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Titanium dioxide (rutile) particle uptake from the rat GI tract and translocation to systemic organs after oral administration

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Abstract

Titanium dioxide (rutile) particles of nominal size 500 nm were administered as a 0.1 ml dose of a 2.5% w/v suspension (12.5 mg kg^{-1}) to female Sprague Dawley rats, by oral gavage daily for 10 days. Organs and tissues such as Peyer's patches, small intestine, colon, mesentery network and nodes, peritoneal tissue, liver, spleen, heart and kidney were removed for histology, scanning electron microscopy, and spectroscopic analysis for titanium, using the technique of inductively coupled plasma atomic emission spectroscopy. Histological and chemical analysis proved the presence of titanium dioxide particles in all the major tissues of the gut associated lymphoid tissue (GALT), and demonstrated that 500 nm tianium dioxide particles were translocated to systemic organs such as the liver and the spleen. Titanium dioxide particles were also found in the lung and peritoneal tissues, but were not detected in the heart or the kidney. The uptake of inert particulate matter, such as titanium dioxide, used in pharmaceuticals and food poses the question whether insolubility and inertness necessarily guarantees their innocuous nature.

Key words: Titanium dioxide; Organ distribution; Particle uptake

1. Introduction

The possibility of the uptake and translocation of inert particulates across the gut wall after oral administration has several toxicological and pharmaceutical consequences. The use of insoluble materials as chemical additives in the pharmaceutical and food industries (Weiner, 1988) perhaps needs some re-evaluation. Evidence for the transport of colloidal particles across the gut wall is mounting. Pontefract et al. (1973), Gerhart et al. (1981) and Henderson et al. (1986) demonstrated gastrointestinal uptake of asbestos and coal particles. Particulate emissions during mining, storage and combustion of coal, and asbestos inhalation and subsequent swallowing and particulate contamination of drinking water causes there to be legitimate concern about the absorption of these materials. On chronic administration, particles may accumulate in organs such as the liver and

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spleen and, through inhalation, in the lungs. Pontefract et al. (1973) showed the presence of 0.2–2 μm long asbestos fibres in air and city drinking water and in a number of beverages, such as beer. Their conclusion, after finding one fibre 23.55 μm long in the blood of a rat, was that smaller fibres pass through intestinal wall by pinocytosis and larger fibres pierce the gut like a needle. Gerhart et al. (1981) observed, after chronic administration of 125 μm coal particles to the fathead minnow (*Pimephales promelas*), increased mucous globules from goblet cells of the intestinal wall, as a physiological response, consistent with the idea that mucous secretion is a mechanism for lubricating and protecting the gut from abrasion. The occasional piercing of the intestinal wall by a larger particle may be healed by this excessive lubrication.

TiO_2 is used as a dye in tablets and hard gelatin capsule formulations and in cosmetic and medicinal preparations and employed in skin preparations for relief of pruritus and certain exudative dermatoses. It absorbs UV rays and thus is used as a sunscreen (Handbook of Pharmaceutical Excipients, 1986; Reynolds, 1989). Topical use poses few problems. However, environmental exposure to lead titanate zirconate has been cited by Roy et al. (1989), where blood and tissue samples detected lead titanate zirconate in workers producing and using this ceramic compound. Titanium dioxide exposure involving lungs, skin and synovium and other human tissue such as the liver and spleen was investigated by Moran et al. (1991) and Keen and Levison (1992). Using conventional histology and electron microscopy, titanium was identified in human tissues by the

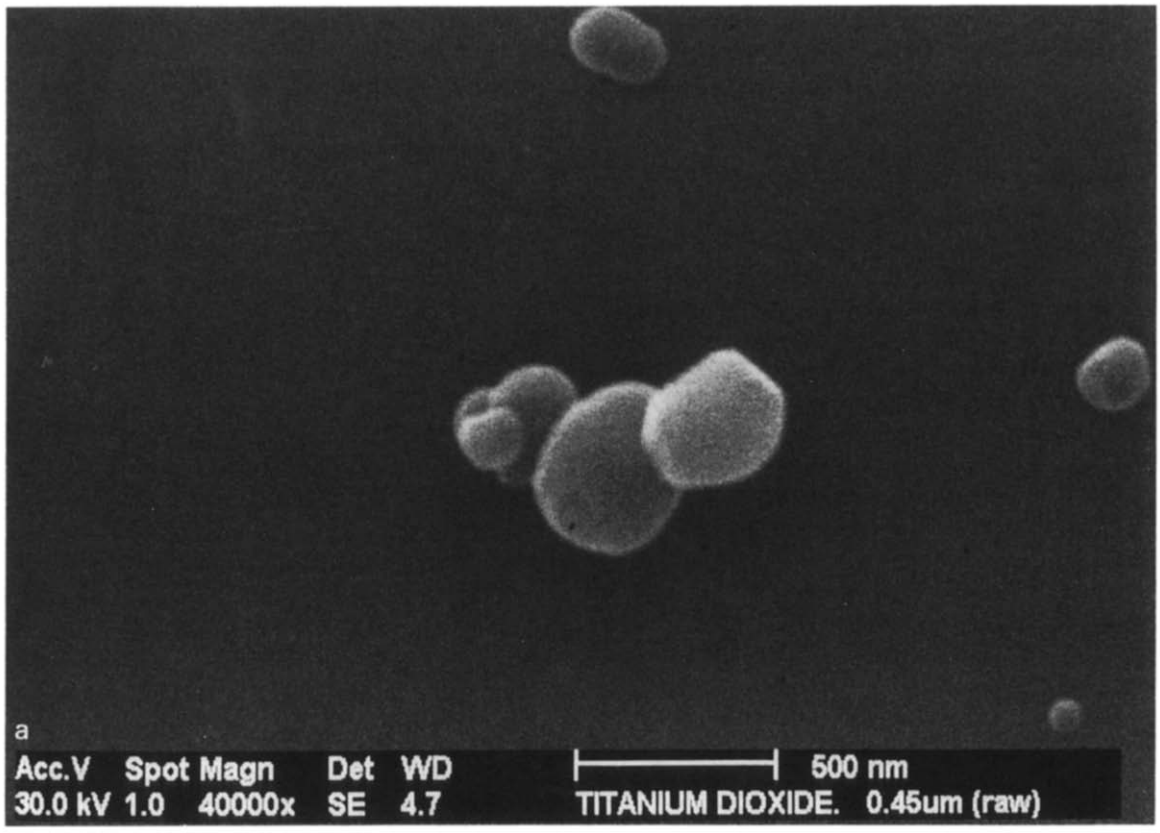


Fig. 1. (a) a scanning electron micrograph of a sample from the titanium dioxide batch used in this study; (b) a titanium dioxide particle at a Peyer's patch in the rat small intestine.

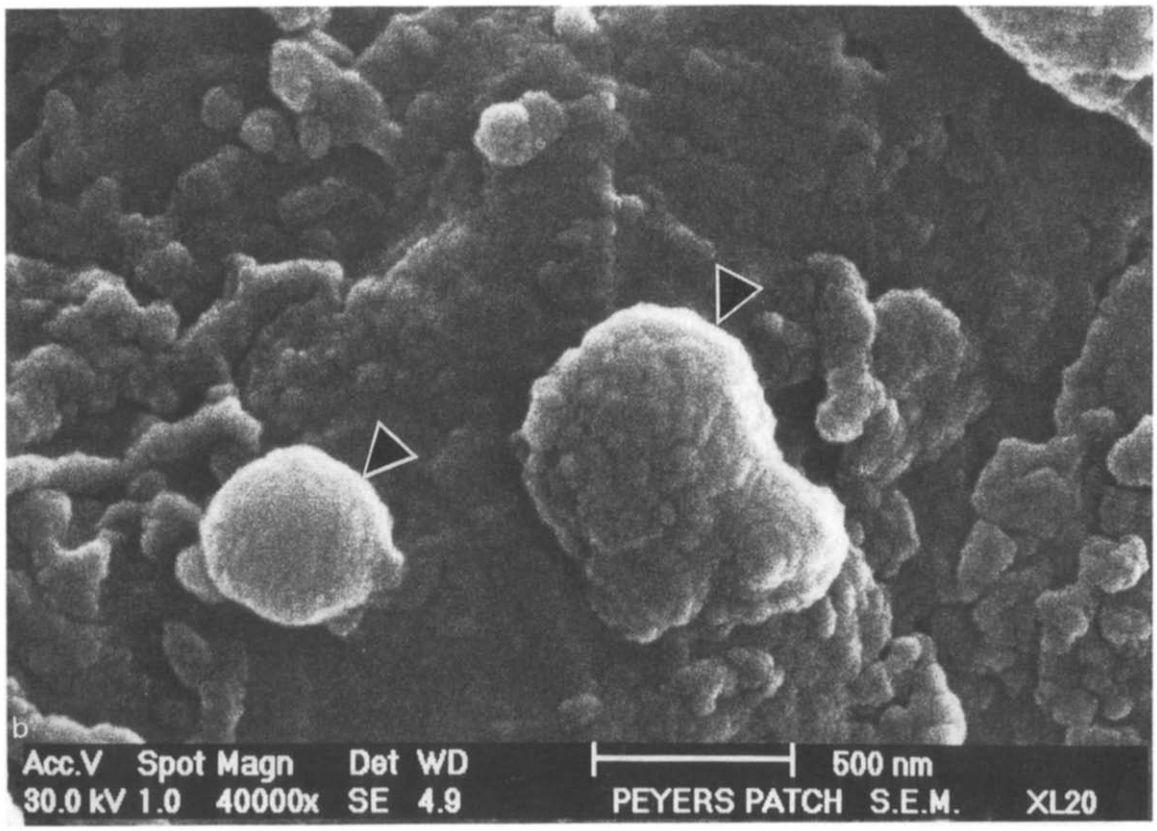


Fig. 1 (continued).

former, who showed that titanium dioxide particle exposure was associated with a lesion characterised by a xanthomatous, granulomatous and necrotizing reaction in the synovium, lungs and skin.

In this paper we have continued our work on the factors affecting the oral uptake and distribution of colloidal particles (Jani et al., 1989, 1990, 1992) with an investigation of the fate of orally administered titanium dioxide particles to shed light on the nature and extent of the phenomenon.

2. Materials and methods

2.1. Particles

Rutile (titanium dioxide) as a dry white powder of nominal size 500 nm was received from Polysciences Ltd (Northampton). A 2.5% w/v suspension was prepared in distilled water and particle size was confirmed by photon correlation spectroscopy to be 475 nm ($\pm$24 nm). Fig. 1a is an electron micrograph of the sample, showing some heterogeneity.

2.2. Animals

Female Sprague Dawley adult rats (average weight 150 g; 12–14 weeks) were used. To six rats a dose of 12.5 mg kg^{-1} (= 0.1 ml volume) titanium dioxide particles was administered by gavage daily for 10 days, a procedure and technique adopted in studies of polystyrene uptake (Jani et al., 1989, 1992). The animals were given free access to water, but food was removed overnight, about 8–10 h before the morning dose. Urine and faeces were collected daily; urine was collected and stored at −70°C to be examined for titanium

Table 1
Comparative observations of uptake and presence of titanium dioxide (rutile) particles (500 nm diameter) in tissues

Peyer's patches	Colon	SI	MN	Peritoneal tissue	Liver	Spleen	Kidney	Heart	Lung
+ +	+ +	+	+ + +	+ +	+ +	+	0	0	+

0, no evidence of uptake and presence; +, very little extent of uptake and presence; + +, moderate extent of uptake and presence; + + +, significant extent of uptake and presence; SI, small intestine; MN, mesentery network and nodes.

later. The animals were weighed daily and kept in individual metabolic cages to ease the collection of urine and faeces and to prevent coprophagia. After the final dose was administered, the animals were kept for 24 h in a particle-free environment to clear the gastrointestinal tract of any unabsorbed particles. Before being killed with ether, the animals were fasted for 15 h to clear the gut of food particles. Stomach, intestine (with mesentery network), colon, peritoneal tissue, liver, spleen, kidney, heart, and lungs were carefully removed, weighed and stored in a 10% v/v for-

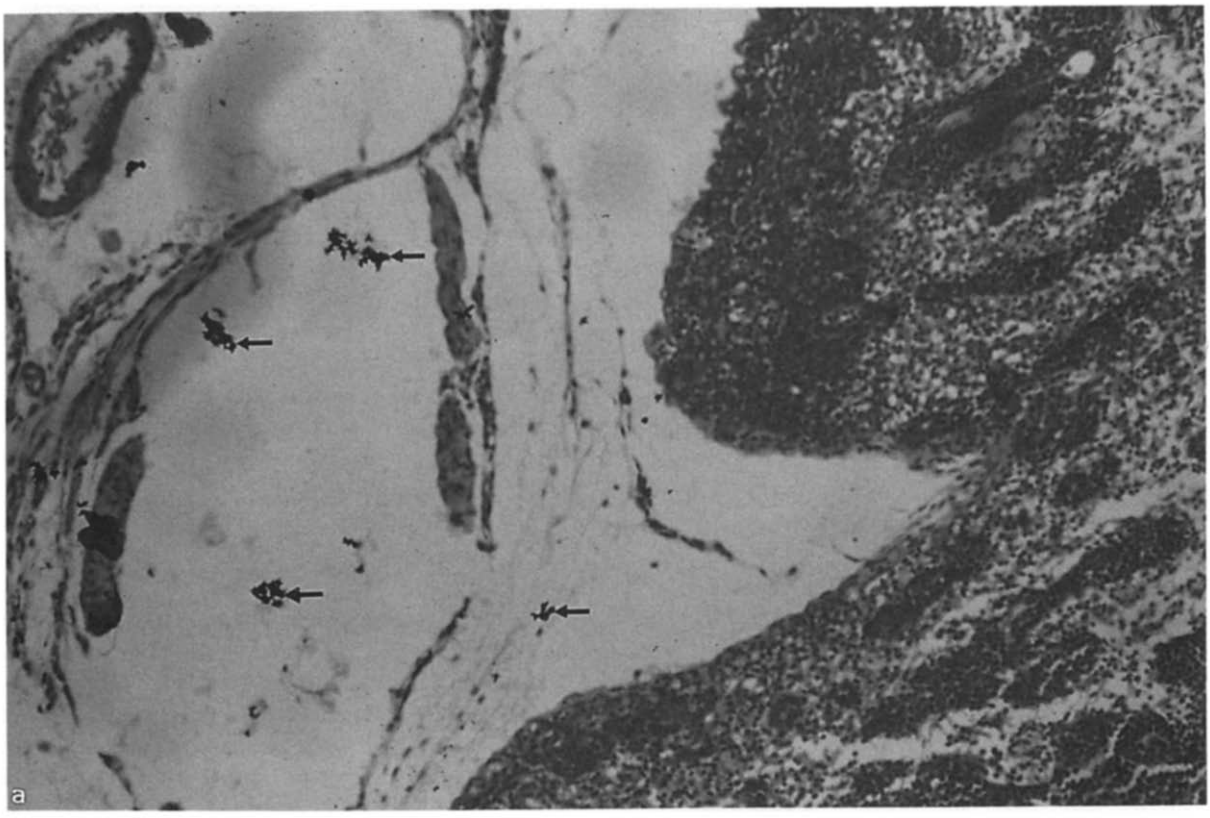


Fig. 2. Photomicrograph (×200) of a conventionally prepared histological slide showing (a) the presence (arrowed) of 500 nm size titanium dioxide particles in the mesentery network; (b) a magnified section of the previous slide (oil immersion) demonstrating the aggregated titanium dioxide particles; the characteristic aggregation of these particles is mainly due to the histological technique of preparation whilst being immersed in 10% formalin. An improved procedure maintaining samples in formalin for a decreased duration, was effected for Fig. 3–5.

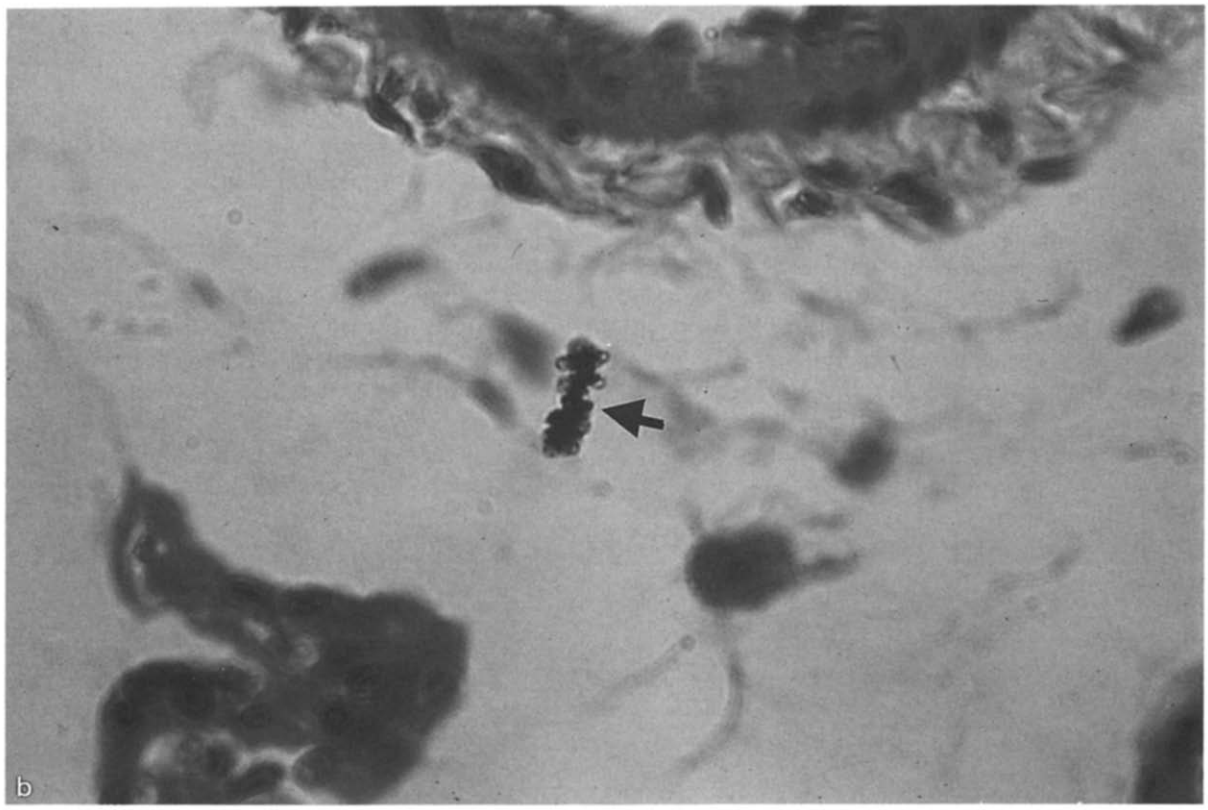


Fig. 2 (continued).

malin solution in individual pots, avoiding cross contamination between samples.

2.3. Histology

After fixation in formalin solution for 24 h., tissues were dehydrated by a series of ascending alcohol concentrations and then finally cleared in Histoclear, and embedded in paraffin wax (56–58°C melting point grade). Sections were cut at 4–6 μm with a Reichert-Jung 2030 rotary microtome using a disposable blade. Organs such as Peyer's patches, liver, mesentery network and nodes were removed, sections prepared and examined for TiO_2 particles, by staining with haematoxylin and eosin prior to examination, under a Nikon Microphot FXA optical microscope.

2.4. Scanning electron microscopy

Peyer's patches were removed and fixed in 2.5% v/v glutaraldehyde in 0.1 M sodium cacodylate buffer for 24 h and post-fixed in 1% w/v osmium tetroxide and dehydrated with a series of ascending alcohols and critical point dried from acetone. Tissues were examined and photographed under a Philips XL20 Scanning Electron Microscope.

2.5. Spectroscopic analysis

Inductively coupled plasma (ICP) atomic emission spectrometry has been used for the detection of trace amounts of the elements which are difficult to analyse by atomic absorption spectropho-

tometry through production of oxides (Mignardi et al., 1990; Minoia et al., 1990; Van Loon and Barefoot, 1992). The organs of four rats were freeze-dried and analyzed for titanium dioxide by ICP atomic emission spectroscopy. First the freezed-dried tissue samples were dry-ashed at 700–1000°C in a platinum crucible, then fused in potassium hydrogen sulphate and leached in dilute sulphuric acid. The resulting solution was analyzed for titanium. The extraction and analysis was carried out (on blinded samples) by Rooney Laboratories Ltd, Basingstoke (Hants). The percentage recovery from, and the background of titanium dioxide in, tissues was established by the use of a positively spiked tissue sample. Recovery of titanium was calculated as 96.2% (±2.8%), and background titanium dioxide was found to be less than 0.25 ppm per sample.

3. Results and discussion

Fig. 1b shows a titanium dioxide particle (arrowed) in the vicinity of a Peyer's patch. Histological results are summarised in Table 1. Titanium dioxide particles were noted in the granular areas of Peyer's patches (PP), in the main body of the mesentery nodes as well as in the connective tissues of the mesentery network. They were also present in the sinusoidal cells of the liver. Uptake

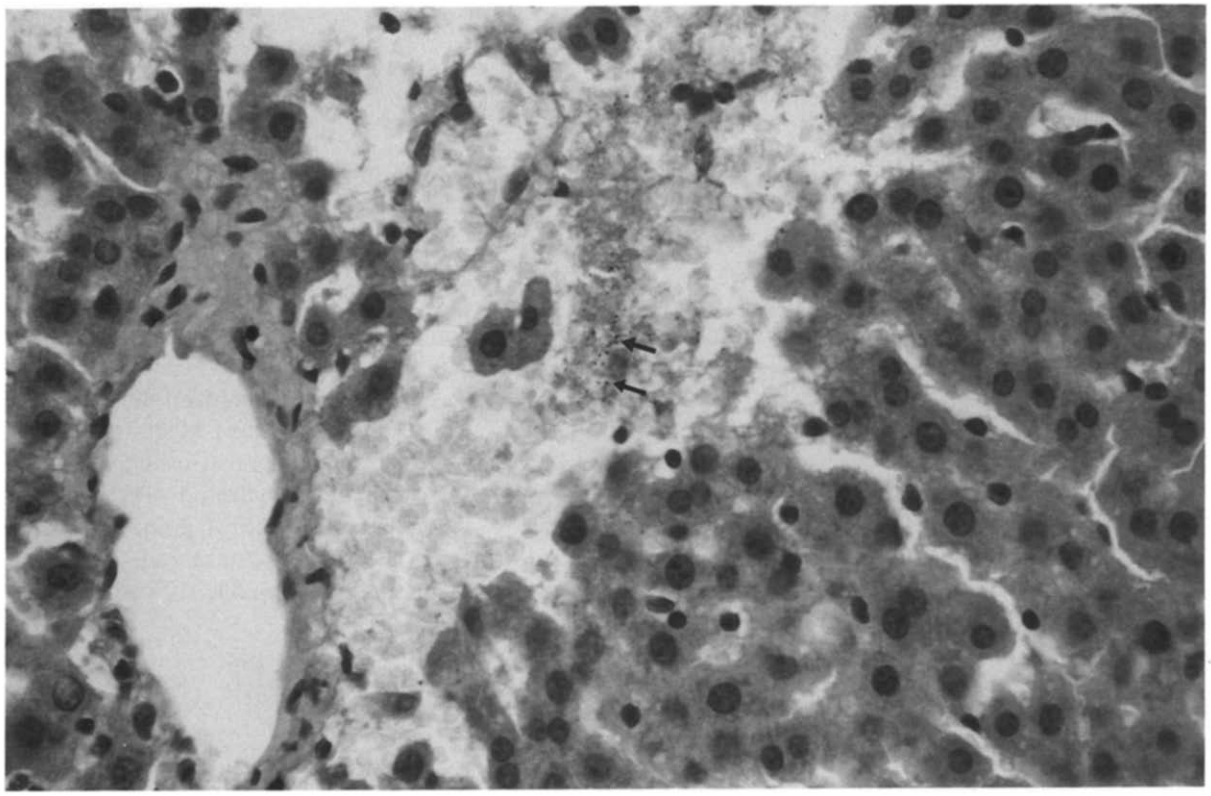


Fig. 3. Photomicrograph (×200) of a conventional histological slide of a part of the rat liver, showing distinct accumulation of the 500 nm size titanium dioxide particles in the extracellular matrix and sinusoidal spaces of the liver (see arrows).

Table 2
Titanium dioxide levels in tissues of rats after TiO_2 (nominal size 500 nm) administration (12.5 mg kg^{-1} per day for 10 days)

Organ (g ± SD) ($n = 4$)	TiO_2 (ppm)	TiO_2 (μg) (total)	% uptake of the dose administered	$\mu g\ g^{-1}$ of tissue (% of the dose)
Blood (1 ml)	2.5	5.25	0.02	5.25 (0.02)
Colon (3.987 ± 0.531)	600	1125	4 %	282.13 (1.13)
Peyer's patches and mesentery network and nodes (1.308 ± 0.051)	600	714.28	2.86	545.92 (2.18)
Small intestine (4.037 ± 0.1549)	14	27.5	0.11	6.81 (0.03)
Stomach (2.032 ± 0.2434)	11	14	0.06	6.89 (0.03)
Liver (6.87 ± 1.182)	350	600	2.4	87.34 (0.35)
Lungs (1.098 ± 0.077)	160	300	1.2	273.17 (1.09)
Peritoneal tissue (3.994 ± 0.505)	195	170	0.68	42.56 (0.17)
Spleen (0.464 ± 0.0211)	9.5	3.75	0.02	8.09 (0.03)
Heart (0.739 ± 0.046)	18.25	10.5	0.04	14.39 (0.06)
Kidney (2) (1.619 ± 0.136)	1.55	2.05	< 0.01	< 0.005 (2.04×10^{-5})
Total	1961.8	2972.33	11.9	1272.56 (5.041)

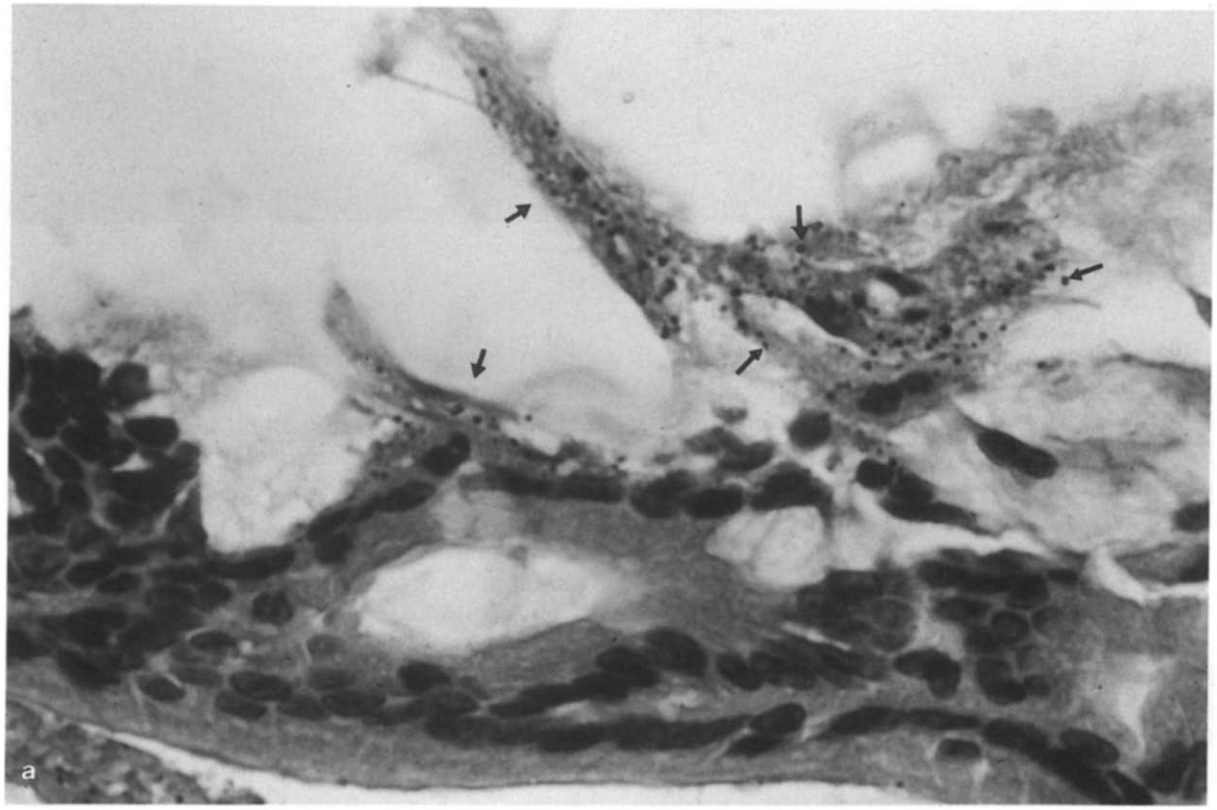


Fig. 4. (a) A photomicrograph (×600) of the subserosal layer of the rat colon showing the presence of aggregated titanium dioxide particles (arrowed). Unlike the small intestine's Peyer's patches, the colon has diffuse lymphoid tissue which is also capable of phagocytosis and uptake of particulate matter. (b) Photomicrograph (×300) of section of Peyer' patch lymphoid tissue prepared from rat treated with 500 nm titanium oxide particles orally for 10 days. Particles are seen as aggregates on the surface and in the cavity of the tissue (arrowed).

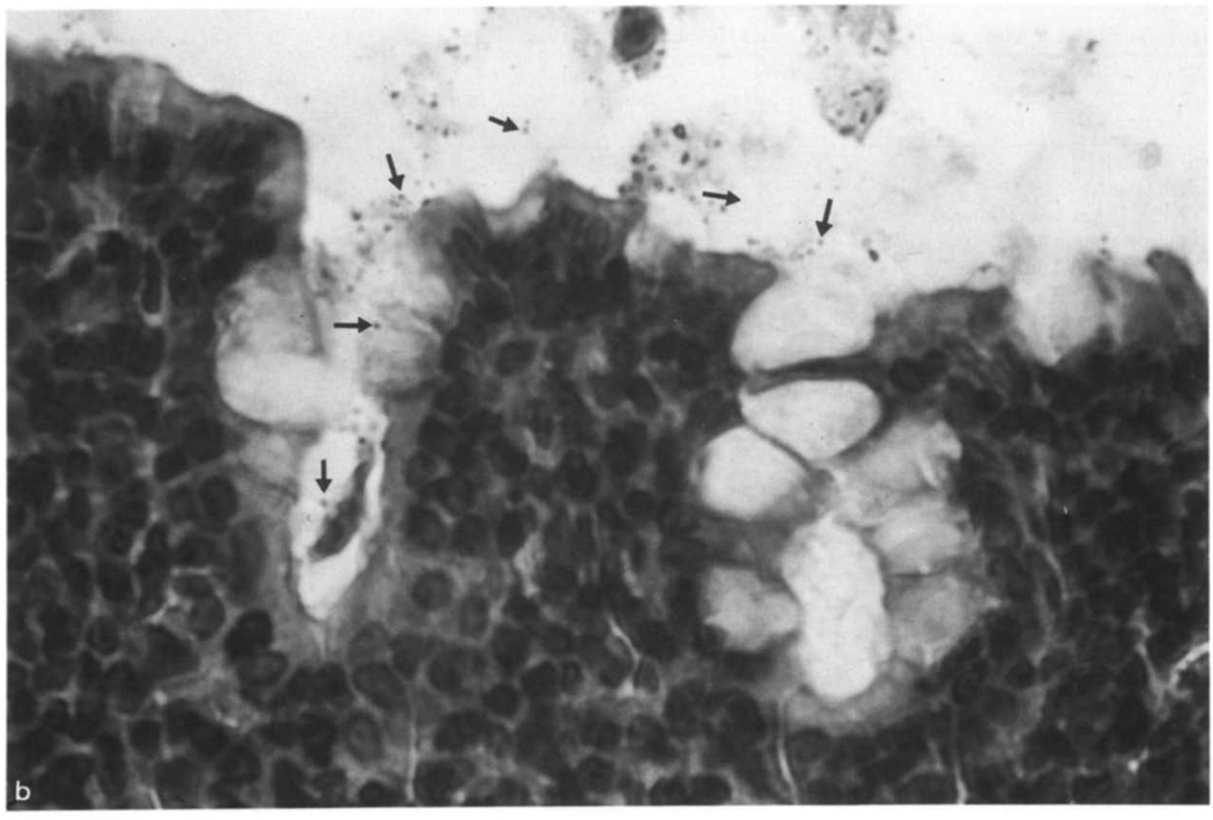


Fig. 4 (continued).

of cadmium by rat everted intestinal sacs (Ohta et al., 1989) shows similar deposition in the granular cells of the intestinal microvilli and Paneth cells. Kidney and heart tissues showed no particles. Although TiO_2 was seen in lung macrophages, this could be explained by the adhesive nature of the TiO_2 particles.

Fig. 2 shows titanium dioxide particles in the mesentery network. Fig. 3–5 also clearly show titanium dioxide particles in tissues such as liver, lymphoid tissues in the colon and the mesentary network near the gut, respectively. Titanium was found both by histology and by analysis in peritoneal tissue.

In the gastrointestinal tract, there is a marked contrast between the levels of TiO_2 found in lymphoid tissue such as Peyer's patches, mesentery network and nodes, and the non-lymphoid tissues of the small intestine (see Fig. 6). The colon having lymphoid tissues such as the appendix and diffuse aggregates shows a high titanium level, 4.5% of the administered dose being associated with colonic tissue. Comparing equal amounts of tissues, i.e., g per g (Table 2 and Fig. 6b), Peyer's patches, mesentery network and lymph nodes from each rat show higher levels of titanium compared to colon, liver or the non-lymphoid small intestine. Peyer's patch tissue was taken from the small intestine plus about 1 cm either side of the patches. Tissues such as the lungs showed the presence of titanium dioxide, and particles have traversed as far as the spleen. The total systemic uptake of the particles if the values of blood, Peyer's patches, liver, lungs, spleen, peritoneal tissue and heart are added, but omitting the values for the colonic tissue, absorption is calculated to be 6.5%, comparable to the data on absorption of submicron polystyrene la-

tex obtained by us (Jani et al., 1990). Only the concentrations of titanium determined in tissues not in direct contact with the lumen of the gut are used in this computation to avoid bias due to trapped or adsorbed particles. Peyer's patch values are included because of the evidence of uptake of particles by Peyer's patches and their translocation to the serosal side of the patch. Titanium dioxide particles (density approx. 3.9 g ml^{-1}) are heavier than polystyrene latex (density around 1.031 g/ml and zeta potential −73 mV) with a zeta potential of −54.3 mV, both factors potentially giving a comparative advantage over the latex, the higher density encouraging association with the gut wall in the intestine and the lower charge greater affinity for Peyer's patch cells.

Millet bran, which is unusually high in silica, is consumed in Northern China, where O'Neill et al. (1982) have demonstrated considerable silica fragments in the mucosa surrounding oesophageal tumours. Dense pigments, mainly of inorganic complex salts of the alum type, the material originating from aluminium-magnesium silicate and found chiefly in the terminal Peyer's patches of the colon, have been noted in patients during colonoscopy and autopsy (Urbanski et al., 1989). Electron microscopical and X-ray microanalysis examination carried out by Nwokolo and coworkers (1992) of upper gastrointestinal biopsies

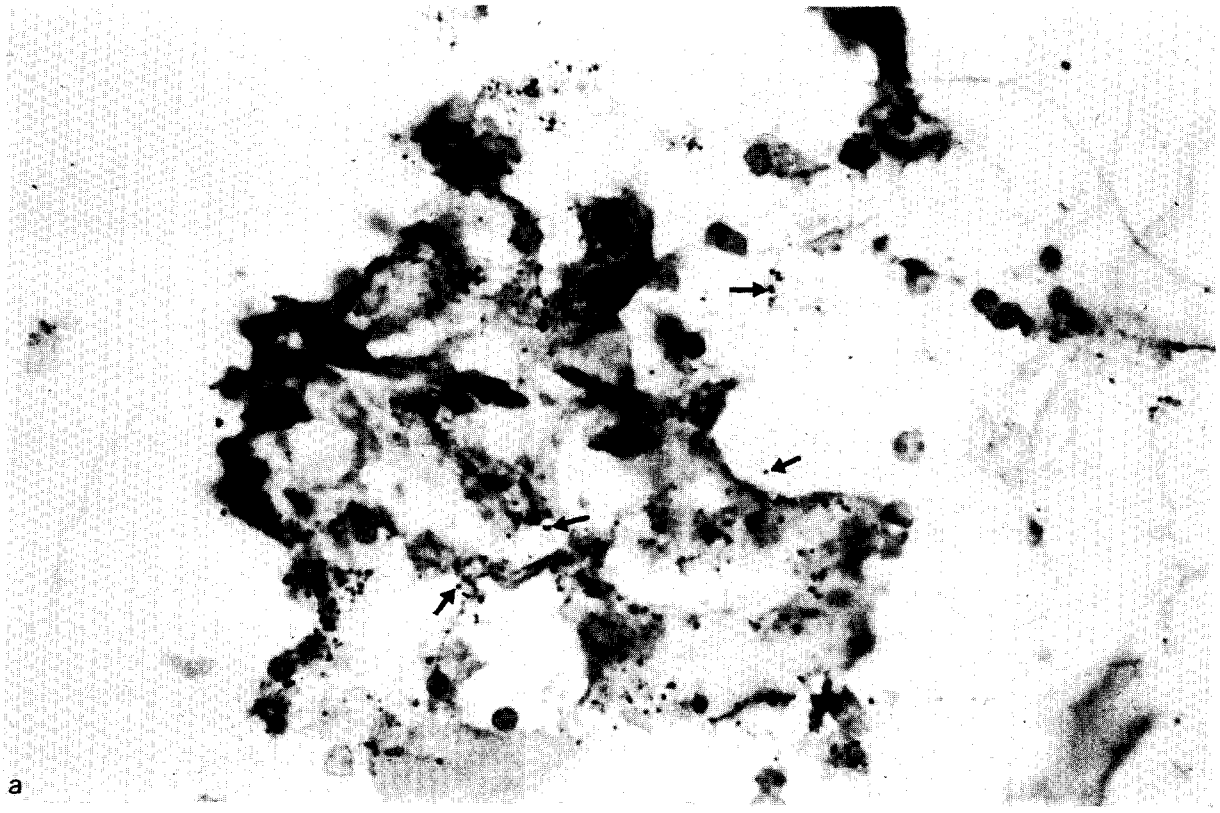


Fig. 5. (a) Photomicrograph (×200) of a mesentery connective tissue network, showing 500 nm titanium dioxide particles as black pigments, which translocate towards the lymph nodes; (b) a distinctive section (×100) of mesentery network, near the gut, depicting several regions of aggregated titanium dioxide particles (arrowed) administered orally for 10 days. (c) A highly magnified section (×600) of panel (b) showing clearly the presence of 500 nm titanium dioxide particles, with blood vessel nearby containing erythrocytes.

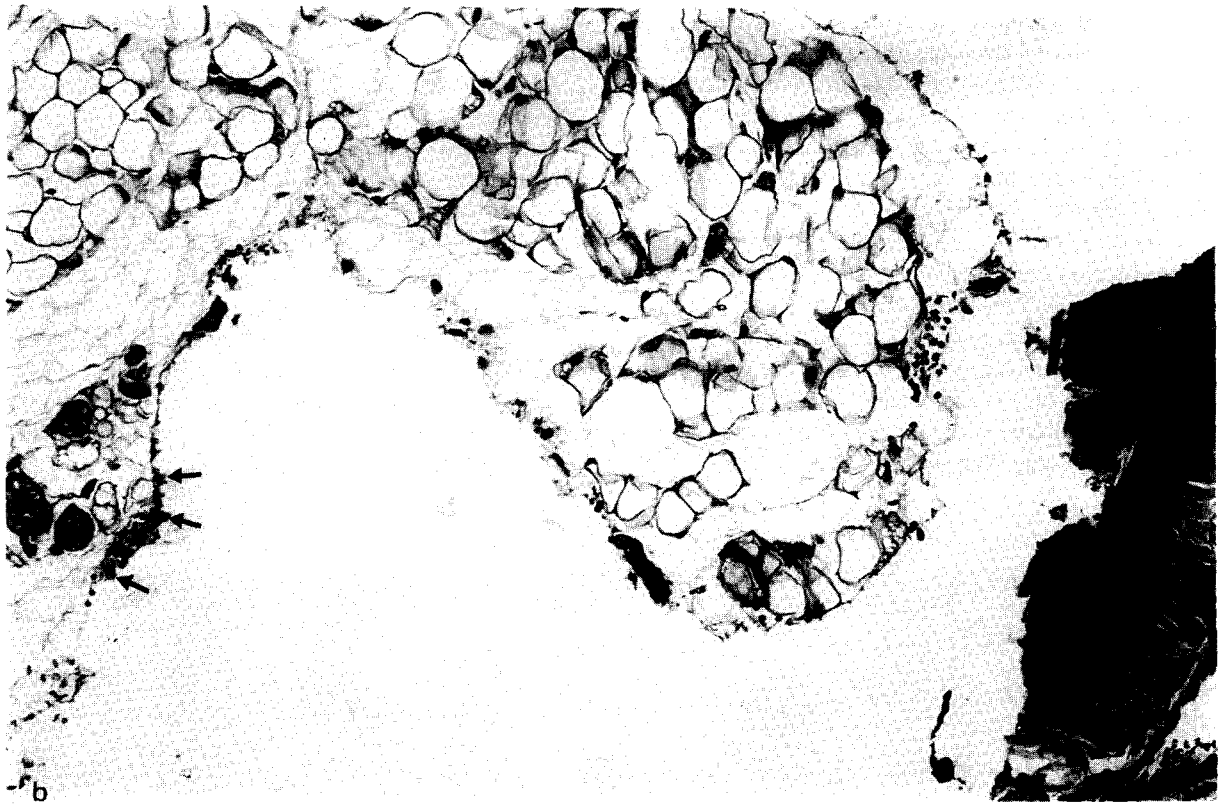
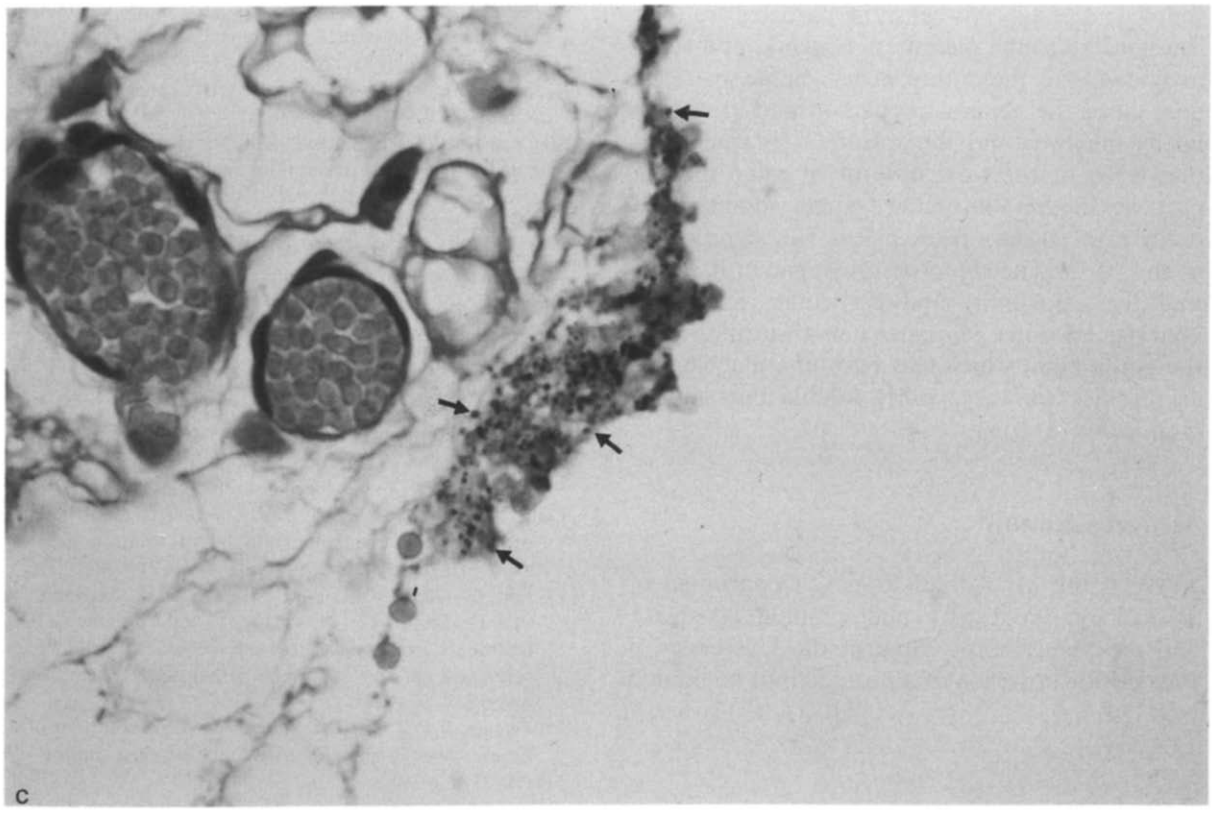


Fig. 5 (continued).

detected electron-dense particles of bismuth in the mucosa of the upper gastrointestinal tract, 30–60 min after oral dosing with tripotassium dicitratobismuthate or bismuth salicylate. These workers observed in all patients who had been dosed with tripotassium dicitratobismuthate transmucosal penetration of bismuth particles in the gastric antral mucosa but did not find any bismuth penetration after oral dosing with bismuth salicylate. Insoluble crystalline matter has been detected in the granuloma tissues and Peyer's patch tissues of patients with Crohn's disease (Shepherd et al., 1987; Shepherd and Levison, 1990; Roge et al., 1991). Sullivan (1990) has suggested that the aetiology of Crohn's disease is associated with the ingestion of insoluble substances such as salts of calcium phosphates, silicates and silica gel from toothpaste and other sources.

4. Conclusions

Many different particulate materials find their way into the gut, from inhaled or swallowed dust, to insoluble drugs and materials in pharmaceutical formulations like titanium dioxide, aluminium and bismuth salts. Without quantifying the extent of uptake, the question whether uptake of colloidal carriers implies anything of potential therapeutic or indeed toxicological significance cannot be answered. We have shown here that particle uptake in the gastrointestinal tract takes place principally via Peyer's patches, rich in lymphatic



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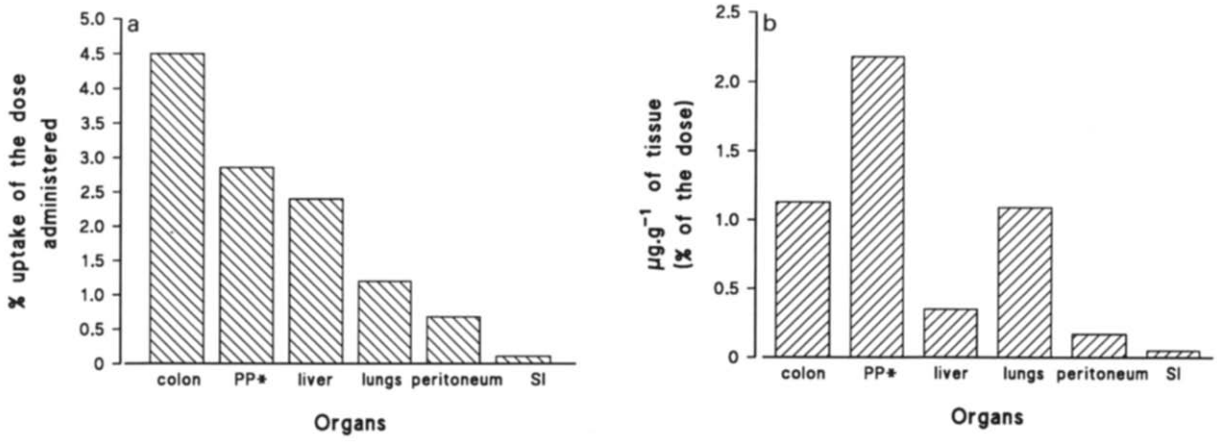

Fig. 6. Histogram of levels of titanium calculated (a) as the percentage of the administered dose accumulated in organ or tissue after 10 days daily administration of titanium at 12.5 mg kg^{-1} or (b) weight of titanium dioxide per unit weight of tissue, showing the higher relative concentrations in tissues such as Peyer's patches (PP, Peyer's patches; SI, small intestine).

supply and phagocytic cells; the particles are then translocated to the mesentery network, and accumulate in the mesentery nodes. Some particles then enter the general circulation and are taken up by the liver and the spleen. It is calculated that 6.5% of the total dose of titanium dioxide particles in the 500 nm size range administered orally over 10 days takes place. The significance of this, if any, needs to be investigated; it seems that the insolubility and particulate nature of materials does not guarantee non-absorption from the gut, a point which may have pharmacological significance for very poorly soluble but sub-micron forms of drugs.

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