breath of rats which have either been given single oral doses of Antabuse (or diethyldithiocarbamate), or are subjects in chronic-feeding tests.

Detailed reports of these studies will be made later.

#### REFERENCES

- <sup>1</sup> C. E. Cox, H. D. SISLER, AND R. A. SPURR, Science, 114 (1951) 643.
- <sup>2</sup> P. W. DALE AND F. G. EBAUGH, Am. J. Med. Sci., 220 (1950) 103.
- <sup>3</sup> E. J. Conway, Microdiffusion Analysis and Volumetric Error, Crosby Lockwood and Son, Ltd., London (1947).
- <sup>4</sup> G. Domar, A. Fredga, and H. Linderholm, Acta Chem. Scand., 3 (1949) 1441.
- <sup>5</sup> H. LINDERHOLM AND K. BORG, Scand. J. Clin. Invest., 3 (1951) 96.

Received June 9th, 1952

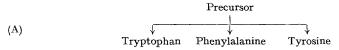
# SPARING EFFECTS IN THE BIOSYNTHESIS OF THE AROMATIC AMINO-ACIDS IN ESCHERICHIA COLI

by

## ERNST D. BERGMANN, SARAH SICHER, AND BENJAMIN E. VOLCANI

Weizmann Institute of Science, Rehovoth, and Scientific Department, Israeli Ministry of Defence, Tel-Aviv (Israel)

From experiments, mainly with multiply deficient mutants of *E. coli*, it has been concluded<sup>1</sup> that the bacteria synthesize tryptophan, phenylalanine and tyrosine from a common precursor:



On the other hand, it has been shown<sup>2</sup> that in the animal body phenylalanine is converted into tyrosine, and it has been assumed that in *E. coli* tryptophan is transformed into phenylalanine<sup>3</sup> and the latter to tyrosine<sup>4</sup>, so that a "straight" scheme appears possible:

(B) 
$$Precursor \rightarrow Tryptophan \rightarrow Phenylalanine \rightarrow Tyrosine \rightarrow Metabolite of Tyrosine$$

A decision between the two alternatives appears possible by the study of "sparing effects" in this system. Scheme (B), and only Scheme (B) demands that in a tryptophan-deficient mutant both phenylalanine and tyrosine will exert a sparing action on tryptophan—in a phenylalanine-deficient mutant only tyrosine on phenylalanine, whilst in a tyrosine-deficient mutant neither of the other amino acids should show a sparing effect. The experiments reported here have proved scheme (B) to be probable.

(i) A tryptophan-deficient mutant of E. coli (19-2), which did not grow on DL-phenylalanine or DL-tyrosine alone, gave full growth, when the combination of 12  $\gamma$ /ml of DL-tryptophan and 40  $\gamma$ /ml of DL-phenylalanine was applied, and an even more luxuriant growth than with tryptophan alone, when to 12  $\gamma$ /ml of DL-tryptophan 20  $\gamma$ /ml of L-tyrosine was added.

(ii) A phenylalanine-deficient mutant of  $E.\ coli$  (M83–5), which does not utilize DL-tryptophan alone and is also not significantly stimulated by the addition of DL-tryptophan to various levels of DL-phenylalanine (1, 4 and 10  $\gamma$ /ml, giving 2, 38 and 50 % of full growth), responded to L-tyrosine in the following manner: tyrosine alone did not stimulate growth, but when 600  $\gamma$ /ml of tyrosine was added to 2  $\gamma$ /ml of phenylalanine, the growth rate rose from 20 to 65 % of full growth. Larger quantities of tyrosine did not exert any additional effect.

(iii) In a tyrosine-deficient mutant (M83-9), DL-tryptophan (up to 1000  $\gamma$ /ml) did not show any effect, either alone or in combination with quantities of L-tyrosine which gave 20 or 50% of full growth, and DL-phenylalanine (600  $\gamma$ /ml) caused only very slight increases (5-12%) at 16 and 25% of full growth (produced by 1 and 2 $\gamma$ /ml of L-tyrosine).

Supporting evidence for the validity of the "straight" scheme (B) can be derived from analogous experiments with the inhibited mutants. As an example, the results obtained with p-aminophenylalanine (PAPA) as inhibitor<sup>5,6,7</sup> may be quoted.

(iv) The tryptophan-deficient mutant, the PAPA-inhibition of which is competitively reversed by DL-tryptophan, was stimulated to full growth already by small quantities of DL-phenylalanine or L-tyrosine, added at growth levels of o and 30% (i.e. in presence of 4 y/ml of DL-tryptophan and 200 and 40 y/ml of PAPA). Both phenylalanine and tyrosine exert thus a sparing effect on tryptophan.

(v) The phenylalanine-deficient mutant, which gives 14 and 30% of full growth in presence of 400  $\gamma$ /ml of PAPA and 8 and 14  $\gamma$ /ml of DL-phenylalanine, respectively, is stimulated up to 60 and 90% of full growth by addition of DL-tryptophan. 8 \(\gamma/\text{ml}\) of \(\overline{L}\)-tyrosine increased the growth from 27 to 100%, when added to 14  $\gamma$ /ml of DL-phenylalanine and 400  $\gamma$ /ml of PAPA, and 2  $\gamma$ /ml of L-tyrosine raised it from 10 to 95% of full growth, when employed in conjunction with  $8\gamma/ml$  of DL-phenylalanine and 400  $\gamma/ml$  of PAPA. Thus, the inhibitor stimulates the utilization of tryptophan by the mutant and enhances the sparing effect of tyrosine.

(vi) In the PAPA-inhibited tyrosine-deficient mutant, addition of pL-phenylalanine to suboptimal quantities of L-tyrosine gave better growth than L-tyrosine alone. In presence of 1600 \(\gamma/\text{ml}\) of PAPA, e.g., the combination of  $\gamma \gamma/ml$  of L-tyrosine and  $2 \gamma/ml$  of DL-phenylalanine gave 120% of the growth, a level which could be achieved with 10  $\gamma$ /ml of L-tyrosine. The inhibitor, therefore,

actually stimulates the cellular faculties of the bacterium.

In conclusion, it therefore seems possible to assume that, in addition to the "branched" scheme described by Davis1, a "straight" scheme in the synthesis of the aromatic amino acids also exists.

A detailed account of these experiments including a study of other inhibitors and of a triply deficient mutant of E. coli, will be published elsewhere.

#### ACKNOWLEDGEMENT

The mutants used in this investigation have been kindly supplied by Dr B. D. DAVIS.

#### REFERENCES

- <sup>1</sup> B. D. Davis, Experientia, 6 (1950) 41.
- <sup>2</sup> See, e.g., S. Undenfriend, J. Cooper, and C. T. Clark, Federation Proc., 101 (1951) 0262. <sup>3</sup> E. Beerstecher Jr. and W. Shive, J. Biol. Chem., 164 (1946) 52.
- 4 E. BEERSTECHER Jr. AND W. SHIVE, J. Biol. Chem., 167 (1947) 49.
- <sup>5</sup> J. H. Burckhalter and V. C. Stephens, J. Am. Chem. Soc., 73 (1951) 56.
- <sup>6</sup> E. Frieden, H. C. Stansel, and K. Dittmar, Federation Proc., 10 (1951) 184.
- <sup>7</sup> B. E. Volcani, S. Sicher, and E. D. Bergmann, unpublished results.

Received April 27th, 1952

# LE RÔLE DU NOYAU CELLULAIRE DANS LES OXYDATIONS ET LES PHOSPHORYLATIONS

par

## J. BRACHET

Laboratoire de Morphologie animale, Faculté des Sciences de l'Université libre de Bruxelles (Belgique)

Nous avons montré récemment<sup>1</sup> que la consommation d'oxygène de fragments énucléés d'amibes se maintient à son taux normal pendant une dizaine de jours; rapprochant ce fait d'observations de Mazia et Hirshfield établissant que l'énucléation diminue fortement et rapidement la pénétration du 32P dans les amibes, nous avions conclu que l'enlèvement du noyau provoquerait une interruption dans le couplage entre les oxydations et les phosphorylations.