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Intestinal Uptake of Particulate Material by Dexamethasone-treated Rats: Use of a Novel Technique to Avoid Intestinal Mucosal Contamination

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Abstract

The aim of this study was to investigate the effect of immune suppression on the uptake of particles across the wall of the intestine and the dissemination of the particles to systemic organs.

Normal and dexamethasone-immunosuppressed rats were dosed orally with 0·5 mL distilled water or fluorescent polystyrene latex particle suspension containing $2{\cdot}33 \times 10^9$ 2-μm diameter particles. One hour after particle dosing, the animals were killed by CO_2 asphyxiation. The intestinal tissues and systemic organs were sampled for particle quantitation. To avoid contamination by particles adherent to intestinal mucosa the epithelium of intestinal tissue samples was removed before quantification. The number of fluorescent particles in tissues was determined by fluorescence microscopy of digests of selected samples. The uptake of particulate material across the intestinal wall was significantly ($P < 0{\cdot}05$) increased in rats treated with dexamethasone but the number of particles transferred to systemic organs did not differ from values found for control animals.

The results suggest that although dexamethasone increased intestinal permeability the apparatus or mechanisms involved in particle transport to distal sites were not affected during immune suppression.

Under normal conditions, the mucosal defence system of the gastrointestinal tract comprises non-immune (gastric acid, proteolysis, mucus, peristalsis and intestinal flora) and immune (secretory IgA and phagocytic cells) mechanisms which prevent adherence of particulate material to the mucosal epithelial cells, which are thereby shielded from pathogenic effects (Udall & Walker 1987; Sanderson & Walker 1994). However, several studies have demonstrated that a variety of particulate matter, both viable and non-viable, can be translocated across the wall of the intestine (Trier 1991; Kato & Owen 1994; Simon et al 1995). Previous studies suggested that gut-associated lymphoid tissue is the major route of uptake of particles translocated from the intestinal lumen into the intestinal submucosa; the particles pass through the unique epithelial cells, M cells, on the dome of gut-associated lymphoid tissue (Neutra & Kraehenbuhl 1994; Jepson et al 1996). There is also evidence that the villous epithelium in rats is capable of uptake of particles of various sizes (Matsumo et al 1983; Jani et al 1989; Florence et al 1995; Hodges et al 1995; Simon et al 1995). In addition, there are reports indicating the involvement of immune accessory cells in the uptake of particles (Matsumo et al 1983; Wells et al 1988; Nicklin & Miller 1989; Liu & Macpherson 1993; Trout & Lillihoi 1993), although the contribution of the immune system to uptake of particulate matter is not fully elucidated.

Roy & Walsh (1992) demonstrated that the immunosuppressive drug dexamethasone reduced the size of intestinal lymphoid domes and follicles. Their work showed that dexamethasone depleted the number of M cells and lymphocytes in Peyer's patches. In addition, dexamethasone can reduce the number and phagocytic function of macrophages (Lortie et al 1990; Nakamura et al 1996) and it can suppress neutrophil function (Bober et al 1995).

Macrophages are thought to be primarily involved in particle conveyance (Matsumo et al 1983; Wells et al 1988). We hypothesized that

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dexamethasone might alter the uptake of particulate matter across the intestinal wall and the translocation of the particles from the intestine to systemic organs. The purpose of this study was to examine the effect of dexamethasone on the transfer of orally administered 2-μm diameter fluorescent polystyrene latex particles from the intestine to systemic and lymphoid organs, by quantifying the number of particles found within these tissues.

Materials and Methods

Animals and procedures

Male CD Sprague–Dawley rats (Charles River, Kent, UK), 270–300 g, eight-weeks-old, were housed and acclimatized under standard conditions for one week before the study. All animal treatment was performed by a licensed investigator in accordance with the Animals (Scientific Procedures) Act (1986).

Animals were given subcutaneous dexamethasone 21-phosphate, disodium salt (Sigma, Poole, UK), at doses of 0·012 ($n=5$), 0·3 ($n=6$), 1·0 ($n=5$) and 3·0 mg kg^{-1} ($n=11$) for three consecutive days. Preliminary studies have shown these doses of dexamethasone to be immunosuppressive in Sprague–Dawley rats, as shown by a reduction in the numbers both of antibody-forming cells and of total white blood cells, a reduction of lymphoid organ weight, and histopathological findings showing reduced cellularity in lymphoid organs (unpublished data). Concurrent control animals were given normal saline, (control 1 (normal saline and latex particles), $n=11$; control 2 (normal saline), $n=7$). On the last day of dexamethasone treatment all the animals were fasted for approximately 16 h. Animals in the control 1 and dexamethasone-treated groups were then given, by gavage, 0·5 mL of a suspension of non-ionic monodispersed, fluorescent-labelled polystyrene latex particles, $2{\cdot}139 \pm 0{\cdot}05$ μm diameter (Polysciences, Northampton, UK) containing $2{\cdot}33 \times 10^9$ particles. Animals in control group 2 were given distilled water by gavage. One hour after administration of particles or distilled water the animals were killed by CO_2 asphyxiation.

Examination of tissue samples

To minimize contamination by fluorescent particles the skin of each rat was removed before opening of the abdomen and surgical instruments used for dissecting animals were thoroughly cleaned between each autopsy. Disposable materials were used for each autopsy. Again, to minimize any risk of contamination with fluorescent particles, internal organs were collected before the intestinal tissues.

Femoral bone-marrow nucleated cells were collected. The left femur was excised, the ends cut off and the marrow cells flushed with 2·5 mL phosphate-buffered saline into a collecting vessel. Bone-marrow samples were immediately placed on ice. The bone-marrow nucleated cells were counted by use of a Serono Diagnostics model 9010 blood-cell counter.

For assay of particles, samples of brain, heart, thymus, lung, liver, spleen, kidney, testis and mesenteric lymph nodes were taken from each animal. To remove the gut contents, the entire intestine was excised and its lumen flushed twice with ice-cold phosphate-buffered saline (40 mL), containing (mM) 96 NaCl, 1·5 KCl, 8 KH_2PO_4, 5·6 Na_2HPO_4, pH 7·4. After removal of the gut contents, tissue samples were taken from each animal. Tissue samples consisted of three Peyer's patches from the most proximal region, near the pyloric sphincter, and the most distal region, near the caecum, of the small intestine. Although Peyer's patch tissues were carefully dissected free from adjacent tissues, a small proportion of villous tissue and underlying musculature was probably included in the tissue samples excised. Samples of the small intestine adjacent to excised Peyer's patches were carefully taken from proximal and distal regions of the small intestine and also from the ascending colon. The samples of tissues from the intestine and internal organs were weighed separately. All internal organ samples were fixed in 70% industrial methylated spirit.

Removal of non-translocated particles (epithelium stripping)

To exclude non-translocated particles adherent to the mucosal surface of the intestinal epithelium, intestinal epithelial cells were dissociated from segments of the intestine by a modification of the method of Fox et al (1985) and Tepperman et al (1993). Each tissue sample was weighed in a separate container immediately after excision and then washed in its own container for 2 h in ice-cold phosphate-buffered saline before epithelial stripping. Each sample of intestine from each control and particle-treated rat was washed twice in ice-cold phosphate-buffered saline and in ice-cold phosphate-buffered saline plus 1 mM dithiotreitol, to remove intestinal mucus (Ferraris et al 1992). Each washing of tissue in buffer was performed for 30 min. Before changing the buffer, the tissue sample was agitated in a vortex mixer for 30 s. After washing, each tissue sample was transferred to a separate plastic tube containing citrate buffer (mM, 96 NaCl, 1·5 KCl, 8 KH_2PO_4, 5·6 Na_2PO_4, 27 $Na_3C_6H_5O_7.2H_2O$, pH 7·4; 10 mL) to loosen the

attachment of the epithelial cells (Ferraris et al 1992). The tubes were placed in a shaking water bath (37°C, 80 rev min^{-1}) for 15 min.

For intestinal epithelial cell dissociation, samples of the intestinal tissues were then removed from the citrate buffer and transferred to individual clean plastic tubes containing Hanks balanced salt solution (Gibco Laboratories) supplemented with 1·5 mM EDTA and 0·5 mM dithiotreitol, pH 7·4 (epithelial-cell-dissociating solution). The samples were then placed in a shaking water bath (37°C, 80 rev min^{-1}) for 60 min. The removal of calcium by EDTA and citrate results in loss of protein mediated, calcium-dependent adhesion of cells to each other and to the basement membrane (Ferraris et al 1992). The tissues were removed from the epithelial-cell-dissociating solution, extensively washed with phosphate-buffered saline (2 × 30 min) and blotted dry. After each incubation and each washing period the tissue samples were agitated in a vortex mixer for 30 s to remove adherent particles.

Particle quantification

After epithelial stripping half of each tissue sample was prepared for histological examination and the other half was weighed and placed in 70% industrial methylated spirit for 24 h. The fixed intestine and internal organ samples and bone-marrow cell samples were solubilized in potassium hydroxide (15%, 10 mL) for at least four days (Simon et al 1995). Each sample of solubilized tissue was filtered through a black-back gridded filter (Millipore, UK; pore size 0·8 μm) which was then placed on a cleaned glass slide and mounted in Hydromount (National Diagnostics, Atlanta, GA). Separated sets of glass slides and cover slips were used for each control and experimental sample. Particles on filters were readily detected against the black background of the filter by fluorescence microscopy and were counted. The number of particles detected in each sample was expressed as the number of particles (g tissue)$^{-1}$ (fresh weight). The intensity of fluorescence of the particles had previously been shown not to be affected by the alcohol fixation or by treatment with potassium hydroxide.

Technique control of intestinal tissue for quantification of non-translocated particles

Intestinal tissue from animals in control group 2 was treated in exactly the same way as described for tissue from particle-treated rats. To assess the number of particles that might have adhered to the tissue samples during the epithelial cell dissociation and washing steps, i.e. particles released by the dissociation technique which might then have adhered to the newly exposed submucosal tissues, intestinal tissue from undosed animals was excised, weighed and incubated in the epithelial-cell-dissociating solutions previously used for the corresponding tissue from dosed animals. Tissue from animals in control group 2 was referred to as technique-control tissue. After epithelial cell dissociation for 60 min, technique-control tissue was washed, fixed, solubilized and examined for the number of particles as previously described for the experimental tissue.

Intestinal permeability test

Two groups of six rats were housed under the same conditions as for the particle-uptake study and were allowed to acclimatize for one week. Rats in the control group were treated with normal saline subcutaneously for three consecutive days. Rats in the experimental groups were injected subcutaneously with dexamethasone 1 or 3 mg kg^{-1} for three consecutive days. On the last day of dosing with normal saline or dexamethasone rats were fasted for 16 h before further treatment. Rats were anaesthetized (sodium pentobarbital, 60 mg kg^{-1}, i.p.) and intestinal permeability was measured by a modification of the method described by Chen et al (1996). Rats in the control and dexamethasone-treated groups were given intestinal permeability test solution (1 mL) containing 5 mg fluorescein isothiocyanate (FITC)-dextran, 3000 MW (molecular probes), intraduodenally. One hour after FITC-dextran dosing portal blood was taken from each rat using a heparinized needle and syringe. Plasma samples were analysed for FITC-dextran using fluorescence spectrometry (Hitachi F-2000, Japan) at an excitation wavelength of 480 nm and an emission wavelength of 520 nm.

Statistical analysis

Quantitative data were compared by means of the non-parametric Kruskal–Wallis test. $P < 0.05$ was considered as indicative of statistical significance.

Results

Particle uptake

The number of particles taken up in the tested segments of the epithelium-stripped intestine obtained from the proximal and distal small intestine and colon of rats treated with dexamethasone (3 mg kg^{-1}) was generally significantly higher than in rats previously treated with normal saline alone and dosed with particles ($P < 0.05$). Uptake of particulate matter into intestinal tissues of immunosuppressed rats was generally increased by increasing the dose of dexamethasone (Table 1).

Table 1. Numbers of 2-μm latex particles detected in the intestinal tissues of rats treated with dexamethasone or normal saline for three consecutive days and then dosed orally with $2{\cdot}33 \times 10^9$ particles or with distilled water.

Particle treatment	Proximal Peyer's patches	Distal Peyer's patches	Proximal small intestine	Distal small intestine	Colon
Normal saline + distilled water	0±0	0±0	0±0	0±0	0±0
Normal saline + particles	1025±558 ($2{\cdot}76 \times 10^{-6}$)	7591±3268 ($2{\cdot}75 \times 10^{-5}$)	6423±5185 ($7{\cdot}39 \times 10^{-4}$)	17958±15805 ($1{\cdot}87 \times 10^{-3}$)	9304±5631 ($6{\cdot}05 \times 10^{-4}$)
Dexamethasone 0·012 mg kg^{-1} + particles	3743±2652 ($1{\cdot}01 \times 10^{-5}$)	563±269 ($2{\cdot}04 \times 10^{-6}$)	5621±4453 ($6{\cdot}46 \times 10^{-4}$)	1320±562 ($1{\cdot}38 \times 10^{-4}$)	1294±493 ($8{\cdot}41 \times 10^{-5}$)
Dexamethasone 0·3 mg kg^{-1} + particles	4871±3362 ($1{\cdot}01 \times 10^{-5}$)	6048±2260 ($2{\cdot}19 \times 10^{-5}$)	5985±1247 ($6{\cdot}88 \times 10^{-4}$)	13709±12227 ($1{\cdot}43 \times 10^{-3}$)	1912±718 ($1{\cdot}24 \times 10^{-4}$)
Dexamethasone 1·0 mg kg^{-1} + particles	2638±744* ($7{\cdot}10 \times 10^{-6}$)	5982±2243 ($2{\cdot}17 \times 10^{-5}$)	56349±30976 ($6{\cdot}48 \times 10^{-3}$)	9161±4479 ($9{\cdot}56 \times 10^{-4}$)	11533±10562 ($7{\cdot}5 \times 10^{-4}$)
Dexamethasone 3·0 mg kg^{-1} + particles	36646±19137* ($9{\cdot}86 \times 10^{-5}$)	8302±3344 ($3{\cdot}01\% \times 10^{-5}$)	104313±59915* (0·01)	11694±6460 ($1{\cdot}22 \times 10^{-3}$)	25436±12428* ($1{\cdot}65 \times 10^{-3}$)

Tissue samples were taken 1 h after particle dosing. The number of particles is shown for each region of the epithelium-stripped intestinal tissues examined. Results are presented as the number of particles (g tissue)$^{-1}$ (mean ± s.e.m.). The numbers in brackets are the amount (%) of dose taken from each whole region of the intestinal tissue. *$P < 0{\cdot}05$, significantly different from result for control rats treated with normal saline and latex particles.

Table 2. Numbers of 2-μm latex particles detected in the epithelium-detached fraction of various regions of samples of intestinal tissue from rats.

Particle treatment	Proximal Peyer's patches	Distal Peyer's patches	Proximal small intestine	Distal small intestine	Colon
Normal saline + distilled water	0±0	0±0	0±0	0±0	0±0
Normal saline + particles	170±35	25470±19478	19817±15881	30220090±2988150	5012±3334
Dexamethasone 0·012 mg kg^{-1} + particles	448±304	204±79	2414±1752	2187±1487	638±339
Dexamethasone 0·3 mg kg^{-1} + particles	4817±3362	6048±2260	5985±1247	13709±12227	1912±718
Dexamethasone 1·0 mg kg^{-1} + particles	2683±744	5982±2243	56349±30976	9161±4479	11533±10526
Dexamethasone 3·0 mg kg^{-1} + particles	14589±10931	6228±4193	1046660±973235	24719±12333	3789±1183

The rats were the same as those used to obtain the data presented in Table 1. Results are presented as the number of particles (g tissue)$^{-1}$ (mean ± s.e.m.).

Large numbers of particles, including adherent non-translocated particles, were detected in samples of detached intestinal epithelium (Table 2).

Particles were not detected in control rats treated with normal saline (Table 1). Small numbers of particles were found in technique-control intestinal tissues corresponding to all particle-treated tissues. The numbers of particles found in those samples did not exceed 1% of the numbers in experimental tissues and were ignored (Table 3).

Histological examination of intestinal samples showed that the overlying epithelium from the Peyer's patches and the epithelial cells from the villous tip to the lower villous-upper crypt region were completely removed by the epithelial-cell-dissociation procedures. The numbers of particles detected in systemic organs of rats treated with the highest dose of dexamethasone did not differ significantly from those in particle-treated control rats or in rats given the lower doses of dexamethasone (Table 4).

Comparison of the numbers of particles taken up and transported into lymphoid organs, namely thymus, spleen, mesenteric lymph nodes and bone marrow, of rats treated with various doses of dexamethasone and of particle-treated control rats did not show any statistically significant differences (Table 4).

Intestinal permeability

Administration of dexamethasone (1 and 3 mg kg^{-1}) led to a significant increase ($P < 0{\cdot}05$) in intestinal permeability compared with that of FITC-dextran-treated control rats. The permeability of the intestine of rats given the higher dose of dexamethasone (3 mg kg^{-1}) was greater than for

Table 3. Numbers of particles detected in technique-control tissues taken from undosed rats incubated in the epithelial-cell-dissociating solution of corresponding tissues derived from dexamethasone-treated rats.

Particle treatment	Proximal Peyer's patches	Distal Peyer's patches	Proximal small intestine	Distal small intestine	Colon
Normal saline + particles	53 ± 27	186 ± 111	61 ± 36	37 ± 20	19 ± 9
Dexamethasone 0·012 mg kg^{-1} + particles	8 ± 8	8 ± 8	8 ± 6	21 ± 18	3 ± 2
Dexamethasone 0·3 mg kg^{-1} + particles	49 ± 41	80 ± 42	42 ± 13	89 ± 55	4 ± 2
Dexamethasone 1·0 mg kg^{-1} + particles	40 ± 40	39 ± 28	167 ± 118	412 ± 385	38 ± 15
Dexamethasone 3·0 mg kg^{-1} + particles	235 ± 139	116 ± 45	325 ± 167	44 ± 18	10 ± 3

Results are presented as the number of particles (g tissue)$^{-1}$ (mean ± s.e.m.).

Table 4. Quantification of the dissemination of latex particles conveyed from the intestine to the internal organs of rats treated with dexamethasone or normal saline and orally dosed with $2{\cdot}33 \times 10^9$ particles or distilled water.

Treatment	Brain	Heart	Lung	Liver	Kidney	Testis	Thymus	Spleen	Mesenteric lymph nodes	Bone marrow
Normal saline + distilled water (n = 7)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Normal saline + particles (n = 5)	11·3 ± 3·9	3·4 ± 1·3	11·2 ± 4·3	16·5 ± 7·7	5·9 ± 1·6	6·7 ± 1·9	33·0 ± 25·0	26·1 ± 14·1	12·3 ± 7·0	0·3 ± 0·2
Dexamethasone 0·012 mg kg^{-1} + particles (n = 5)	14·8 ± 7·8	2·8 ± 1·8	5·0 ± 2·7	12·8 ± 8·6	4·2 ± 2·5	0·8 ± 0·2	10·0 ± 9·0	6·4 ± 2·9	1·8 ± 1·1	0·8 ± 0·6
Dexamethasone 0·3 mg kg^{-1} + particles (n = 6)	2·7 ± 1·5	7·5 ± 3·6	9·8 ± 3·6	4·7 ± 4·5	4·8 ± 2·5	7·8 ± 2·6	14·2 ± 6·6	16·7 ± 9·9	9·6 ± 6·5	0·5 ± 0·5
Dexamethasone 1·0 mg kg^{-1} + particles (n = 5)	8·8 ± 11·1	7·6 ± 5·9	9·0 ± 2·9	9·0 ± 6·1	11·4 ± 3·1	8·8 ± 4·1	39·3 ± 24·9	8·4 ± 7·0	4·2 ± 3·1	0·8 ± 0·4
Dexamethasone 3·0 mg kg^{-1} + particles (n = 11)	17·6 ± 7·2	9·5 ± 2·6	22·7 ± 7·8	7·0 ± 0·9	16·2 ± 7·3	10·9 ± 5·1	45·7 ± 30·6	30·4 ± 13·7	18·7 ± 11·4	1·6 ± 0·8

Tissue samples were taken 1 h after particle dosing. Data are presented as the number of particles (g tissue)$^{-1}$ (mean ± s.e.m.) except for bone marrow, for which data are presented as number of particles per 10^7 nucleated cells counted (mean ± s.e.m.).

Table 5. Intestinal permeability to FITC-dextran (molecular weight 3000) of rats dosed subcutaneously with normal saline or with 1 or 3 mg kg^{-1} dexamethasone for three consecutive days.

Treatment	Plasma FITC-dextran (μg mL^{-1})
Normal saline	0·77 ± 0·10
Dexamethasone 1 mg kg^{-1}	1·32 ± 0·18*
Dexamethasone 3 mg kg^{-1}	1·37 ± 0·14*

Plasma levels of FITC-dextran are shown as mean ± s.e.m., n = 6 for each group. *$P < 0{\cdot}05$, significantly different from control rats.

those treated with the lower dose of dexamethasone (1 mg kg^{-1}) but the difference was not significant (Table 5).

Discussion

It is known that viable and non-viable particles can be translocated across the gastrointestinal epithelium (Trier 1991; Kato & Owen 1994) and disseminated to distant sites of the body (Jani et al 1989; Florence et al 1995; Mathiowitz et al 1997; Simon et al 1997). Although there is increasing evidence of the involvement of immune accessory cells in the uptake of particulate matter (Matsumo et al 1983; Wells et al 1988; Nicklin & Miller 1989;

Liu & Macpherson 1993; Trout & Lillihoi 1993), the precise mechanisms underlying the involvement of cells of the immune system are not understood.

The aim of this study was to examine the role of the immune system in uptake of particulate matter and transportation to distal sites using the immunosuppressive drug dexamethasone. There is evidence suggesting that dexamethasone alters gut-associated lymphoid tissues by reducing the size of lymphoid domes and follicles. Dexamethasone depletes M cells, a major route of particle passage, and lymphocytes (Roy & Walsh 1992), and can also suppress the phagocytic function of macrophages (Lortie et al 1990; Nakamura et al 1996), which have been shown to be crucial accessory cells in uptake of particulate matter (Matsumo et al 1983; Wells et al 1988; Nicklin & Miller 1989; Liu & Macpherson 1993; Trout & Lillihoi 1993). There is evidence that dexamethasone depletes lymphocytes in the peripheral blood (unpublished data). As uptake of particulate matter and dissemination to distal organs occurs rapidly (Sass et al 1990; Jenkins et al 1994; Thomas et al 1996), it seems that macrophages might not suffice to account for this process because they are present in relatively small numbers. It has been claimed that CD4+ and CD8+ lymphocytes are involved in uptake of particulate matter (Trout & Lillihoi 1993).

In this study 2-μm polystyrene latex particles were detected within the tested segments of the proximal and distal small intestine and in the colon during immunosuppression. The particles detected represent the absolute numbers of particles translocated across the intestinal epithelium, as non-translocated particles adherent to the mucosa were excluded by epithelial stripping. Large numbers of particles were recovered in the epithelium-detached fraction. The numbers of particles within the epithelium-detached samples varied widely. The numbers of particles in the intestinal wall tissue of rats treated with the higher dose of dexamethasone (3 mg kg^{-1}) were generally higher than in the tissue of control rats and of those receiving the lower dose of dexamethasone (1 mg kg^{-1}). However, this was not so for tissues in the distal region of the small bowel. Relative larger proportions of particles were found to accumulate in each region of the intestinal tissues than the systemic organ tissues, presumably because of the dexamethasone-induced increase in intestinal permeability, as demonstrated here. Because the uptake of particles by intestinal tissues obtained from rats treated with 0·012, 0·3 or 1 mg kg^{-1} dexamethasone was not significantly different, intestinal permeability tests were performed on animals given high doses of the drug, i.e. 1 and 3 mg kg^{-1}. Spitz et al (1994) reported that subcutaneous administration of dexamethasone, 5·3 mg kg^{-1}, for two consecutive days induced increased intestinal permeability in female Fisher rats. Moreover, the translocation of particles across the intestinal epithelium might, therefore, be a passive, dexamethasone-mediated process via the formation of ostia on the luminal surface of gut-associated lymphoid tissue (Roy & Walsh 1992).

Although dexamethasone reduces the number and phagocytic function of macrophages (Lortie et al 1990; Nakamura et al 1996), a small number of particles was transported to distal organs. Their passage to such tissues might be a function of the remaining lymphocytes and macrophages. It is surprising that the total numbers of particles transferred to systemic organs was in the same range as in the control animals. This might be because the greater numbers of translocated particles in the intestinal wall tissues in rats treated with dexamethasone are exposed to a lower number of remaining immune accessory cells. Another possible explanation is that some proportion of the dosed particles in the intestine might be transported to distal organs by direct circulation within blood vessels and lymphatics, not via phagocytes (O'Hagan 1990).

These results show the effect of immune disturbance induced by dexamethasone on the uptake of particulate matter across the wall of the intestine and the translocation of the particles from the intestine to the systemic organs. The precise mechanisms involved and the importance of the immune system in the uptake of particulate matter and its dissemination requires further investigation.

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