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1,2-Dibromoethane—Effect on the metabolism and ultrastructure of *Escherichia coli*

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1,2-DIBROMETHANE (1,2-DBE) has a wide household and industrial distribution. It is used most extensively as a pesticide and as an anti-knock agent in leaded gasolines. Recently the mutagenicity[1–5]* of 1,2-DBE and its ability to alkylate the guanine moiety*† of DNA were demonstrated. In view of the potential health hazard created by a mutagenic environmental agent, the effects of 1,2-DBE on living cells was further investigated. The present report is concerned with the effects of this agent on the growth of *Escherichia coli*.

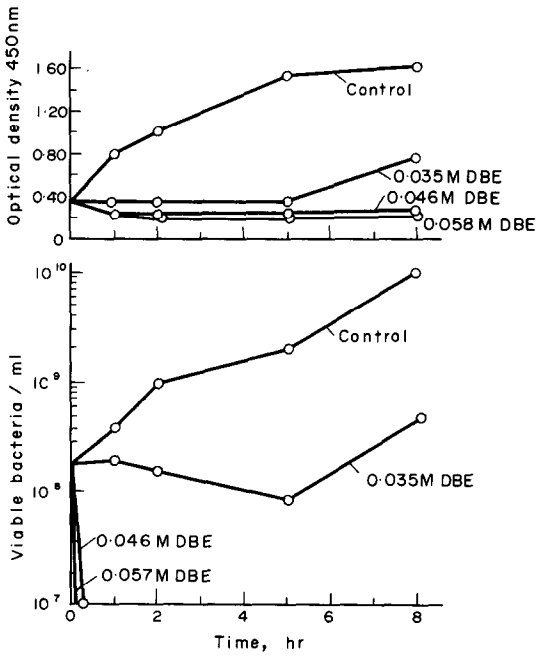

FIG. 1. Effect of DBE on growth of bacteria. Bacteria (*E. coli* K-12) in medium HA[10] were brought to the exponential growth phase, at which time portions of the cultures were distributed into flasks containing premeasured amounts of DBE. The cultures were incubated at 37° with aeration and at intervals portions of each culture were withdrawn for determination of turbidity (450 nm) and enumeration of viable cells.

* H. Brem, A. B. Stein and H. S. Rosenkranz, *Cancer Res.*, in press.
† G. A. Carden, III, H. Brem and H. S. Rosenkranz, manuscript in preparation.

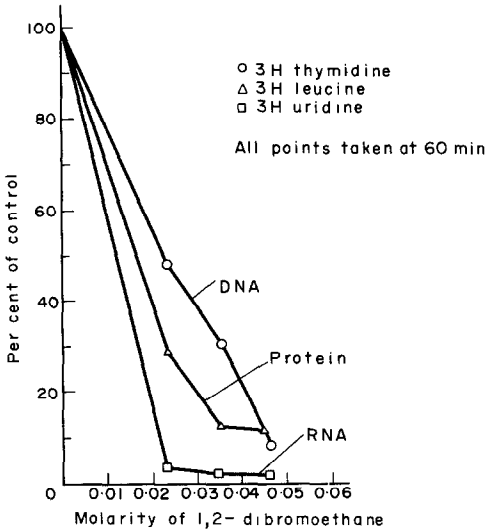

FIG. 2. Effect of DBE on the metabolism of *E. coli*. Bacteria in medium HA[10] were brought to the exponential growth phase, at which time the bacteria were distributed into flasks containing radioactive precursors and various amounts of DBE. At the end of 1 hr of incubation at 37°, samples were withdrawn for determination of radioactivity incorporated into DNA, RNA and proteins. The precursor for DNA was ^{3}H-thymidine ($6{\cdot}9 \times 10^{-8}$M, 11·3 Ci/m-mole); "cold" uridine ($1{\cdot}4 \times 10^{-4}$M) was also added to inhibit and repress thymidine phosphorylase;[15] for RNA, ^{3}H-uridine (2×10^{-4}M, 2 Ci/m-mole) and, for proteins, ^{3}H-lysine ($5{\cdot}4 \times 10^{-5}$M, 0·15 Ci/m-mole) were used.

The various techniques used in the study of metabolism and ultra structure have been previously described.[6-9] Additional experimental details are given in the tables and legends to the figures.

Exposure of bacteria to levels of DBE exceeding 0·04 M resulted (Fig. 1) in cellular death accompanied by cell lysis (as determined by decreased turbidities of the cultures). A DBE concentration of 0·035 M was bacteriostatic for the first hr and bactericidal for the next 4 hr. After 5 hr of incubation, bacterial growth resumed (Fig. 1). Resumption of growth was probably due to exhaustion of the DBE from the medium and multiplication of the survivors. When DBE was replenished at 4 hr, bacterial growth did not resume. Of all the metabolic processes tested, RNA synthesis was the most susceptible to inhibition by DBE (Fig. 2), followed by inhibition of protein and DNA synthesis. Thus at 0·023 M DBE, RNA, protein and DNA synthesis were inhibited by 97, 74 and 52 per cent respectively. The DNA of cells exposed to DBE was not extensively degraded as determined by acid precipitability (Table 1).

Bacteria exposed to 1,2-DBE undergo significant ultrastructual alterations (Fig. 3). There is a decrease in the number of ribosomes; those that remain are diminished in size. The typical convoluted cell wall

TABLE 1. STABILITY OF DNA OF DBE-TREATED BACTERIA*

Time (min)	Radioactivity (cpm/ml) Control	DBE-treated
0	30,492	28,810
60	28,778	28,820
130	27,312	28,494

* Bacteria were grown for several generations in the presence of ^{3}H-thymidine (0·5 μCi/ml) and cold uridine ($1{\cdot}4 \times 10^{-4}$M). At the beginning of the experiments, the cells were distributed into flasks containing premeasured amounts of DBE (0·035 M). At intervals, portions from each culture were withdrawn for determination of radioactivity remaining alkali-stable and acid-precipitable.

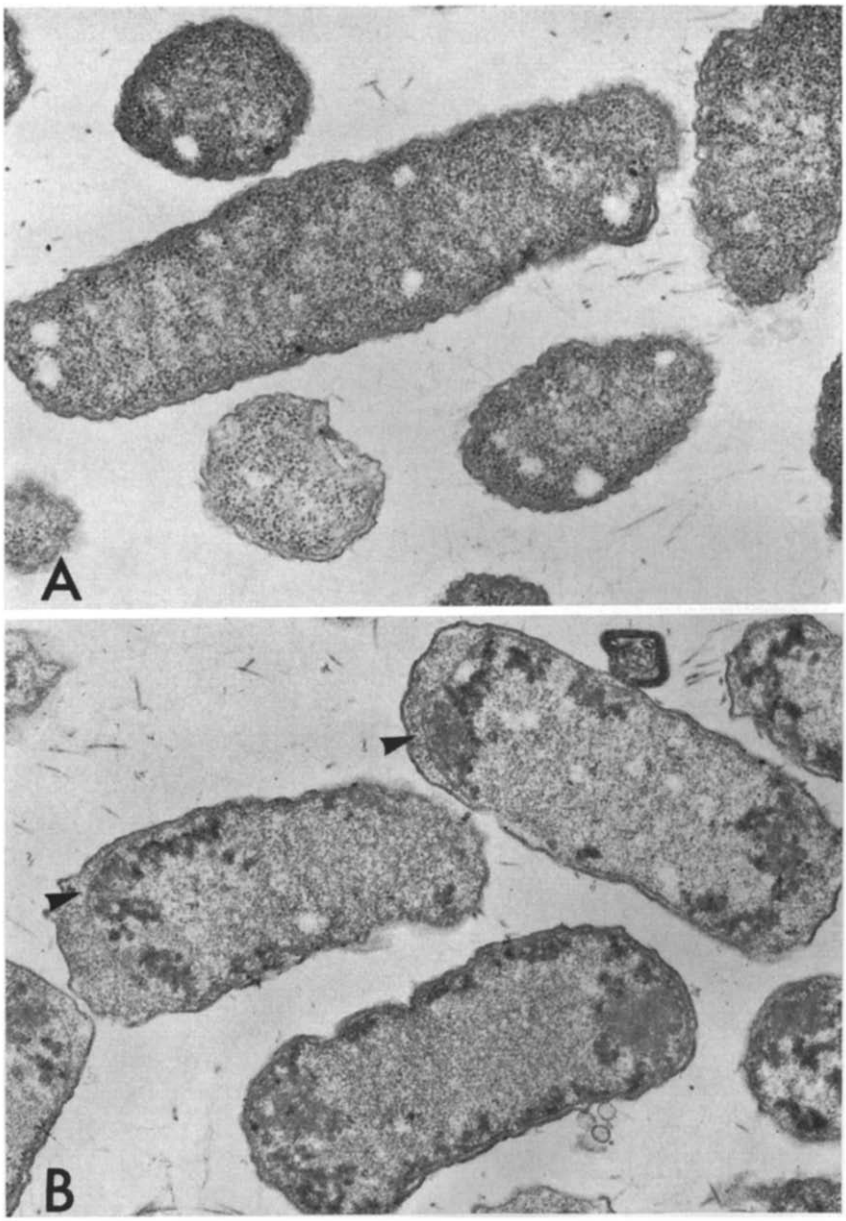

FIG. 3. Appearance of normal bacteria (A) and of bacteria exposed to 0·035 M DBE for 3 hr (B). The arrows show retraction of the cytoplasmic membrane from the cell wall (×37,400).

of Gram-negative bacteria becomes smooth in appearance. In places, the cytoplasmic membrane is clearly separated from the cell wall and electron-dense material has accumulated at the periphery of the cell, at or near the cytoplasmic membrane.

The present observations suggest that 1,2-DBE acts at the cell envelope. This finding is in keeping with the role of DBE as a lipid solvent. Since it is now known that many of the cell's regulatory mechanisms operate from the cytoplasmic membrane—a structure dramatically affected by DBE—it is not surprising that an effect of the drug on these structures also affects macromolecular biosynthetic processes. Although the present findings suggest that DBE acts on the external cell structure, they do not exclude the possibility that other cellular sites are also involved. The nature of the material accumulating at or near the cytoplasmic membrane of DBE-treated cells remains to be determined; however, its electron-dense character suggests that it may be a nucleic acid, perhaps the DNA that accumulates while RNA and protein syntheses are blocked. Although 1,2-DBE is an alkylating agent[12–15] with mutagenic[1–5,*] properties, the present findings indicate that this is not the sole basis of its biological activity. Many of the effects of DBE on the external cell structure can be explained on the basis of its lipophilic properties. Because of this quality and its alkylating potential, it would seem that DBE might not be excluded from the brain barrier. In view of the strong positive correlation between ability to induce mutations and to cause cancer, it might be hypothesized that 1,2-DBE possesses a neurotropic carcinogenic potential. This possibility deserves further consideration because of the widespread use of 1,2-DBE. The level of 1,2-DBE present in the environment is presumably much lower than those used in the present study; any extrapolation of the present data to humans exposed to environmental levels of this agent must, therefore, be tempered by this concentration difference.

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