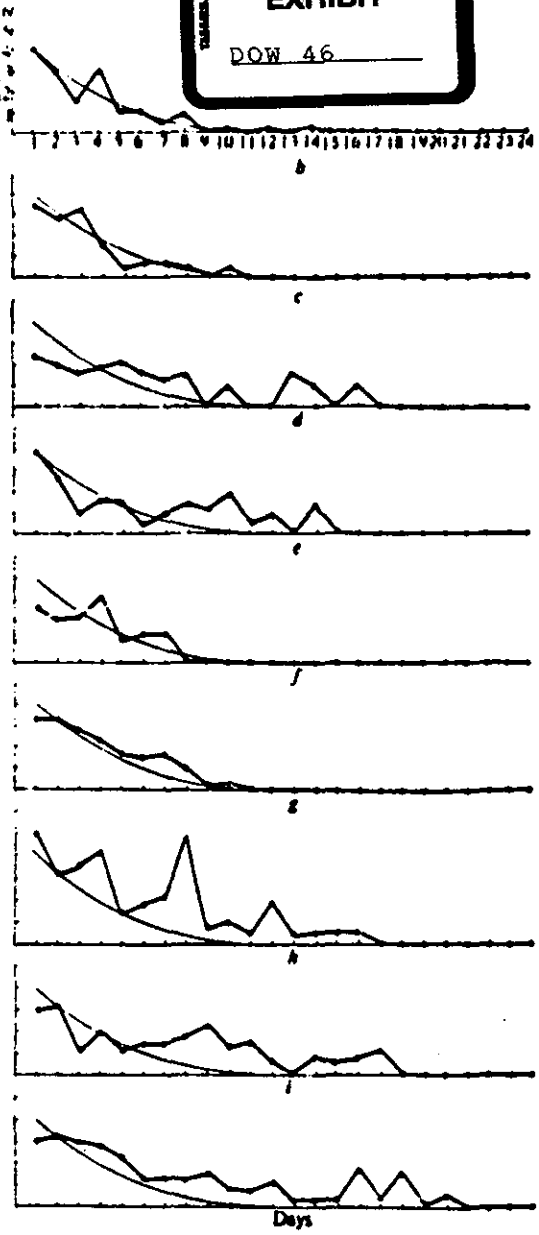

Fig. 2 Ventricular rates of the control hearts compared with the first 23 d for the test hearts.

seem reasonable to continue with further conventional clinical trials. If a new drug is depressant to the foetal myocardium above a certain concentration, it would be unwise to exceed this in a subsequent clinical trial. If the substance were to affect only young foetal hearts it might still be safe in late pregnancy.

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Penetration of Asbestos through the Digestive Tract of Rats

MESOTHELIOMAS of the pleura and peritoneum[1-3] have been linked with the inhalation of asbestos fibres. We have shown[4] that asbestos fibres are present in drinking water, beer, wine and other beverages. Asbestos fibres can penetrate the mucosa of the stomach and intestine[5] and Telnchi and Rubenstone[6] have found asbestos material in a gastric carcinoma. Godwin and Jagatic[7], reporting on mesotheliomas of the pleura caused by asbestos, noted that asbestos particles were widely distributed in various tissues. They suggested that particles can migrate from the lung through the blood and lymphatic system to all parts of the body. In the light of these reports we surmised that asbestos consumed orally can pass through the gut into the blood stream and accumulate in various tissues.

To determine if this is the case we injected a suspension of asbestos fibres into the stomachs of rats. The suspension was prepared by shaking 2.5 g of chrysotile asbestos (Johns-Manville No. 7RFO2) in 500 ml of water in a graduated cylinder. The suspension was allowed to settle for 30 min and the top 250 ml, which contained about 9.4×10^9 (1 fibre) fibres ml^{-1}, was drawn off. Most of these fibres are 0.2 μm to 2.0 μm long (Fig. 1). To ensure that fibres passed through the digestive tract without rats inhaling any of them, the asbestos was introduced into the gastrointestinal tract by opening the abdomen under anaesthesia and injecting the fibres directly into the stomach. Two to 4 d later the rats were killed and blood and tissues analysed for asbestos. To ensure that any fibres on the rat hair could not contaminate the organs removed during surgery, the rats were first thoroughly vacuumed and washed with double distilled water and methanol. During the removal of tissues wet towels were placed over the animals and around the incisions. Uncontaminated blood was drawn from the orbital sinus using a capillary tube.

To avoid formation of excess ash, which interfere with examination of specimens under the electron microscope, the tissues were first solubilized with soluene (Packard Instrument Company); the fibres were then centrifuged down, washed with methanol and ashed as previously described[4]. The ash was taken up in 1 ml of distilled water which had been filtered through a 0.2 μm filter, and 5 μl of this mixture was dropped on hydrophilic carbon-coated electron microscope grids supported on a ring peg. The grids were dried in a closed glass container (to avoid air contamination) under a heat lamp and were examined at magnifications of 20,000 and 80,000 with an electron microscope. The presence of chrysotile asbestos fibres was verified by electron diffraction. They were counted and the numbers corrected for dilution to determine the number in the original 1 ml sample. Results of recovery of asbestos fibres from rats treated in this way are shown in Table 1.

Rats in group 1 were given 9.4×10^9 fibres (1 ml) and killed 2 d later; those in group 2 received 94×10^9 fibres and were killed 4 d later. No asbestos fibres were detected in the blood of non-injected rats (controls) but 4.65×10^6 g^{-1} (a statistically significant increase) were found in the first group.

This is the first time direct physical evidence for the presence of asbestos fibres in the blood has been obtained. Four days after treatment the amount of asbestos in the blood of rats in group 2 had dropped to 1.19×10^6 g^{-1} even though these rats received ten times as much asbestos. This indicates that blood

Table 1 Numbers of Asbestos Fibres Recovered from Treated and Non-Treated Rats

	Controls mean*		Group 1† mean*		Group 2‡ mean*	
Blood	0.00	—	4.65¶	1.02	1.19	0.77
Spleen	2.33	0.67§	4.11	0.49	3.45	1.20
Omentum	2.46	0.46	2.74	0.87	18.25	7.79
Heart	2.09	0.41	—	—	2.28	0.40
Brain	0.05	0.02	0.31‖	0.19	0.29**	0.11
Lungs	1.06	0.26	1.80	0.54	1.74	0.26

* Average of five rats in all cases except where noted. † Animals injected with 9.4×10^9 fibres and tissues examined 2 d later. ‡ Animals injected with 94×10^9 fibres and tissues examined 4 d later. § Standard error of the mean. ‖ Four rats only. ¶ $P < 0.01$; ** $P < 0.05$.
(The figures in the table are fibres $g^{-1} \times 10^{-6}$.)

can clear itself of the fibres. Tissues such as the spleen, heart and lung also seem to have, to a lesser extent, the ability to clear themselves of the asbestos fibres, as the figures obtained from rats in group 2 indicate. They are lower than those for group 1 rats even though they received a larger dose, but the tissues had longer to clear themselves. Indeed, recent work in our laboratory with radioactive asbestos indicated that some tissues, such as the lungs, can reduce their fibre content by half in 2 d. This clearing action could possibly have quite an effect on the numbers of fibres found in the liver and kidney of the rats but unfortunately, because of the considerable amount of ash present in these organs even after soluene treatment, we could not examine these specimens under the electron microscope.

Another factor may contribute to the higher fibre count obtained for the organs of rats in group 1 compared with animals in group 2, namely, that there were four times as many fibres in the blood of rats in group 1. The residual blood in the tissues on removal from the animal would of course contribute to the total fibre count of a particular tissue.

This factor could have created the anomaly in the fibre counts in brains. In the brains of treated rats this was about six times higher than in the controls, and the increase in fibre count is statistically significant for rats in group 2 but not, oddly, for rats in group 1 even though there were more fibres in these animals. The greater number of fibres per gram in the blood of rats in group 1 could be responsible for the larger standard error in the counts of rats in group 1 and therefore did not allow the values obtained to be significant.

The brain seems to have little ability to clear itself of asbestos fibres, but the omentum has even less. This tissue, which

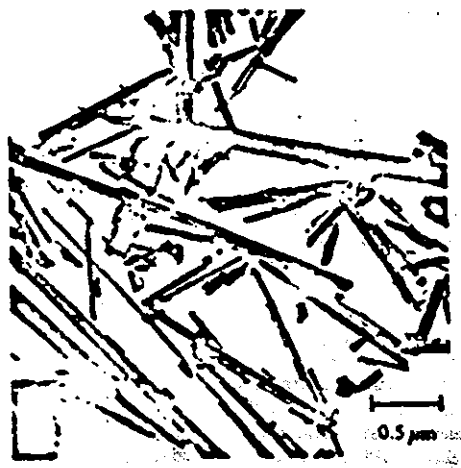

Fig. 1 Electron micrograph of the suspension of asbestos fibres injected into the rat stomach. Note the varying sizes of the bundles of fibres present.

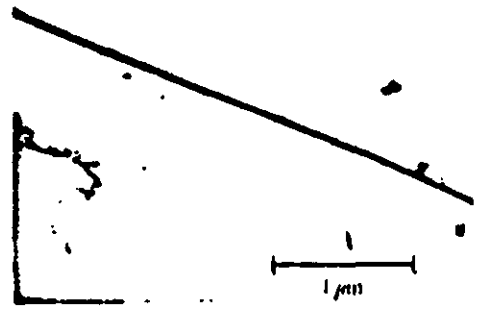

Fig. 2 Small bundle of asbestos fibres from the peritoneum of a rat. This bundle is at least 15 μm long.

surrounds the small intestine, appeared to accumulate most asbestos. Although the asbestos fibre content of the omentums in rats in group 2 may seem rather high (one extreme count contributed to the high standard error) the omentum does not seem to respond as do the other tissues. As well as having little ability to clear itself, fibre accumulation in the omentum may take place much more slowly than it does in other tissues. As the omentum consists partly of peritoneum and partly adipose tissue, asbestos fibres may enter the peritoneum by direct penetration of the intestinal wall. Concerning the prolonged retention of asbestos fibres in the omentum in women asbestos workers, Keal[8] found a higher incidence of peritoneal cancer than of lung cancer.

The amount of asbestos found in the tissues of the controls is higher than we had anticipated, considering the age of the rats and the number of fibres they might have consumed in their drinking water[4]. But we do not know how much was in their feed or air. We have analysed the tissues of some people who died of natural causes and found somewhat similar levels. In one person there was twice as much asbestos in the brain as in the brains of our control rats and about one quarter as much in the omentum, but the omentum content was still higher than the brain.

Most of the fibres found were about 0.2–2.0 μm long but some measured 15 μm (Fig. 2) and one fibre in the blood of a rat was 23.55 μm long. We do not know how the fibres pass through the intestinal wall. Some long ones may pierce the gut like a needle, whereas pinocytosis may account for the absorption of the small ones.

We demonstrated earlier the presence of asbestos fibres in air, snow, city drinking water and a number of beverages[4]. The experiment described here shows that fibres of a similar size administered into the stomach of rats appear in the blood and accumulate in various tissues in the body.

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