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Blood clearance and organ deposition of intravenously administered colloidal particles. The effects of particle size, nature and shape

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Summary

The blood clearance and organ deposition of polystyrene and cellulose (DEAE) particles have been studied in the rabbit using labelled material and the technique of gamma scintigraphy in order to investigate the importance of particle size, shape and nature. Small (1.27 μm diameter) polystyrene microspheres were taken up by the reticuloendothelial system of the liver, while large polystyrene particles (15.8 μm diameter) were mechanically filtered by capillary beds of the lungs. Cellulose microspheres and fibres were also taken up into lung tissue. Large cellulose fibres, 30 μm diameter, proved to be toxic whereas large cellulose microspheres were well tolerated. The implications for drug targeting are discussed.

Introduction

Particles in the colloidal size range and above (0.5–30 μm) are administered intravenously, either in the form of extraneous particulate contamination or by deliberate design as in radionuclide diagnostic imaging or for the delivery of pharmacological or nutritional agents.

All parenteral products will contain some extraneous particulate matter and previous reports (Illum et al., 1978a, b; Illum 1980; Groves and de Malka, 1976) have considered the origins and amounts contained in injectable fluids. Limits for such contamination are laid down in pharmacopoeial monographs. The presence of

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particles in infusion solutions is generally considered to be hazardous and certain contaminants have been associated with serious conditions such as the development of tumours (Groves and de Malka, 1976).

In contrast, intravenous products may deliberately contain many millions of small particles, for instance, particulate radio-pharmaceuticals are used routinely in diagnostic procedures in man. Radiolabelled macroaggregates and microspheres of human serum albumin are employed as agents for lung perfusion imaging and sulphur colloid is used as a liver imaging agent (Davis and Taube, 1978; Klingensmith et al., 1978). Radioactively labelled microspheres of polymeric materials find use in measurements of regional blood flow in experimental animals (Archibald, 1975; Schroeder et al., 1978) and degradable microspheres have been studied as potential drug delivery systems for the intravenous and intra-arterial administration of drugs; for instance, antitumour agents (Kato et al., 1981; Oppenheim, 1981).

Early studies have indicated that the number of administered particles as well as their size and nature are of importance. Large particles (greater than about 10 μm diameter) will be trapped in the lungs by mechanical filtration while smaller particles will be cleared by the reticuloendothelial system. This involves phagocytosis by cells at a variety of tissue sites but especially those in the liver and the spleen (Kanke et al., 1980). The effect of particle number and composition has been considered by Davis and Taube (1978). The acute toxicity in rat was found to range from 154,000 to 705 particles per gram body weight as the particle diameter changed from 13.5 to 90.7 μm. Wilkins and Myers (1966) studied the importance of surface characteristics using modified polystyrene latex. Negatively charged material was taken up by the liver while positively charged material showed an initial appreciable accumulation in the lungs and a later accumulation in the spleen. The results were discussed in terms of the electrophoretic behaviour of the particles both in buffer solution and in serum.

Spherical particles are useful as model systems; however, particulate contamination in infusion fluids is often in the form of fibres. A communication by Purkiss (1975) has described the injection of cellulose fibres into mice, and reports histological evidence for the deposition of particles in lung, liver as well as kidney.

The purpose of this study was to investigate systematically the effect of particle size, nature and shape on blood clearance and subsequent organ deposition following intravenous administration of particulate systems to the rabbit. Polystyrene and modified cellulose particles labelled with a gamma-emitting radionuclide, ^{131}I, were chosen and the various changes in clearance and deposition were followed using a variety of experimental procedures.

Materials and methods

Materials

Polystyrene microspheres (1.27 and 15.8 μm mean diameter) were obtained from Dow Diagnostics, Indianapolis, IN, and the particle size was checked using a Coulter Counter (Model TAII, Coulter Electronics, Luton). The particles were

surface-labelled with ^{131}I using the procedure described by Huh et al. (1974). A suspension of microspheres in [^{131}I]sodium iodide solution (70 M Bq) was exposed to a cobalt-60 source for at least 3 h to give a total radiation dose of approx. 50 kGy. The unbound iodide was removed by dialysis. In vitro and in vivo experiments indicated that the integrity of the label was satisfactory although slow leaching of the iodide-131 label did occur over extended periods of time (Mills et al., 1982).

DEAE-cellulose microspheres (DEAE-Sephacel) were obtained from Pharmacia Fine Chemicals, Uppsala. The material had a particle size range of 40–160 μm diameter and was provided in a pre-swollen condition in an aqueous ethanol dispersion. DEAE-cellulose fibres were obtained from Merck, Darmstadt in the form of material for column chromatography. This was separated into different size fractions by air eluriation (Zig-Zag classifier). Two size fractions (5 μm and 30 μm nominal diameter) were selected for animal experiments. Photomicrography showed that the two particle size distributions were broad.

DEAE-cellulose is a basic ion-exchange material with a binding capacity of approximately 0.7 Meq./g. It can be labelled effectively with anionic dyes such as [^{131}I]rose bengal (380 mg/g in normal saline at 25°C). DEAE-cellulose particles were suspended in normal saline and sterilized by autoclaving. Suitable quantities of material (60 mg $\simeq 10^8$ particles) were labelled by the addition of approximately 2–6 MBq of [^{131}I]rose bengal solution (0.29 mg/ml) (Amersham International) and allowed to equilibrate for 18 h. The binding of the rose bengal to the DEAE-material was found to be extremely efficient in vitro over a range of pH values (4–9) and ionic strengths. However, incubation of labelled DEAE-cellulose microspheres with rabbit plasma indicated that some of the rose bengal was released rapidly. This is believed to be due to the binding of plasma proteins to the surface of the particles and thereby displacing the rose bengal. The large molecular weight of the protein prevented its penetration into the cellulose matrix. Thus it was expected that [^{131}I]-labelled DEAE-material would release free [^{131}I]rose bengal upon administration. Control experiments were therefore conducted using appropriate doses of [^{131}I]rose bengal solution.

Animal experiments

New Zealand White female rabbits (weight range 2–4 kg) were randomly divided into groups of 3. The various particulate systems were injected rapidly via the marginal ear vein (10^6 particles, 1.8–2.6 MBq per dose). The distribution of the particles in various body organs was followed using external scintigraphic imaging (MaxiCamera II-Gamma Camera, International General Electric Company of New York, Hayes). A dynamic study (each image of 60 s duration) was recorded for 10 min from the time of injection. Static images of the distribution of the particles were recorded over a period of 10 days. The data were recorded and processed using a dedicated computer system (Gammascope, Link Systems, High Wycombe). Blood samples were removed at suitable time intervals from the contralateral ear and were analyzed for radioactivity using a gamma counter (Intertechnique CG 4000 gamma counter, Intertechnique, Uxbridge). Eleven days after administration the animals were killed and organs removed. The total activity in different organ sites was

determined using a large sample volume well-type gamma-counting system (EG and G Ortec, Bracknell). Small samples of the organs were taken for histological analysis. These samples were fixed, dehydrated and embedded in wax. Microtome sections were cut at 6 μm and stained with haematoxylin eosin.

Results

Gamma scintigraphy

Computer processed gamma camera images of lung and liver regions of the rabbit are shown in Fig. 1. The shapes and positions of these organs are distinctive (Hardy and Wilson, 1981) and it was therefore possible to define regions of interest for subsequent data analysis using the computer. (Note that for the lung field the separation of the two lungs is discernable.) A set of typical distribution curves (for the first 10 min following administration) is shown in Figs. 2 and 3. The curves have been corrected for differences in administered activity. The small polystyrene particles (1.27 μm) circulated through the lungs but soon became localized in the liver (Fig. 2). The larger particles were trapped in the lungs within 1–2 min of administration irrespective of their nature (Fig. 3).

The retention of the various particles at the deposition sites was followed by analyzing the static images obtained over a 10-day period. The data presented in Fig. 4 have been corrected for differences in administered activity, radioactive decay and the slow leaching of the label from the particles (by applying a correction factor calculated from the whole body clearance of total activity). It is clear that no significant redistribution of the particles from their deposition sites occurred during

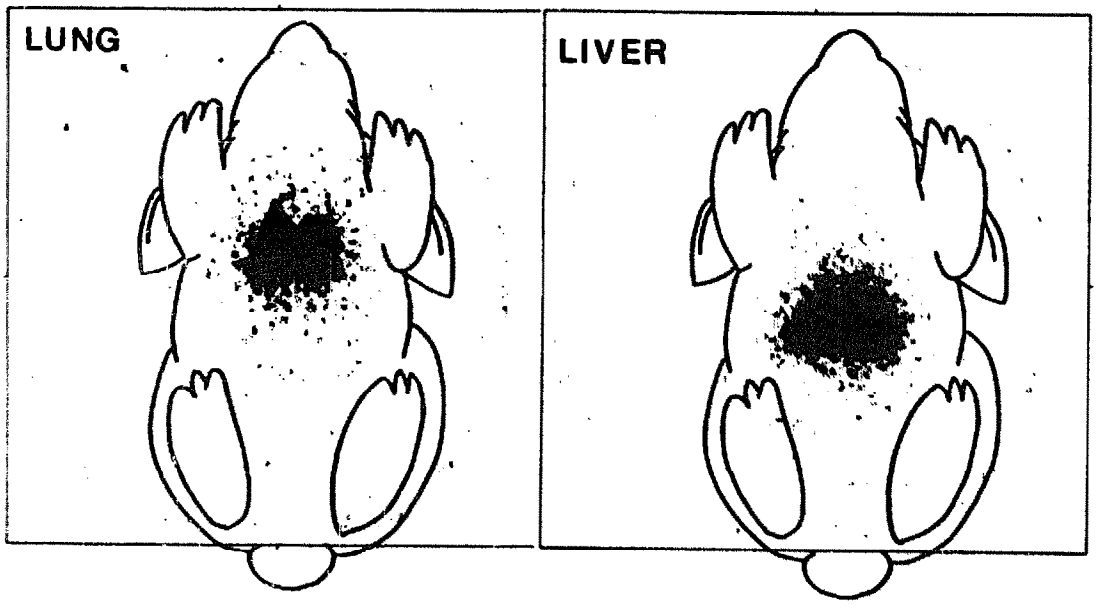

Fig. 1. Scintiscans of rabbits 10 min after administration of radiolabelled polystyrene microspheres. Lung: 15.8 μm diameter. Liver: 1.27 μm diameter.

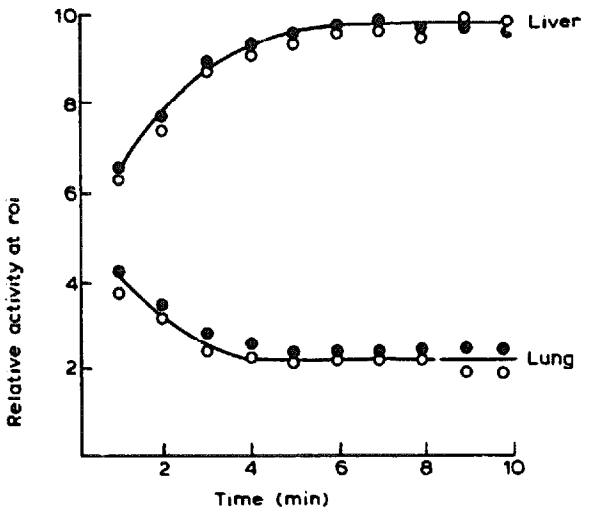

Fig. 2. Activity–time profiles for liver and lung regions following intravenous administration of radiolabelled polystyrene microspheres 1.27 μm diameter (data for two rabbits corrected for initial dose).

the 10-day time period. Those animals dosed with particles labelled with [^{131}I]rose bengal did demonstrate some transient activity in the abdominal region. However, this was probably due to free [^{131}I]rose bengal which is known to be excreted by the liver into the bile following intravenous administration (Taplin et al., 1955).

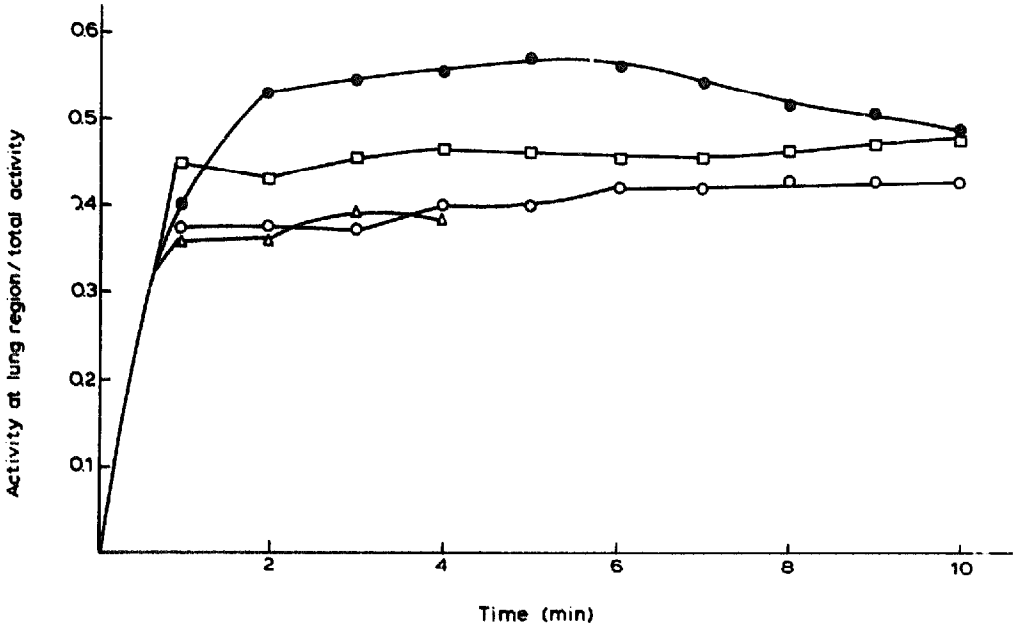

Fig. 3. Activity–time profiles for lung region following intravenous administration of radiolabelled particles (mean values, data corrected for different doses of radioactivity). Polystyrene microspheres, 15.8 μm diameter, ●; DEAE-cellulose microspheres, □; DEAE-cellulose fibres, 5 μm diameter, ○; and 30 μm diameter, △.

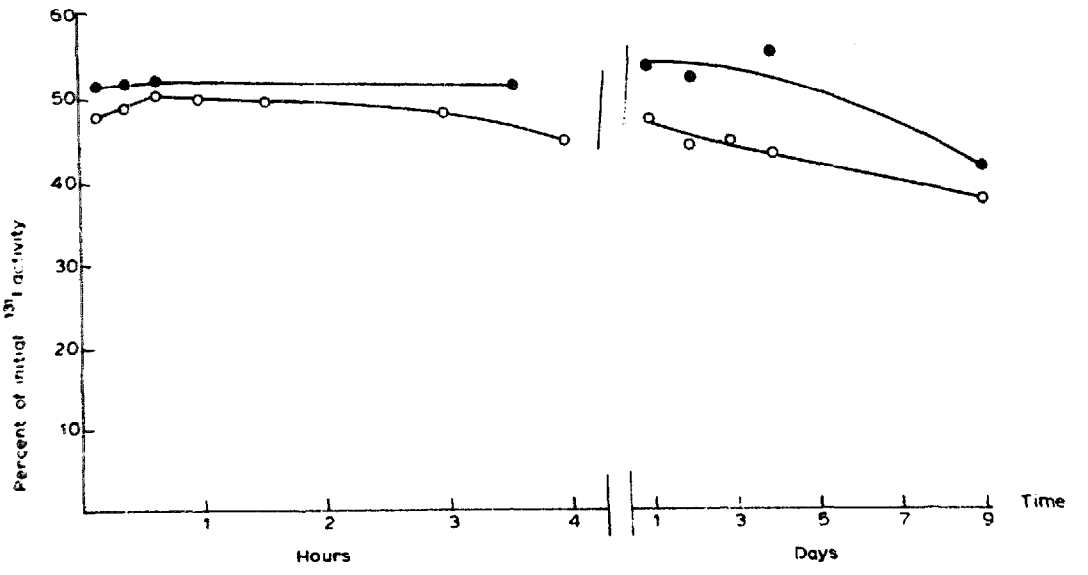

Fig. 4. Activity–time profiles for different regions of interest showing the retension of intravenously administered labelled polystyrene particles (data corrected for different initial doses, radioactive decay and leaching of the label). Polystyrene microspheres, ●, 1.27 μm diameter in liver region; polystyrene microspheres, ○, 15.8 μm diameter in lung region.

The two animals which received DEAE-cellulose fibres of 30 μm diameter died within 4 min of the intravenous injection following an acute toxic response (tachycardia, dyspnoea, dystaxia).

Blood activity–time profiles

The amount of activity in the blood (expressed as a percentage of the administered dose) was small, even within a few minutes after administration of the particulate systems (Fig. 5). This indicated that the uptake into organ sites was very rapid. The blood activity–time profiles for the particulate systems studied showed significantly different patterns, which were dependent on the nature of the particles rather than their size. Considering the polystyrene systems, the level of activity for the smaller particles was low but rose to a peak at 300 min after administration and then fell. A similar profile was given by the 15.8 μm diameter polystyrene particles except that the initial activity was higher and the peak was at 150 min after administration. Such data indicated that the polystyrene particles were cleared very rapidly upon injection but that some re-location fragmentation or release of label might have occurred.

The blood activity–time profiles for the DEAE-cellulose systems conformed more closely to a bi-exponential pattern of clearance. There were no peaks in the curves and the half-time for the initial part of the curve was of the order of 1 or 2 min. The second exponential phase had a half-time of the order of 200 min. This second phase is thought to reflect the leaching of the [^{131}I]rose bengal label from the particles rather than to redistribution or clearance. (Illum and Davis, 1982).

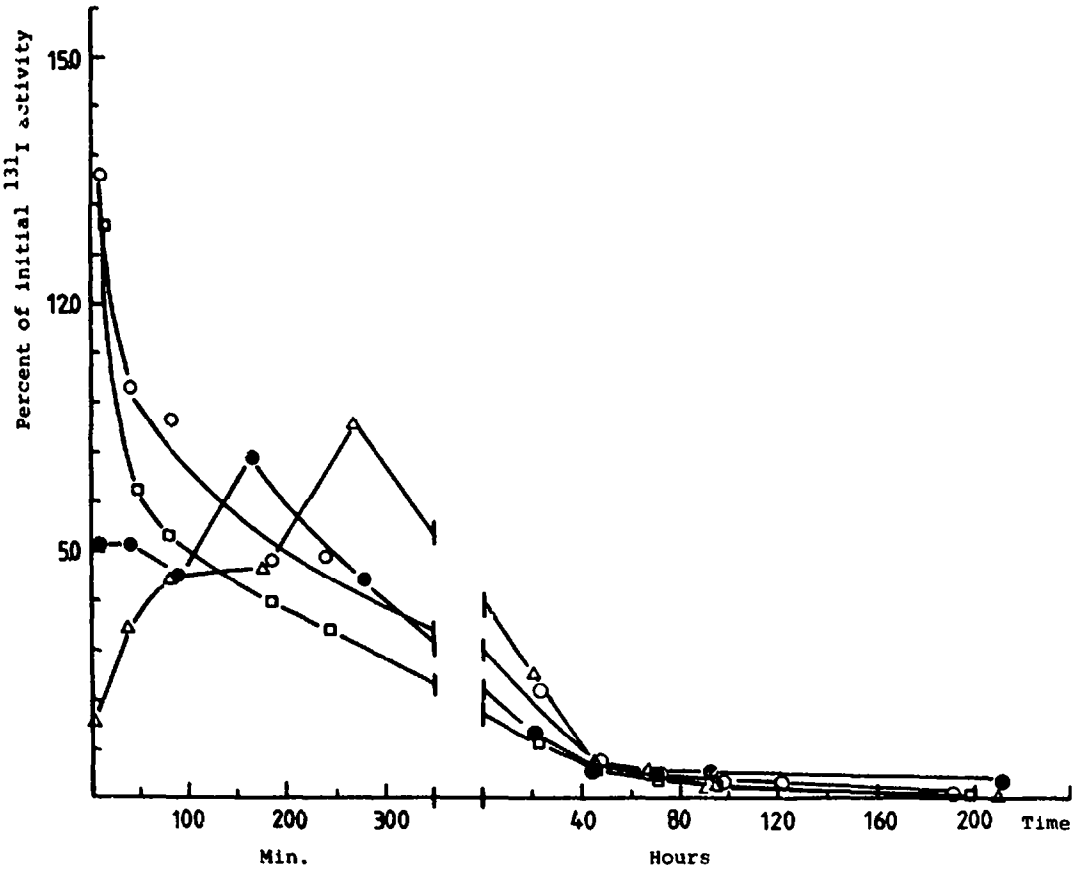

Fig. 5. Blood-activity–time profile following the intravenous administration of labelled particles (mean values). Polystyrene microspheres, △, 1.27 μm diameter; ●, 15.8 μm diameter; ○, DEAE-cellulose microspheres; □, DEAE-cellulose fibres, 5 μm diameter.

Organ distribution

The animals were killed 11 days after administration, specific organs (lungs, liver, spleen, heart and kidneys) were removed and the residual radioactivity determined. The results are presented in Table 1 and shown in histogram form in Fig. 6. In general the activity in the heart was generally low. (The highest activity was obtained for the rabbits that received the large DEAE-cellulose fibres that led to massive pulmonary embolism and death.)

The observed distribution of activity indicated that the 1.27 μm diameter polystyrene particles were localized in the liver with small quantities in the spleen and the lungs. In contrast the 15.8 μm diameter polystyrene particles were found mainly in the lungs. The levels in the liver were low and no activity was found in the spleen. These levels of activity coincided well with the observed distributions of the particles obtained using gamma scintigraphy.

The DEAE-cellulose microspheres were largely taken up into the lung although some activity in the liver and kidney was observed. The small DEAE-cellulose fibres were largely sequestered by the lungs but a significant proportion of activity was

TABLE I

RADIOACTIVITY (^{131}I) IN INDIVIDUALLY COUNTED INTACT ORGANS 11 DAYS FOLLOWING INTRAVENOUS ADMINISTRATION OF VARIOUS TYPES OF PARTICLES. THE RESULTS ARE GIVEN IN PERCENT AND PERCENT PER GRAM ORGAN OF TOTAL ACTIVITY IN COLLECTED ORGANS.

Type of particles injected	Lungs		Heart		Liver		Spleen		Kidneys	
	%	% per g	%	% per g	%	% per g	%	% per g	%	% per g
Polystyrene microspheres (1.27 μm)	5.9	0.63	0.0	0.00	90.2	0.88	3.8	5.10	0.1	0.01
	5.6	0.59	0.0	0.00	87.7	1.31	6.1	5.67	0.0	0.01
	0.4	0.03	0.0	0.00	96.4	0.97	3.1	1.82	0.1	0.01
mean	3.9	0.42	0.0	0.00	91.4	1.05	4.3	4.20	0.1	0.01
Polystyrene microspheres (15.8 μm)	78.4	6.19	0.2	0.03	4.2	0.04	0.0	0.00	2.2	0.12
	98.9	8.38	0.0	0.00	0.7	0.01	0.0	0.00	0.3	0.01
mean	88.7	7.28	0.1	0.02	2.5	0.03	0.0	0.00	1.3	0.06
DEAE fibres (~5 μm)	84.8	8.61	0.0	0.00	9.1	0.07	0.0	0.00	5.9	0.38
	80.9	6.51	0.5	0.09	9.1	0.06	0.4	0.23	9.2	0.31
	55.0	4.39	0.0	0.00	19.5	0.24	2.3	1.46	23.2	1.10
mean	73.6	6.50	0.2	0.03	12.6	0.12	0.9	0.56	12.8	0.50
DEAE * fibres (~30 μm)	0.8	0.72								
	0.3	0.04								
	0.6 **	0.38 **								
	42.7	7.67	1.8	0.24	50.7	0.39	0.0	0.00	3.8	0.18
	53.7	3.83	1.0	0.10	40.7	0.22	0.0	0.00	4.5	0.16
mean	48.1 ***	5.80 ***	1.4	0.17	45.7	0.31	0.0	0.00	4.2	0.17
DEAE microspheres (40–160 μm)	93.1	7.37	0.2	0.02	3.4	0.03	0.1	0.13	3.2	0.11
	95.5	6.54	0.1	0.01	2.5	0 03	0.2	0.19	1.7	0.09
	89.4	5.94	0.0	0.00	7.5	0.06	0.0	0.00	3.0	0.12
mean	92.7	6.61	0.1	0.01	4.5	0.04	0.1	0.11	2.6	0.11

* The rabbits injected died after 4 min.

** Abnormal lung.

*** Normal lung.

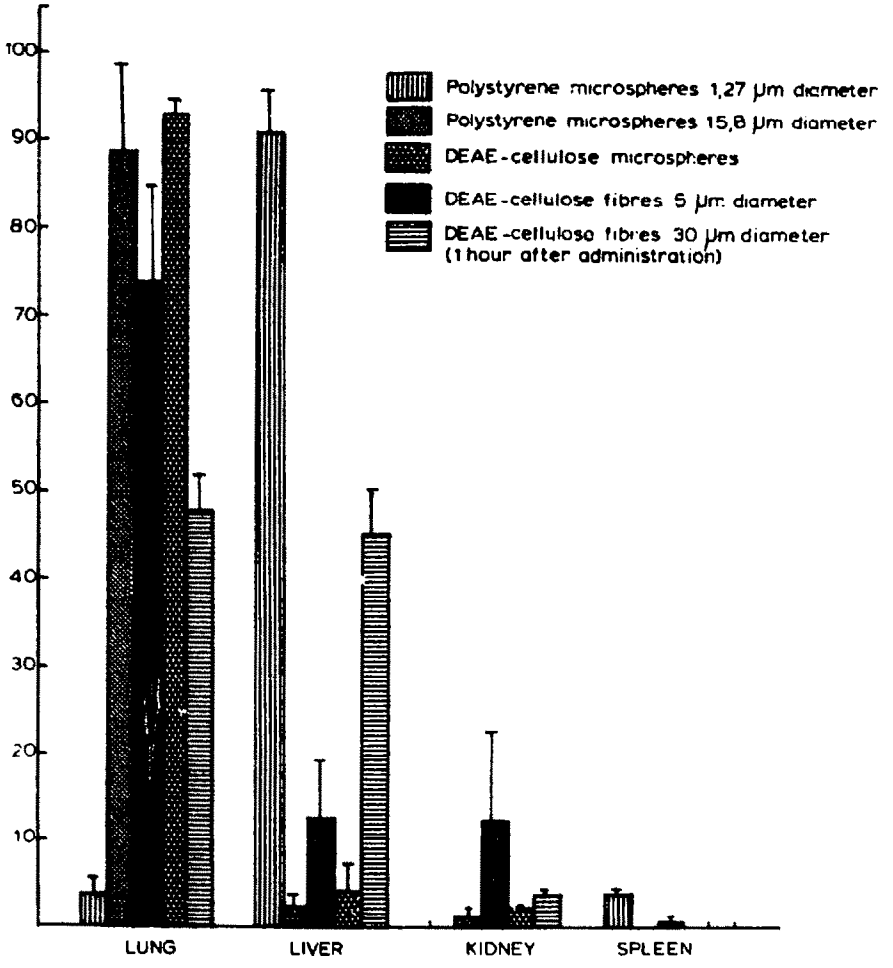

Fig. 6. Distribution of particulate systems in various organs 11 days after intravenous administration.

found in the liver. The activity in the kidney was also significant. The large DEAE-cellulose fibres had a fatal effect in the two rabbits tested. Within 4 min the animals died of pulmonary embolism. On post-mortem examination the right lung of each animal was found to be fully inflated and abnormal. This lung was found to have low activity. The normal lung had much higher levels of activity. Clearly the particle had caused blockage of the main pulmonary vessels on the right side of the lung. The liver contained 50% of the activity and there were also significant levels in the kidneys and heart. Thus the fibres were partly trapped in one of the lungs and partly circulating in the blood pool.

Histology

Histological examination demonstrated the presence of the larger particles (DEAE-cellulose microspheres (40–160 μm), DEAE-cellulose fibres (5 μm and 30 μm) and polystyrene microspheres (15.8 μm)) in the lungs (Fig. 7) and it was striking that the large particles were able to deposit deeply into the capillary beds of the lungs (Davis, 1975). Particulate material was not detected in the other organs

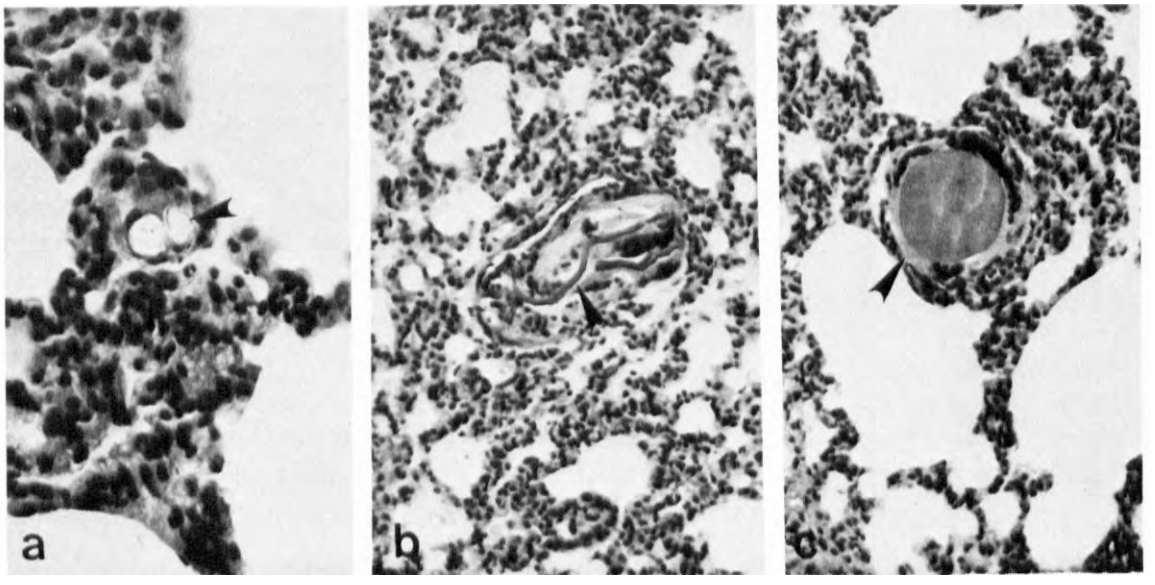

Fig. 7. Photomicrographs showing (arrowed) the deposition of inert fibres and spheres within the lungs. a: 15.8 μm diameter polystyrene microspheres; ×300. b: 5 μm diameter DEAE-cellulose fibres; ×150. c: 40–60 μm DEAE-cellulose microspheres; ×150.

examined. The liver certainly contained the activity associated with the injected dose of the smaller polystyrene particles (1.27 μm) but these were not detected within the organ by light microscopy. This negative finding may be due to an inability to resolve intracellular polystyrene particles of this size or reflect a loss of particles during histological processing.

Discussion

The reticuloendothelial system of the body has a major role in removing small foreign particles from the circulation. Phagocytic cells are found in many different sites but the Kupffer cells in the liver are of particular importance. The response of the reticuloendothelial system to foreign particles is influenced by factors such as particle size, charge and the nature of the surface.

Colloidal sulphur particles tagged with technetium 99-m have been used for some years for liver imaging (Chandhuri et al., 1973).

Previous workers have demonstrated that the clearance of polystyrene microspheres from the circulation after intravenous administration is dependent upon particle size (Kanke et al., 1980). Spherical particles less than 7 μm in diameter were deposited in the liver, while particles larger than 7 μm were filtered mechanically and retained for prolonged periods in the lungs.

Thus in the present study the smaller polystyrene microspheres behaved as "classical" colloidal particles and were taken up by the Kupffer cells of the liver. The larger polystyrene particles were lodged in the capillary beds of the lungs since they were larger than the critical size for passage through the pulmonary vascular bed. A

rapid uptake of large particles into the lungs was observed by Kanke et al. (1980) who also reported the redistribution of small polystyrene divinyl benzene microspheres from the lung to the liver within a few minutes following intravenous administration. These workers in addition commented on maxima in blood activity–time profiles similar to those shown in Fig. 5. However, they were unable to provide a suitable explanation for the phenomenon.

The DEAE-cellulose particles were all taken up by the lung, irrespective of the particle size. The particles of a nominal 5 μm diameter were effectively cleared by mechanical filtering. Clearly the shape of a particle as well as its size is of importance. Spherical particles would be expected to travel through capillary beds more easily than fibres which could become entrapped through a "log-jam" effect. The larger cellulose fibres (30 μm nominal diameter) were fatal to the two rabbits studied. Death was caused by obstruction of the pulmonary vessels. In contrast the large DEAE-cellulose microspheres were well tolerated even though they were of 40–160 μm diameter and all rabbits survived until the end of the experimental period with no evidence of adverse reactions.

Large degradable microspheres formed by gelatin and containing pharmacological agents have been used by Yoshioka et al. (1981) for the specific delivery of the anti-cancer agent, mitomycin C, to the lung. The present results support the view that large microspheres could find use for the selective delivery of drugs to the lungs, although toxicity consideration must not be ignored.

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