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Jejunal ultrastructural changes induced by kidney bean (*Phaseolus vulgaris*) lectins in rats

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Summary. Rats maintained for a period of 5 days on a diet containing purified lectins extracted from a Brazilian variety (called 'Jalo') of white kidney beans (*Phaseolus vulgaris*) developed marked ultrastructural changes in the epithelium of the proximal jejunum, while both pair-fed and ad-libitum-fed controls did not. The jejunal absorptive cells of rats fed a diet containing lectins exhibited conspicuous abnormalities of the microvilli. They were shorter, slightly thicker, irregular and more sparse; some were bi- or tri-furcated, sharing a common base of implantation. A slightly disorganized terminal web was present below the brush border. The supranuclear cytoplasm of a great number of cells exhibited large cytolysosomes. Comparison with the results of pair-feeding suggests that purified bean lectins have a direct causative role in the pathogenesis of absorptive cell changes in the jejunal villi of rats. The possible pathogenic mechanism of these lesions is discussed.

Keywords: *Phaseolus vulgaris*, lectins, jejunal ultrastructure, kidney beans

Rats given diets containing raw kidney beans or purified bean lectins show marked growth inhibition associated with low food intake when compared to ad-libitum-fed control animals (Liener 1962; Kakade & Evans 1966; Jaffé & Lette 1968). In addition, these diets cause striking pathological changes in the epithelium of the small intestinal villi in the rat (Pusztai *et al.* 1979 *a*, *b*; King *et al.* 1982; Sotelo *et al.* 1983). These changes are similar to those found in malnourished children with severe restriction in the intake of both protein and calories (Brunser *et al.* 1976). Previous reports do not indicate whether the morphological changes are the result of a direct toxic effect of biochemically active factors, e.g. lectins, in beans, on the small intestine and/or are the result of associated malnutrition. The present study was undertaken therefore, to examine the ultrastructure of the proximal jejunum in rats fed a basic diet containing purified kidney bean lectins and to compare it with that in pair-fed control and ad-libitum-fed control animals.

Materials and methods

Male rats of the Wistar strain, weighing an average of 96 g, were used. They were allocated into three groups of four animals and maintained on the following diets for a period of 5 days. Group A (ad-libitum control) received a basic diet *ad libitum*; group B (kidney bean lectins) received the same basic diet except that purified bean lectins were added accounting for 1% (1 g/100 g) of the diet at the expense of corn starch; and group

C (pair-fed control) was pair-fed with group B and received equal amounts of the basic diet. All animals were allowed free access to tap water. The animals were housed in individual cages and fed the basic diet in stainless steel feeding dishes and water in glass drinking tubes. They were weighed daily and their dietary consumption was recorded. The basic diet contained (g/100 g): casein, 10.0; soybean oil, 8.0; fibre, 4.0; corn starch, 73.0; salt mixture (National Research Council 1978), 4.0; and vitamin mixture (Manna & Hauge 1953), 1.0.

The lectins were extracted from a toxic Brazilian variety of white kidney beans (*Phaseolus vulgaris*), called 'Jalo' (Mancini Filho & Lajolo 1981). This was obtained from the Experimental Station Lavras (Institute of Agricultural Research of the State of Minas Gerais, Brazil). The beans were cleaned, ground in a mill, passed through a 0.08-mm mesh sieve, and suspended in saline solution (0.85% NaCl) in a ratio of 1:5. After 2 h stirring at room temperature, the suspension was centrifuged at 5000 r/min for 20 min, and the pH adjusted to 4.2 with 0.2N HCl. The sediment was discarded and the pH of the supernatant adjusted to 7.0 and then treated with celite–bentonite (1:1) to eliminate trypsin inhibitor (Kakade & Evans 1966). Following overnight shaking, the supernatant was separated and precipitated with 50–75% saturated ammonium sulphate. The lectins were purified on a thyroglobulin–sepharose 4B column (Matsumoto & Osawa 1972).

All animals were killed under ether anesthesia by exsanguination from the abdominal aorta. Small pieces of tissue from the proximal jejunum were rapidly removed and fixed by immersion in 3% glutaraldehyde solution in 0.1M phosphate buffer (pH 7.3) for 3 h. They were then fixed in 1% osmic acid in 0.1M phosphate buffer for 1–2 h, dehydrated in ascending concentrations of acetone, and embedded in araldite. Semithin sections used for light microscopy were stained with toluidine blue. Areas of the middle and upper thirds of the villi were selected for electron microscopy. Ultrathin sections were cut with a diamond knife in a Sorvall MT5000 ultramicrotome and double stained with uranyl acetate and lead citrate. The grids were examined on a Zeiss EM 109 electron microscope at 80 kV.

The significance of the difference between 2 mean values were assessed by Student's *t*-test (Snedecor & Cochran 1967).

Results and discussion

During the experimental period animals in control group A grew well (growth rate: 2.7 g/day/rat). Rats in group B fed a diet containing purified kidney bean lectins showed low food intake and a progressive loss of body weight (growth rate: 1.0 g/day/rat) similar to that of respective pair-fed controls (growth rate: 0.4 g/day/rat), thus indicating that the growth inhibition in rats given lectins was attributable to reduced food intake (Table 1).

When examined by electron microscopy the epithelial cells from both control rats of group A and pair-fed controls of group C appeared normal. The microvilli were numerous, slender and uniform, and the terminal web was well organized. The cellular organelles, such as the nucleus, mitochondria, rough and smooth endoplasmic reticulum and Golgi complex appeared normal. The jejunal absorptive cells of rats fed a diet containing purified lectins exhibited conspicuous alterations of the microvilli. They were more sparse, shorter, slightly thicker and irregular; some were bi- or tri-furcated, sharing a common base of implantation and they were covered by a glycocalyx. A slightly disorganized terminal web was present below the brush border, but other cell organelles appeared normal (Figs 1 & 2). Large cytolysosomes containing lipid droplets, myelin bodies and cellular organelles such as mitochondria and ribosomes were present in the supranuclear cytoplasm of many cells (Fig. 3).

These results clearly show that rats maintained for 5 days on a semi-synthetic diet

Table 1. Body weights (initial and final), growth rates and diet intake of rats from group A (ad-libitum-fed control), group B (diet containing kidney bean lectins) and group C (pair-fed control)

Group	Body weight (g)		Growth rate (g/day/rat)	Diet intake (g/day/rat)
	Initial	Final		
A	96.2±1.31	109.7±1.25	2.70	11.65±1.35
B	96.0±1.29	91.0±0.82	−1.00	4.20±0.21
C	95.5±2.32	93.5±3.47	−0.40	4.20±0.21
Significance of difference				
A×B	NS	$P<0.001$		$P<0.01$
A×C	NS	$P<0.025$		$P<0.01$
B×C	NS	NS		NS

The values are mean±SE. Means calculated using data from four rats.

containing purified lectins extracted from a Brazilian variety (called 'Jalo') of white kidney beans (*Phaseolus vulgaris*) develop ultrastructural changes of the absorptive cells of the proximal jejunum by comparison with pair-fed and ad-libitum-fed control animals. The electron microscopic appearance of the absorptive cells of both pair-fed and ad-libitum-fed controls was normal. By pair-feeding the animals, in order to control nutritional variables, we were able to conclude that the alterations observed in the epithelium of the proximal jejunum of experimental animals were directly induced by the lectins.

Similar ultrastructural changes of absorptive cells of the proximal jejunum have been reported previously in rats fed diets containing raw kidney beans or purified kidney bean lectins (Pusztai *et al.* 1979*b*; King *et al.* 1982) or raw escumite beans (Sotelo *et al.* 1983) by comparison with ad-libitum-fed control animals.

The significance of our ultrastructural findings is difficult to interpret. They are nonspecific and show close similarities to those seen in human jejunal biopsies obtained from patients with tropical sprue (Mathan *et al.* 1975) and infantile marasmic malnutrition (Brunser *et al.* 1976). Since the absorptive surface of the intestinal epithelium of chickens treated with the protein synthesis inhibitor cycloheximide, exhibits changes similar to those described here (Lecount & Grey 1972), it is likely that microvillus abnormalities are the morphologic manifestation of a modulation in luminal plasma membrane protein content and turnover, caused by the lectins.

The increased number of cytolysosomes observed in the absorptive cells of rats fed the bean lectin diet may indicate foci of sublethal intracellular injury, caused by noxious agents including lectins penetrating the cells as a result of permeability changes in the lectin-modified plasma membrane of the microvilli. Cytolysosome formation can be induced by many noxious agents including trauma, X-rays, ultraviolet radiation, chemical agents, hypoxia, endotoxin shock and virus infections (Ericsson 1969). It is also possible that the low food intake and reduced body-weight gain of lectin-fed rats are systemic effects partly produced by internalized lectins and other intestinal toxins. On the other hand, increased numbers of cytolysosomes are found during partial or total starvation due to disturbances in cell metabolism, and these disappear on realimentation (Desai 1971).

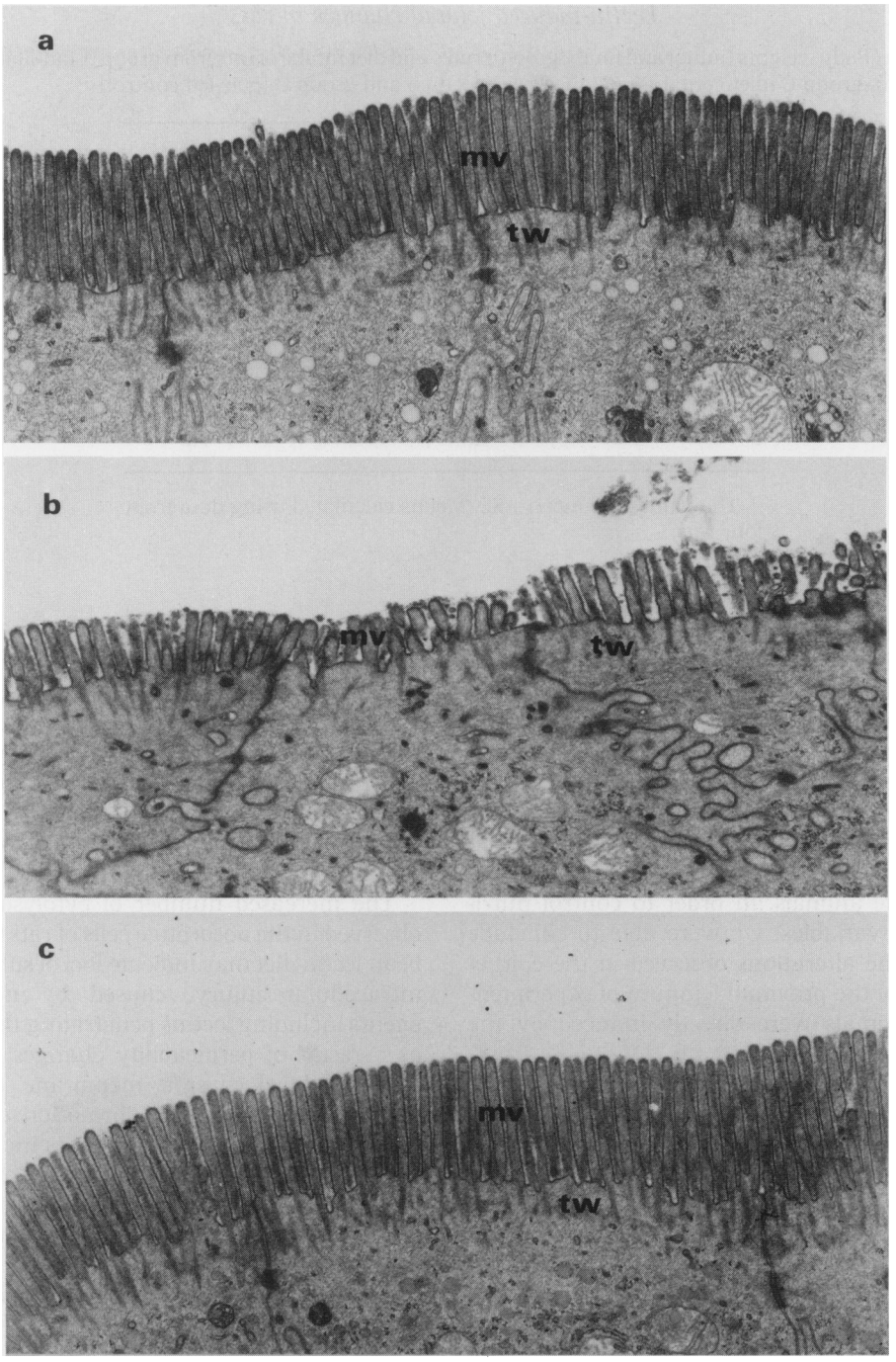

Fig. 1. Electron micrographs. Apical portions of absorptive cells from the middle one-third of jejunal villi in rats fed a semisynthetic basic diet *ad libitum* (group A), showing normal microvilli and terminal web (*a*); in rats fed the same basic diet containing 1% purified kidney bean lectins (group B), showing shorter, slightly thicker, more sparse and irregular microvilli and a slightly disorganized terminal web (*b*); and in group C rats pair-fed with group B, showing normal microvilli and terminal web (*c*). mv, Microvilli; tw, terminal web. × 14 300.

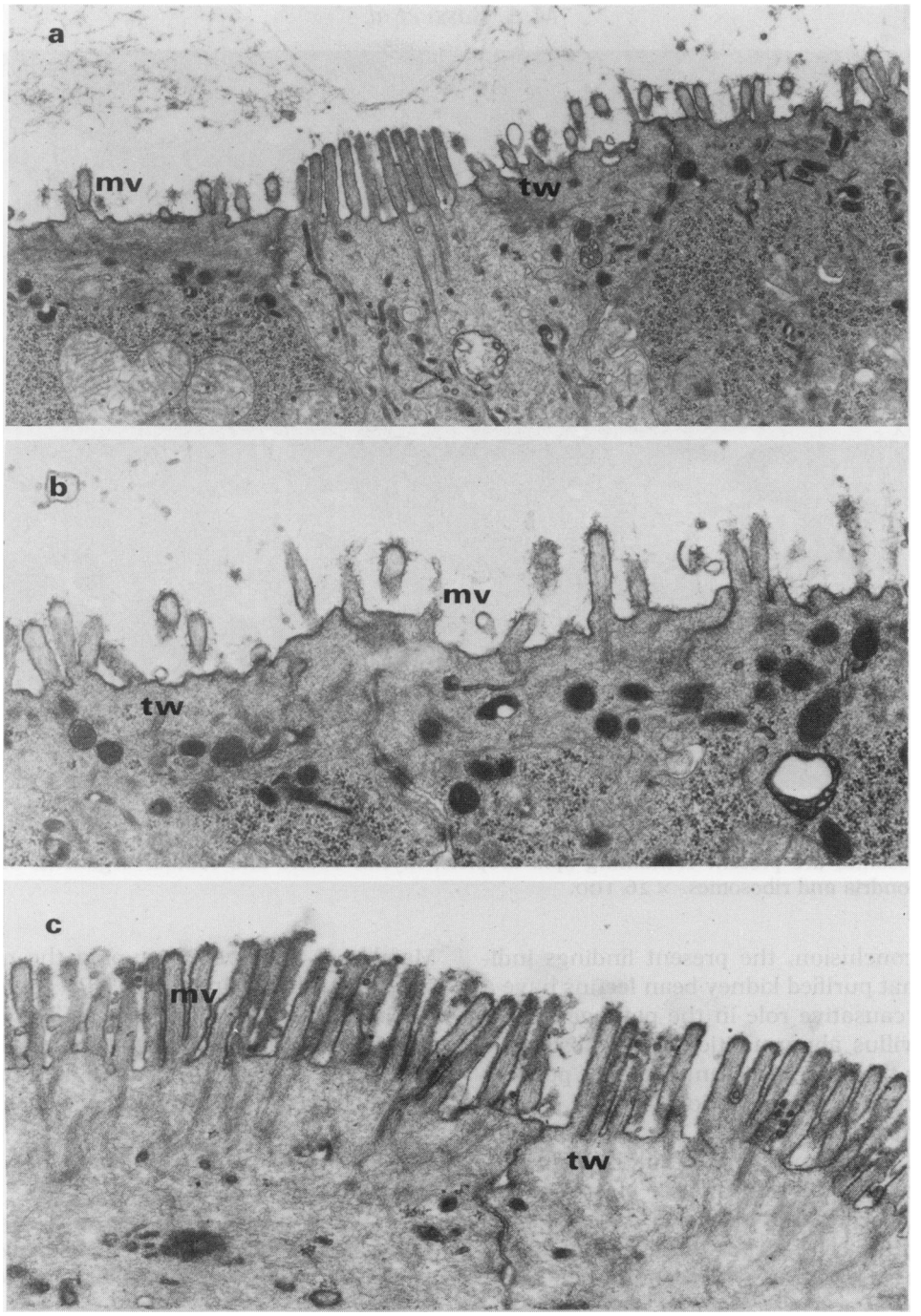

Fig. 2. Electron micrographs. Apical portions of absorptive cells from the middle one-third of jejunal villi in rats fed a diet containing purified kidney bean lectins. The microvilli are more sparse, shorter, slightly thicker and irregular. Some are bi- or tri-furcated, sharing a common base of implantation. A slightly disorganized terminal web can be seen. mv, Microvilli; tw, terminal web. (*a*) × 14 300; (*b*) × 26 100; (*c*) × 26 100.

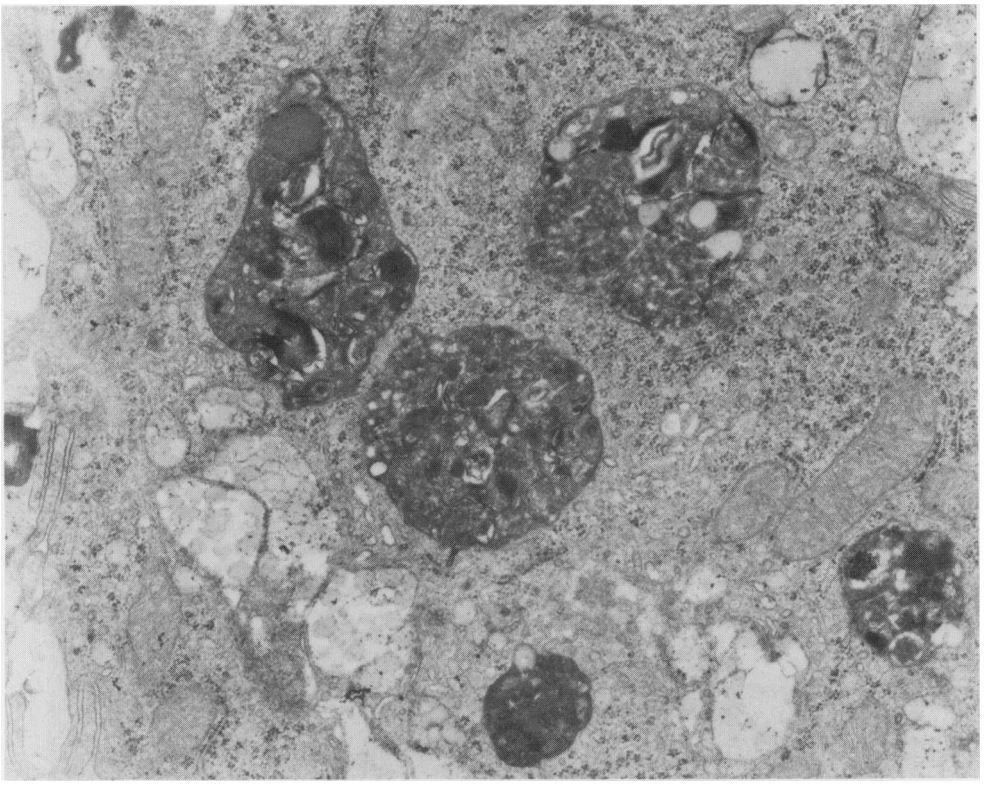

Fig. 3. Electron micrograph showing the supranuclear cytoplasm of an absorptive cell from the middle one-third of a jejunal villus in a rat fed a diet containing purified kidney bean lectins. Numerous cytolysosomes are present containing lipid droplets, myelin bodies and cellular organelles such as mitochondria and ribosomes. × 26 100.

In conclusion, the present findings indicate that purified kidney bean lectins have a direct causative role in the pathogenesis of microvillus abnormalities of the absorptive cells in the proximal jejunum of rats, possibly as a result of modulations in the protein content and turnover of the plasma membrane. This in turn could result in permeability changes in the luminal plasma membrane, facilitating increased access to the absorptive cells of intraluminal toxins, including the lectins, which could then cause both local and systemic toxicity.

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