

# Effects of Ingested Chrysotile on DNA Synthesis in the Gastrointestinal Tract and Liver of the Rat

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The effects of chrysotile on DNA synthesis in the gastrointestinal tract of the rat were studied by measuring the uptake of tritium-labeled thymidine; whole stomach, small intestine, colon liver were removed, cleaned, and homogenized and the DNA was isolated and assayed for tritium label.

A dose-response study indicated that 2 weeks after a 5 mg/kg dose of chrysotile, DNA synthesis was increased in the small intestine and colon and reduced in the liver; synthesis was reduced in the small intestine 2 weeks after a 500 mg/kg dose. Following a 100 mg/kg dose of chrysotile, a transient increase in DNA synthesis was noted in the stomach and small intestine at 1 and 7 days, respectively, besides increased synthesis in the colon from 28 to 63 days. These data suggest that asbestos penetrates the gastrointestinal mucosa and influences regulation of DNA synthesis in the gastrointestinal tract.

## Introduction

Recent studies have revealed the presence of asbestos fibers, both chrysotile and amphiboles, in samples of city drinking water and in processed beverages from several countries (1, 2). Possible sources of such contamination include asbestos-cement conduits, widely used in city water mains, and asbestos filters, used in the clarification of beverages.

There is ample evidence that asbestos fibers migrate away from the site of initial entry in the body. Studies with rats have demonstrated that ingested chrysotile fibers penetrate the intestinal mucosa, where they have been identified by electron microscopy (3), and migrate into the blood and surrounding viscera, especially the omentum (4). Subcutaneous injection of asbestos in mice is followed by its extensive dissemination and development of pleural

mesotheliomas (5). Further studies have demonstrated migration of asbestos fibers away from their injection site to sites of apparently selective accumulation in lymphoid tissues and also in the pleura and peritoneum, where inflammatory and proliferative changes as well as mesotheliomas developed subsequently (6, 7).

An excess of esophageal, gastric, colonic, and rectal cancer has been reported for insulation workers occupationally exposed to chrysotile (8). Exposure of these organs may have resulted from hematogenous or other dissemination of asbestos from the lung or from swallowing chrysotile-laden sputum. Recovery of labeled crocidolite in the feces of rats has been demonstrated shortly after exposure by inhalation (9).

Perhaps the earliest indication of neoplastic transformation is the deregulation of cell proliferation followed by an acceleration of DNA synthesis (10). The purpose of this preliminary study was to determine the early effects of ingested chrysotile on the steady-state DNA synthesis in the gastrointestinal tract and

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liver of the rat. Particular emphasis was directed to the stomach and the colon in view of the relatively high incidence of cancer in these organs following occupational exposure to asbestos (8).

### Materials and Methods

UICC standard chrysotile A was prepared by using standard techniques, by grinding an aqueous suspension in a Waring Blender for 30 min followed by an ethanol rinse and air drying (11). Electron microscopic examination of the asbestos preparation demonstrated a varied fiber composition, including apparently single fibers besides partially splayed bundles (Fig. 1); a wide range of fiber lengths was also evident.

Young adult male Charles River CD strain rats, weighing 300–450 g, were divided into eight test groups, each of five animals, and a combined control group of 21 animals. Chrysotile suspensions in normal saline were

administered by gavage on a single occasion in volumes of 1.4 to 1.6 ml, depending on body weight; controls received saline only. In a dose-response study, three groups of rats were gavaged with 5, 100, or 500 mg/kg of chrysotile and sacrificed 2 weeks subsequently. In a time-response study, the rates of DNA synthesis were examined in five groups of rats at sequential intervals from 1 day to 9 weeks following gavage with 100 mg/kg chrysotile.

Animals were given food and water *ad libitum* until 8:00 A.M. of the day of sacrifice. All thymidine uptake studies were performed between 9:00 A.M. and 1:00 P.M. One hour before sacrifice, animals were injected intraperitoneally with thymidine-methyl- $H^3$  (specific activity 5 Ci/mmol, Amersham/Searle) at a dosage of 0.25  $\mu$ Ci/g body weight. DNA synthesis was determined by measurement of the amount of tritiated thymidine incorporated into tissue DNA during 1 hr.

After sacrifice, the gastrointestinal tract,

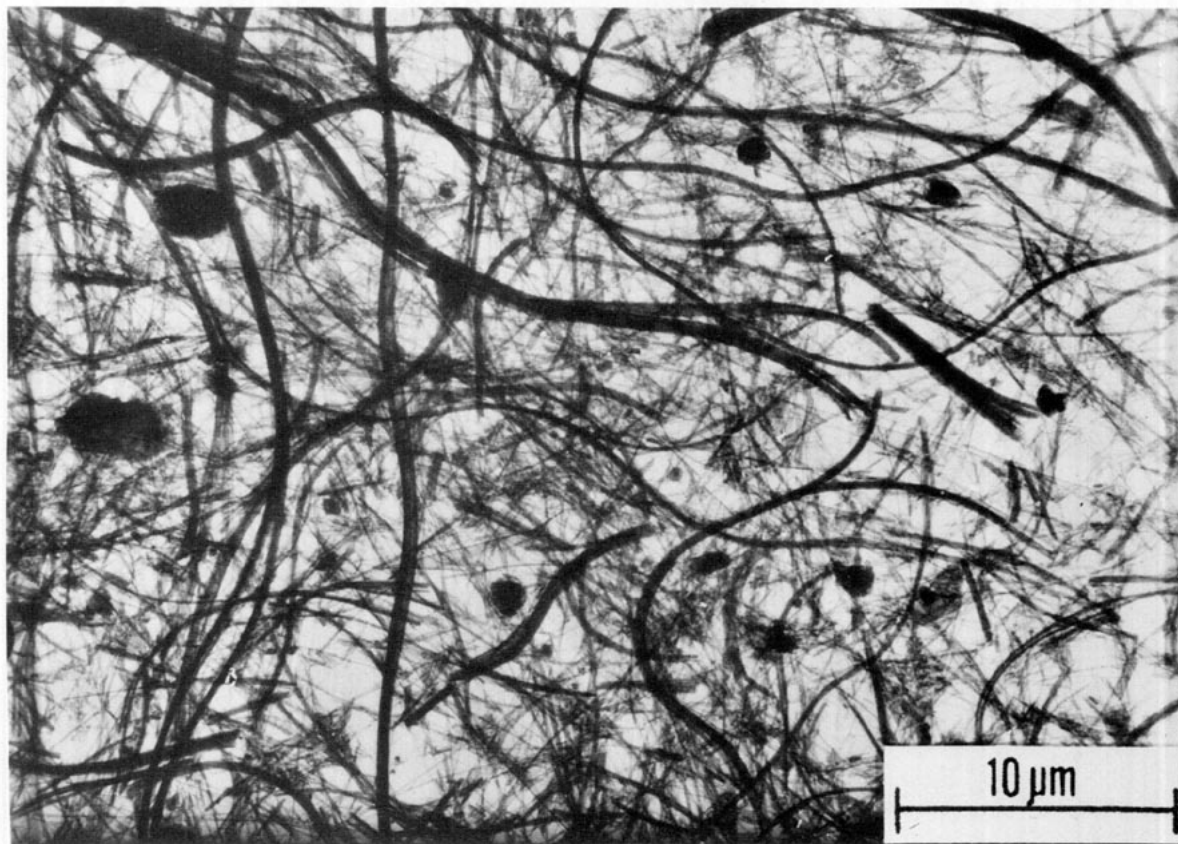


FIGURE 1. Electron micrograph of the chrysotile preparation 2150X

liver, and other organs were removed. Immediately after removal, the stomach and small and large intestines were thoroughly rinsed free of their contents with normal saline. Then, the entire stomach, small intestine from the pylorus to cecum, large intestine from the cecum to the rectum, and liver were homogenized in 0.32M buffered sucrose (20% w/v) with a motor-driven Teflon pestle and glass homogenizer. DNA was isolated from triplicate aliquots of homogenate by modification of previously described procedures (12); homogenates were washed twice with cold 0.2M PCA and once with 95% ethanol, followed by alkali digestion overnight with 1N NaOH to obtain a relatively RNA-free pellet. The DNA pellet was twice hydrolyzed with 0.5M PCA at 70°C for 20 min, centrifuged at 14,000g, and the supernatant filtered through Whatman #1 paper to exclude particulate matter which would otherwise affect counting accuracy. Deoxyribose was then quantitated by the diphenylamine reaction (13). The tritium label was quantitated by liquid scintillation analysis of aliquots of the 0.5M PCA extracts prepared in a cocktail containing 80 ml Spectrafluor PPO-POPOP Liquid Scintillator (Amersham/Searle), 480 ml Triton X-100 (Amersham/Searle), and 1440 ml scintillation grade toluene (Fisher Scientific). Counting efficiency of the Nuclear Chicago Liquid Scintillation System 6801 was determined daily by internal standardization with toluene-<sup>3</sup>H (New England Nuclear).

## Results

No mortality or significant weight loss occurred during the study.

The effects of a single dose of chrysotile, at 5, 100, or 500 mg/kg, on DNA synthesis 2-weeks subsequently are indicated in Figures 2-5. Thymidine incorporation in the stomach wall at these dosages did not differ significantly from control values ( $p > 0.10$  (Fig. 2). Thymidine uptake 2 weeks after a 5 mg/kg dose was significantly greater in the small intestine ( $p < 0.05$ ) and colon ( $p < 0.01$ ), but lower in the liver ( $p < 0.02$ ) than in the corresponding controls (Figs. 3-5). Thymidine uptake in the small intestine (Fig. 3) was lower 2 weeks following a 500 mg/kg dose than in controls ( $p < 0.10$ );

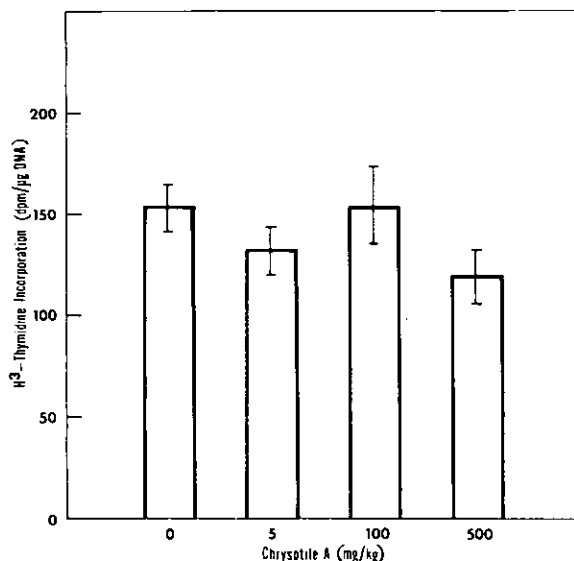


FIGURE 2. Thymidine uptake in rat stomach 2 weeks after chrysotile treatment. Each bar represents the mean of 5 (experimental) or 21 (control) animals  $\pm$  SEM.

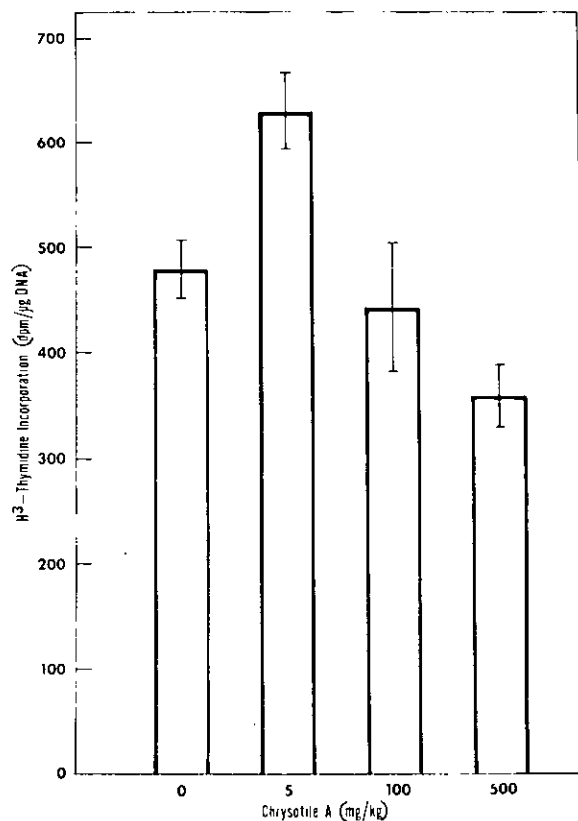


FIGURE 3. Thymidine uptake in rat small intestine two weeks after chrysotile treatment. Each bar represents the mean of 5 (experimental) or 21 (control) animals  $\pm$  SEM. ( $p < 0.05$ ) for the 5 mg/kg dose and the 500 mg/kg dose ( $p < 0.10$ ).

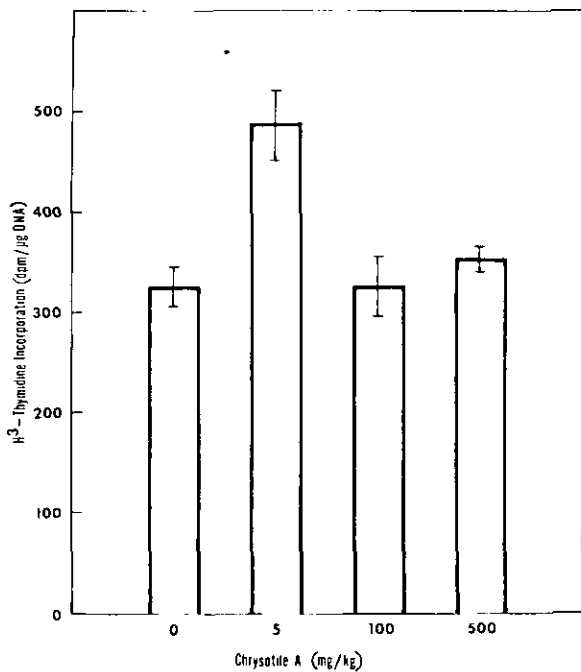


FIGURE 4. Thymidine uptake in rat colon at two weeks after chrysotile treatment. Each bar represents the mean of 5 (experimental) or 21 (control) animals  $\pm$  SEM. The difference from control is significant ( $p < 0.01$ ) for the 5 mg/kg dose.

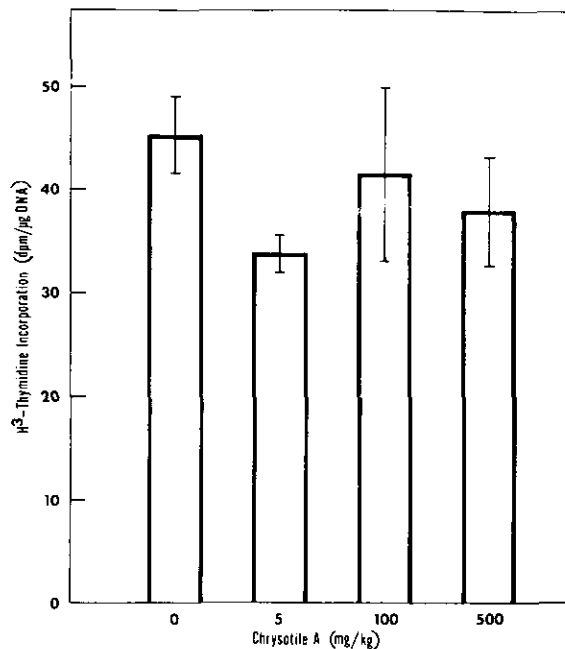


FIGURE 5. Thymidine uptake in rat liver two weeks after asbestos treatment. Each bar represents the mean for 5 (experimental) or 21 (control) animals  $\pm$  SEM. The difference from control is significant ( $p < 0.02$ ) for the 5 mg/kg dose.

however, results for the 100 mg/kg dose in the small intestine and for the 100 and 500 mg/kg dosages in liver, stomach, and colon did not differ significantly from controls.

Thymidine uptake in the stomach, small intestine, and liver 1, 7, 14, and 28 days after a single 100 mg/kg dose of chrysotile and in the colon at 1, 7, 14, 28, and 63 days is shown in Figures 6–9. Incorporation in the stomach one

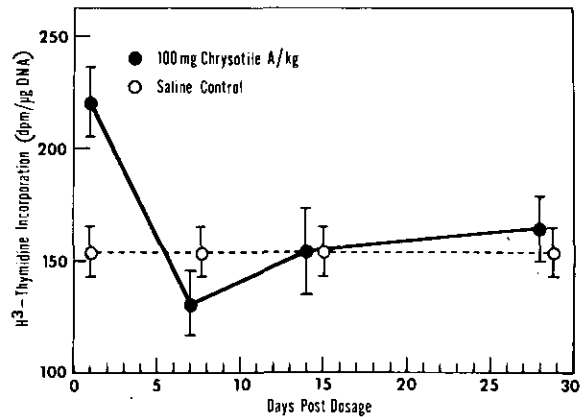


FIGURE 6. Thymidine uptake in rat stomach following a single 100 mg/kg dose of chrysotile. Each point represents the mean for either 21 (control) or 5 (experimental) animals  $\pm$  SEM. The difference from control is significant ( $p < 0.01$ ) for the 1st day after treatment.

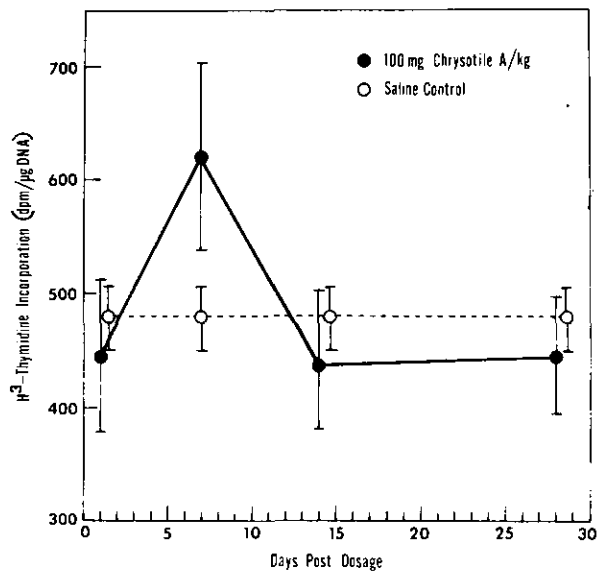


FIGURE 7. Thymidine uptake in rat small intestine following a single 100 mg/kg dose of chrysotile. Each point represents the mean for either 21 (control) or 5 (experimental) animals  $\pm$  SEM. The difference from control is significant ( $p < 0.10$ ) for the 7th day after treatment.

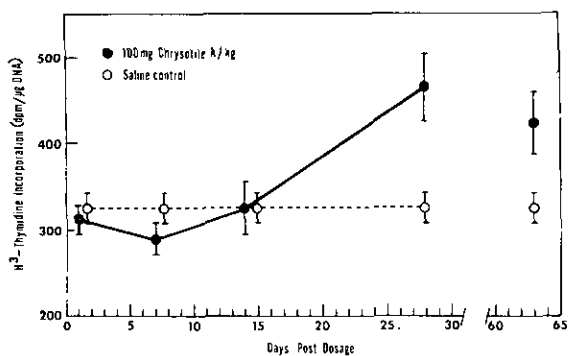


FIGURE 8. Thymidine uptake in rat colon following a single 100 mg/kg dose of chrysotile. Each point represents the mean for either 21 (control) or 5 (experimental) animals  $\pm$  SEM. The difference from control is significant for the 28th day ( $p < 0.01$ ) and the 63rd day ( $p < 0.02$ ) after treatment.

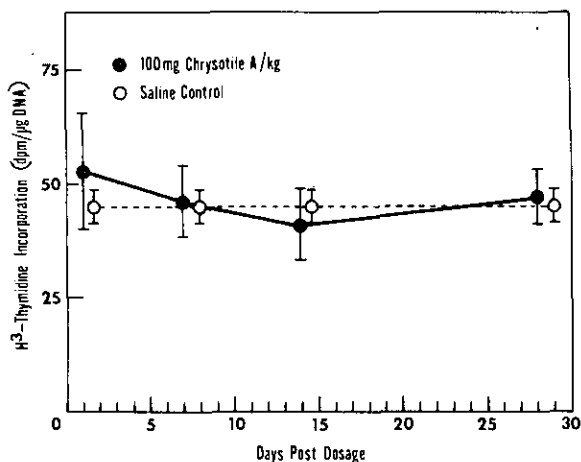


FIGURE 9. Thymidine uptake in rat liver following a single 100 mg/kg dose of chrysotile. Each point represents the mean for either 21 (control) or 5 (experimental) animals  $\pm$  SEM.

day after dosing was significantly greater than in controls ( $p < 0.01$ ); however, uptake at 7, 14, and 28 days did not differ significantly from controls (Fig. 6). Additionally, increased uptake in the small intestine was noted 7 days after dosing ( $p < 0.10$ ) (Fig. 7). Uptake in the colon was significantly greater at 28 days ( $p < 0.01$ ) and 63 days ( $p < 0.02$ ) after chrysotile dosing, while the 1, 7, and 14 day data were within normal limits (Fig. 8). Liver DNA synthesis was consistent at all intervals and did not differ significantly from controls (Fig. 9).

## Discussion

Very little information is available on the effects of asbestos on DNA synthesis or repair in any tissue. Natural chrysotile administered intratracheally stimulates a high rate of DNA synthesis in the rat pleura within 5 days subsequently (14). In contrast, inhalation of quartz dust by rats caused a significant and prolonged reduction in lung DNA content (15).

This preliminary study indicates that transient early and delayed alterations in the mitotic activity of the gastrointestinal tract cells occur following chrysotile ingestion. These effects are both dose-dependent and time-dependent.

Transient increases in the mitotic activity of the whole stomach and small intestine and delayed increases in colon mitotic activity occur following single dosage with 100 mg/kg of chrysotile. Since entire organs were used in this investigation, the cell populations affected have not been defined. Turnover rates of 3–6.4 days for rat stomach epithelium (16) and of 3 days for rat colon have been reported, although a second cell population of colonic mucosal cells is known to turnover at a much slower rate (17).

The failure to detect dose-response effects in the stomach at 14 days (Fig. 2) may be due to its more early response, as demonstrated by increased thymidine uptake 1 day after dosing with 100 mg/kg of chrysotile (Fig. 6). In the small intestine, the stimulatory effects of the 100 mg/kg dosage at 7 days reverted to normal limits by 14 days, whereas effects of the 5 mg/kg dosage (Fig. 3) were apparent at 14 days; the 500 mg/kg dose was inhibitory at 14 days. A contrasting effect was found in the colon, where thymidine incorporation was found to be elevated 14 days after a 5 mg/kg dose of chrysotile (Fig. 4), while a similar response to a 100 mg/kg dose was delayed until 28 days (Fig. 8).

Differences in the response of DNA synthesis in different segments of the alimentary tract to ingested asbestos may reflect anatomical and other factors, such as variations in pH and motility, which may influence the interaction of chrysotile fibers and the epithelial lining.

Our data suggest that asbestos fibers penetrate the mucosal layer of the gut and either enhance mitosis by interaction with

nuclear DNA, through some unknown mechanisms, or cause accelerated cell death and thus stimulate a mitotic burst of replacement cells. Persorption of very large particles, such as diatoms, cellulose particles, and starch granules through the intestinal mucosa is now well recognized (18); particles between 5 and 120  $\mu\text{m}$  in diameter can be regularly recovered from body fluids. Most of the chrysotile fibers used in the present investigation are within this size range.

## Summary

The effects of chrysotile on DNA synthesis in the gastrointestinal tract of the rat were studied by measuring the uptake of tritium-labeled thymidine; whole stomach, small intestine, colon, and liver were removed, cleaned, and homogenized, and the DNA was isolated and assayed for tritium label.

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