Effects and distribution of intravenously administered cellulose particles in mice

Foreign particulate matter in intravenous fluids has been shown to originate from a number of sources. Garvan & Gunner (1963) implicated rubber bungs as the cause of contamination by a number of foreign substances: one major contaminant was cellulose fibres. Consequently, Garvan & Gunner (1964) examined the lungs of rabbits after the animals had received intravenous fluids. Histological changes, capillary and arterial granulomas were present within the lungs. Every granuloma contained fragments of cellulose. Post-mortem sections of human lungs from patients having received intravenous therapy in their terminal illness showed results similar to those in the rabbit experiments.

Articles by Ho (1967) and Gross (1967) outlined the possible danger of particulate matter in solutions for intravenous use. Gross stated, that, at our present state of knowledge there is little or no conclusive evidence of untoward clinical symptoms, although known biological reactions to particles are clinically undesirable and needless. In recognition of the danger of particulate matter contaminating infusion solutions, the British Pharmacopoeia 1973 introduced a limit test in addition to the visual examination previously required.

This study sets out to track the distribution and physiological effects of one major contaminant, cellulose, upon brain, heart, lung, liver, spleen and kidney.

(1) *Preparation of cellulose injection.* The cellulose fibres (Courtaulds Limited, Viscose Division, Manchester) were 1½ denier bright viscose continuous tow filaments with an approximate cross sectional diameter of 15 μm.

The viscose rayon tow was cut into lengths of 1–2 cm and embedded, with fibres vertical, in hard paraffin wax. Sections were cut using a histological microtome set at 1·0 μm. The cellulose particles, which were of widely differing sizes, were recovered by melting the paraffin section, centrifuging, and washing repeatedly in ether until free of paraffin. The final washings in ether were filtered through a grade 'O' glass filter to remove larger particles produced. The ether was evaporated off and the dry cellulose resuspended 2·5 mg ml^{-1} in sodium chloride injection 0·9%, in sealed glass ampoules and sterilized by autoclaving.

From each batch of injections prepared, one ampoule was taken and the particle size distribution of the cellulose determined by Coulter Counter. The average mean diameter of the cellulose particles was 20·5 μm and the standard deviation 1·68.

(2) *Procedure for injection of mice.* Eight groups of 5 mice were injected weekly with 0·1 ml of injection of sodium chloride containing 2·5 mg ml^{-1} of cellulose, via the tail veins. One group of ten were similarly injected with injection of sodium chloride 0·9% as a control.

At weekly intervals from one to seventeen weeks, mice were killed and the brain, heart, lung, liver, spleen and kidney were removed and examined histologically. After fixation of the dissected organs in 10% formal saline, paraffin blocks were made by normal histological processes. Paraffin sections of the organs were then cut to 5 μm, stained with haemotoxylin and eosin and mounted.

The prepared slides were viewed under polarized light using a Carl Zeiss polarizing microscope set at an angle of rotation of 15°. A similar procedure was followed using normal light for the detection of granulomas in the stained tissue.

In all lung sections from 1–17 injections, granulomas containing cellulose were found. No granulomas were found in the spleen and the results of the sections from the liver, brain, and heart were uncertain. Granulomas containing cellulose were found on two occasions in the kidney (Fig. 1A), one accompanied by a foreign body

giant cell. None of the sections from the control mice showed any granulomas in any organ examined.

The incidence of particles reflecting polarized light occurring in the same plane of focus as the tissue from mice injected with cellulose was greater than the controls. Such particles appeared in the sections of heart, kidney, brain and liver. Their presence cannot be disregarded since the production of granulomas requires time.

The frequency of the granulomas in lung and kidney must be considered in relation to the amount of tissue examined. The thickness of each section was 5 μm and the average area for each was 20 mm^2. Thirty-three sections of kidney were examined, the total volume of tissue examined being 2·0 mm^3, in which two granulomas were found. The absence of granulomas in other tissues cannot, at present, be taken as an indication that they do not occur. The small amount of cellulose seen in the selected tissue indicates that most cellulose injected remains in the blood vessels (Fig. 1B).

Jonas (1965) considered the effects of introducing a particle into the radial vein of a mammal. He suggested that the particle will continue to travel to the right side of the heart via the systemic venous system. Since the calibre of veins increases as they near the heart, the particle is not likely to become lodged. The particle then progresses to the right atrium, the right ventricle and finally, to the pulmonary artery. The calibre of arteries decreases as they branch to the tissue and organs.

The pulmonary artery terminates in a massive capillary bed in the lung. These capillaries have a diameter of 7–12 μm and thus a theoretical particle greater than 7–12 μm would be trapped in the vascular bed. If the lodged particle inhibits the

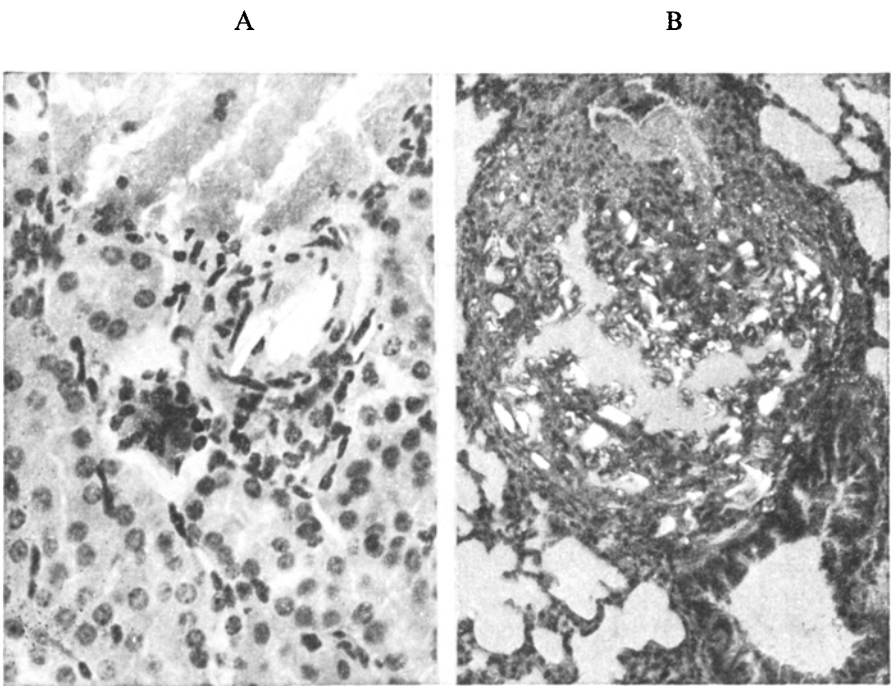

FIG. 1A. Mouse kidney after five injections of cellulose (2·5 mg ml^{-1}) showing two cellulose particles. The formation of a granulma can be seen and a foreign body giant cell is present. The largest particle is 33·7 μm. Magnification $\times$370.

FIG. 1B. Mouse lung tissue after 7 injections of cellulose (2·5 mg ml^{-1}) showing the massing of cellulose in the tissue and blockage of long vessels. Magnification $\times$ 145.

normal activities of the tissue, cellular damage or tissue death may result. Not all particles larger than 7–12 μm are filtered out by the lung due to the existence of arterial-venous shunts in the lung.

Prinzmetal, Ornitz & others (1948) demonstrated that glass beads up to 390 μm may pass through the pulmonary-capillary bed and reach the systemic circulation. The site of lodgement is again relative to size as in the case of selective X-ray and diagnostic media. In the present experiments most cellulose particles which entered the circulatory flow were trapped in the lungs, with a large number of resulting granulomas. The particles trapped were not limited in size; one particle 33·7 μm long and 13·0 μm wide became lodged in the mouse kidney forming a granuloma (see Fig 1A).

It is possible that large particles use arterial-venous shunts to enter the systemic circulation and may be of more importance than smaller particles not held by the normal filtering action of the lung.

Food and Drug Administration experiments (see Jonas, 1965 ref.) with beagle dogs showed that injected filter paper fragments could obstruct blood vessels. In the present experiment the spectacular effects of cellulose partly obstructing vessels in the lung can be seen (see Fig 1B). Obstruction of veins at the site of injection also occurred causing necrosis and degenerative changes in the mouse tail, especially at the tip.

Thus it is possible that particulate matter could obstruct the blood vessels causing platelet aggregation with possible formation of emboli and cause neoplastic and degenerative changes. Pulmonary arterial lesion, cotton fibrils in brains of patients who died following cerebral angiography and infarction due to cotton fibre embolisms have been reported (Groves, 1973).

Thus, cellulose particles injected into the blood stream of the mouse via the tail veins became lodged in the lung and kidney, with the formation of granulomas. The size of particles entering the systemic circulation is not governed by the size of the diameter of pulmonary bed blood vessels (7–12 μm). A system exists, possibly via A–V shunts, whereby particles greater than 7–12 μm can enter the systemic circulation and become lodged in major organs.

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