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Morphological changes of the villous microvascular architecture and intestinal growth in rats with streptozotocin-induced diabetes

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Summary. Using intact tissues and vascular corrosion casts, morphological changes in the small intestine of streptozotocin-induced diabetic rats present 1 month after onset were investigated using scanning and transmission electron microscopy. Although there were increases in the height of the villi in the jejunum and not in the ileum, characteristic changes in surface morphology in the diabetic rats were observed only in the ileum. Use of a vascular corrosion cast technique clearly demonstrated the prominent thick marginal vessels of the intestinal villi of the control rats. In the diabetic rats, the central arterioles in the villi were dilated both in the jejunum and the ileum. Marginal vessels were small in the ileum but prominent in the jejunum. Using transmission electron microscopy, decreases in diameter and the number of endothelial fenestra were also evident in the marginal vessels of the ileal villi in the diabetic rats. Thus in rats made diabetic, fine structural alterations of the marginal vessels are induced in the villi of the distal ileum, in addition to quantitative changes in the jejunal villi, presumably related to hyperphagia. The marginal vessels seem to be involved in the regulation of microcirculation of the intestinal villi in the rat.

Key words: Diabetes mellitus – Intestinal mucosa – Microcirculation – Electron microscope

Introduction

Although rats with alloxan- or streptozotocin-induced diabetes show a general impairment in growth, their intestinal growth is enhanced and their intestinal villi become enlarged (Jervis and Levin 1966; Schedl and Wilson 1971). When diabetic rats are allowed food ad libitum, they become hyperphagic (Jervis and Levin 1966) and the daily food consumption is increased (Lorenz-Meyer et al. 1977; Miller et al. 1977). Hyperphagia and consequent increased luminal nutrition are suggested to play a role in the development of intestinal hypertrophy occurring in experimental diabetes (Lorenz-Meyer et al. 1977; Young et al. 1982). Miller et al. (1977), however, noted that dietary restricted diabetics showed essentially the same increase in DNA synthesis of the cryptal cells as diabetics eating ad libitum. Activity of disaccharidases, absorption of hexose, certain amino acids, and sodium are increased in diabetics compared with controls (Olsen and Rosenberg 1970; Younoszai and Shedl 1972; Shedl and Wilson 1974), whereas calcium, strontium, and magnesium absorption are depressed (Schneider and Schedl 1972; Miller and Shedl 1976a, b). Thus, much uncertainty exists as to the pathogenesis of the changes.

The morphological changes of intestinal villi in diabetics must be accompanied by the alterations in vascular architecture and morphology, because the microvasculature of villi is closely involved in absorption. Vascular changes are known to occur at an early stage of diabetes (Ditzel and Saglid 1954; Fenton et al. 1979). In another type of intestinal hypertrophy which occurs after intestinal resection, a few studies of blood flow (Touloukian and Spencer 1972; Ulrich-Baker et al. 1986) have revealed that increased circulation to the remnant of intestine was involved in the change. Bohlen and Hankins (1982) reported arteriolar dilatation in the intestinal submucosa and muscle layer of diabetic rats but microvascular changes in the villi were not given attention. Little is known

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of the microvascular changes of villi in cases of intestinal hypertrophy.

The present study was designed to reveal differences in morphological changes in the villi between the jejunum and ileum of the diabetic rats, to gain insight on possible factors responsible for the changes. Particular attention was focused on changes in microvasculature of the villi.

Materials and methods

Two month-old male Wistar rats, and initially weighing approximately 200 g, were used. The 30 rats were divided into two groups of equal number. A single intravenous administration of a freshly prepared solution of streptozotocin (65 mg/kg in 0.1 M citrate buffer, pH 4.5) was given and a diagnosis of diabetes was established by persistent hyperglycaemia (>300 mg/dl), glycosuria, and impaired growth. The control rats were given a corresponding volume of citrate buffer intravenously. The rats were housed two per cage, in an air conditioned room, and were fed rat cubes and water ad libitum for one month.

One month later the examinations were made. To avoid diurnal variations, all the rats were killed between 10 a.m. and noon. Eleven pairs of control and diabetic rats were used for intact tissue examinations and 4 pairs for the vascular corrosion cast/SEM study.

For scanning electron microscopy (SEM) the abdomen was opened under ether anaesthesia and intestinal sites were sampled: (1) proximal jejunum, 3 cm distal from the ligament of Treiz, and (2) distal ileum, 3 cm proximal from the ileocoecal junction. After being opened along the mesenteric side, the samples were pinned flat on dental wax plates, washed with a jet of saline from a medicine dropper to remove the intestinal contents and surface mucus, and were immediately fixed with half strength Karnovsky's fixative (1.6% paraformaldehyde and 1.7% glutaraldehyde buffered with 0.1 M cacodylate, pH 7.4). Care was taken to avoid distortion by stretching. The specimens were flooded with the same fixative for 30 min at 4° C. After the initial fixation, the flattened tissue was cut into small pieces with a razor blade, under a dissecting microscope, 0.5 by 2 mm longitudinally for SEM, and 1 by 1 mm for transmission electron microscopy (TEM), respectively. The specimens were transferred to the same and to fresh fixative overnight at 4° C. They were then rinsed several times with 0.1 M cacodylate buffer (pH 7.4), dehydrated in a graded series of ethanol, and dried in a Hitachi HCP-2 critical point dryer. The dried specimens were then mounted on aluminium stubs for SEM, using double-sided adhesive tapes, and oriented with lateral villous surfaces toward the edge of the stub. The specimens were coated with a 20 nm thickness of platinum in a SEM coating system (SC-500, Emscope), and examined using a Hitachi S-430 scanning electron microscopy at an accelerating voltage of 20 KV.

In the preparation of vascular corrosion casts the abdomen was opened under ether anaesthesia and a cannula was inserted into the superior mesenteric artery. The superior mesenteric vein was cut for outflow. After a thorough irrigation with heparinized saline, half strength Karnovsky's fixative was perfused. The casting medium of partially polymerized methacrylate resin (Murakami 1971) was then injected manually at a rate of 0.1 ml/sec until the intestine turned red and resin dripped continuously from the superior mesenteric vein. After complete polymerization of the resin, the two intestinal sites described were removed and corroded in 30% KOH. The casts were rinsed with several changes of tap water, and then frozen. Using a dissecting microscope, they were cut into pieces using a razor blade, to obtain a luminal view. The trimmed specimens were dried in air, mounted, and coated with platinum for subsequent observation by SEM.

For transmission electron microscopy (TEM), after the initial fixation, intestinal tissues were further fixed in half strength Karnovsky's fixative for 2 h at 4° C, rinsed in 0.1 M cacodylate buffer (pH 7.4), and then fixed in 1% osmium tetroxide in the same buffer for a further 2 h. After fixation, they were dehydrated in a graded series of ethanol and embedded in epoxy resin. Thick sections (1 μm) were cut with a sapphire knife on a Porter-Blum MT-1 microtome, and stained with toluidine blue for light microscopy. Thin sections (60–80 nm) were cut with a diamond knife, stained with 2% uranyl acetate and lead citrate, and examined using a Hitachi H-300 transmission electron microscope.

Table 1. SEM analysis of the height of the villi (Mean±SD, $n=11$)

	Control rat	Diabetic rat	Statistical comparison
Jejunum	338.8±23.0 μm	469.0±32.7 μm	$P<0.01$
Ileum	182.6±17.7 μm	183.6±18.8 μm	NS

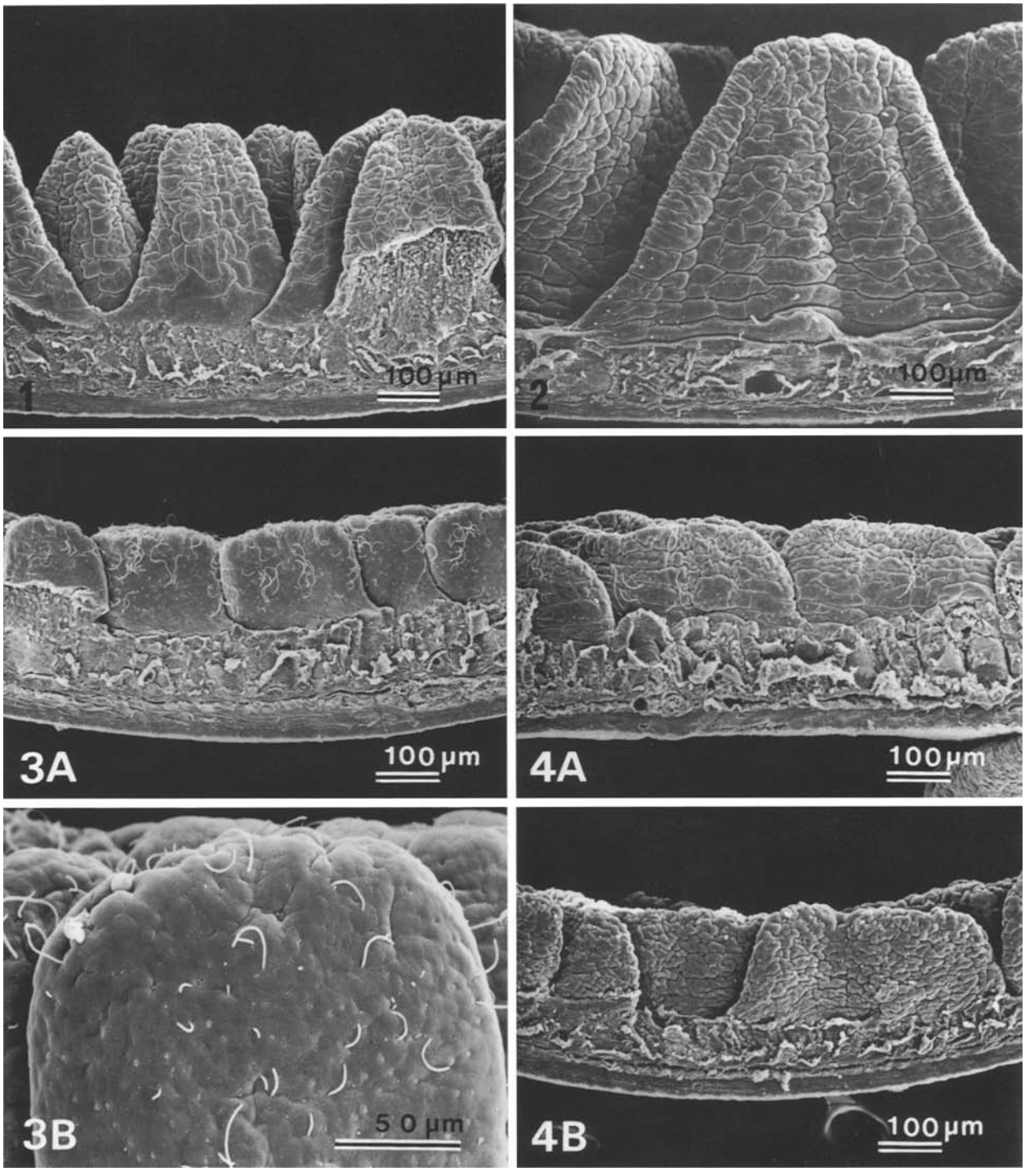
NS: not significant

Results

On SEM, from the side view of the intestinal wall, the height of the villi was measured and the ultrastructure of the villous surface was examined.

In both control and diabetic rats, jejunal villi were tongue-shaped (Figs. 1, 2) and had surface convolutions (Pfeiffer 1971). The jejunal villi of the diabetic rats appeared markedly enlarged (Fig. 2) when compared with findings in controls (Fig. 1) and the height was significantly increased in diabetics (Table 1). The surface of the villi appeared to be the same in contour in tissues from both control and diabetic rats, and had a cerebral gyrus-like pattern (Fig. 1, 2).

In the ileum, the villi were ridge-shaped in the controls (Figs. 3A, B). Filamentous organisms, which are thought to be the Arthromitaceae (Chase and Erlandsen 1976), were often observed on the surfaces of the villi, in the tissues from both control and diabetic rats (Figs. 3A, B, 4A). The ileal villi appeared wider (Figs. 4A, B) than the controls (Fig. 3A), though the height of the villi remained unaffected in the presence of diabetes (Table 1). There was a marked difference in the appearance of the villous surfaces between control and diabetic rats. In the controls, the villous surface was generally smooth (Figs. 3A, B). In the diabetic rats, marked convolutions were present throughout the entire surface, albeit variable in de-

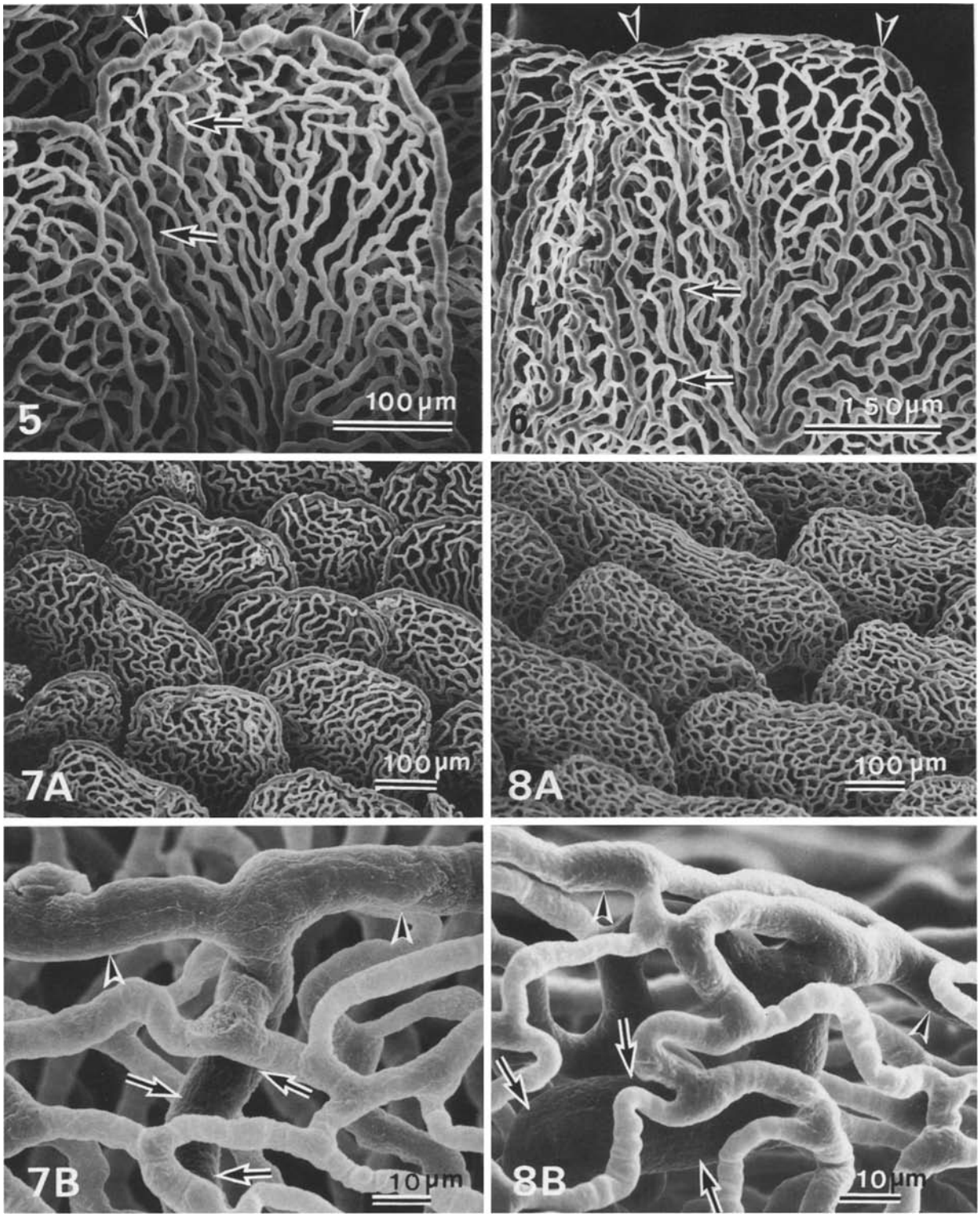


Figs. 1–4. SEM views of the rat intestinal wall. Note changes in size and surface characteristics of the villi from diabetics. **1** Control rat, Jejunum ×100. **2** Diabetic rat, Jejunum ×100. **3A, 3B** Control rat, Ileum ×100, ×400. **4A, 4B** Diabetic rat, Ileum ×100, ×100

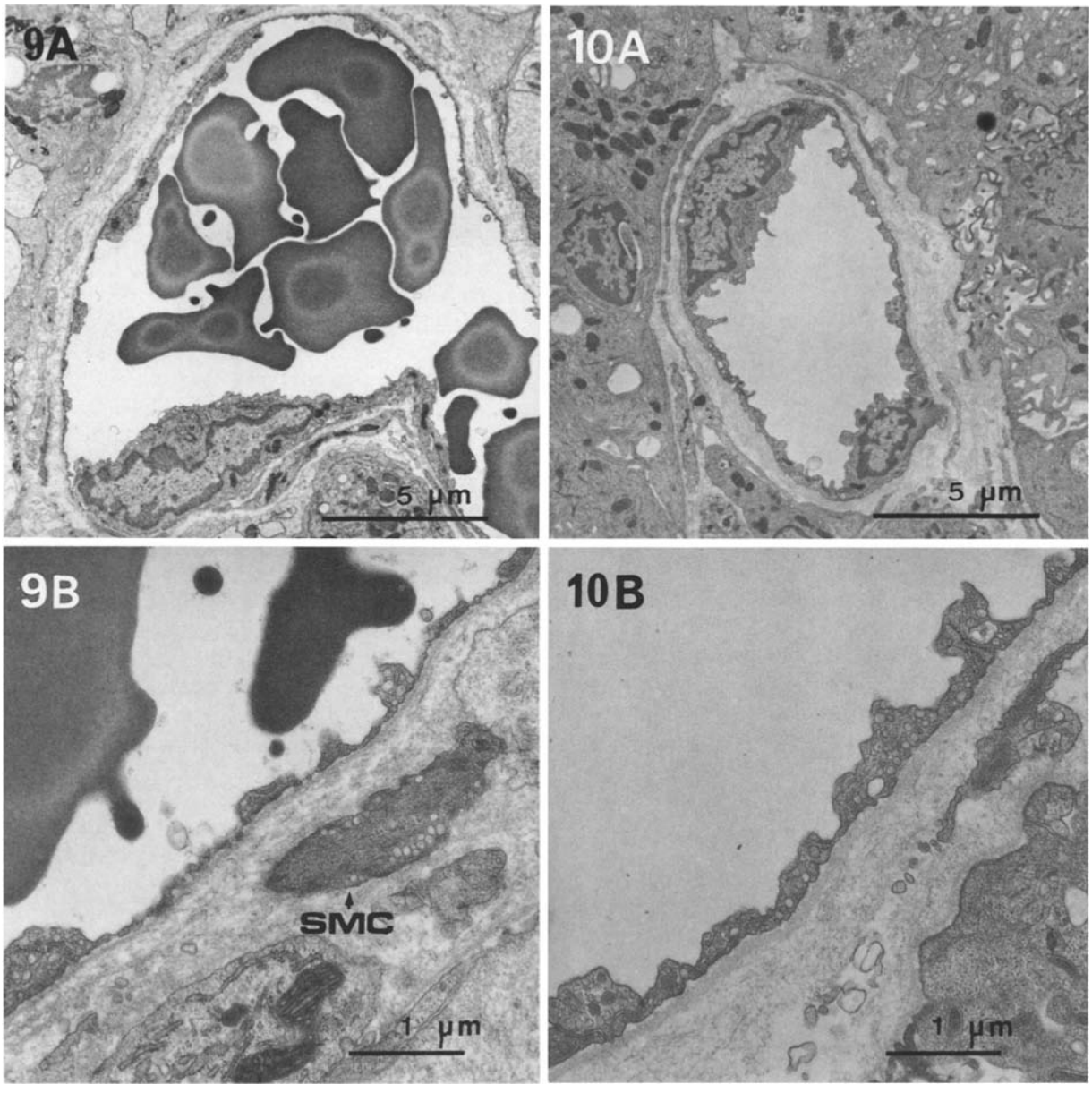
gree. The surface appearances varied from cobble stone-like (Fig. 4A) to rough granular (Fig. 4B).

In the control rats, the vascular architecture of the villi was basically the same in both the jejunum and ileum, and conformed with the fountain-pattern described by Mall (1887) (cited by Jacobson and Noer 1952) and Ohashi et al. (1976).

In the jejunal villi, one central arteriole branched at the tip of villi into two prominent thick marginal vessels in a T- or Y-shape (Fig. 5)



Figs. 5–8. SEM views of vascular corrosion casts of the intestinal villi. Prominent thick marginal vessels are obscured in the ileal villi from diabetics. **5** Control rat, Jejunum ×200. **6** Diabetic rat, Jejunum ×150. **7A, 7B** Control rat, Ileum ×100, ×1000. **8A, 8B** Diabetic rat, Ileum ×100, ×1000. *arrow*: central arteriole, *arrow head*: marginal vessel



Figs. 9–10. TEM views of a marginal vessel in transverse section at the tip of the ileal villus. The marginal vessel is small in diameter with a reduced number of endothelial fenestra in the diabetics. Thickening of the basement membrane of the endothelial cell is also evident. **9A, 9B** Control rat ×5000, ×17000. **10A, 10B** Diabetic rat ×5000, ×17000. *SMC*: smooth muscle cell

giving rise to a capillary network which in turn was collected into para-axial venules. In the ileum, one or sometimes two central arterioles were observed in wide ridge-shaped villi and the central arterioles were generally branched into two prominent large marginal vessels in a T-shape at the tip (Figs. 7A, B). There were no marginal vessels connecting directly to the collecting venule without diminishing in size. This finding confirmed the results of other observations in the rat (Mohiuddin 1966; Ohashi et al. 1976). The arterio-venous shunt as described by Spanner (1932) was not found.

In the diabetic rats, the basic fountain-pattern of Mall was maintained both in the jejunal and ileal villi. In the jejunal villi, as in control rats, marginal vessels were prominent (Fig. 6) as seen in the control jejunal villi (Fig. 5). In the ileal villi, however, the marginal vessels were less prominent (Fig. 8A) and appeared much smaller in size (Fig. 8B) when compared with the findings in the control rats (Figs. 7A, B).

Table 2. Inner diameter of the central arteriole in the villus (Mean ± SD, $n = 40$)

	Control rat	Diabetic rat	Statistical comparison
Jejunum	12.7 ± 1.1 μm	17.1 ± 1.6 μm	$P < 0.01$
Ileum	10.4 ± 1.2 μm	15.6 ± 1.8 μm	$P < 0.01$

To investigate the haemodynamic changes in the villi of diabetic rats, the inner diameter of the central arteriole was measured at the straight portion below the branching site, in 10 central arterioles of the villi both in the jejunum and ileum of all the rats.

Table 2 shows that central arterioles of diabetic rats were dilated in both the jejunal and ileal villi, compared with findings in the controls.

On TEM it was evident that in the control villi, the marginal vessel was wide enough to accommodate up to six erythrocytes, while most of the capillaries could accommodate no more than two, findings similar to the result of light microscopic observations by Mohiuddin (1966). The marginal vessels revealed unique ultrastructural features in that they had a large number of endothelial fenestra (more than 20 per cross section) (Fig. 9A), and smooth muscle cells were sporadic around the endothelial cells (Fig. 9B).

In the jejunal villi from the diabetics, no distinct ultrastructural change was observed in the marginal vessels. In the ileal villi, there were marked ultrastructural changes in the marginal vessels. The marginal vessel was small in diameter and the endothelial layer and basement membrane were generally thickened. Only a few fenestra (less than 5 per cross section) were observed in the endothelial cells (Figs. 10A, B), though no distinct ultrastructural change was observed in smooth muscle cells around endothelial cells of the marginal vessels.

Discussion

The present study revealed that changes of the intestinal villi of diabetic rats differed between the jejunum and ileum. In the ileum, unusual prominent deep surface convolutions of the villi developed without increase in height of the villi. In contrast, in the jejunum this height was significantly increased, though the surface morphology remained unchanged. The diameter and number of the endothelial fenestra of the marginal vessels at tips of the villi were decreased in the ileum, whereas the vessels remained unchanged in the jejunum.

The close relationship between the epithelium and endothelial ultrastructure has been demonstrated in rat intestinal capillaries (Milici and Bankston 1981), in which maturation of intestinal epithelium is concomitant with formation of endothelial fenestrations. Intestinal absorptive epithelial cells originate at the base of the crypts as immature proliferative cells, differentiate as they migrate up the crypts and onto the villi and are finally extruded from the villous tips (Cheng and Leblond 1974), where the marginal vessels are observed. Decrease in the number of endothelial fenestra of the marginal vessel may lead to a disturbance in the exchange of substances between the arterial blood and the enterocytes at the villous tips. This would result in a disturbance in maturation of the absorptive cells at the villous tips and induce abnormal cell kinetics in ileal cell turnover, as suggested by Miller et al. (1977) and would also produce abnormal distinct convolutions of the villous surface in the ileum with diabetes. Although the relation between the cell kinetics and the villous surface morphology is poorly understood, prominent surface convolutions with dysplasia in the colon have been shown using SEM (Shields et al. 1985). The marked surface convolutions of the villi without increase in height, revealed three-dimensionally by SEM in our study are likely to reflect the light microscopic findings of Miller et al. (1977). They found that increase in the number of epithelial cells in the ileal villi of diabetic rats was not accompanied by an increase in height of the villi. Thus, marginal vessels at the villous tips were suggested to be actively involved in regulating the cell kinetics of the intestinal absorptive cells along the villi.

Endothelial fenestrations of the visceral capillaries change in number in response to the environment in vivo and in vitro (Mak and Lieber 1984; Milici et al. 1985). Modification of the blood flow alters the ultrastructure of the fenestration of the endothelium in the hepatic sinusoid in the rat (Nopanitaya et al. 1976). Elevated portal pressure and hypoxia in the sinusoids reduced the number of fenestrations (Nopanitaya et al. 1976). In diabetic humans and laboratory animals, the earliest phases of hyperglycaemia are associated with vasodilatation followed by a progressive vasoconstriction (Poulsen and Nielsen 1976; Katz and Janjan 1978). In the initial phase of dilatation, only the smallest arterioles constrict in the wing of living diabetic bats (Bohlen and Hankins 1983). Thus, a decrease in diameter of the marginal vessel may lead to a

decrease in the number of endothelial fenestra of the marginal vessel in the diabetic ileal villi. If there is constriction of the marginal vessel in the earlier stage of diabetes, elevated pressure and hypoxia in the vessel would occur and the number of fenestra would diminish. We suggest that the marginal vessels contract actively, as deduced from the finding that the marginal vessel is a large capillary with rich fenestrated endothelia but resembles the metarteriole described by Fernando and Movat (1964), because it is provided with scattered smooth muscle cells. Recent in vivo video microscopic studies on the microcirculation of the intestinal villi in the rat (Holliger et al. 1983; Neff et al. 1985) support our concept. Physiologically marginal vessels at the villus tip in the rat have been described as distributing arterioles (Bohlen 1980, 1984) or considered to be meta-arterioles (Holliger et al. 1983).

We also noted a significant dilatation of the central arterioles in the villi both in the jejunum and ileum of the diabetic rats. This observation may reflect either the dilatation phase of diabetes or the intestinal absorptive hyperaemia within the mucosal vasculature (Chou et al. 1976). Intestinal vessels also dilate in case of absorptive hyperaemia (Bohlen 1980). In the jejunum, marginal vessels and the villous surface morphology remained unchanged, therefore, dilatation of the central arterioles can be explained by sustained absorptive hyperaemia due to hyperphagia and the consequent increase in luminal nutrition. In the ileum, marginal vessels were affected by diabetes and there were changes in the villous surface morphology. Therefore, dilatation of the central arterioles in the ileal villi of diabetic rats may also be due to diabetes.

Miller et al. (1977) proposed that the ileum was more sensitive to the effects of diabetes, perhaps because the fascicles accompanying the ileal mesenteric vasculature were longer than those serving the proximal jejunum (Schmidt et al. 1983). We speculate that in the jejunum, diabetes-related changes are masked by hyperphagia. In the ileum, on the other hand, the changes are not masked by hyperphagia because most nutrients are absorbed well before the terminal ileal site we examined. Constriction of the marginal vessels with diabetes is compensated for in the jejunum by mechanisms related to an absorptive hyperaemia, in which case the marginal vessels are dilated (Bohlen 1980). The present finding of an increase in height of the villi, presumably due to hyperphagia (Lorenz-Meyer et al. 1977) observed only in the jejunum, supports our proposal.

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