROSSI, C. R. (1957). Sintesi dell'ossalacetato e utilizzazione del piruvato nell'avitaminosi H. *Lo Sperimentale*, **107**, 243.
- SCHMIDT, G. & THANNHAUSER, S. J. (1945). A method for the determination of deoxyribonucleic acid, ribonucleic acid and phosphoproteins in animal tissues. *J. biol. Chem.* **161**, 83.
- SCHNEIDER, W. (1945). Phosphorus compounds in animal tissues. I. Extraction and estimation of deoxypentose nucleic acid and pentose nucleic acid. *J. biol. Chem.* **161**, 293.
- SHIVE, W. & RODGERS, L. L. (1947). Involvement of biotin in the biosynthesis of oxal-acetic and α -ketoglutaric acids. *J. biol. Chem.* **169**, 453.
- SKODA, J. & SORM, F. (1958). Accumulation of nucleic acid metabolites in *Escherichia coli* exposed to the action of 6-azauracil. *Biochim. Biophys. acta*, **28**, 659.
- STRAUSS, R. R. & MOAT, A. G. (1958). A role for biotin in yeast glycolysis. *J. biol. Chem.* **233**, 765.
- SUND, R. F., RAVEL, J. M. & SHIVE, W. (1958). Ornithine-citrulline enzyme synthesis in biotin-deficient cells of *Streptococcus lactis*. *J. biol. Chem.* **231**, 807.
- VOLKIN, E. & CARTER, C. F. (1951). The preparation and properties of mammalian ribonucleic acids. *J. Amer. chem. Soc.* **73**, 1516.
- WRIGHT, L. D., CRESSON, E. L., SKEGGS, H. R., WOOD, T. R., PECK, R. L., WOLF, D. E. & FOLKERS, K. (1952). Isolation of crystalline biocytin from yeast extract. *J. Amer. chem. Soc.* **74**, 1996.
- WYATT, G. R. (1951). The purine and pyrimidine composition of deoxypentose nucleic acids. *Biochem. J.* **48**, 584.