tween the groups as have measurements of cholesterol and lipoproteins by other investigators using different technics.

1. McDonald, H. J., Bermes, Jr., E. W., Spitzer, R. H., *Fed. Proc.*, 1955, v14, 733.

2. Lillie, R. D., Burtner, H. J., *J. Histochem.*, 1953, v1, 8.

3. Swahn, B., *Scand. J. Clin. Lab. Invest.*, 1952, v4, 98.

4. Barr, D. P., *Minn. Med.*, 1955, v38, 788.

5. ———, *Circ.*, 1953, v8, 641.

6. Chapman, I., Goldbloom, A. A., Mirrer, G., Eiber, H. B., *Arch. Path.*, 1956, v61, 357.

7. Lewis, L. A., Page, I. H., *Circ.*, 1953, v1, 707.

8. Steiner, A., Kendall, F. E., Mathers, J. A. L., *ibid.*, 1952, v5, 605.

9. Eiber, H. B., Goldbloom, A. A., Deutschberger, O., Chapman, I., Loewe, W. R., *Geriatrics*, 1955, v10, 213.

10. Wertlake, P. T., Wilcox, A. A., Haley, M. I., Peterson, J. E., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 163.

Received May 9, 1958. P.S.E.B.M., 1958, v98.

Temperature-Sensitive Requirement for Methionine or p-Aminobenzoic Acid in Strain of *Escherichia coli*.* (24162)

THOMAS J. BIRD† AND JOSEPH S. GOTS

Department of Microbiology, School of Medicine, University of Pa., Philadelphia

It has been known for at least 50 years(1) that naturally occurring strains of *Escherichia coli* can be cultured readily in simple synthetic media lacking specific growth factors. A simple source of carbon and energy such as glucose, glycerol or lactic acid and inorganic ammonium salts as sole source of nitrogen suffice for growth of the majority of *E. coli* strains. Occasional strains have been isolated, unable to grow in a minimal salts-glucose medium(2). This report deals with a naturally occurring strain of *E. coli* which behaves like the species prototype when grown at room temperature (21-24°C), but will not grow at 37°C unless the medium is supplemented with either methionine or p-aminobenzoic acid (PABA). This anomalous behavior is apparently associated with an abnormal reaction whereby homocysteine interferes with the role of PABA in synthesis of methionine.

Materials and methods. Escherichia coli, strain WM-13, was one of several strains isolated from clinical material at the Hospital for Women's Medical College of Pennsylvania and obtained through the courtesy of the late Dr. L. J. Kimmelman. It was identified as *E. coli* on the basis of morphology, lactose fermentation and typical IMViC reactions. It is susceptible to the action of coliphages T1, T3, T6 and T7, but resistant to T2, T4 and T5. It serves as sensitive indicator strain for temperate phages *lambda* and *w2*(3), and can be lysogenized by these phages. Growth requirement is unchanged by lysogenization. When lysogenized with *lambda* phage it loses its sensitivity to lysis by T1 and T7 and production of mature *lambda* phage can be induced with ultraviolet irradiation or with L-azaserine. Growth analyses were carried out in the salts-glucose (S-G) medium of Gots and Chu(4). The medium was inoculated to final concentration of 10^5 cells/ml with a bacterial suspension harvested from overnight culture in nutrient broth and washed twice by centrifugation with minimal S-G media. Growth was recorded by turbidimetric readings with a Klett-Summerson photoelectric colorimeter equipped with green filter (#54).

Results. When incubation was carried out at 37°C, strain WM-13 was unable to grow in unsupplemented S-G medium. When medium was supplemented with either casein hydrolysate (0.1%) or with a mixture of B vitamins, even greater growth was obtained than in nutrient broth. Addition of single amino

* This work has been aided by a grant from the U. S. Atomic Energy Commission.

† Present address: Dept. of Bacteriology, Stritch School of Medicine, Loyola University, Chicago, Ill.

acids immediately revealed a specific growth requirement for methionine. Effective, though incomplete, restoration of growth was also obtained with leucine and α-aminobutyric acid. The effect of the B vitamin mixture could be completely replaced by the single addition of p-aminobenzoic acid (PABA).

To rule out the possibility that the alternate response to methionine or PABA might be due to a mixed population, tests for homogeneity of population were made by colony counts on solid media. The same number of colonies was obtained when the culture was plated on nutrient agar or on minimal agar containing either methionine or PABA. Unsupplemented controls showed no colonies even at inoculum size of 10^8 bacteria/plate. When these plates were kept at room temperature for 24 hours colonies appeared on the unsupplemented plates of same size and number as those which had grown on supplemented plates at 37°C. Ability of strain WM-13 to grow in the unsupplemented S-G medium at room temperatures was readily confirmed in liquid media. Summary of the nutritional behavior of this strain at the 2 temperatures is given in Table I. At room temperatures strain WM-13 is indistinguishable from the average non-exacting *E. coli* strains usually encountered; only at 37°C is its alternate requirement for methionine or PABA manifested.

Table I also shows inability of known precursors of methionine to substitute for methionine. This indicates that the requirement for methionine is associated with a metabolic disturbance at the terminal conversion of homocysteine to methionine. Homocysteine, and to a lesser extent, homoserine proved inhibitory to normal growth at room temperature (Table I). Homocystine and homocysteine thiolactone were as effective as homocysteine in this inhibition.

TABLE I. Growth Response of *Escherichia coli*, Strain WM-13.

Additions	μg/ml	Growth 37°C	Growth 21-24°C
None	0	0	120
DL-methionine	20	105	103
L - "	20	103	102
D - "	20	45	112
L-cysteine	20	0	119
DL-homoserine	20	0	66
Cystathionine	40	0	104
DL-homocysteine	20	0	0
DL-homocysteine thiolactone	20	0	0
DL-homocystine	20	0	0
Vit. B_{12}	.1	0	116
p-aminobenzoic acid	.01	109	113
DL-leucine	10	65	102
Alpha-aminobutyric acid	10	82	107

Growth is recorded as turbidity readings after 24 hr.

TABLE II. Reversal of Homocysteine Inhibition by Methionine.

DL-homocysteine (μg/ml)	Growth (turbidity) DL-methionine None	DL-methionine 20 μg/ml
.0	160	160
12.5	5	150
25.0	0	155
50.0	0	135

Growth is recorded as turbidity readings after 24 hr at room temperature.

Inhibition by homocysteine was completely nullified by addition of methionine (Table II). Thus, homocysteine imposed a growth requirement at room temperature, which mimicked growth requirement at 37°C. PABA was also able to provide growth in the presence of homocysteine but, unlike methionine, activity of PABA depended on concentration of homocysteine. In the case of methionine, as long as the minimal amount necessary for optimal growth (20 μg/ml) was present, homocysteine was inactive over a wide range of concentrations. With PABA, the minimal amount for optimal growth at 37°C was 0.005 μg/ml. Growth in the presence of 50 μg of homocysteine/ml could not be obtained at room temperature until the concentration of PABA reached 0.1 μg/ml. Further increase of PABA to 10 μg/ml was required to prevent inhibition by 200 μg homocysteine/ml. Though this suggests a competitive type of relationship between PABA and homocysteine, a frank quantitative demonstration of such a relationship could not be obtained.

When a washed suspension of WM-13 cells was plated on unsupplemented media, derivatives (about $3/10^8$ cells) which no longer required methionine could be isolated after 3-4 days incubation at 37°C. These isolates were resistant to inhibition by homocysteine. Similarly, derivatives isolated from survivors of

homocysteine inhibition at room temperature no longer required methionine for growth at 37°C. This indicates that the methionine requirement at 37°C and inhibition by homocysteine at room temperature are alternate manifestations of a common metabolic lesion and that the lesion may be corrected by mutation. The mutant so obtained behaves like the average *E. coli* prototype.

Discussion. The unique nutritional behavior of strain WM-13 appears to be focused around a metabolic disorder in that function of PABA which is concerned with synthesis of methionine from homocysteine. Complete derangement of PABA metabolism is unlikely since formation of other end-products of PABA function (*e.g.*, purines, thymine, serine, etc.) remains intact. Ability of methionine alone to replace completely a requirement for PABA has previously been shown with mutants of neurospora(5-6) and under conditions of inhibition by 2-chloro-4-aminobenzoic acid(7). It is known that the synthesis of methionine is the first reaction to become limiting when PABA function is even partially inhibited(8-9).

The patterns of antagonism exerted by methionine and PABA on inhibition by homocysteine suggest that homocysteine, or a product derived from homocysteine, interferes with a cofactor (CoF) derived from PABA and that this interference, even if partial, prevents formation of methionine. This would create an anomalous and chaotic situation whereby homocysteine could prevent its own conversion to methionine. Isolation of homocysteine-resistant mutants which no longer require methionine indicates that inhibition by homocysteine and requirement for methionine are alternate manifestations of a common metabolic disorder. The temperature sensitivity of the requirement might well be due to an acceleration of metabolic activities which could lead to increased formation of endogenous homocysteine or increased conversion of homocysteine to an active inhibitor. Since homocysteine is not inhibitory to the average *E. coli* strain, the anomaly of strain WM-13 may be due to the possession of a deleterious reaction whereby homocysteine is diverted from its role as a precursor of methionine to the production of the inhibitor. Such a reaction would be either normally absent in the *E. coli* prototype or under suppressive control by a regulatory device. A mutation in WM-13 which would lead to the loss of the homocysteine by-pass would restore the typical *E. coli* growth pattern by allowing an economical utilization of homocysteine.

Both leucine and α-aminobutyric acid are structural analogues of homocysteine. As such, they could act as antimetabolites in activation of homocysteine and thus prevent formation of the inhibitor and conserve endogenous homocysteine for methionine formation. This would explain the ability of leucine and α-aminobutyric acid to support growth.

Summary. A strain of *Escherichia coli* (strain WM-13), isolated from clinical material, was unlike the average wild-type strains of *E. coli* in that it could not grow in a synthetic salts-glucose medium. Growth could be obtained only by supplementing the medium with either methionine or p-aminobenzoic acid. Except for partial stimulation by leucine and α-aminobutyric acid, none of the other naturally occurring amino acids or B vitamins could support growth. Growth requirement was evident only when cultivation was carried out at 37°C. At room temperature (21-24°C) full growth was obtained in the unsupplemented minimal medium, but addition of homocysteine again imposed a requirement for methionine or p-aminobenzoic acid. The temperature-sensitive growth requirement is apparently the result of a deleterious reaction, involving homocysteine as a substrate, which interferes with the function of p-aminobenzoic acid in the biosynthesis of methionine.

1. Dolt, M. L., *J. Inf. Dis.*, 1908, v5, 616.
2. Rowley, D., *J. Gen. Microbiol.*, 1953, v9, 37.
3. Hughes, I. M., Gots, J. S., *Bact. Proc.*, 1954, p48.
4. Gots, J. S., Chu, E. C., *J. Bact.*, 1952, v64, 537.
5. Strehler, B. L., *ibid.*, 1950, v59, 105.
6. Sankar, D. V. S., *Nature*, 1958, v181, 630.
7. Wang, C. H., King, T. E., Cheldelin, V. H., Christensen, B. E., *J. Biol. Chem.*, 1951, v188, 753.
8. Winkler, K. C., deHaan, P. G., *Arch. Biochem.*, 1948, v18, 97.
9. Shive, W., *Ann. N. Y. Acad. Sci.*, 1950, v52, 1212.

Received May 13, 1958. P.S.E.B.M., 1958, v98.