Short Communications and Preliminary Notes

THE PRODUCTION OF CARBON DISULFIDE FROM TETRAETHYLTHIURAM DISULFIDE (ANTABUSE) BY RAT LIVER

by

CARTER D. JOHNSTON AND CLAUDIA S. PRICKETT*

Division of Pharmacology, Food and Drug Administration
Federal Security Agency, Washington, D.C. (U.S.A.)

COX, SISLER AND SPURR[1] recently reported that tetramethylthiuram disulfide was enzymatically decomposed by spores and mycelia of several fungi, with the production of a volatile compound identified as CS_2. This finding prompted us to investigate the action of rat liver homogenates on tetraethylthiuram disulfide (Antabuse), a compound of current interest in the therapy of chronic alcoholism[2]. Such homogenates also decomposed Antabuse with the formation of CS_2.

The reaction was conducted in the outer chamber of a CONWAY microdiffusion unit[3]. The water-insoluble Antabuse was most easily added to the liver as an alcoholic solution (0.07 M), using a volume calculated to give 5 μ moles of the compound per 250 mg fresh weight of tissue, and contained in 1 ml of homogenate. The balance of 2 ml final volume of incubation mixture consisted of the other additions as described below.

Liberated CS_2 was trapped in a dibutylphthalate solution of dimethylamine, triethanolamine, and Cu^{++} in the inner well of the unit. The contents of the well were transferred to a colorimeter tube and diluted to volume with ethanol. The optical density of the yellow cupric dimethyldithiocarbamate solution was measured at 440 mμ with a Beckman model B spectrophotometer. From 0.1 to 1 μ mole of CS_2 per 10 ml final volume may be determined in this manner with an error of $\pm$ 2 %.

CS_2 was also identified independently by trapping it in alcoholic KOH and precipitating the yellow cupric xanthate after acidification and addition of Cu^{++}.

Homogenates heated for 30 minutes at 100° lost their ability to decompose Antabuse, and extensive dialysis also destroyed the activity.

The decomposition takes place in two steps: (1) the reduction of Antabuse to diethyldithiocarbamate (DEDTC) (which has been reported previously in blood and urine in *in vivo* studies[4,5] and confirmed in this laboratory), and (2) the breakdown of the DEDTC to CS_2 and diethylamine. The rate of the second step is dependent on pH, since the dithiocarbamate ion first hydrolyses to the unionized acid which breaks down spontaneously. From buffer experiments it could be shown that the rate of CS_2 formation at pH 7.3, although quite low, was directly proportional to the concentration of DEDTC.

It was consequently of interest to find that CS_2 production was increased by triphosphopyridine nucleotide (TPN, $3.5 \cdot 10^{-5}$ M) and glucose-6-phosphate (0.01 M), in the presence of Mg (0.02 M), and nicotinamide (0.02 M), using a phosphate buffer at pH 7.3. In a typical experiment 0.7 μ moles of CS_2 per hour was produced from 5 μ moles of Antabuse, compared with 0.16 μ moles of CS_2 in the abscence of TPN and glucose-6-phosphate. When glutamate (0.01 M) and diphosphopyridine nucleotide (10^{-4} M) were substituted in the reaction mixture 0.21 μ moles of CS_2 were liberated. The actual incubation time was 30 minutes.

An attractive hypothesis is that the Antabuse is enzymatically reduced by $TPNH_2$ (from the glucose-6-phosphate dehydrogenase system), with the result that sufficiently high concentrations of DEDTC are built up to give an accelerated overall CS_2 production.

In vivo experiments carried out by one of us (C.S.P.) have also demonstrated CS_2 in the exhaled

* Part of this material is from a thesis submitted to the Graduate School of Georgetown University, Washington, D.C., in partial fulfilment of the requirements for the Ph. D. degree.

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

1 C. E. Cox, H. D. Sisler, and R. A. Spurr, *Science*, 114 (1951) 643.
2 P. W. Dale and F. G. Ebaugh, *Am. J. Med. Sci.*, 220 (1950) 103.
3 E. J. Conway, *Microdiffusion Analysis and Volumetric Error*, Crosby Lockwood and Son, Ltd., London (1947).
4 G. Domar, A. Fredga, and H. Linderholm, *Acta Chem. Scand.*, 3 (1949) 1441.
5 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[1] that the bacteria synthesize tryptophan, phenylalanine and tyrosine from a common precursor:

Precursor

(A) ↓ ↓ ↓

Tryptophan Phenylalanine Tyrosine

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

(B) Precursor → Tryptophan → Phenylalanine → Tyrosine →
→ 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 γ/ml of DL-tryptophan and 40 γ/ml of DL-phenylalanine was applied, and an even more luxuriant growth than with tryptophan alone, when to 12 γ/ml of DL-tryptophan 20 γ/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 γ/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 γ/ml of tyrosine was added to 2 γ/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 γ/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 γ/ml) caused only very slight increases (5–12%) at 16 and 25% of full growth (produced by 1 and 2 γ/ml of L-tyrosine).