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Quantitative and microautoradiographic study on mouse intestinal distribution of polycyanoacrylate nanoparticles *

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Summary

Polyhexyl-[3-^{14}C]cyanoacrylate nanoparticles were perorally administered to mice. After 90 min, 4, 8, 24, 48 h, and 6 days the animals were killed and the intestine was divided into 24 sections. The radioactivity distribution between neighbouring sections in individual animals was very irregular and could vary by up to a factor of 1000. The overall distribution, however, was more homogeneous with a distinct accumulation in the sections before the ileo-cecal junction after 90 min and in the cecum after longer time periods. The amount of radioactivity dropped to 30–40% of the 90 min value within 4–8 h and to 5% 24 h after dosing. Radioactivity (0.04%) was still detectable after 6 days. Histological investigations revealed radioactivity adjacent to the brush border, inside goblet cells, and in muscle cells up to 6 days after administration. The histogical analysis also gave evidence that labelled material was translocated to the circulation.

Introduction

Maincent et al. (1984, 1986) reported an increased bioavailability of vincamine in rabbits after peroral administration – namely 164% relative to solution – by binding of the drug to polyhexyl cyanoacrylate nanoparticles. A similar increase in peroral bioavailability was reported in dogs by Damgé et al. (1987) after incorporation of Lipiodol, an iodized oil, into polyisobutyl-2-cyanoacrylate nanoparticles in comparison to a Lipiodol emulsion. The question arises: why is the peroral bioavailability enhanced with nanoparticles? Although uptake of colloidal particulate materials in the intestine (persorption) was observed by Volkheimer (1972) for starch corns and other very small particles, and although nanoparticles were detected qualitatively associated with the lamina propria by scanning electron microscopy by Aprahamian et al. (1986) and also were found in intercellular spaces of the mucosa (Damgé et al., 1987), results obtained by our laboratory suggest that persorption of nanoparticles does not occur at a high level (Nefzger et al., 1984). Macroautoradiographic studies by Couvreur et al. (1986) showed an association of nanoparticles with the

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gut wall after peroral administration. For this reason, Maincent et al. (1986) put forward the hypothesis that the above-mentioned enhanced bioavailability of vincamine in comparison to a solution was caused by an increase in the intensity and/or duration of the contact of the drug with the gut wall due to the binding to nanoparticles.

The following quantitative and microautoradiographic study was carried out to obtain more insight into the intestinal distribution of polycyanoacrylate nanoparticles.

Materials and Methods

Preparation of [^{14}C]polyhexylcyanoacrylate nanoparticles

Hexyl-[3-^{14}C]cyanoacrylate (Amersham Int., U.K.) was dispersed by means of mechanical stirring at 4°C in 100 ml of an aqueous solution containing 50% 0.1 M HCl, 0.2% w/v Pluronic F68 (Serva, Heidelberg, F.R.G.) and 1% w/v dextran 70 (Pharmacia, Uppsala, Sweden). After 4 days, polymerization was complete and the pH was adjusted to 7.0 with NaOH. The resulting suspension was lyophilized and the powder was stored at 4°C. Immediately before use, the lyophilizate was resuspended in water containing 0.5% Pluronic F68 by ultrasonication. The nanoparticle preparation thus produced was investigated by transmission electron microscopy using negative staining with phosphotungstic acid. Nanoparticles with sizes varying between 200 and 300 nm were observed.

Animal experiments

Male albino mice strain ICR were used with an average weight of 20 g. Nanoparticles were administered perorally respectively intragastrically with a ball-point syringe to groups of 4–5 animals for each time point. The volume used was 0.25 ml/10 g of b.wt. equivalent to 2.7 mg of polymer with a radioactivity of 2.5 μCi. After certain time intervals, namely 90 min, 4 h, 8 h, 24 h, 48 h and 6 days, animals which were fasted for the last night, were killed by decapitation. The whole gut was excised, extended on a centimeter-scale and divided into 24 portions of 1–1.5 cm length. The cecum was used as an internal indicator, carrying always sample numbers 19, 20 and 21.

Scintillation counting

Aliquots of about 50 mg from each segment of the gut were dissolved in 1 ml of Omnisolve (LKB Products) at 50°C for 4 h. After bleaching with 0.5 ml of 30% H_2O_2 at 30°C for 1 h, the samples were set up in 10 ml Optiphase MP (LKB Products) and counted in a Packard Tricarb 2000 A scintillation counter.

Electron microscopic autoradiography

Likewise, small aliquots of the same segments of the gut as used for scintillation counting were fixed for 1 h in freshly prepared 2.5% glutaraldehyde in 25 mM cacodylate buffer, pH 7.2. Already during fixation, the tissue was minced with a razor blade to very small pieces. Following aldehyde fixation, the samples were washed with buffer, osmicated and embedded in Araldite ACM (Fluka AG, Buchs, Switzerland) after dehydration with graded ethanol.

Only samples from gut pieces with confirmed radioactivity clearly above the background were selected for further processing for electron microscopic autoradiography. The detailed method for handling ultrathin sections is described by Waser et al. (1987).

Results

A quantitative determination of the radioactivity in intestinal sections after peroral administration of ^{14}C-labelled polyhexyl cyanoacrylate nanoparticles showed that the particles were distributed very irregularly (Fig. 1). The whole intestine was divided into 24 sections of approximately equal size. The radioactivity between neighbouring sections varied by up to a factor of 1000.

After 90 min, the highest radioactivity was found in the sections before the cecum, whereas after all other time periods (4 h, 8 h, 48 h, and 6 days) the highest radioactivity was observable in the cecum (Fig. 2).

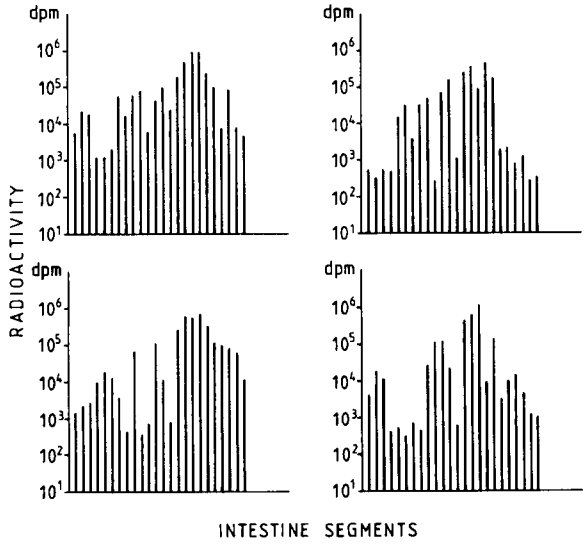


Fig. 1. Radioactivity distribution in the intestines of individual mice 90 min after peroral administration of polyhexyl-[3-^{14}C]cyanoacrylate nanoparticles. The intestine was divided into 24 equal sections.

The total radioactivity recovered in the intestine after 90 min amounted to about 60–80% of the administered dose. However, it has to be considered that this value represents a very rough extrapolation, since aliquots of the intestinal sections were set aside for the histological examinations and contained unknown quantities of radioactivity. Since this amount may be very variable as shown above and therefore may be different from the same sections used for quantitative determinations, the recovery value cannot be estimated more exactly.

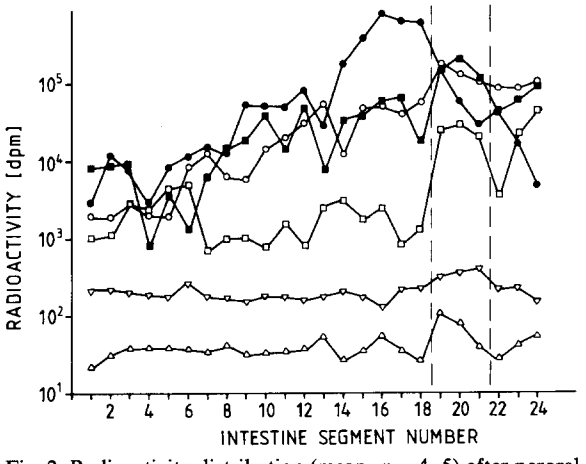


Fig. 2. Radioactivity distribution (mean, $n = 4–5$) after peroral administration of polyhexyl-[3-^{14}C]cyanoacrylate nanoparticles to mice. Times: ●, 90 min; ○, 4 h; ■, 8 h; □ 24 h; ▽ 48 h; and △, 6 days after administration. The intestine was divided into 24 equal sections. The frame (broken vertical lines) marks the cecum.

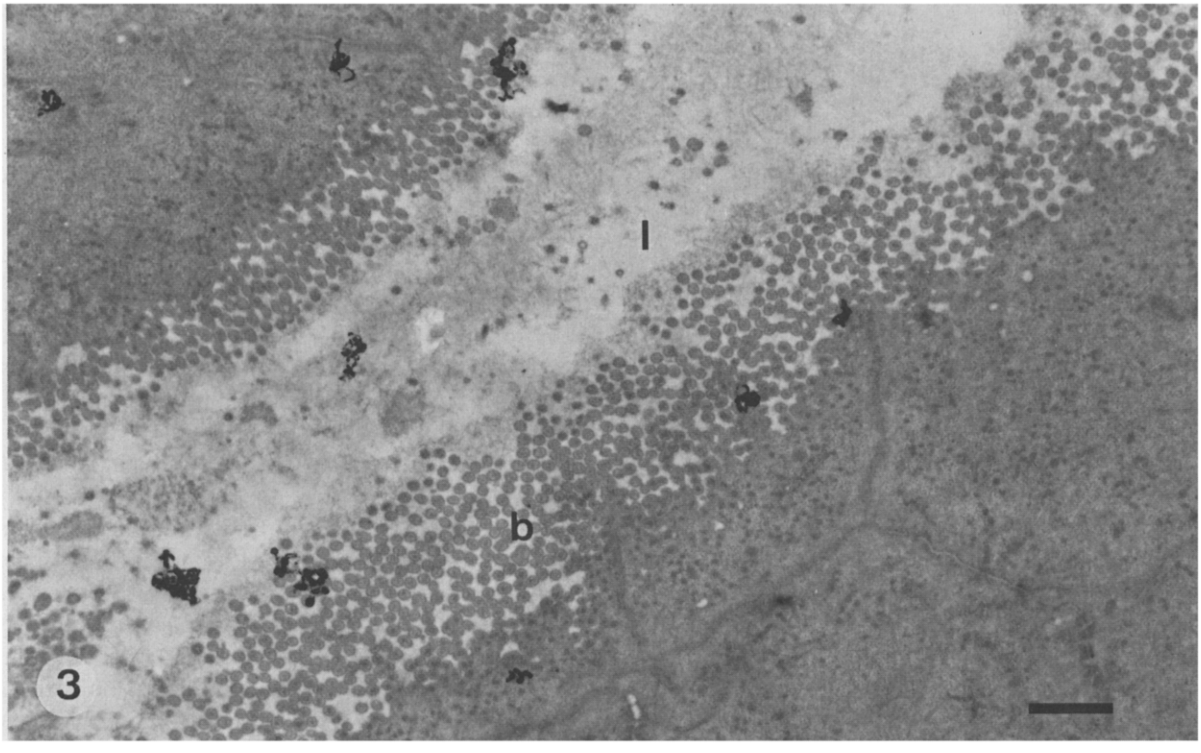
Nevertheless, the comparison of the radioactivity recovery after longer time periods with the 90 min value (Table 1) shows that there was a rapid elimination within 90 and 240 min and then stayed approximately constant for another 240 min. After 24 h, only 6% and after 48 h only 0.2% of the 90 min radioactivity was left. Surprisingly, 0.04% of the 90 min radioactivity was still detectable after 6 days.

The dynamics of the pathway and the retention of the ^{14}C-labelled polyhexyl cyanoacrylate carrier material in the digestive system of mice after intragastric administration was also followed by means of histological analysis by electron microscopy. With the intent to analyze only those nanoparticles that were closely in touch with the brush border membrane or that were already taken up into the mucosa and muscle cells, conventional methods in preparing tissue samples were used, thus implementing the removal of free or loosely bound particles during processing. Under these conditions, the autoradiographic observations confirmed the presence of radioactivity throughout the whole gut, e.g. from the jejunum to the colon including the cecum. Over a period of 6 days, at time points 90 min, 4, 8, 24 and 48 h, and 6 days, radioactivity could be found – although in minor

TABLE 1

Recovery of radioactivity (mean ± S.D.) in the intestine after different time intervals after peroral administration of polyhexyl-[3-^{14}C]cyanoacrylate nanoparticles

Time	dpm		% of 90 min value
	mean	S.D.	
90 min	3.32×10^5	0.98×10^5	100
4 h	1.27×10^5	0.31×10^5	38
8 h	1.42×10^5	0.62×10^5	43
24 h	1.93×10^4	2.44×10^4	5.8
48 h	6.27×10^2	2.85×10^2	0.19
6 d	1.20×10^2	0.30×10^2	0.04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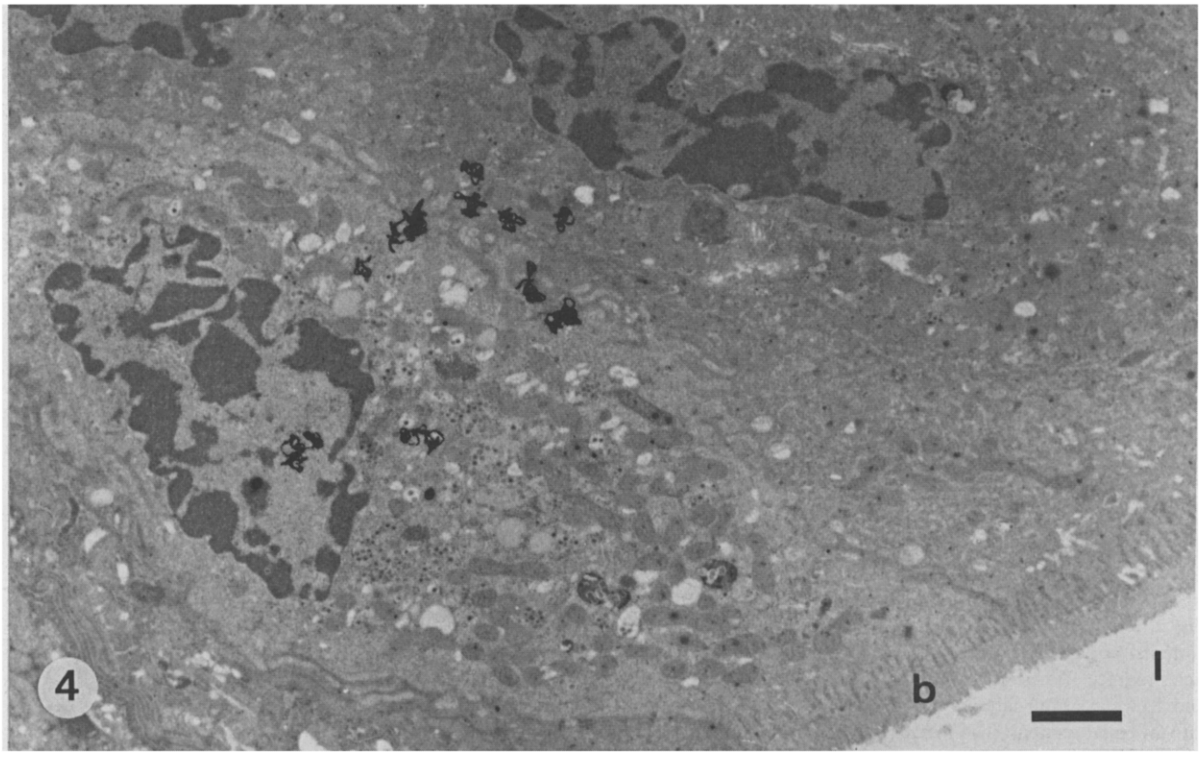
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quantities – in contact with the brush border membranes (Figs. 3, 5, 8), incorporated into the underlying cell layers (Figs. 4, 5, 7, 8), and, rather unexpectedly, in goblet cells (arrow in Fig. 6). Furthermore, as a very rare event, there was some evidence that the labelled material can be translocated to the circulation.

Discussion

The irregular distribution of individual doses of polyhexyl cyanoacrylate nanoparticles in the intestine was surprising, because the autoradiographs of Couvreur et al. (1986) indicated a homogeneous layer of nanoparticle radioactivity associated with the gut wall. However, as shown in Fig. 1, the variations appear to be totally random and were different from animal to animal. This irregular distribution pattern is an indication that nanoparticles may travel in a 'slug' form within clots of mucin as observed with other dosage forms, although rats and mice do not produce a lot of mucin and therefore are poor models for this phenomenon (Gruber et al., 1987). Nevertheless, no particular association with certain gut parts or with special tissue or cells was observable apart from the higher levels before the cecum after 90 min or in the cecum at later times (Fig. 2). This latter distribution pattern is also similar to that observed with other dosage forms (Davis et al., 1984, 1986; Gruber et al., 1987) and indicates a retention of the nanoparticles probably together with other intestinal contents at the ileo-cecal junction (valva ileocaecalis) around 90 min postdosing before discharge into the cecum. A cecal retention is later observable for up to 6 days.

Although the gastrointestinal transit time is often described as being 20–30 h for rats and mice (Gruber et al., 1987), the nanoparticles were retained only at a level of 30–40% for 8 h and about 5% for 24 h (Table 1). Despite the individual fluctuations discussed above, the overall distribution through the intestine was rather homogeneous, becoming more homogeneous after longer times after dosing. The latter properties combined with their relatively higher retention in the cecum suggest that nanoparticles may offer some advantages for the delivery of certain drugs (Gruber et al., 1987).

The histological investigations indicate some absorption of radioactivity confirming earlier findings by Nefzger et al. (1984) and Aprahamian et al. (1986). Unfortunately, electron microscopical studies did not allow the determination of whether the observed radioactivity resulted from nanoparticles or from degradation products. The histological analysis also does not reveal any particular absorption pathway.

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Fig. 3. Electron micrograph showing localization of radioactivity in the ileum of mice 8 h after peroral administration of polyhexyl-[3-^{14}C]cyanoacrylate nanoparticles. Nanoparticles are adsorbed to the villi of the ileum. b = brush border; l = lumen of the gut. Bar = 1 μm.

Fig. 4. Electron micrograph showing localization of radioactivity in the colon of mice 24 h after peroral administration of polyhexyl-[3-^{14}C]cyanoacrylate nanoparticles. Radioactivity is incorporated into muscle cells in a typical cluster formation. b = brush border; l = lumen. Bar = 1 μm.

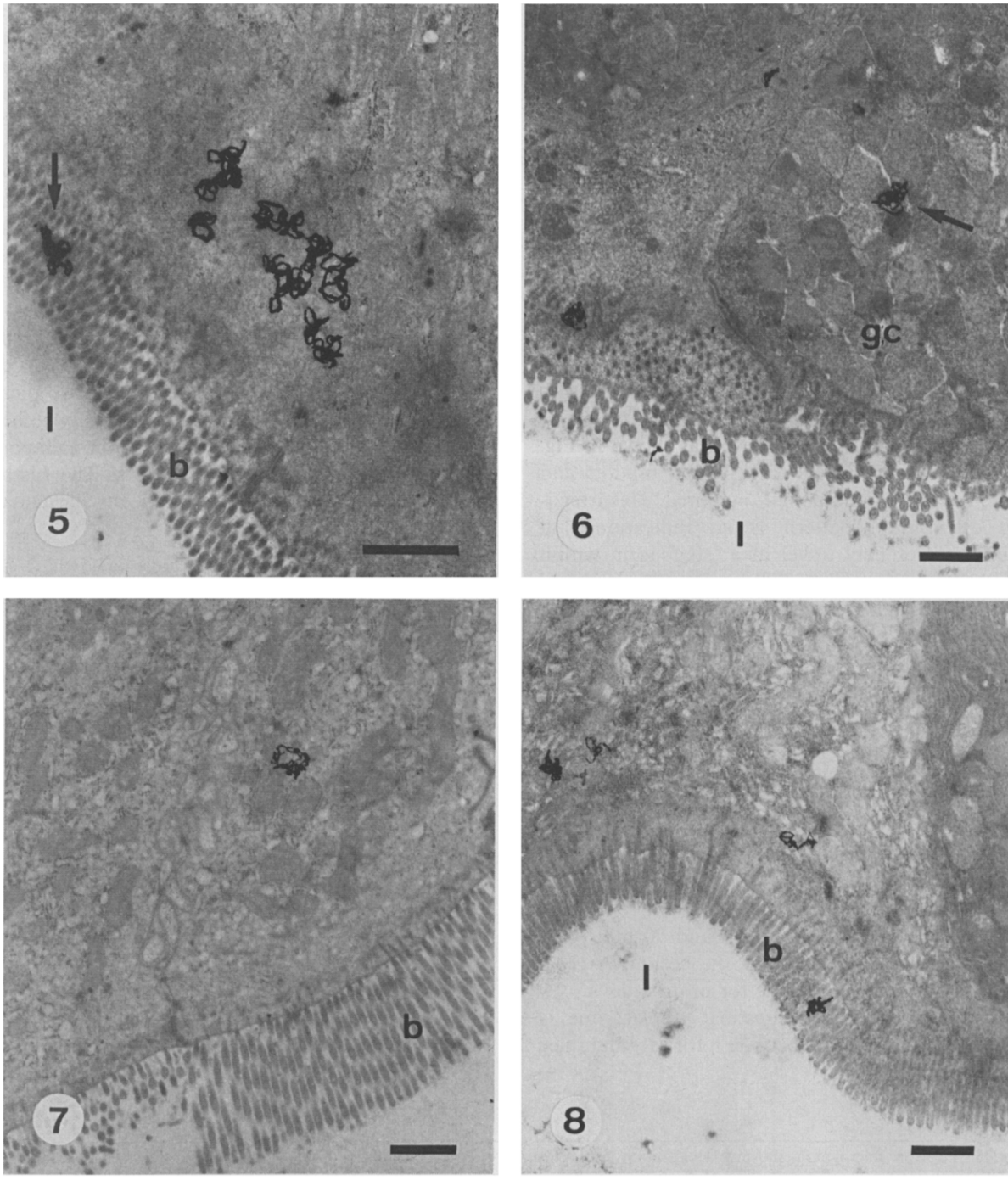

Fig. 5. Electron micrograph showing localization of radioactivity in the colon of mice 4 h after peroral administration of polyhexyl-[3-^{14}C]cyanoacrylate nanoparticles. Radioactivity is already detectable in muscle cells in the form of clusters or adjacent to the brush border (arrow). b = brush border; l = lumen. Bar = 1 μm.

Figs. 6–8. Electron micrographs showing localization of radioactivity in the ileum of mice after peroral administration of polyhexyl-[3-^{14}C]cyanoacrylate nanoparticles. Radioactivity is observable inside goblet cells of the ileum (arrow) after 8 h (Fig. 6), inside muscle cells after 24 h (Fig. 7) and 6 days (Fig. 8). gc = goblet cell; b = brush border; l = lumen of the gut. Bar = 1 μm.

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