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Mutagenesis of *Escherichia coli* (CSH50) by Asbestos (41954)

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Abstract. The mutagenic character of richterite asbestos was detected in *Escherichia coli* strain CSH50, using a modified Ames test. Two sets of experimental plates which contained naturally occurring asbestos were used, to one set of which S-9 rat liver homogenate was added. Control plates contained no asbestos. Mutant colonies appeared in significantly greater frequency in both experimental sets as compared with controls, and experimental plates with rat liver homogenate contained the greatest number of mutant colonies. It is postulated that enzymes contained in rat liver metabolize some unknown mutagen introduced with the natural asbestos sample other than the inert richterite fibers themselves. This finding, together with the unlikelihood that bacterial cell walls could be easily penetrated by asbestos fibers, tends to negate fiber penetration as the mechanism of mutagenesis. The mutagenic mechanism operative in *E. coli* (CSH50) may be applicable to animal cell systems.

The United States Department of Labor Occupational Safety and Health Administration designated asbestos as a human carcinogen in 1975 (1). Subsequent hypotheses have been proposed to explain how asbestos causes mutation in animal cells, but few have been studied extensively. Because of its inert nature, asbestos cannot interact chemically with cells. It is furthermore unclear why other fibrous materials much like asbestos, such as fiberglass, are not carcinogenic.

One possible mutagenic mechanism is that of fiber penetration through the cell membrane resulting in physical damage to DNA. It appears that fiber size and shape are critical to achieve mutation. Longer, thicker fibers cause fibrosis of the lung, whereas finer fibers are more strongly tumorigenic (2). Stanton and others (3) have shown that fibers must have diameters < 1.5 μm and lengths > 8 μm to be tumorigenic. Other fiber forms are normally not produced in these dimensions. Pernis and Governa (4) have also suggested that cell contact with a fiber induces redistribution of membrane immunoglobulins and other glycoprotein components. This association may disturb cell regulation and cause carcinogenesis without actual fiber penetration.

An additional possible mutagenic mechanism may depend upon impurities found in asbestos samples. Carcinogenic hydrocarbons or heavy metals such as chromium or nickel, occurring naturally or derived from processing, may adhere to the surfaces of fibers (2). A final possible mutational mechanism is based on the increased incidence of cutaneous warts in persons who handle asbestos, suggesting that asbestos may facilitate virally induced carcinogenesis (5).

The study reported here sought to determine whether mutagenesis occurred in *Escherichia coli* (CSH50) after exposure to asbestos, in a modified Ames test. This type of investigation was performed, in part, to minimize fiber penetration as a means of stimulating mutagenesis, since the greater thickness of the bacterial cell wall, in comparison to the animal cell membrane, was viewed as a means of substantially discouraging mechanical penetration. If mutations were amplified in bacteria after asbestos exposure, some mechanism other than mechanical penetration of fibers would thus appear to be operative. The possibility that heavy metals present in asbestos could be responsible for its associated carcinogenesis, though unlikely in bacteria (6), has not been ruled out by the study reported herein. Furthermore, hydrocarbons or other undetermined impurities present in the natural asbestos could account for any mutagenic properties of asbestos observable in bacteria.

Materials and Methods. The asbestos sample used in this investigation was obtained from the Allamore Talc District of west Texas. It is an amphibole mineral called richterite, an alkali-rich analogue of tremolite (7). The richterite fibers were taken from the natural rock, ground by hand with mortar

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and pestle, and then autoclaved before introduction into media plates. An electron micrograph of the prepared richterite asbestos appears in Fig. 1.

A modified Ames test (8) was used to detect asbestos-induced mutations. The procedure was similar to that used by Yavornitzky and Trzeciak (9) in their studies of the mutagenic effects of hair dyes and saccharin on *E. coli* (CSH50). Instead of *Salmonella typhimurium* as used in the original Ames test (8), *E. coli* (CSH50) served as the test organism in the study reported here. The strain was obtained from Donald S. Dean of Baldwin-Wallace College, Berea, Ohio. *E. coli* (CSH50) was used because it bears no

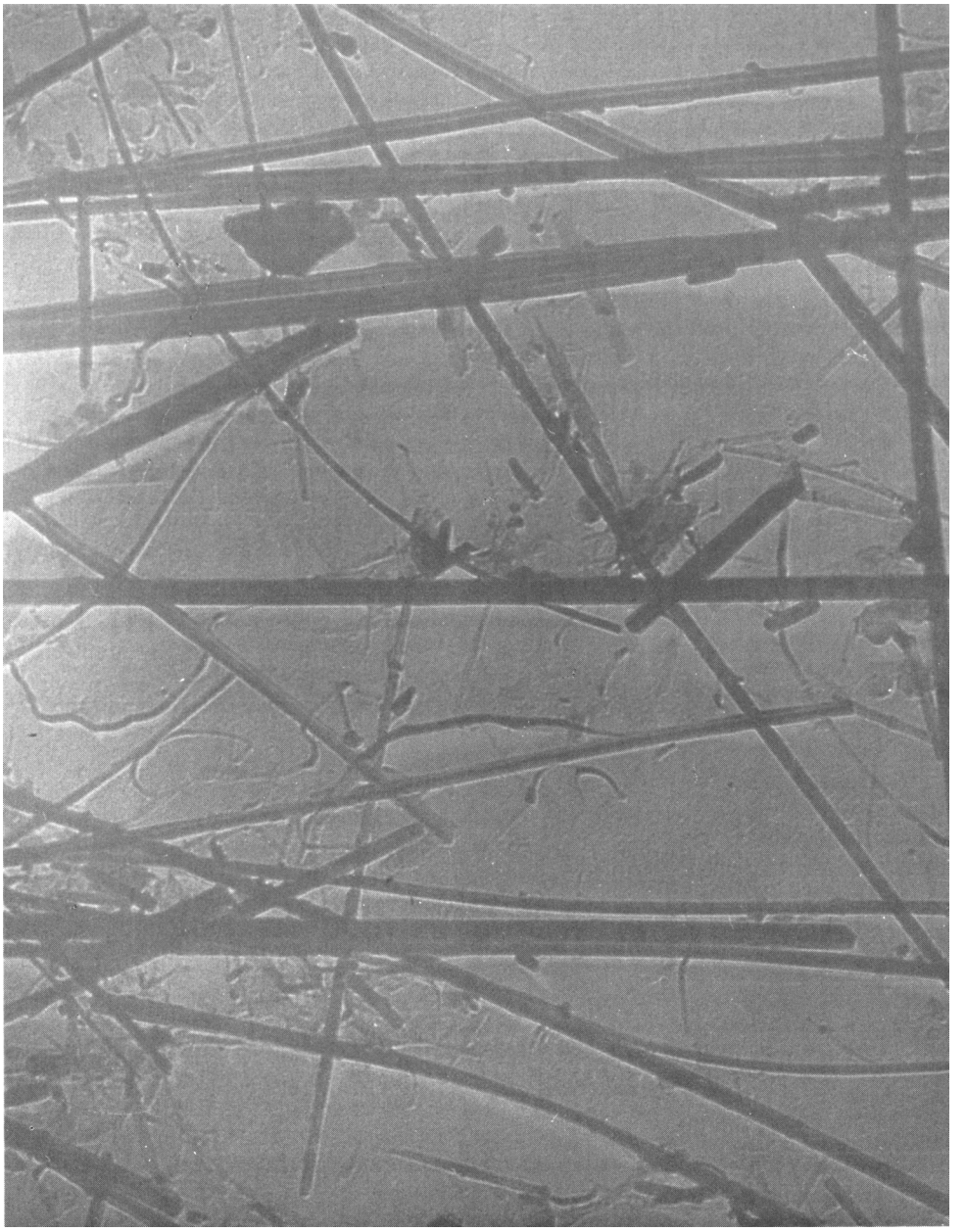

FIG. 1. Electron micrograph of richterite asbestos. ×52,500.

mutation which alters the structure of the cell wall unlike the strain used by Ames. A fully intact cell wall is significant to this study to reduce the possibility that any resulting mutation could be due to fiber penetration. *E. coli* (CSH50) has a phenotype of Ara^-, Lac^-, Pro^-, Str^r, B_1^- (9). This strain bears a deletion mutation which prevents the production of an enzyme required for the synthesis of proline and can only be grown on a medium supplemented with proline. On rare occasions the mutant strain undergoes spontaneous mutation restoring production of proline. This mutation is detected by plating the revertant bacteria on a medium lacking proline, with resulting growth. If mutagens are added to the proline-deficient medium, revertant colonies occur with much higher frequency. *E. coli* (CSH50) regains proline prototrophy when a mutation enables it to bypass the block in proline biosynthesis via the arginine pathway, as described by Kuo and Stocker (10).

The S-9 rat liver homogenate, required for supplying enzymes responsible for genesis of mutagenic chemical forms as originally described by Ames (8), was prepared according to the procedure outlined by Yavornitzky and Trzeciak (9).

Inocula of the bacterium were added to three different sets of petri plates. The control plates all lacked asbestos but half contained rat liver homogenate (0.5 ml of a 1:15, v:v, homogenate prepared in phosphate buffer solution). Two sets of experimental plates were used. One contained asbestos (0.01 g/plate) without rat liver homogenate; the other contained both asbestos and rat liver homogenate. Each plate was poured so as to have separate bottom and top agar layers. The bottom agar contained a minimal medium for *E. coli* (CSH50), consisting of a 20% glucose solution, Bonner–Vogel E medium, and 0.5 ml of a 1% solution of vitamin B_1, but lacked proline. The top agar contained 0.5 ml of 0.5 m*M* proline solution, sufficient for bacterial growth for a few generations during which mutagenesis could occur. The top agar also contained richterite asbestos and rat liver homogenate in appropriate experimental plates. Following the addition of the rat liver homogenate and the ground richterite asbestos to the melted top agar, inocula containing approximately 1.6×10^6 cells in 0.1 ml of nutrient broth were then added to the top agar. The top agar was then poured and swirled onto solidified bottom agar. After 2 to 4 days of incubation at 37°C, spontaneous and chemically induced mutants appeared. The easily visible colonies were larger than those of the nonmutated strain. The procedure was verified by using saccharin, in concentrations ranging from 1.0×10^{-4} to 1.0×10^{-2} mg/ml, as the mutagenic agent before application of asbestos.

Results. Four trials were conducted, each with 12 petri plates. The results of these trials are summarized in Table I. Mutant colonies appeared with significantly greater frequency in both experimental sets in comparison to the control set; the experimental set containing rat liver homogenate showed the greatest number of mutant colonies.

Statistical differences between sets of data were tested by standard Student *t* tests (11). The resultant *t* values, means, variances, and *P* values are shown in Table II. All three sets

TABLE I. RESULTS OF MUTAGENIC ASBESTOS ON *E. coli* (CSH50)

Plate	Plate content	Mutant colonies by trial			
		1	2	3	4
Control set					
1	*E. coli*	11	26	12	15
2	*E. coli*	8	13	20	9
3	*E. coli* Rat liver	19	17	12	13
4	*E. coli* Rat liver	16	14	10	22
Expt set 1					
5	*E. coli* Asbestos	38	43	46	28
6	*E. coli* Asbestos	29	32	31	48
7	*E. coli* Asbestos	41	38	35	42
8	*E. coli* Asbestos	33	25	39	30
Expt set 2					
9	*E. coli* Asbestos Rat liver	189	173	118	153
10	*E. coli* Asbestos Rat liver	132	111	149	167
11	*E. coli* Asbestos Rat liver	96	211	203	183
12	*E. coli* Asbestos Rat liver	126	146	121	124

TABLE II. STATISTICAL ANALYSIS OF DATA BY STUDENT'S *t* TEST

	$\bar{x}$	S^2	t^a		
Control set					
Without liver	14.3	36.5			
With liver	15.4	15.4	7.70		
Expt set 1	36.1	46.1		10.89	
Expt set 2	150.1	1190.8			12.97

[a] All *t* values correspond to $P < 0.001$.

of data were found to be significantly different, with *P* values of <0.001 in all comparisons between experimental and control sets.

Discussion. The findings indicate that richterite asbestos in its natural state is mutagenic for *E. coli* (CSH50) under the experimental conditions described. Further examination may elucidate mutational mechanisms. Fiber penetration should have been significantly hindered in this experimental system by the bacterial cell wall, so that mutational changes could more likely be attributed to impurities in the asbestos sample or to some other mechanism of induction. These naturally occurring impurities are likely to be hydrocarbon contaminants as mentioned earlier (2).

Experimental set 2, with rat liver homogenate, is of particular interest. Because of the inert nature of asbestos, it can be assumed that rat liver homogenate must affect some chemical substance other than asbestos fibers in the sample, resulting in metabolic byproducts responsible for mutation. This assumption does not exclude facilitation of the action of such chemical mutagens by asbestos.

This study does not eliminate the possibility that mutation has resulted from fiber penetration. Fiber penetration could have been responsible for the increased number of mutant colonies noted in Experimental set 1, but it is equally possible that some less potent chemical mutagen accompanying the asbestos may have been responsible. The study clearly indicates that fiber penetration is likely not the primary mutagenic mechanism in *E. coli* and that at least one other mechanism is operating. The origin of asbestos-induced carcinogenesis in animal systems may be attributable to factors other than fiber penetration, the induction model currently favored.

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