J. Anat. (1996) 189, pp. 553–559, with 1 figure Printed in Great Britain

Gastrointestinal uptake and translocation of microparticles in the streptozotocin-diabetic rat

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(*Accepted 2 July 1996*)

ABSTRACT

Uptake and translocation of particulates across the mucosal barrier of the gastrointestinal (GI) tract is now generally recognised but the effect of pathophysiologically induced changes on this process is less well established. This study evaluated the effect of diabetes mellitus on GI absorption of particles, comparing particle localisation and particle loading in different microanatomical sites of the primary organ (small intestine) and possible particle translocation pathways to selected secondary organs (mesenteric lymph nodes, liver, spleen) in normal and streptozotocin-induced diabetic animals. Fluorescent polystyrene latex particles (~ 2 μm diameter) were fed orally to young adult Sprague–Dawley rats and quantitative bulk tissue and morphological techniques used to chart particle transit across the small intestine to secondary organs 0.5 h postadministration. In the normal animal, epifluorescence and confocal laser scanning microscopy provided confirmatory evidence for particle absorption within the primary organ and transport to other sites in the body. By contrast, in the diabetic animal, particle translocation and peripheral distribution were reduced with ~ 30% decrease in particle loading in the epithelial/nonepithelial tissue compartments. This could be a consequence of gastric retention and altered intestinal motility and permeability which are known to be associated with diabetes.

Key words: Drug delivery systems; diabetes mellitus; intestinal motility.

INTRODUCTION

Particle uptake across the mucosal barrier of the gastrointestinal (GI) tract is an area of general interest, notably in the toxicological and pharmaceutical sectors (O'Hagan, 1990; Kreuter, 1991; Couvreur & Puisieux, 1993; Florence & Jani, 1993). There is increasing evidence that the peroral administration of drug-loaded particles could provide useful drug delivery systems. Previous studies have established the mucosal uptake of latex microparticles and their translocation to mesenteric lymph nodes (Jani et al. 1989, 1990; O'Hagan, 1990; Kreuter, 1991; Hodges et al. 1995). Although it is known that alterations in intestinal absorption may occur with variation in physiological condition and with disease (Fedorak, 1990), information remains limited on the effect on particle absorption of pathophysiologically-induced changes (O'Hagan, 1990; Kreuter, 1991). The present study investigates this issue in rats following experimental streptozotocin-induced diabetes, previously shown to be associated with effects on intestinal transport (Miller et al. 1977; Fedorak, 1990). To probe diabetes-induced effects on particulate movement across the mucosa of the small intestine, the passage of ~ 2 μm diameter fluorescent polystyrene latex particles, administered orally to diabetic and nondiabetic rats, has been charted quantitatively across the mucosal barrier of the gastrointestinal tract to the mesenteric lymph nodes, liver and spleen.

MATERIALS AND METHODS

Nonfasted male Sprague–Dawley rats, aged 7–8 wk and weighing ~ 250 g, were maintained on standard laboratory food and tap water, available ad libitum. All animal treatments were carried out by a licensed investigator in accordance with regulated procedures under the Animals (Scientific Procedures) Act (1986). Experimental and control groups were formed by random assignment.

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Table 1. *Length and internal circumference measurements of the small intestine in diabetic, citrate buffer-treated and nontreated rats**

Animal group	Length of intestine (cm)	Internal circumference (mm)
Diabetic[a]		
Experimental	131.4±0.52	55.8±2.01
Control	131.0±0.52	53.6±2.01
Citrate buffer-treated[b]		
Experimental	102.1±0.47	32.9±0.22
Control	100.4±0.47	32.5±0.22
Nontreated		
Experimental	105.8±2.46	31.9±0.37
Control	103.6±2.46	33.5±0.37

* Data express the mean and standard error of the mean of values of length (in cm) and the mean internal circumference (in mm) of the small intestine: n = 3 rats per group; n = 10 sections per animal; n = 3 readings per section. [a]Intestine significantly longer and greater in internal circumference in diabetic than in citrate buffer and nontreated animals; [b]no significant difference in intestinal length and internal circumference between citrate buffer and nontreated animals.

Diabetes was induced in ~ 4 wk-old animals (n = 12) given a single 0.1 ml intraperitoneal injection of freshly prepared streptozotocin (Sigma Chemical Co., Poole, UK) in citrate buffer, pH 4.5 (46 mg/kg) (Joslin, 1985). Age-matched nondiabetic animals received either citrate buffer only (n = 12) or no treatment (n = 12). Blood glucose concentration was determined prior to treatment and monitored at 2 d intervals over a 2 wk period following streptozotocin treatment, using a reflectance photometer and glucose reagent strips (Ames Division, Miles Laboratories Inc., Slough, UK). Animals were considered diabetic at blood glucose levels of 14 mmol/l or greater.

At ~ 6 wk of age, *experimental* groups of diabetic, citrate buffer, or nontreated animals (n = 6 animals per group) were dosed intraorally with 0.25 ml particle suspension (dose administered 1.95×10^9 particles) of plain (nonionic), monodisperse fluorescent polystyrene latex (1.80±0.087 μm diameter) microparticles (Polysciences Inc., Warrington, PA, USA). Age-matched *control* groups of diabetic, citrate buffer, or nontreated animals (n = 6 animals per group) were dosed intraorally with 0.25 ml sterile double-distilled water. Animals were killed by carbon dioxide asphyxiation 0.5 h after dose administration. Tissue samples were taken from unfixed material for bulk tissue (maceration) analysis, and from whole animal perfusion-fixed material for morphological analysis. Precautions were taken to minimise contamination by ambient fluorescent particles as previously described (Hodges et al. 1995).

Bulk tissue analysis

The mesenteric lymph nodes, liver (right lobe) and spleen (middle region) were excised before the small intestine, to prevent contamination with gut luminal contents. The proximal third segment of the small intestine was isolated and the Peyer's patch regions (PPRs) dissected and pooled. All tissue samples were weighed, digested in 15 ml of 15% KOH and incubated at 60 °C for 2–3 d. The macerates were then diluted to 25 ml with 2% KOH and vortexed for 1 min; 20 aliquots (1 μl) were counted by epifluorescence microscopy, the average number of particles per 1 μl aliquot established, and an extrapolated value for the number of particles per gram of tissue calculated. The results are given as mean values of counts from n = 3 rats from each experimental and control group of animals.

Morphological analysis

Following whole animal perfusion-fixation with 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) at ambient temperature, mesenteric lymph nodes, liver (right lobe) and spleen (middle region) were excised first. The small intestine was then removed, its length measured and a proximal sample taken ($\sim \frac{1}{9}$ of total length). This segment was dissected into Peyer's patch-containing (PPC) full-circumference rings. These were washed in three changes of 0.1 M sodium cacodylate buffer to remove as much as possible of the luminal contents. Propidium iodide-stained (0.001 μg/ml in 0.1 M sodium cacodylate buffer for 20 min) 14 μm-thick cryosections were prepared and mounted without dehydration, in Gelvatol (Cairn Chemical, Chesham, Bucks, UK). Particle uptake into different tissue sites of PPC full-circumference cryosections of proximal small intestine, and their transit into mesenteric lymph nodes, liver and spleen was assessed by epifluorescence and confocal scanning laser microscopy. Coded slides were spot checked by a second observer. Results are given (1) as the mean number of particles per tissue site (averaged from total particle counts per tissue site from n = 10 sections); and (2) as the percentage of the total particle numbers per tissue compartment (averaged from total particle counts per tissue compartment/total particle counts from all tissue compartments × 100, n = 10 sections).

Full circumference rings of proximal small intestine devoid of Peyer's patch were wax embedded and 7 μm sections cut and stained with haematoxylin and eosin. The perimeter length of every 10th section (n = 3

observations per section; n = 10 sections per group) was established from tracings using an Optilab image analysis package (Graftek, Meudon-La-Forêt, France) and the mean internal (mucosal) circumference then calculated.

Statistical analysis

All data were tested statistically using 1-way analysis of variance (ANOVA) followed by Scheffé F test of multiple comparisons between pairs of means as appropriate at a significance level of 5%.

RESULTS

Comparative measurements of the small intestine showed both the total length of the small intestine to be significantly longer and the internal circumference to be significantly larger in the diabetic than in the citrate buffer or nontreated groups (Table 1).

Mean particle numbers obtained from direct counts of tissue macerates from different body sites are summarised in the Figure. Bulk tissue analysis of the proximal Peyer's patch regions showed a marked difference in particle numbers between the 3 groups of experimental animals: a significantly greater number of particles ($P < 0.05$) was observed in diabetic animals (1.90×10^8) compared with citrate buffer-treated (2.10×10^6) or nontreated animals (2.75×10^6). By contrast, maceration data from mesenteric lymph nodes showed significantly greater particle numbers ($P < 0.05$) in citrate buffer-treated (4.90×10^6) and nontreated (4.60×10^6) animals compared with diabetic animals (4.40×10^4). Liver showed similar significant differences in particle numbers across the experimental groups (citrate buffer: 7.70×10^4; non-

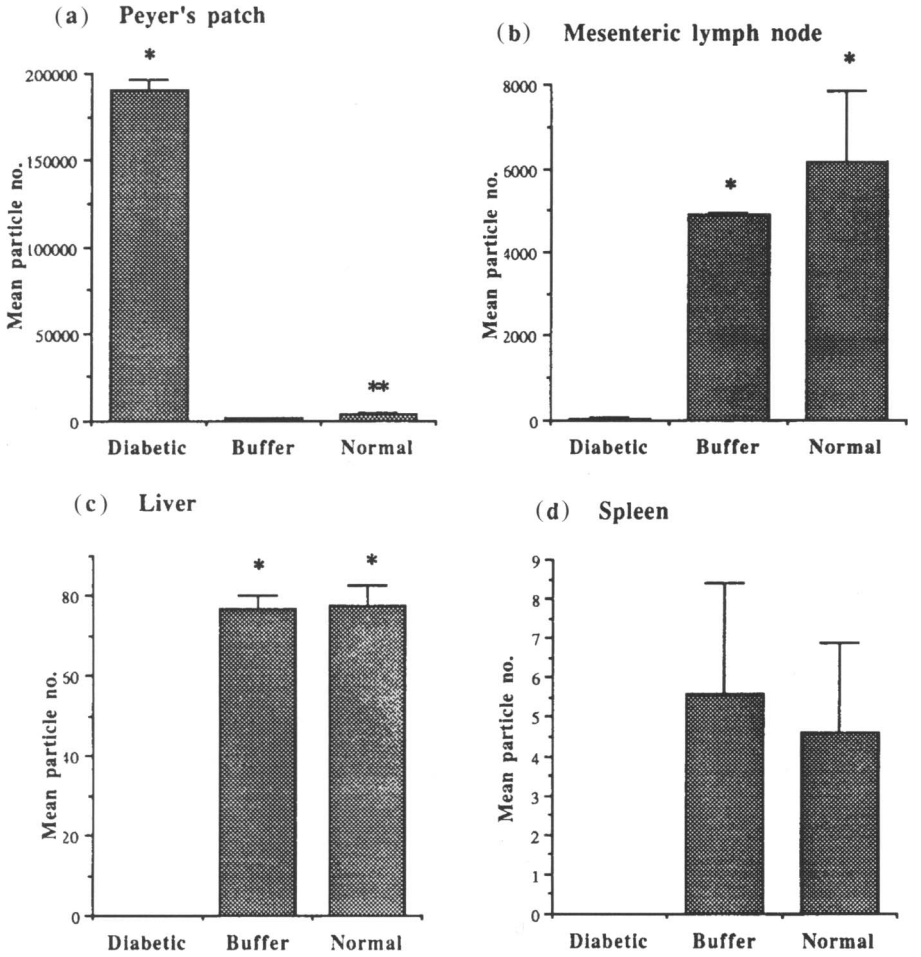

Fig. Particle numbers (y axis) in Peyer's patch regions (from proximal region of the small intestine), mesenteric lymph nodes, liver and spleen following diabetes or citrate buffer treatment or nontreatment of young adult rats. The data represent the number of particles per gram of tissue. Each bar gives the mean and S.E.M. of values obtained from groups of pooled Peyer's patches (*a*), pooled mesenteric lymph nodes (*b*), liver (*c*) and splenic tissue (*d*) from n = 3 rats for each experimental group. No particles were detected in control diabetic, citrate buffer-treated or nontreated tissues from n = 3 rats per group. [a]Significantly greater particle numbers in PPRs of diabetic than of buffer or nontreated animals; [b]significantly greater particle numbers in PPRs of nontreated than in buffer-treated animals; [c]significantly greater particle numbers in mesenteric lymph nodes and in liver of buffer or nontreated than of diabetic animals.

Table 2. *Quantitative tissue distribution of 1.80 μm latex particles in Peyer's patch-containing full circumference tissue rings of proximal small intestine in diabetic, citrate buffer-treated and nontreated rats*

Experimental group	Particle count[1]		
	Diabetic animals	Citrate buffer-treated animals	Nontreated
Tissue site			
Lumen[a]	163.0 (110–232)	40.3 (21–65)	38.8 (22–55)
Mucosal surface			
Enterocyte	17.0 (4–43)	16.7 (3–24)	15.3 (2–19)
Goblet cell[b,c]	0.1 (0–1)	4.23 (3–24)	8.20 (2–19)
Villous region			
Enterocyte	10.2 (0–23)	11.6 (3–24)	7.90 (2–19)
Goblet cell[d]	0.03 (0–1)	1.80 (0–6)	0.77 (0–4)
Lamina propria	0.03 (0–1)	0.76 (0–5)	0.20 (0–3)
Blood capillary	—	—	—
Cryptal region			
Epithelium	2.90 (0–14)	1.70 (0–7)	1.40 (0–7)
Pericryptal stroma	0.10 (0–3)	0.50 (0–4)	—
Peyer's patch			
FAE[c,e]	—	0.26 (0–2)	1.30 (0–6)
Lymphoid tissue	0.06 (0–2)	1.00 (0–6)	6.00 (0–14)
Deeper layers			
Submucosa	—	—	—
Blood vessels	—	—	—
Muscularis externa	—	—	—
Serosa	—	—	—
Mean total	193.4	78.9	79.9
Mean tissue total	13.3	17.7	17.6

[1] Data express the mean number (top set of figures) and the range (bottom set of figures) of particles counted per tissue site per section: n = 3 rats per experimental group; n = 10 sections per animal. [2]Mean total in section is the mean total number of particles per section: n = 10 sections per animal. [3]Mean tissue total is the mean of total numbers of tissue-associated particles per section: n = 10 sections per animal. [a]Significantly greater particle numbers in diabetic than in buffer or nontreated animals; [b]significantly lower particle numbers in diabetic than in buffer or nontreated animals; [c]significantly lower particle numbers in buffer than in nontreated animals; [d]significantly lower particle numbers in diabetic than in buffer-treated animals; [e]significantly lower particle numbers in diabetic than in nontreated animals.

Table 3. *Comparison of particle content in different tissue compartments of Peyer's patch-containing full circumference tissue rings of proximal small intestine from diabetic, citrate buffer-treated and nontreated rats*

Experimental group	Diabetic animals	Citrate buffer-treated animals	Nontreated animals
	Particle count[2]		
Tissue site[1]			
Luminal[a]	180.1	61.2	62.3
Epithelial	13.1	15.4	11.4
Nonepithelial[b]	0.2	2.3	6.2
	Percentage of total[3]		
Luminal	93.1	77.6	80.0
Epithelial	6.8	19.5	14.3
Nonepithelial	0.1	2.9	7.8

[1] The luminal compartment equates to lumen, intervillous, and mucosal surface sites; the epithelial compartment equates to all epithelial tissues of the PPC ring; and the nonepithelial compartment equates to all nonepithelial tissues of the PPC ring. [2]Data express the mean numbers of particles per section; n = 10 sections per animal. [3]Data express particle content as a percentage of the total particle count: figures are based on the mean numbers of particles per tissue compartment per section/mean of total numbers of particles per section × 100: n = 3 rats per experimental group. [a]Significantly greater particle numbers in diabetic than in buffer or nontreated animals; [b]significantly lower particle numbers in diabetic and buffer-treated animals than in nontreated animals.

treated: 7.80×10^4; diabetic: no detectable particles). No significant difference in particle content was observed across splenic tissue of citrate buffer-treated (5.60×10^3), nontreated (4.50×10^3) or diabetic (no detectable particles) animals. No particles were detected in tissues sampled from control diabetic, citrate-treated, or nontreated animals given sterile distilled water.

Morphological analysis identified fluorescent microparticles in various microanatomical sites of the PPC rings of proximal small intestine, the mesenteric lymph nodes, the liver and the spleen (Tables 2–4). Serial optical sectioning by confocal microscopy provided unequivocal evidence that particles were located within the tissue section and had not been artefactually deposited on the surface of the section.

Quantitative data from microscopic counts of fluorescent microparticles in diabetic, citrate buffer-treated and nontreated animals showed that particle loadings in different organs and microanatomical sites varied across the experimental groups (Tables 2–4). In the small intestine (Tables 2, 3) particles were found primarily localised in the lumen and along the mucosal surface with greater particle numbers per section in the diabetic (163) than in the citrate buffer-treated (40.3) or nontreated (38.8) animals. Most tissue-associated particles were present within the epithelial layer, predominantly within enterocytes: particle loadings per section were comparable in all 3 experimental groups (diabetic 17; citrate buffer 16.7; nontreated 15.3). No particles were detected in the

Table 4. *Quantitative tissue distribution of 1.80 μm latex particles in mesenteric lymph node, liver and spleen of diabetic, citrate buffer-treated and nontreated rats**

	Experimental group		
	Diabetic animals	Citrate buffer-treated animals	Nontreated
Organ/tissue site			
Mesenteric lymph node			
Lymphoid tissue	0.63 (0.4)	2.70 (0–5)	2.90 (0–5)
Lymphatic vessels	—	—	—
Blood vessels	0.67 (0–3)	2.20 (0–6)	2.30 (0–5)
Capsule/subcapsular sinus	0.47 (0–2)	0.83 (0–3)	0.80 (0–2)
Mean total**	1.77	5.73	6.00
Liver			
Hepatocyte	—	0.53 (0–4)	0.40 (0–3)
Sinusoid	—	1.17 (0–5)	0.87 (0–4)
Central vein	—	0.80 (0–4)	0.50 (0–3)
Portal triad	—	0.10 (0–1)	0.33 (0–1)
Mean total**	—	2.60	2.1
Spleen			
Red pulp	—	—	0.10 (0–2)
White pulp	—	—	—
Trabecular vein	—	—	—
Mean total**	—	—	0.10

* Data express the mean number (top set of figures) and the range (bottom set of figures) of particles counted per tissue site per section; n = 3 rats per experimental group; n = 10 sections per animal. **Mean total is the average total number of particles counted per section; n = 10 sections per animal.

follicle-associated epithelium (FAE) of diabetic animals. All 3 experimental groups showed particles in Peyer's patch lymphoid tissue with a significantly lower number per section in diabetic (0.06) than in nontreated (6.0) animals. No particles were identified in the submucosa, blood vessels, muscularis or serosa in any of the 3 groups.

Particles were found in the mesenteric lymph nodes (Table 4) of all 3 experimental groups and in 3 of the 4 tissue sites examined, namely the capsule, subcapsular and blood sinuses of the node but not in the lymph vessels. Particle loadings were lower in the mesenteric lymph node tissues of diabetic animals. In liver, particles were present only in citrate buffer and nontreated animals: the majority of particles were found in the sinusoids with lower numbers observed in the central veins, hepatocytes and branches of the portal triads. No particles were observed in the splenic tissue of diabetic or citrate buffer-treated animals; a few particles were observed in the red pulp area of spleen in the nontreated group.

DISCUSSION

The growing evidence for passage of particulate matter, including viable bacteria, from the gastrointestinal tract through the mucosa (O'Hagan, 1990; Kreuter, 1991) has directed attention to the effect of pathophysiological conditions on the particle translocation process (Deitch, 1994; Van Leeuwen et al. 1994). Discussion here focuses on 2 main aspects of the work, namely: (1) confirmation of particle absorption and transport to other sites of the body in nondiabetic animals; and (2) diabetes as a possible example of a pathophysiologically-induced disturbance of particle uptake and translocation.

The nondiabetic groups of animals (citrate buffer and nontreated) provide data from maceration and microscopic analyses of specimens that identify particle uptake into the tissues of the small intestine and transport to the mesenteric lymph nodes, liver and, to a minor extent, spleen. These data clearly corroborate earlier findings (Jani et al. 1989, 1990; O'Hagan, 1990; Couvreur & Puisieux, 1993; Florence & Jani 1993; Hodges et al. 1995). However, some discrepancies in the data are apparent, perhaps due to variations in the methods of analysis. The microscopic data show that a high proportion of particles may remain within the luminal compartment of the small intestine. This probably leads to overestimation of particle counts from macerated intestinal tissues, reflecting the difficulty both in dissecting the lymphoid epithelial domes without any adjacent villous tissue and in completely removing luminal contents. By contrast, fewer differences are detected in the secondary organs where the trends are similar for both methods of analysis. The more detailed quantitative data established from microscopy of sectioned tissues establishes differences of up to several orders of magnitude between the numbers of particles absorbed in the primary organ and those found in the secondary organs.

Direct comparisons of particle content in different microanatomical sites of the small intestine between the diabetic, citrate buffer and nontreated groups show in diabetic animals: (1) significantly greater numbers in the luminal compartment and higher, though nonsignificant, numbers in the cryptal epithelium; (2) significantly lower numbers associated with goblet cells and follicle-associated epithelium; and (3) lower, though not significantly so, numbers in

the pericryptal stroma and lymphoid tissue. Particle numbers in the villous epithelium are not altered significantly in diabetic animals but there is a drop in the numbers gaining access to the villous stroma: lower numbers of particles are detected, overall, in the nonepithelial compartment of diabetic and of citrate buffer-treated animals than of nontreated animals. The percentage of particles located within individual tissue compartments of the small intestine is lower both in the epithelial and nonepithelial compartments of animals in the diabetic group in comparison with nondiabetic animals. In the latter group, particle percentages are higher in the epithelial compartment and lower in the nonepithelial compartment of the citrate buffer-treated animals than in the nontreated animals.

Several conclusions may be drawn from the above comparisons. Although epithelial uptake remains essentially unaffected, there is clear evidence that the diabetic condition reduces the transepithelial passage of ~ 2 μm latex particles across the GI tract and their transit to secondary organs. This is reflected by reduced numbers of particles in the nonepithelial tissues of the diabetic compared with nondiabetic animals. Physiological alterations in the diabetic small intestine such as gastric retention and reduction in intestinal motility (Bergström & Norrby, 1988; Mathison & Davison, 1988) may be contributory factors, with particles gaining access to cells of the epithelial compartment at a later time-point in diabetic than in nondiabetic animals. The reduced transmucosal passage of particles as a consequence of reduced intestinal motility has also been suggested in earlier studies (Volkheimer, 1977). However, the results of studies on gastric motility in diabetes are conflicting and in short-term diabetic rats, the intestinal transit has been found to be significantly accelerated compared with controls (Lysy et al. 1995). The diabetic condition can lead to increased density of the basal lamina underlying the GI mucosa (Mantle et al. 1989) and this may be a further factor limiting particle translocation into deeper villous regions. The increases in length and internal circumference of the diabetic small intestine corroborate previous findings (Mantle et al. 1989) and confirm the effects of diabetes on the animals. However, there is no direct evidence in the literature to indicate whether such changes in intestinal diameter and length in short-term diabetic rats are due to hypertrophy and/or hyperplasia. The greater intestinal mass is not reflected by enhanced particle uptake across the GI mucosa at the specific point sampled in this study; if an estimate is made of the effect of any change in gut length by multiplying the length and particle uptake for all groups, the increase in length is not sufficient to counteract the downward trend in particle uptake in the diabetic group. Furthermore, although there is evidence that the diabetic state (Fedorak, 1990) may increase transport processes, the lower particle loadings found in the nonepithelial compartments of the diabetic animals suggest involvement of some other mechanism(s) affecting the transmucosal passage of particles.

Whatever the basis for the substantial reduction in particle absorption and peripheral distribution seen, overall, in these animals, the diabetic syndrome provides an example of pathophysiologically-induced changes in the particle absorptive capacity of the GI mucosa.

ACKNOWLEDGEMENTS

The authors are grateful to the Radiation Protection Research Programme of the Department of Health, London, UK for support. The excellent assistance of Dr J. S. McCullough, Mr K. Lee, Ms O. O'Shea and Mr C. Ferris is gratefully acknowledged.

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