

### Folic Acid, Vitamin B<sub>12</sub> and Nucleic Acid Synthesis in *Lactobacillus casei*

A RELATIONSHIP between folic acid and certain constituents of nucleic acids in micro-organisms has been known for some time<sup>1</sup>. This has been ascertained more directly by the recent demonstration<sup>2</sup> that partial deficiency of folic acid in a medium otherwise favourable for rapid growth of *Lactobacillus casei* results in impairment of the synthesis of deoxyribonucleic acid but not of ribonucleic acid. According to Stokes<sup>3</sup>, folic acid may function in an enzyme system responsible for the synthesis of thymine, which in turn is used for forming nucleic acids. Presumably, this takes place through its deoxyriboside thymidine with involvement of vitamin B<sub>12</sub><sup>4</sup>, the mediation of which in nucleic acid synthesis has also been shown<sup>5</sup>. This and other functional relationships between folic acid and vitamin B<sub>12</sub><sup>6</sup> suggested an investigation of their effects, singly and together, on nucleic acid metabolism.

With *L. casei* (ATCC 7469) grown in a medium<sup>7</sup> varied with respect to concentrations of folic acid (Lederle) and vitamin B<sub>12</sub> (crystalline, Merck), some results of a typical experiment are set forth in the accompanying table. Nucleic acids were extracted by Schneider's procedure<sup>8</sup>. Ribonucleic acid was determined by the orcinol method<sup>9</sup> and deoxyribonucleic acid by the diphenylamine reaction<sup>9</sup> in suitable aliquots of this extract.

Supplement	Ribonucleic acid* (per cent)	Deoxyribonucleic acid* (per cent)	Cell mass* (gm. per lit.)	0.1 N acid† per 10 c.c. medium (c.c.)
Folic acid 5 µgm./100 c.c.	9.09	1.87	0.21	2.5
" " 200 " "	8.42	2.82	0.48	12.14
Vitamin B <sub>12</sub> 5 " "	9.39	1.25	0.21	2.26
" " 200 " "	9.50	2.93	0.24	7.50
Folic acid + vitamin B <sub>12</sub> 5 µgm. each/100 c.c.	10.26	2.26	0.20	2.38
" " " 20 " "	9.74	2.50	0.30	4.76
" " " 200 " "	9.28	3.82	0.68	12.43

\* 24-hr. growth; † 72-hr. growth

As may be expected, folic acid enhances the synthesis of deoxyribonucleic acid. Similar results are obtained with vitamin B<sub>12</sub> although, from growth and acid production, it is seen that it could not completely replace folic acid as a growth factor for *L. casei*. The interesting observation, however, is that there is a pronounced, and more or less all-round additive, effect of folic acid and vitamin B<sub>12</sub>.

Other related metabolic activities are being studied.

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### Methionine and the Formation of Pigment by Yeasts

YEAST 47<sup>1</sup> grows well in a defined salts-glucose-ammonia medium, containing *p*-aminobenzoic acid, biotin, pantothenic acid and inositol<sup>2</sup>. This medium, supplemented with 500 µgm. of L- (or 1,000 µgm. of DL-) methionine per ml., but containing suboptimal concentrations (0.003–0.04 mµgm./ml.) of D-biotin, yields yeast crops after 72–96 hr. at 28° which are unusual in that they are pink in colour. Other conditions being suitable, the colour is not formed (1) if any other amino-acid is substituted for methionine; (2) if the concentration of D-biotin is raised to 0.08 mµgm./ml.; (3) in the presence of optimal biotin, but suboptimal pantothenate, *p*-aminobenzoic acid or inositol. Formation of the pink pigment is thus associated with the presence of methionine and with a relative deficiency of biotin.

If, however, *p*-aminobenzoic acid is omitted from the medium and replaced by adenine, methionine and histidine<sup>1,2</sup>, no pigment is formed; nor is it possible to induce pigment formation in a strain of *Schizosaccharomyces octosporus* examined in these laboratories<sup>3</sup> which requires adenine, methionine and histidine for growth. Again, under conditions otherwise suitable, but in the presence of 25 µgm. of adenine hydrochloride per ml. of medium, pigment formation by yeast 47 is almost, if not entirely, suppressed. These observations, which suggest that a precursor

of adenine is a further factor in pigment formation, are possibly related to those of Lindgren<sup>4</sup>, who associate the formation of a pink pigment by an artificially induced mutant of *Saccharomyces cerevisiae* with the interaction of excess methionine with a precursor of adenine (formed as the result of a 'genetic block' in adenine synthesis) and with other substances.

Pigment formation by yeasts under the conditions described is not confined to yeast 47, having been induced in strains of *Schizosaccharomyces pombe*, *Saccharomyces ludwigii*, *Saccharomyces carlsbergensis* and five single-cell strains of brewer's yeasts (*S. cerevisiae*). Certain of these strains form pigment in absence of exogenous methionine, possibly because relatively large amounts of methionine are synthesized by the cell.

It is also noteworthy that, wherever the yeast crops are pink, a diazotizable amine (not *p*-aminobenzoic acid) is present in the fermented medium. The amine is not present when the nutritional environment is such that normal, creamy-white yeast crops are obtained.

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