Glycoconjugate Journal (1995) **12**: 22–35

Lectins and also bacteria modify the glycosylation of gut surface receptors in the rat

ARPAD PUSZTAI[1], STANLEY W. B. EWEN[2], GEORGE GRANT[1], WILLY J. PEUMANS[3], ELS J. M. VAN DAMME[3], MARIE E. COATES[4] and SUSAN BARDOCZ[1]

[1] *The Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, UK*

[2] *Department of Pathology, University of Aberdeen Medical School, Aberdeen AB1 2ZX, UK*

[3] *Catholic University of Leuven, Laboratory for Phytopathology and Plant Protection, Willem de Croylaan, B-3001 Heverlee, Belgium*

[4] *School of Biological Sciences, University of Surrey, Guildford, Surrey GU2 5XH, UK*

Received 19 April 1994, revised 11 July 1994

Oral exposure to lectins or the presence or absence of bacteria in the rat small intestine were shown by histological methods using anti-lectin antibodies or digoxigenin-labelled lectins to have major effects on the state of glycosylation of lumenal membranes and cytoplasmic glycoconjugates of epithelial cells. Taken together with the dramatic effects of exposure to lectins on gut function, metabolism and bacterial ecology, this can be used as a basis for new perspectives of biomedical manipulations to improve health.

Keywords: Lectins, digoxigenin-lectins, small intestine, glycosylation, rats, gut bacteria

Abbreviations: DIG, digoxigenin-labelled; POD, peroxidase-labelled; Spf, specific pathogen-free; TBS, Saline (0.9% w/v NaCl solution) buffered with 0.05 M Tris-glycine, pH 7.8; PAP, antibody-peroxidase-antiperoxidase. For lectins see Table 1.

Introduction

The epithelium of the small bowel is composed of a monolayer of absorptive enterocytes interspersed with other minor cell types: mainly goblet cells, producing mucins, and enteroendocrine cells, synthesizing and secreting peptide hormones. The small intestinal epithelium is organized into two functionally and morphologically distinct compartments: the crypts, where the stem cells proliferate and differentiate, and the villi, where absorption and digestion occur [1] by the fully differentiated and mature apical cells. During migration along the crypt-villus axis, there is a continuous change in the cellular membrane; its protein composition, the pattern and activity of the enzymes expressed in it and the state of glycosylation of its components go through distinct phases of development [1–4] before they are extruded into the lumen. Thus, it is generally believed that the glycosyl side-chains of membrane proteins of crypt cells are usually of the oligomannose type, whereas the fully mature cells on the villi express complex glycosyl side-chains.

To direct surface proteins from the site of synthesis in the cytoplasm to the plasma membrane, they must go through several steps of glycosylation [2,4] and this varies in different species [5]. However, within any one species glycosylation depends mainly on the stage of differentiation and maturation of the cells, their position along the crypt-villus axis and location along the gastrointestinal tract, and the age and blood group specificity of the animal. Furthermore, as bacterial digestive enzymes continuously remove a large part of secreted mucinous glycoconjugates in animals with a conventional microflora (conventionally grown animals), while these accumulate under germ-free conditions [6], the intestinal nitrogen metabolism in conventionally grown and germ-free rats is different [7]. Accordingly, bacteria in the gut are expected to affect the state of glycosylation of lumenal receptors.

Many lectins, regardless of whether they are from plants or bacteria, are potent exogenous growth signals and some can also mimic the action of metabolic hormones [8]. Their biological activity is a direct consequence of lectin function: through recognition and binding to specific carbohydrates of receptors on surface membranes, they send signals and deliver messages to cells. Since interaction between lectins and the gut depends on their specific recognition of membrane glycans projecting into the lumen, one of the reasons for the differences in the ability of lectins to bind to the gut wall is due to this variation in the glycosylation of lumenal membrane components.

There is evidence that lectins which bind avidly are readily endocytosed and/or transcytosed [8] and because of their mito-

genicity, they increase the rate of crypt cell proliferation, speed up epithelial turnover [8] and are also expected to change the glycosylation of membrane glycans. Unfortunately, comprehensive information on the carbohydrate structure of surface receptors is not available at present and even less is known about the effects of different diets or lectins or the presence/absence of bacteria on glycosylation. Therefore the main aim of the present work was to obtain a low resolution map of glycosylation in the small intestine by histological staining techniques at the light microscope level to identify the terminal parts of different carbohydrate side-chains of membranous, cytoplasmic and secreted surface components.

Two main approaches were used: in a first approach, sections were taken from the small intestine of rats after exposure to lectins, such as GNA (*Galanthus nivalis* agglutinin; strictly specific for terminal α1-3 mannosyl groups), Con A (*Canavalia ensiformis* agglutinin; a less well-defined mannose/glucose-specific lectin) and PHA (*Phaseolus vulgaris* agglutinin; specific for complex glycosyl residues). Terminal structures of gut surface glycosyl side-chains in these sections were determined by reaction with a panel of digoxigenin-labelled lectins (DIG-lectins) and compared with those of control rats. This method was expected to furnish us with information on membrane glycosylation in control, well-fed rats and how this was changed by acute or chronic oral exposure to lectins and the presence/absence of bacteria. In a second and complementary approach, small intestinal sections taken from rats after acute or chronic exposure to GNA, Con A or PHA, were not only examined with digoxigenin-labelled histochemical reagents as to which types of surface glycans they expressed but were also tested with appropriate anti-lectin antibodies to see whether the lectins did actually bind to appropriate glycosyl groups *in vivo*. Information on the changes in glycosylation induced by lectins in the presence or absence of bacteria coupled with that of their effects on small intestinal function might provide rational approaches for improving the digestive/absorptive function and bacterial ecology of the gut.

Materials and methods

Lectins

PHA and GNA were purified and characterized as described before [9]. Con A and a rabbit antibody against this lectin were from Sigma (UK). DIG-lectins and a peroxidase-labelled (POD)-Fab fragment of anti-digoxigenin rabbit antibody preparation were from Boehringer Mannheim UK Ltd. The labelled lectins used in this study, with their sugar specificities, are given in Table 1.

Table 1. Lectins of digoxigenin-labelled lectins used in this study and their sugar specificity.

Lectin	*Specificity*
Con A (*Canavalia ensiformis*)	D-mannose/glucose
GNA (*Galanthus nivalis*)	α1-3 D-mannosyl-
PHA (*Phaseolus vulgaris*)	complex glycosyl-
AAA (*Aleuria aurentia*)	α1-6 fucose
MAA (*Maackia amurensis*)	NeuAc α2-3Gal/GalNAc
SNAI (*Sambucus nigra*)	NeuAc α2-6Gal/GalNAc
PNA (*Arachis hypogaea*)	Gal β1-3GalNAc in the absence of terminal sialic acid
WGA (*Triticum vulgaris*)	β1-4GlcNAc- oligomers, also weak reaction with neuraminyl-terminals

Animal experiments

Conventionally grown spf (specific pathogen-free) inbred Hooded-Lister rats of the Rowett colony (groups of four, average weight 80–90 g; individually housed) were intragastrically intubated with solutions of individual lectins (5 mg; acute exposure) or fed for 10 days on fully balanced diets containing 9.3% (w/w) lactalbumin and 0.7% individual pure lectins, such as GNA, Con A or PHA. Control rats were pair-fed on a diet containing 10% (w/w) lactalbumin [9]. On the morning of the 10th day, rats were given 2 g of their respective diets and killed by ether anaesthesia 2 h later. The abdomen was cut open, the small intestine (and other tissues) was removed, and two sections of 2 cm each, 5 and 7 cm from the pylorus were taken for histology and lectin-histology respectively. All other tissues were taken, freeze-dried and weighed. Rats in acute experiments were killed 1 h after intubation but otherwise treated in the same way as those from the feeding experiments.

Identical experiments were carried out with barrier-raised and maintained germ-free Hooded-Lister rats which were originally derived from the same strain as the Rowett colony. They were fed diets irradiated in a cobalt-60 unit receiving a minimum dose of 5 Mrad. The rats had free access to sterile water containing vitamins K and B_{12}. All manipulations were carried out in isolators equipped with an air filtration device and maintained under slight positive pressure. Otherwise the procedures used were as with the conventionally grown rats described above. Sterility was ensured by microbial testing of faeces samples every 24 h.

The nutritional evaluation of the results will be described elsewhere.

Histology

Pieces (about 2 cm) of the small intestine, usually about 5 and/or 7 cm from the pylorus were taken at the end of the experiments, fixed in 4% buffered formalin, embedded in paraffin wax and sectioned at 3 μm. The sections were dewaxed in two changes of xylene and rinsed in alcohol. Endogenous peroxidase activity was blocked by exposure to methanol-hydrogen peroxide for 15 min.

Digoxigenin-lectin staining The sections were incubated with 0.1% (w/v) trypsin at pH 7.8 (saline buffered with Tris-glycine; TBS), 37°C for 30 min. Following a rinse in running

tap water for 5 min, the sections were placed in two changes of TBS, 5 min each time. The sections were immersed in normal sheep serum, diluted five times with TBS, for 30 min and then in a solution of the appropriate DIG-lectin diluted 1:100–1:1000 in TBS at room temperature for 30 min. After three washes in TBS, 10 min each time, the sections were reacted with anti-digoxigenin (POD-Fab fragments) diluted 1:200 with TBS in the presence of the appropriate sugar hapten for 60 min at room temperature. The inclusion of the haptenic sugar at this stage ensured that the anti-digoxigenin antibody reacted with digoxigenin of the bound DIG-lectin and not the lectin with the glycosyl side-chains of the IgG molecule by a non-immune reaction. The peroxidase reaction was developed in 3,3′-diaminobenzidine solution, counterstained in Haematoxylin for 3 min and mounted. DIG-lectin staining was abolished by the appropriate haptenic sugar.

Antibody-peroxidase-antiperoxidase (PAP) staining After inhibition of endogenous peroxidase and trypsinization, the sections were reacted with a solution of the appropriate anti-lectin antibodies in the presence of carbohydrate haptens according to the specificity of the lectin, followed successively by the link antiserum and PAP serum. The label was visualized with 3,3′-diaminobenzidine and the sections were counterstained with Haematoxylin.

Results

Changes in glycosylation after oral exposure to lectins of conventionally grown and germ-free rats

Terminal mannose residues In normal conventionally grown control rats there were only a few terminal α-linked mannosyl residues on the villus brush-border lumenal membrane, but even these disappeared completely after feeding with diets containing GNA or Con A (no staining with DIG-GNA and DIG-Con A, Fig. 1a and c, respectively; Table 2). In agreement with this, on first *in vivo* exposure to GNA, the binding of this lectin to the brush-border membranes of small intestinal villi of conventionally grown rats was slight (Fig. 2a) but gradually increased on extended dietary exposure (shown by reaction with anti-GNA antibodies, followed by PAP staining; Fig. 2c). Moreover, on combined exposure to PHA and GNA, there was a rapid development of GNA binding (Fig. 2b).

In contrast to conventionally grown rats, brush-border membranes of germ-free control rats contained small numbers of terminal mannosyl groups as shown by a slight but definite membrane staining of the villus enterocytes by DIG-Con A (Fig. 1d). In keeping with this, the binding of GNA to brush-border membranes *in vivo* was more extensive in germ-free than in conventionally grown rats on a first exposure to this lectin (Fig. 2d). The *in vivo* binding patterns of Con A to brush-border membranes was similar to those of GNA (results not given).

In contrast to their absence from the membrane, the concentration of α-mannosylated glycoproteins in the cytoplasm of enterocytes was appreciable, at least in some regions of the small intestinal villi (Table 3). Thus, in control rats there was cytoplasmic staining by the strictly mannose-specific DIG-GNA but it was confined to cells at the villus-crypt junction (Fig. 1a; Table 3) whereas after chronic exposure to PHA-diets, not only did the staining become more intense but also the mannosylated glycoconjugates spread up to the tip of the villi (Fig. 1b). The cytoplasmic staining patterns obtained with the less specific DIG-Con A were less consistent (Table 3). Furthermore, changes in the concentration of cytoplasmic glycans with mannosyl terminals of conventionally grown rats fed on Con A or GNA were less extensive compared with those on PHA-diet (staining with strictly mannose-specific DIG-GNA; not illustrated).

Table 2. Intensity of surface staining with digoxigenin-labelled lectins of brush-border membranes of the small intestine of conventionally grown rats fed diets with either added lectins or lactalbumin for 10 days.

	Diets											
	Lactalbumin			*PHA*			*GNA*			*Con A*		
Digoxigenin-labelled lectins	*Epithelium*			*Epithelium*			*Epithelium*			*Epithelium*		
	Tip	*Base*	*Crypt*	*Tip*	*Base*	*Crypt*	*Tip*	*Base*	*Crypt*	*Tip*	*Base*	*Crypt*
Con A	tr	–	–	tr	–	–	–	–	–	–	–	–
GNA	–	–	–	–	–	–	–	–	–	–	–	–
AAA	+p	tr	tr	+p	+p	+p	tr	–	+p	+p	+p	+p
MAA	++	+	+p	++	++	++	+	tr	tr	++p	–	+
SNAI	tr	tr	–	tr	–	–	tr	–	–	tr	–	–
PNA	++	++	++	+++	++	++	+	tr	+	+	tr	tr
WGA	++	+	+	++	+	++	+	+	+	+	tr	+
PHA	++	tr	+	+++	+p	++	++	+	++	+++	tr	+

Staining intensity is expressed on an arbitrary scale from –, no staining; tr, traces; +, ++, +++, increasing positive staining.
p, patchy staining.

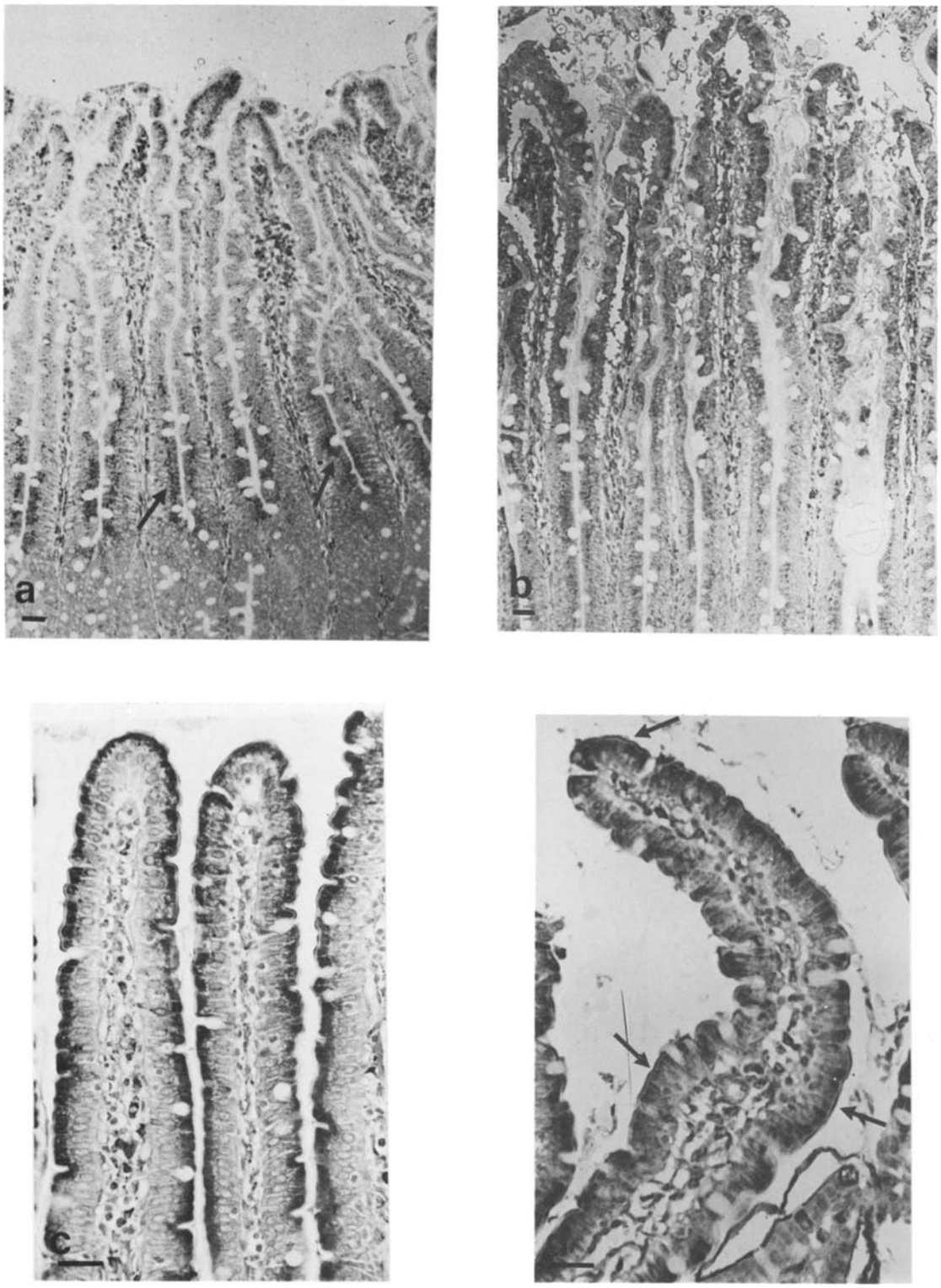

Figure 1. Changes in terminal mannosylation on oral exposure to lectins. **(a)** Cytoplasmic mannosyl glycoconjugates are confined to the villus-crypt junction in control rats (see arrow) but **(b)** they occur generally in villus cells right up to the tip of the villi after PHA treatment, as shown by staining with DIG-GNA. Furthermore, **(c)** the staining of the villi by DIG-Con A is exclusively cytoplasmic in conventionally grown control rats, but in germ-free rats **(d)** there is also some staining of the villus brush-border membrane glycoconjugates (see arrow). Bar = 25 μm.

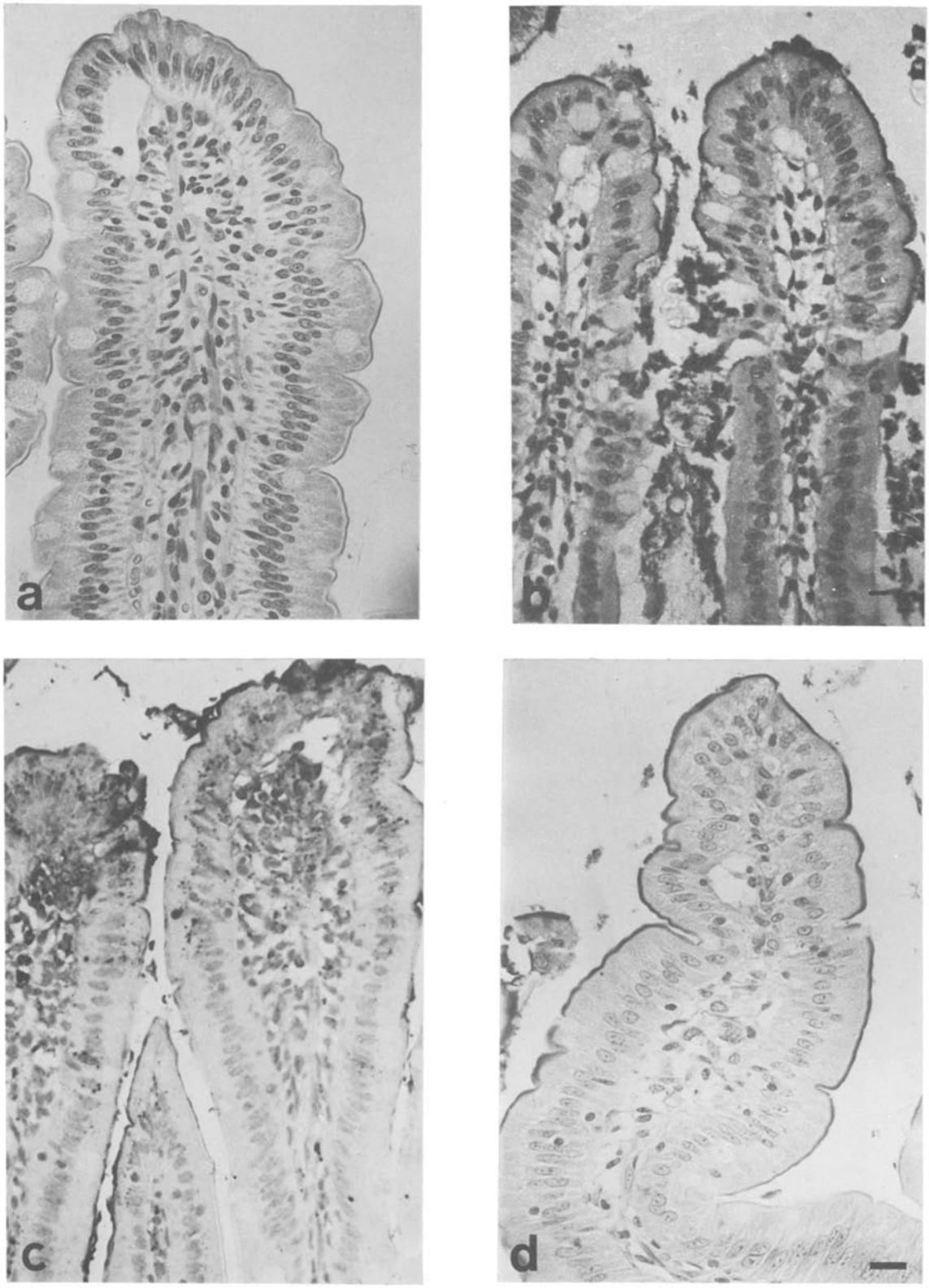

Figure 2. ***In vivo*** **binding of GNA to the brush-border epithelium in conventionally grown rats.** The presence of GNA on the brush-border membrane on **(a)** acute exposure to GNA; **(b)**after PHA treatment; **(c)** after 6 days feeding on GNA diet and **(d)** in germ-free rats on acute exposure to GNA was shown by reacting the sections with monospecific anti-GNA antibodies followed by PAP staining. Bar = 40 μm.

Table 3. Intensity of cytoplasmic staining with digoxigenin-labelled lectins of brush-border epithelial cells of the small intestine of conventionally grown rats fed diets with either added lectins or lactalbumin for 10 days.

	Diets											
	Lactalbumin			*PHA*			*GNA*			*Con A*		
Digoxigenin-labelled lectins	*Epithelium*			*Epithelium*			*Epithelium*			*Epithelium*		
	Tip	*Base*	*Crypt*	*Tip*	*Base*	*Crypt*	*Tip*	*Base*	*Crypt*	*Tip*	*Base*	*Crypt*
Con A	+++p	+	–	++p	+	–	+p	+	+	++p	+p	–
GNA	–	++P	–	++P	++p	tr	–	++p	tr	+p	tr	–
AAA	+g	+g	tr	trg	trg	+p	+++g	+++g	+	+g	+g	tr
MAA	+g	+g	tr	trg	trg	+p	+g	+g	tr	++g	++g	tr
SNAI	+++	++	+	tr	–	–	++	tr	–	+	–	–
PNA	+g	+g	+gP	+/trg	+/trg	+g	+g	+g	trg	tr	–	–
WGA	+p	tr	+pP	+g	+g	++g	+g	+g	+g	+g	tr	tr
PHA	+++	+	tr	++p/+g	tr	+p	+g	+g	+g	tr	–	–

Staining intensity is expressed on an arbitrary scale from –, no staining; tr, traces; +, ++, +++, increasing positive staining.
p, patchy staining; P, Paneth cell staining; g, goblet staining.

Terminal fucose residues Expression of fucose groups on lumenal membranes of control conventionally grown rats shown by DIG-AAA staining was moderate and confined mainly to the villus tip. This became almost nil after chronic exposure to GNA but increased somewhat with Con A or PHA (Fig. 3c,d). Staining with DIG-AAA of the lumenal membranes of germ-free rats was more extensive (Fig. 4a,c,d) compared with those of the corresponding conventionally grown rats (Fig. 3a,c,d), except for the villi of the GNA group whose membrane staining was slight in both germ-free and conventionally grown animals (Fig. 4b).

The cytoplasm of goblet cells of conventionally grown rats was stained strongly with DIG-AAA although its intensity varied with different treatments. Thus, although in rats fed on control lactalbumin diet (Fig. 3a) the average ratio of villous goblet cells to enterocytes of about 1:10 was similar to that of rats fed on GNA-diet (Fig. 3b), the concentration of fucosyl groups after GNA treatment significantly exceeded that of the lactalbumin controls. In contrast, after chronic exposure to Con A (Fig. 3c) or PHA (Fig. 3d), both the number of goblet cells and the strength of their staining were appreciably reduced. Although in these rats the outline of the goblet cells was still visible, their contents were shown to have been largely emptied.

In germ-free rats, staining of the cytoplasm of goblet cells with DIG-AAA was generally stronger and more uniform than in their conventionally grown counterparts (Fig. 4a–d), except for the GNA-fed rats whose small intestinal villi contained fewer goblet cells and showed less intensive staining for fucosyl terminals than the conventionally grown animals.

Terminal NeuAc α2-3Gal/GalNAc residues Staining of lumenal membranes by DIG-MAA of most rats was slightly more extensive than that with DIG-AAA. However, the villus membrane of the GNA-treated rats stained noticeably less well than that of the other groups, particularly in germ-free rats (Fig. 5b). Furthermore, the cytoplasm of most goblet cells in both germ-free (Fig. 5a,b,d) and conventionally grown (only given for GNA-fed rats; Fig. 5c) rats giving positive staining for terminal fucose residues also reacted with DIG-MAA indicating that they contained both terminally fucosylated and sialylated mucins.

Terminal NeuAC α2-6Gal/GalNAc residues Staining of lumenal membranes with DIG-SNAI was slight and patchy in all rats.

Although the cytoplasm of goblet cells was not stained with DIG-SNAI (Fig. 6), the cytoplasmic concentration of NeuAC α2-6Gal/GalNAc residues was particularly high in villous enterocytes of lactalbumin controls in both germ-free (Fig. 6a) and conventionally grown (Fig. 6b) rats. However, this was greatly reduced on chronic exposure to lectins; staining with DIG-SNAI was slight in Con A-fed rats (Fig. 6c) and became almost nil on treatment with PHA (Fig. 6d). There were no major differences between germ-free and conventionally grown rats.

Terminal Gal β1-3GalNAc residues For staining non-sialylated terminal Gal β1-3GalNAc residues, the sections were reacted with DIG-PNA without pre-treatment with sialidase.

The lumenal membrane of germ-free control rats reacted particularly strongly with DIG-PNA; staining further increased with the conventionally grown lactalbumin-fed rats and even the interepithelial membranes stained strongly giving a reticular appearance to the villus (not shown). A similar effect was seen with PHA-feeding in germ-free (Fig. 7d) and conventionally grown rats (not given). However, in both

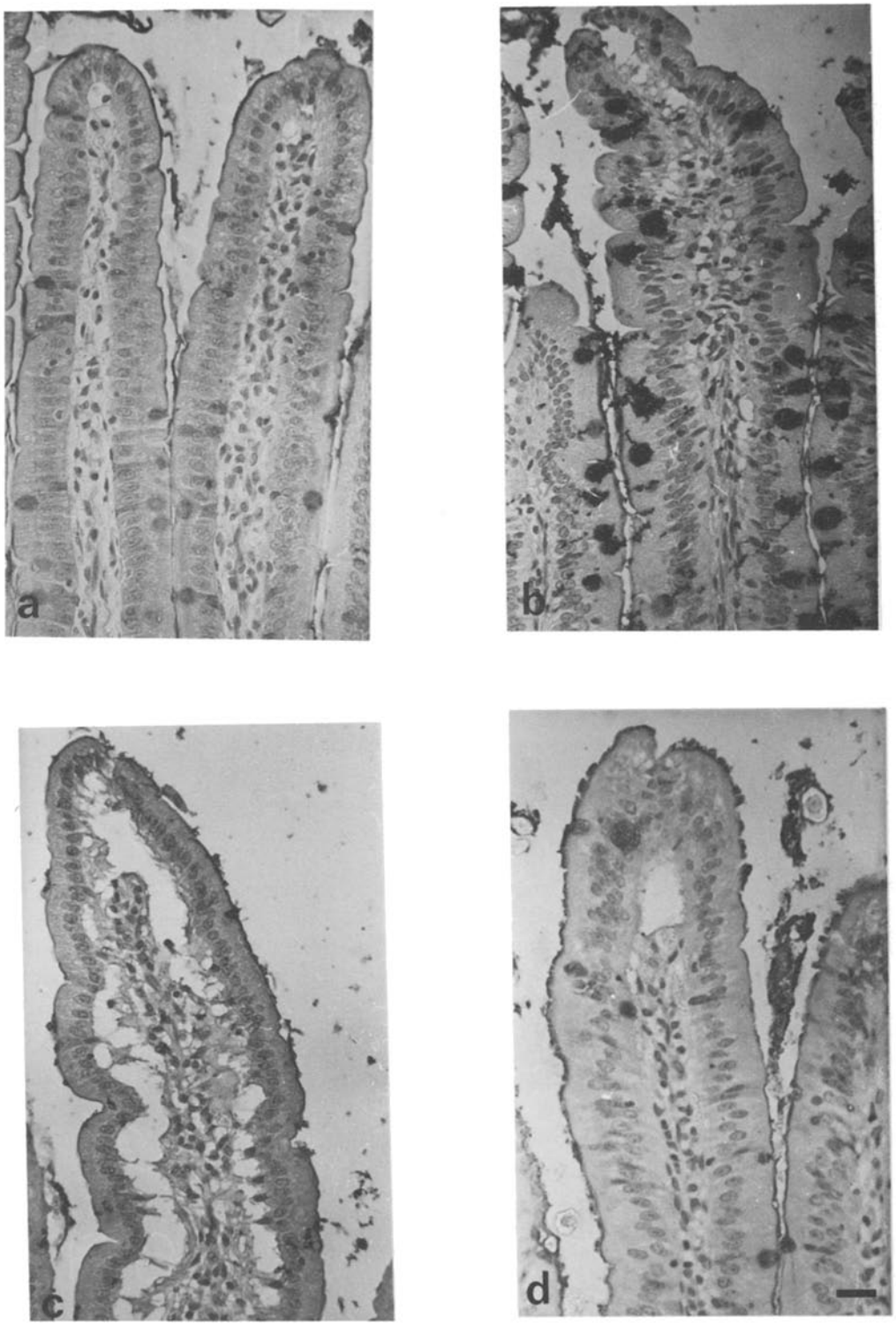

Figure 3. The presence of fucosyl terminals in goblet cell mucins of conventionally grown rats. The sections were stained with DIG-AAA in **(a)** control lactalbumin-fed rats; **(b)** GNA-fed rats; **(c)** Con A-fed rats and **(d)** PHA-fed rats. Bar = 25 μm.

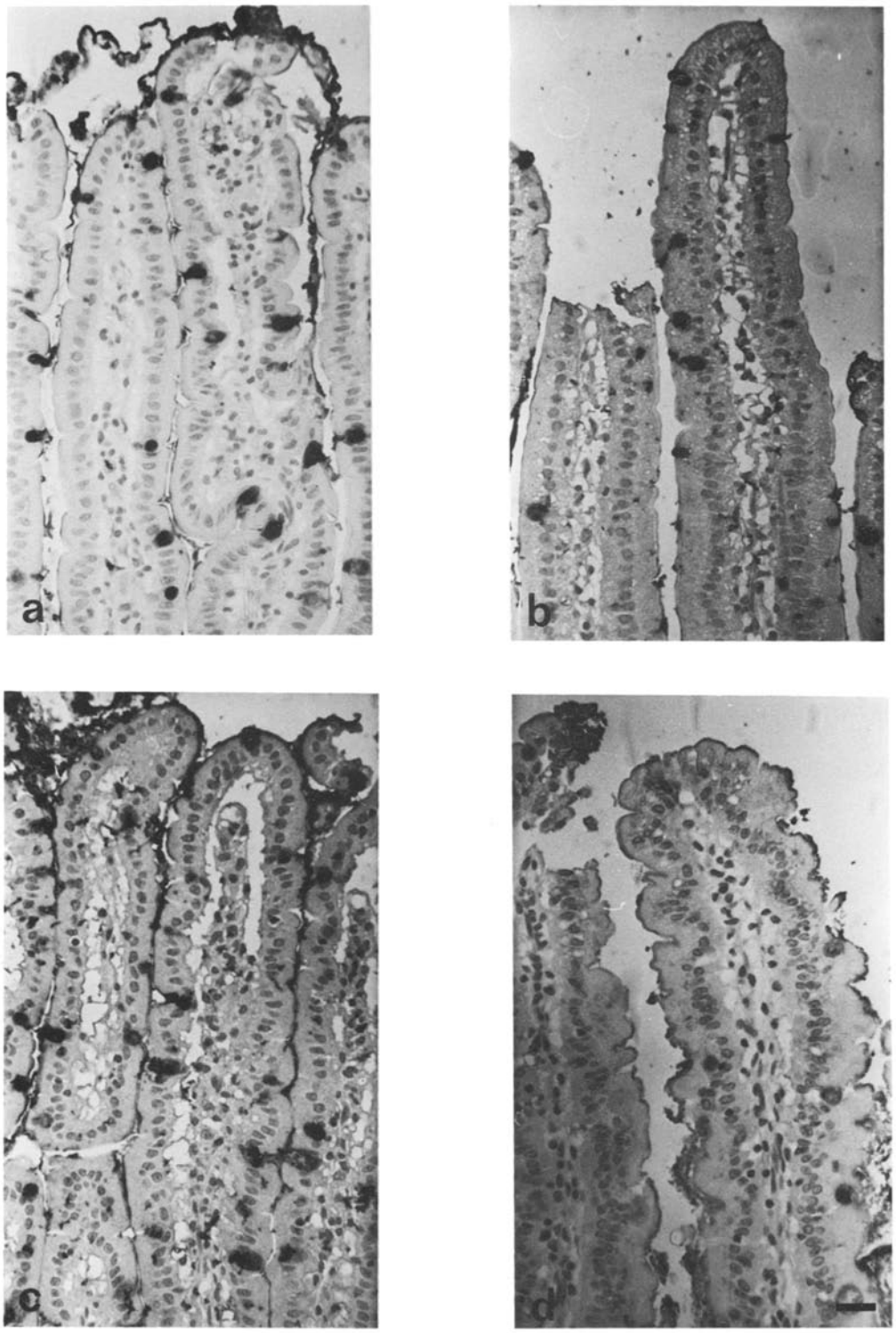

Figure 4. The presence of fucosyl terminals in goblet cell mucins of germ-free rats. The sections were stained with DIG-AAA in **(a)** control lactalbumin-fed rats; **(b)** GNA-fed rats; **(c)** Con A-fed rats and **(d)** PHA-fed rats. Bar = 25 μm.

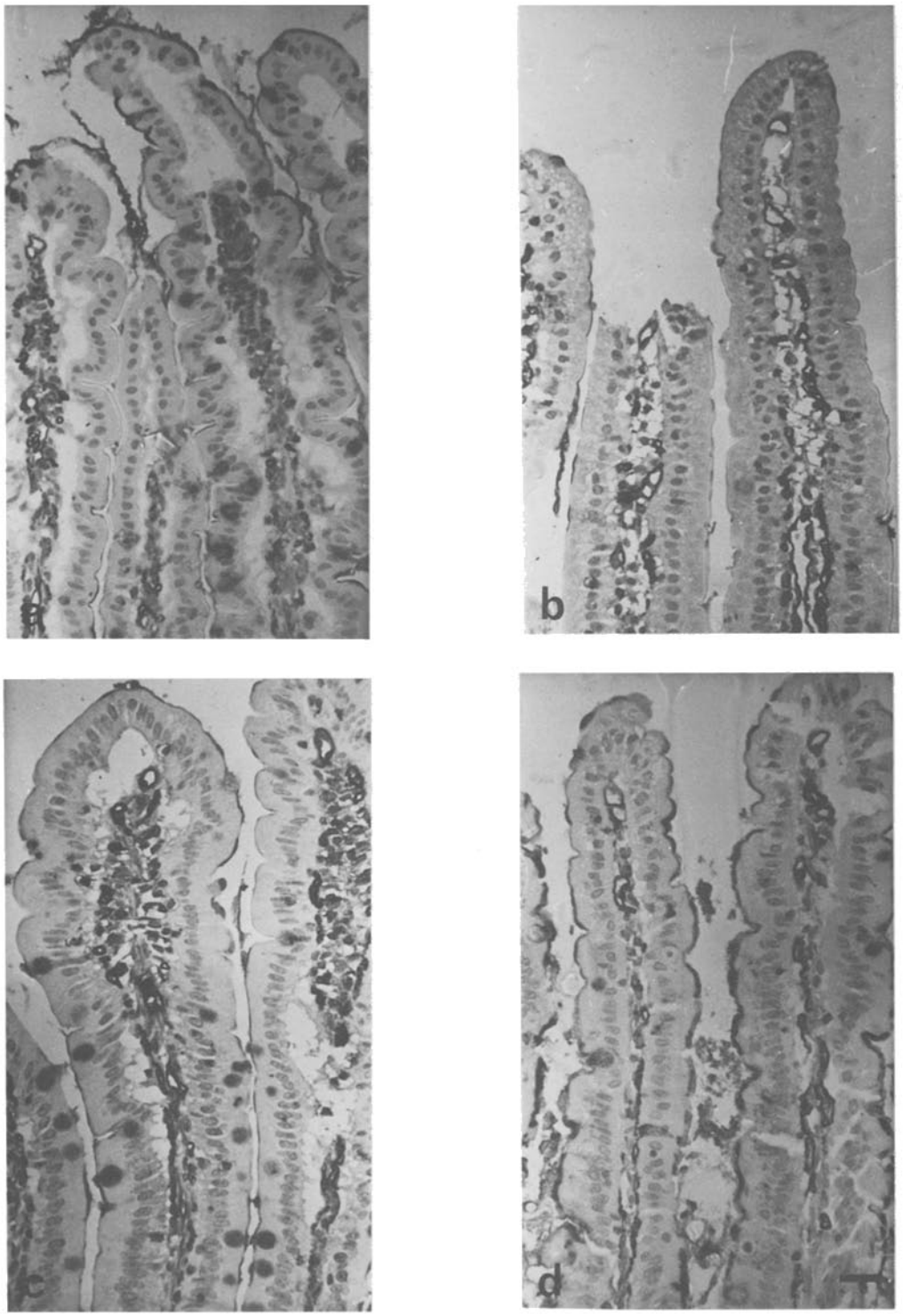

Figure 5. The presence of NeuAC α-2,3Gal/GalNAc in goblet cell mucins of germ-free rats. The sections were stained with DIG-MAA of (**a**) control lactalbumin-fed rats; (**b**) GNA-fed rats; (**c**) Con A-fed rats and (**d**) PHA-fed rats. Bar = 25 μm.

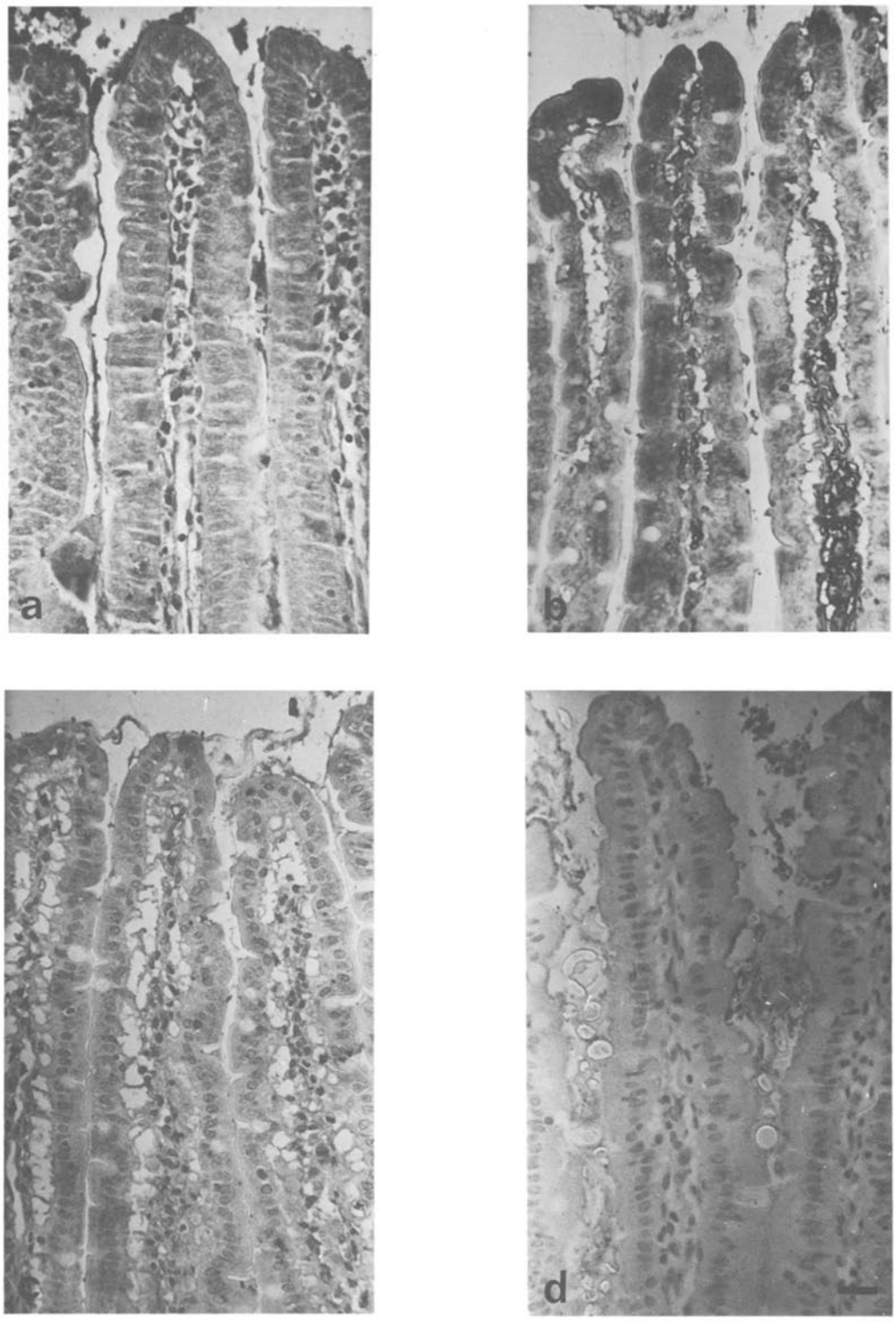

Figure 6. The presence of NeuAC α-2,6Gal/GalNAc on small intestinal villi. The sections were stained with DIG-SNAI of **(a)** germ-free control rats; **(b)**conventionally grown control rats; **(c)** GNA-fed germ-free rats and **(d)** PHA-fed germ-free rats. Bar = 25 μm.

GNA- (Fig. 7b) and particularly Con A-fed rats (Fig. 7c), there was less staining with DIG-PNA and this was not dependent on the presence or absence of bacteria in the gut lumen.

In contrast, the cytoplasm of the epithelial enterocytes was not stained (Fig. 7a), although occasionally the content of goblet cells was slightly stained.

Terminal β1-4GlcNAc residues (and oligomers) Reaction with DIG-WGA was similar to that with DIG-PNA described above. No major differences between germ-free and conventionally grown rats were found with this labelled lectin.

Complex glycosyl side-chains Reaction with DIG-PHA was extensive in all rats (results not given). Although most of the staining was confined to lumenal membranes of the villus epithelium, there was occasionally also slight staining of goblet cells. In addition, DIG-PHA strongly stained the brush-border membrane of the crypt epithelium (not illustrated). However, this was generally true and all DIG-labelled lectins which stained the membranes, goblet cell mucins and/or cytoplasmic glycans on the villi reacted equally well with the glycoconjugates of corresponding structures in small intestinal crypts (results not given).

For facilitating comparisons, the results of DIG-lectin staining with conventionally grown rats have been tabulated in Tables 2 and 3.

Discussion

Although it is commonly accepted that the diet has a major effect on health, with a few exceptions direct experimental evidence for the involvement of food components in the regulation of gut metabolism is generally not available. One of these exceptions is the role of dietary lectins, particularly PHA, in nutrition and metabolism.

PHA is a powerful exogenous metabolic signal and growth factor for the gut (for references see [8]). Because of its stability and avid binding to receptors of endogenous growth factors, hormones and bacteria on the lumenal brush-border membrane, PHA delivers potent messages to the epithelial cells leading to changes in gene expression and cellular metabolism. Although it is not clear whether these are direct lectin effects or the effects are mediated through endogenous growth factors, there is definite evidence for the binding of lectins to cells of jejunal crypts [9]. Therefore, it is possible that the PHA signal may directly stimulate crypt cell proliferation and its dramatic effects on the gut may be a consequence of this. Major changes in the glycosylation of the brush-border membrane and cytoplasmic glycoconjugates can be expected as a consequence of the powerful physiological growth factor activity of PHA.

At high dietary intakes PHA is known to be nutritionally toxic for conventionally grown but not for germ-free rats [8]. As it is a potent growth factor in germ-free- as well as in conventionally grown rats, the direct metabolic effects of PHA may have little to do with its toxicity [8]. Accordingly, it was thought possible that the PHA-induced shifts in glycosylation might be different in the presence or absence of bacteria in the gut lumen.

All other lectins which bind to the small intestinal epithelium affect its metabolism and therefore they are also expected to influence its state of glycosylation. GNA, a strictly mannose-specific lectin, binds to the crypt [9], but its effect appears to be the opposite of PHA. By slowing down the rate of crypt cell proliferation, GNA was shown to induce a decrease in the length and cell numbers of the crypts. Therefore its effects on brush-border glycosylation were expected to be particularly revealing in comparison with that of PHA or Con A, another mannose-specific lectin which is generally regarded as toxic for conventionally grown rats.

Although the resolution of DIG-lectin staining at the light microscope level was inherently low and fine structural features of epithelial cells could not be unequivocally located, the results presented in this paper give an overall view of the state of glycosylation in the lumen of the small intestine. With this technique it was impossible to decide whether the surface glycosyl structures stained with DIG-lectins were an integral part of the cellular membrane or only loosely adhering components derived from sloughed off tip cells. However, the staining clearly illustrated the presence of saccharide structures on the gut wall or in the cytoplasm of the epithelial cells which were available for possible interactions with dietary factors or resident or infecting bacteria. In fact, compared with high resolution electron microscopy, the main strength of this first low resolution experimental approach was that the results give a comprehensive and representative picture of the morphological features of the small intestine on which its proper physiological functioning is dependent.

Glycosylation of small intestinal brush-border membrane and cytoplasmic components is remarkably constant in inbred rats such as the Hooded-Lister rats of the Rowett colony which have been kept in isolation for over 50 years. Although glycosylation is developmentally regulated in rats, changes are minimal with animals of the same age as in the present study. Glycosylation is also dependent on the location of the section along the small intestine. However, sections examined here were taken from the same location in the jejunum. There was also special care taken that the rats selected for the experiments were not injured in any way, were healthy and kept under strict microbiological control. Additionally, as the germ-free rats were of the same origin as the conventionally grown animals, the comparison of their state of glycosylation was expected to be fundamentally sound.

In general the results presented in this paper confirmed and extended some of the commonly held views on the state of glycosylation of small intestinal brush-border membranes and cytoplasmic glycoconjugates of both villi and crypts in weaned conventionally grown rats, well-fed on lactalbumin

or other control diets [10–12]. Thus, the villous brush-border membrane contained mainly complex glycosyl structures and the expression of terminal mannosyl residues in it was vanishingly small. Some of the membrane glycoconjugates were fucosylated and/or α2-3 sialylated [12] but with the low resolution technique used it was impossible to decide whether these were genuine membrane components or at least in part derived from goblet cell mucins absorbed to the glycocalix. In agreement with previous observations [11] α2-6 sialylation was absent on the brush-border membrane or in goblet cells, although these saccharide structures were shown in the present paper to be abundant in the cytoplasm (Fig. 6a,b). Even more intriguingly, staining by DIG-PNA without sialidase pre-treatment showed a high expression of Gal β1-3GalNAc structures (T antigen) on the brush-border membrane (Fig. 7a) which in either sialylated or free form are thought to be more representative for the colonic epithelium than the small intestine [12]. In contrast to the findings of Taatjes and Roth [11], the brush-border membrane of the small intestine of normal well-fed rats contained relatively large amounts of saccharide residues reactive with DIG-WGA which, in addition to its possible staining of some sialylated glycans, may have indicated the presence of at least some hybrid type glycosyl structures [10] with possible GlcNAc terminals.

The main and most important finding of this work was that the state of glycosylation of both membrane and cytoplasmic glycoconjugates was modified by feeding rats on diets containing lectins. Some of these changes were also dependent on the presence or absence of bacteria in the lumen of the gut and, indeed, bacteria themselves could modify the glycosylation of lumenal receptors even in control lactalbumin-fed rats (see for example membrane staining by DIG-Con A of germ-free villi, Fig. 1d, in comparison with the lack of such staining in conventionally grown rats). Even though in the present study the histological techniques used were at best semi-quantitative, some of the differences between germ-free and conventionally grown rats were large enough to be regarded as significant. Thus, goblet cells were usually stained more strongly in germ-free rats (Fig. 4) than in conventionally grown rats (Fig. 3), with the only exception being rats fed diets containing GNA.

Changes in glycosylation caused by lectins were more substantial than those by bacteria. Indeed, in a few cases such as with fucosylated (Figs 3 and 4) or sialylated goblet cell mucins (Fig. 5) or cytoplasmic glycoconjugates containing NeuAC α2-6Gal/GalNAc residues (Fig. 6), oral exposure to highly reactive lectins such as PHA (occasionally even Con A) reduced the concentration of these glycans to virtually nil. Clearly, the removal of a part or most of the protective effects of the surface mucin coat of the brush-border epithelium on continuous oral exposure to lectins may have deleterious consequences for the digestive system. This may in part be offset by the consequent reduction in the number of attachment sites for bacteria but the potential physiological significance of this remains to be demonstrated.

One of the most biologically pertinent changes in glycosylation by oral exposure to lectins was their effect on terminal mannosylation of glycans. Thus, mannosylated glycoconjugates were present almost exclusively in the cytoplasm of epithelial cells of well-fed control rats at the crypt villus junction (Fig. 1a), whereas after feeding diets containing PHA there was a definite shift in location on the villi and cellular concentration. Indeed, in PHA-fed rats most epithelial cells up to the villus tip contained increased amounts of mannosylated cytoplasmic glycoconjugates (Fig. 1b). As glycans of less differentiated epithelial cells are polymannosylated it is possible that with the lectin-induced increase in the rate of crypt cell proliferation and the correspondingly shortened transit time of epithelial cells, the crypt cells penetrated further up the villus than they would do under normal physiological conditions. This shift combined with the brush-border damage caused by PHA may possibly be the main underlying reason for the *E. coli* overgrowth in the rat small intestine after dietary exposure to PHA [13] since the outpouring of mannosylated glycans from the cytoplasm of damaged tip cells (Fig. 2b) may provide additional binding sites for Type-1, mannose-sensitive, fimbriated bacteria. A corollary of this is that the dietary administration of mannose-specific lectins such as GNA successfully blocked the overgrowth of *E. coli* and thus could be used for the prevention of non-specific colitis (Chemical probiosis, [13]).

Dietary exposure to lectins induced other major changes in terminal glycosylation. For example, the strong and mainly membrane staining by DIG-PNA of both germ-free and conventionally grown control rat villi (Fig. 7a) could be substantially reduced by feeding the rats on diets containing mannose-specific lectins such as GNA (Fig. 7b) or Con A (Fig. 7c). In contrast, exposure to PHA highly increased the staining intensity of membranes on the villi. There were similar changes in reactivity with DIG-WGA. Here again feeding PHA diets increased the expression of terminal β1-4GlcNAc on epithelial cell membrane glycoconjugates in both germ-free and conventionally grown rats (not illustrated) in comparison with control rats or those fed on diets containing either GNA or Con A. The biological significance of these changes are unknown at present.

In conclusion, continuous oral exposure of rats to lectins such as PHA, Con A and GNA, induced major changes in the expression of both membrane and cytoplasmic glycoconjugates containing different terminal saccharide structures. Some of the changes were appreciably influenced by bacteria in the small intestinal lumen. Moreover, some of these changes in glycosylation also led to major shifts in the bacterial flora of the small intestine such as were found with the dramatic overgrowth of *E. coli* in its lumen after feeding rats on PHA-diets and its successful blocking by the inclusion of GNA in the diet. In general, these changes fall into one of the following categories:

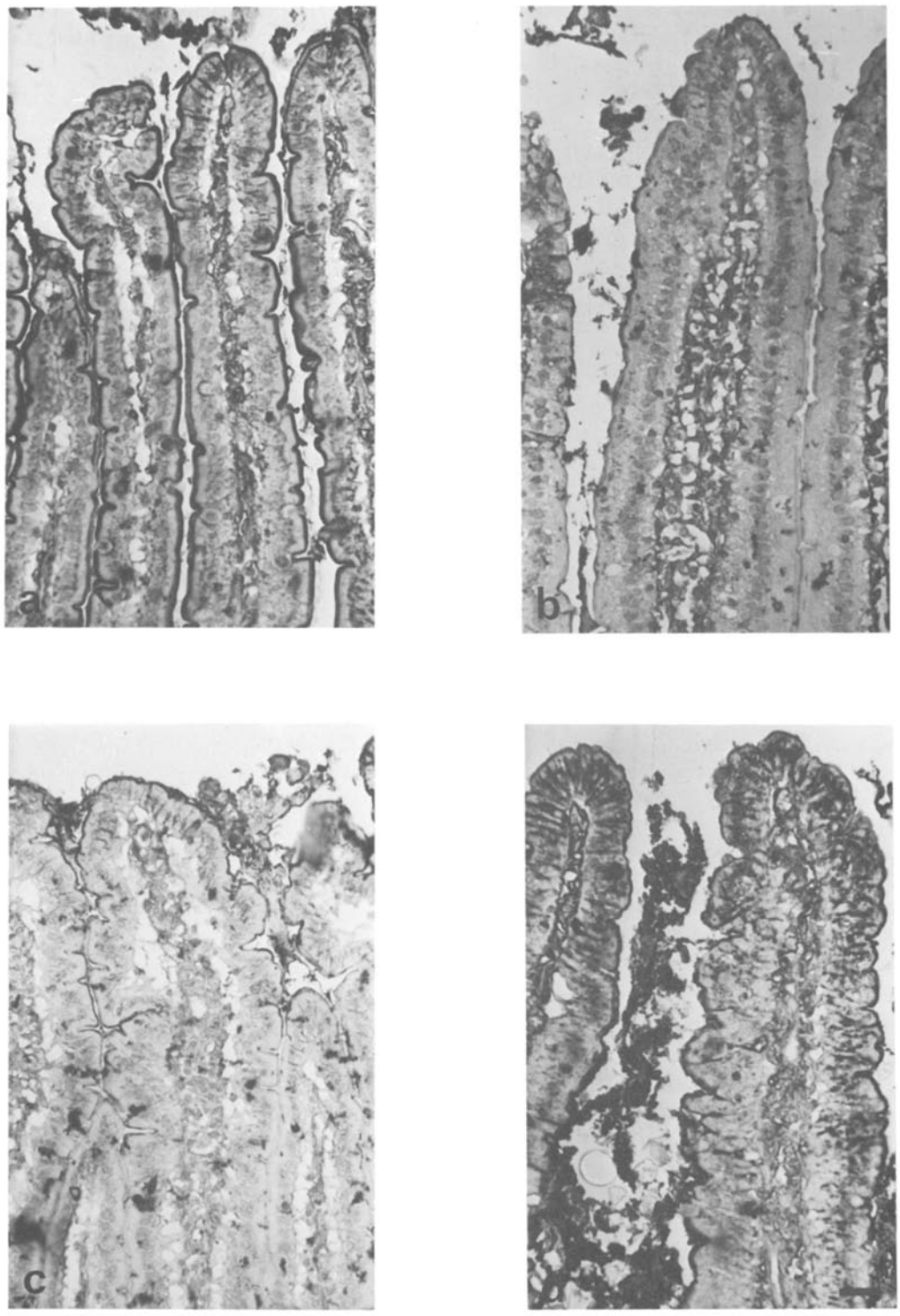

Figure 7. The presence of T antigen-type terminals on the small intestinal villi of germ-free rats. The sections were stained with DIG-PNA without sialidase pre-treatment of **(a)** control rats; **(b)** GNA-fed rats; **(c)** Con A-fed rats and **(d)** PHA-fed rats. Bar = 25 μm.

1. *Changes in glycosylation by displacing endogenous ligands* Endogenous ligands such as Type-1 mannose-sensitive fimbriated bacteria can be displaced from terminal mannosylated glycans of lumenal membranes by mannose-specific lectins revealing previously blocked sugar terminals (see for example Fig. 2c).

2. *Changes in glycosylation resulting from increased rate of crypt cell proliferation* By speeding up cellular turnover, lectins which are growth factors for the gut [8] can increase the proportion of less differentiated, immature epithelial cells on the villi, with the consequent increase in the concentration of terminally mannosylated cytoplasmic glycoconjugates. Other lectin-stimulated metabolic effects can also lead to an increase or decrease in cytoplasmic/membrane components with different terminal glycosyl groups (Figs 6 and 7).

3. *Changes in glycosylation resulting from the effects of lectins on goblet cells* The contents of the goblet cells of both conventionally grown and germ-free rats can nearly be emptied by PHA exposure (Figs 3–5); Con A is almost as powerful in germ-free rats as PHA (Fig. 3c). In contrast, GNA has the opposite effect. Moreover, as shown previously, sialic acid-binding lectins such as SNAI or MAA, can also change the glycosylation of the gut wall by overstimulating and exhausting the mucin synthesizing capacity of goblet cells [14].

Future perspective and practical implications

Changes in glycosylation induced by dietary lectins may lead to either the appearance of new or the removal of existing glycosyl structures on the surface of the gut and consequently, to changes in the binding potential of the brush-border for dietary factors and bacteria. Since a critical step in the bacterial colonization of the gut is the binding of the bacterium to the gut surface through its fimbrial- and/or surface adhesins, the bacterial ecology of the intestinal tract may be altered by changing the expression of the sugar structures on the lumenal surface. Furthermore, the secretion of gut peptide hormones such as cholecystokinin, gastrin and others, can be modulated by changes in the state of glycosylation of brush-border neuroendocrine cells because, depending on which lectin is bound by them, their hormone secretion is stimulated or depressed [8,15]. Lectins can also be used to change the digestive/absorptive functions of the gut by inducing its growth and changing the specific activity of brush-border enzymes (to be published elsewhere) or by stimulating the synthesis and secretion of pancreatic enzymes [14]. Some lectins may also be used to stimulate the secretion of mucinous glycoproteins from small intestinal goblet cells. Alternatively, anti-mitotic lectins or incomplete mitogens, can be used to slow down or stop unwanted cell proliferation in the gastrointestinal tract. Thus, by the use of the appropriate dietary lectin(s) the 'engineering' of the digestive tract for improving its performance and bacterial ecology is now a practical possibility [15].

Acknowledgements

AP is a Senior Research Fellow of The Rowett Research Institute and the main part of the work described in this paper has been carried out during his tenure of a Leverhulme Emeritus Research Fellowship; he was a recipient of the Auber Bequest Award of The Royal Society of Edinburgh. The work was also supported by The Scottish Office Agriculture and Fisheries Department and in part by a grant of the National Fund for Scientific Research (Belgium; F.G.W.O. grant 20059.89 N) to Dr Peumans, who is the Research Director, and Dr Van Damme, who is a Research Assistant of this Fund. Dr Ewen gratefully acknowledges the generous grant from the Scottish Home and Health Department for the purchase of the Joyce-Loebl 'magiscan' image analyser. The work is also part of EEC-FLAIR Concerted Action No. 9 and Concerted Action No. AIRI1-CT92-0569. Special thanks are due to Ms Viv Ronaasen (Guildford) for carrying out the germ-free experiments.

References

1. Johnson LR (1988) *Physiol Rev* **68**:456–502.
2. Cole CR, Smith CA (1989) *Biochem Education* **17**:179–89.
3. Gordon JI, (1989) *J Cell Biol* **108**:1187–94.
4. Shylaja M, Seshadri HS (1989) *Biochem Education* **17**:170–78.
5. King TP, Kelly D (1991) *Histochem J* **23**:43–54.
6. Gordon HA, Bruckner G (1984) In *Laboratory Animal Handbooks 9;* Chapter 9, *The Germ-Free Animal in Biomedical Research* (Coates ME, Gustafsson BE, eds) pp. 193–213. London: Laboratory Animals Ltd.
7. Salter DN (1984) In *Laboratory Animal Handbooks 9;* Chapter 9, *The Germ-Free Animal in Biomedical Research* (Coates ME, Gustafsson BE, eds) pp. 253–63. London: Laboratory Animals Ltd.
8. Pusztai A (1991) *Plant Lectins.* Cambridge: Cambridge University Press.
9. Pusztai A, Ewen SWB, Grant G, Peumans WJ, Van Damme EJM, Rubio L, Bardocz S (1990) *Digestion* **46** (suppl. 2):308–16.
10. Roth J (1987) *Biochim Biophys Acta* **906**:405–36.
11. Taatjes DJ, Roth J (1991) *Int Rev Cytol* **126**:135–93
12. Roth J (1993) *Histochem J* **25**:687–710.
13. Pusztai A, Grant G, Spencer RJ, Duguid TJ, Brown DS, Ewen SWB, Peumans WJ, Van Damme EJM, Bardocz S (1993) *J Appl Bacteriol* **75**:360–68.
14. Pusztai A, Ewen SWB, Grant G, Brown DS, Peumans WJ, Van Damme EJM, Bardocz S (1992) In *Polyamines in the Gastrointestinal Tract* (Dowling RH, Fölsch UR, Löser C, eds) pp. 473–83. Dordrecht: Kluwer Academic Publishers.
15. Pusztai A (1993) *Eur J Clin Nutr* **47**:691–99.