Preferential Association of Glycoproteins to the Euchromatin Regions of Cross-Fractured Nuclei Is Revealed by Fracture-Label

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Abstract. We used fracture-label to establish ultrastructural localization of glycoproteins in cross-fractured nuclei of duodenal columnar and exocrine pancreatic cells. Mannose residues were detected in cell nuclei by labeling freeze-fractured tissues with concanavalin A–horseradish peroxidase·colloidal gold (Con A-HRP·CG) or direct concanavalin A·colloidal gold (Con A·CG); fucose residues were detected with Ulex Europaeus I·colloidal gold (UEA I·CG) markers. Areas of the three main intranuclear compartments (euchromatin, heterochromatin, and nucleolus) exposed by freeze-fracture were determined by automated image analysis. Colloidal gold particles bound to each nuclear subcompartment were counted and the results expressed in number of colloidal gold particles per square micrometer ± SEM. Duodenal and pancreatic tissues fractured and labeled with Con A-HRP·CG complex or direct Con A·CG conjugates showed that the vast majority of Con A binding sites was confined to euchromatin regions with only sparse labeling of the heterochromatin and nucleolus. UEA I labeling of duodenal columnar cells showed that colloidal gold particles were almost exclusively confined to cross-fractured areas where euchromatin is exposed. Trypsinization of the fractured tissues before labeling with Con A and UEA I abolished 95–100% of the original label. Our results show that, within the nucleoplasm, mannose and fucose are residues of glycoproteins preferentially located within the regions of euchromatin.

THERE is evidence for the association of glycoproteins to chromatin. Biochemical studies show the presence of glycoproteins in the chromatin in the nucleus of rat liver cells (23, 52), HeLa cells (59), Novikoff hepatoma (23, 67), Walker 256 carcinosarcoma tumor cells (67), calf thymus (51), and in the macronuclei of *Tetrahymena thermophila* (32). Among the various sugars associated with chromatin, mannose and fucose were the most commonly found (23, 32, 51, 52, 59). Biochemical isolation of these pure nuclear fractions is difficult and subject to contamination, in particular, from components of nuclear envelope (23, 31).

Ultrastructurally, the interphase nucleus reveals three distinct intranuclear regions—euchromatin, heterochromatin, and nucleolus. Cytochemical studies confirm the presence of concanavalin A (Con A)[1] binding sites in the nucleoplasm of rat liver nuclei (54) and indicate the presence of Con A and wheat germ agglutinin binding receptors within the nuclei of ovarian follicular cells (56). These initial studies, however, do not establish the relative distribution of glycoconjugates in euchromatin, heterochromatin, or nucleolus.

In freeze-fracture, membranes are split along their bilayer continuum (11, 43) to reveal a two-dimensional view of fractured membrane halves. During fracture, many cells are also cross-fractured exposing their cytoplasm and nucleoplasm (30, 48). We reasoned that the combination of freeze-fracture and cytochemistry in fracture-label could, therefore, be used to detect glycoconjugates not only in freeze-fractured membrane halves of tissues and cells as demonstrated previously in our laboratory (1–3, 6, 11, 30, 43, 44, 56, 60, 61) but also in cross-fractured intracellular organelles including the nucleus. Here, we use fracture-label to investigate the cytochemical, in situ localization and topographical distribution of glycoconjugates in cross-fractured nuclei of rat duodenal columnar and exocrine pancreatic cells. Since biochemical studies have shown the association of mannose and fucose to chromatin, we use Con A (specific for mannose [40]) and Ulex Europaeus I (UEA I, specific for L-fucose [4, 13]) tagged with colloidal gold as markers for glycoconjugates. We show that Con A and UEA I binding receptors are mainly confined to euchromatin regions, i.e., the domains of the nucleoplasm where replication and transcription take place (12, 17).

[1] *Abbreviations used in this paper:* Con A, concanavalin A; Con A·CG, concanavalin A·colloidal gold; Con A-HRP·CG, concanavalin A-horseradish peroxidase·colloidal gold; CPD-FL, critical-point-drying fracture-label; HRP·CG, horseradish peroxidase·colloidal gold; Pt/C, platinum-carbon; UEA I, Ulex Europaeus I; UEA I·CG, Ulex Europaeus I·colloidal gold.

Materials and Methods

Tissue Preparation

Duodenal segments (with pancreas attached) excised from 150–180 g Sprague-Dawley rats killed by cervical dislocation were fixed by immersion in 2.5% glutaraldehyde in phosphate-buffered saline (PBS, pH 7.4) at 4°C, replaced after 2 h by 1% glutaraldehyde/PBS, and left overnight at the same temperature. The segments were washed three times in PBS and trimmed into ~1-mm cubes. They were then embedded in 30% bovine serum albumin (BSA) and gelled by cross-linking with 1% glutaraldehyde by rapid mixing at room temperature (48, 49). The gels were sliced into blocks ~1 × 2 × 2 mm in size, infiltrated gradually with 30% glycerol in PBS at room temperature, and then frozen in Freon 22 cooled by liquid nitrogen. The frozen gels were stored in liquid nitrogen until processing for freeze-fracture cytochemistry.

Colloidal Gold, Lectin–Gold, and Lectin–Protein–Gold Complexes

Two sizes of colloidal gold were prepared. Colloidal gold particles, 20-nm diam, were prepared according to the method of Frens (16). Colloidal gold particles, 10–13-nm diam, were prepared by a modification of Mühlpfordt's method (39): 100 ml of 0.01% chloroauric acid in bi-distilled water was boiled, and 2 ml of 1% (wt/vol) Na_3-citrate was added. 0.45 ml of 1% (wt/vol) tannic acid was added 2 s later and the solution boiled for 5 min. The pH of the colloidal gold was adjusted with 0.02 M K_2CO_3 to pH 7.2 for horseradish peroxidase·colloidal gold (HRP·CG) and concanavalin A· colloidal gold (Con A·CG), and pH 6.3 for Ulex Europaeus I·colloidal gold (UEA I·CG). HRP·CG and UEA I·CG were prepared by the method of Geoghegan and Ackerman (22) and Roth (55), respectively. For preparation of direct Con A·CG, 1.8 mg of Con A in 1 ml of bi-distilled water was added to 60 ml of colloidal gold, pH 7.2 with gentle stirring. The Con A·CG complex was centrifuged at 500 *g* for 20 min at room temperature. The supernatant was recovered and centrifuged at 60,000 *g* for 45 min at 4°C. The final pellet was resuspended in 3 ml of PBS containing 0.02% polyethylene glycol.

Freeze-Fracture Cytochemistry

Freeze-Fracture. Frozen gels were either fractured in liquid nitrogen with a scalpel (for critical-point-drying fracture-label [CPD-FL]) (8, 48, 49) or fractured by successive crushing in a homogenizer with a glass pestle (for thin-section fracture-label) (45–47). In both cases, the fractured gels were thawed in 1% glutaraldehyde in PBS containing 30% glycerol (vol/vol) and then deglycerinated in 1 mM glycylglycine in PBS containing 3% glycerol for 20 min. After washing once in the same glycylglycine solution and then leaving in PBS for 1 h at room temperature, the gels were labeled.

Cytochemistry. For Con A labeling, gels were incubated in a solution of Con A (0.25 mg/ml PBS) for 1 h at room temperature, washed twice in PBS, and labeled with HRP·CG (22) for 2 h at room temperature (Con A-HRP·CG). Controls were preincubated in 0.5 M methyl-α-D-mannopyranoside in PBS for 1 h at room temperature followed by incubation in Con A solution as above but in the presence of the sugar inhibitor, and then labeled with HRP·CG complex. Some gels were also incubated directly either in a solution of HRP·CG or in Con A·CG for 2 h at room temperature. Since horseradish peroxidase is a glycoprotein (57) and many animal tissues are known to contain endogenous lectin-like substances (58), these experiments were done to find out if horseradish peroxidase interferred with the labeling.

For UEA I labeling, gels were directly incubated for 1 h at room temperature in UEA I·CG complex. Controls were preincubated in 0.2 M L-fucose in PBS for 1 h at room temperature followed by incubation in UEA I·CG as described above in the presence of the sugar inhibitor.

Trypsin Treatment. For trypsin treatment, fractured gels were incubated in a solution of trypsin (2 mg/ml PBS) for 45 min at 37°C. After washing twice in PBS, the gels were labeled with UEA I·CG and with Con A followed by incubation in HRP·CG as described.

Critical-Point-Drying and Formation of Platinum–Carbon (Pt/C) Replicas. Labeled gels, after washing three times in PBS, were osmicated in 1% osmium tetroxide in distilled water for 30 min, dehydrated in graded ethanol, and critical-point dried in ethanol/carbon dioxide (45, 48, 49). The gels were then secured with the fractured face up onto a copper specimen carrier coated with a small strip of double-faced adhesive tape and replicated by Pt/C evaporation. After replica formation, tissue debris was digested in sodium hypochlorite and the replicas were washed three times in distilled water and then mounted on Formvar-coated copper grids. Replicas were examined on a Philips 201 electron microscope (Philips Electronic Instruments, Inc., Mahwah, NJ) operated at 60 kV.

Thin-Section. Labeled gels, after washing three times in PBS, were osmicated in 1% aqueous osmium tetroxide, followed by dehydration through a series of graded acetone for Epon embedding. Thin-sections (pale-gold interference color) were prepared and stained with uranyl acetate and lead citrate.

Quantitative Evaluation

To evaluate the density of Con A and UEA I labeling in the euchromatin, heterochromatin, and nucleolus, electron micrographs were taken in the electron microscope at × 10,000 and then magnified 2.8 times on the final prints. Euchromatin, heterochromatin, and nucleolus (if present on the replicas of cross-fractured nuclei) regions were outlined with a marker on the front as well as on the back of the final enlarged prints. Colloidal gold particles over various regions of the nucleus were perforated with a teasing needle. Perforated holes could be easily counted on the back of the photomicrographs. Using an automated image analysis system (Zeiss Videoplan, Carl Zeiss, Inc., Thornwood, NY) the areas of euchromatin, heterochromatin, and nucleolus were determined. The density of Con A and UEA I binding sites (expressed as the number of gold particles per square micrometer ± SEM) over various regions in the nucleus was then determined. Bar histograms were prepared by a Hewlett-Packard microcomputer (Hewlett-Packard Co., Palo Alto, CA).

Results

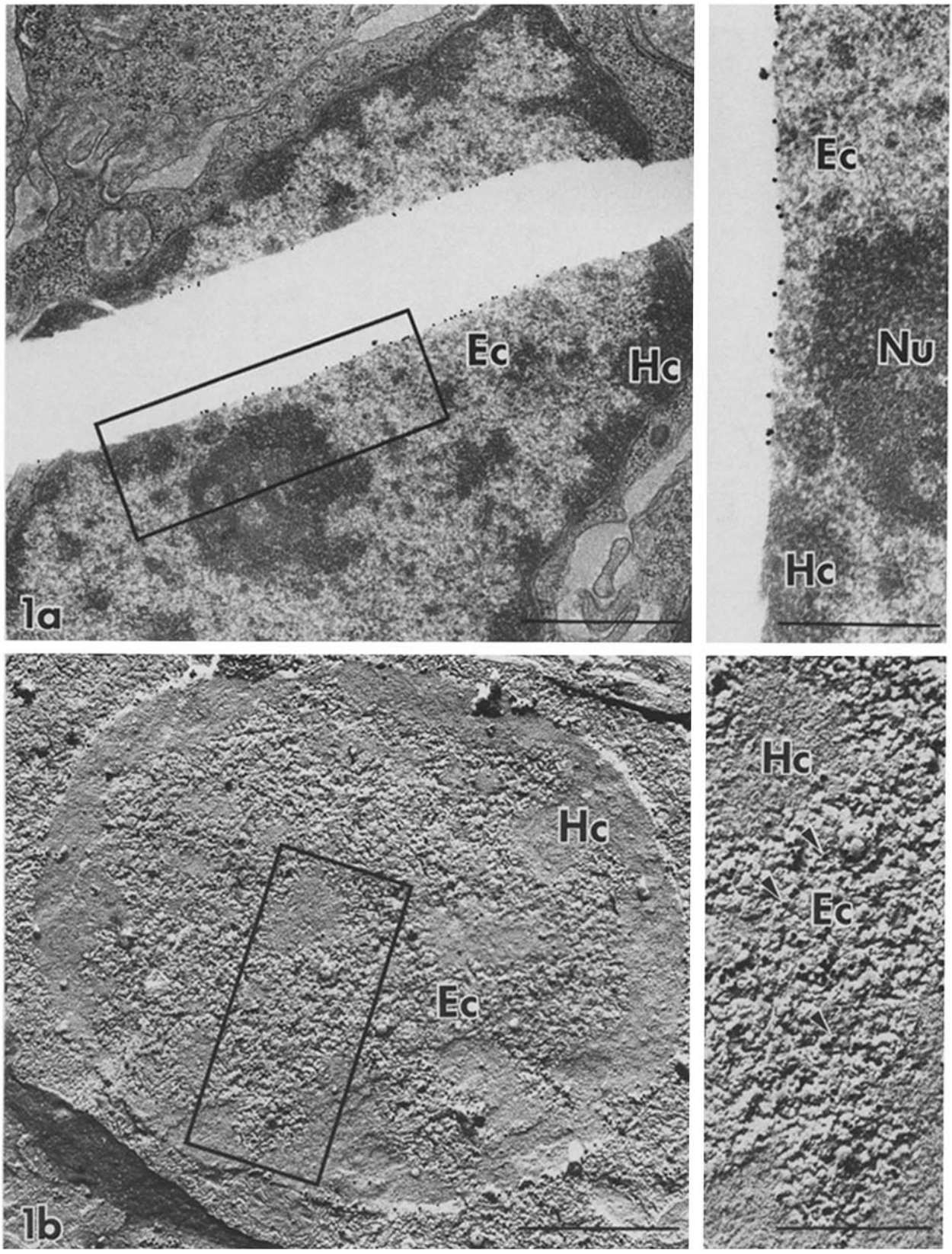
Identification of Euchromatin, Heterochromatin, and Nucleolus in CPD-FL Preparations of Duodenal Columnar and Exocrine Pancreatic Acinar Cells

Three distinct intranuclear regions can be identified in CPD-FL preparations of duodenal columnar and exocrine pancreatic cells: (*a*) euchromatin, which is diffuse and dispersed in thin-sections (Figs. 1*a* and 2*a*), is distinguished by its rough and granular texture (Figs. 1*b* and 2*b*); (*b*) heterochromatin, which has a condensed appearance in thin-sections (Fig. 1*a*), shows a compact and smooth texture in critical-point dried replicas (Fig. 1*b*); and (*c*) nucleolus, which is not observed in every cross-fractured nucleus, also displays a smooth, compact texture similar to that of heterochromatin (Fig. 2*b*).

Freeze-Fracture Cytochemistry

Con A. Thin-sections (Figs. 1*a* and 2*a*) of cross-fractured nuclei of duodenal columnar cells and exocrine pancreatic cells labeled by Con A-HRP·CG or directly by Con A·CG revealed the presence of colloidal gold particles over the cross-fractured surface of nuclei. The label appeared to be almost exclusively confined to cross-fractured euchromatin (Figs. 1*a* and 2*a*). CPD-FL replicas of cross-fractured nuclei showed strong labeling of euchromatin regions and sparse labeling of both heterochromatin and nucleolus (Figs. 1*b* and 2*b*). In CPD-FL preparations, it could be seen that the density of labeling by Con A-HRP·CG (Figs. 1*b* and 2*b*) was always higher than that of labeling directly by Con A·CG (for quantitative data see Figs. 8, *a* and *b*; 9, *a* and *b*).

To determine the specificity of labeling, we incubated samples of fractured duodenal columnar cells in the presence of methyl-α-D-mannopyranoside. Figs. 4 and 5 show typical tracings of the label over regions of euchromatin, heterochromatin, and nucleoli in cross-fractured nuclei of duodenal columnar cells. When tissues were labeled by Con A-HRP·CG (Fig. 4*a*) in presence of the sugar inhibitor (Fig. 4*b*) the labeling intensity of heterochromatin and nucleolus was significantly reduced (94% and 72%, respectively), but that of euchromatin remained higher than expected (43% of the original, see Fig. 8*a* for histogram and data). This suggested to us that the labeling of euchromatin in our study might, in part, be due to direct binding of HRP·CG to components



Figures 1 and 2. Cross-fractured nuclei of duodenal columnar cells (Fig. 1) and exocrine pancreatic cells (Fig. 2) labeled with Con A followed by incubation in HRP·CG complex (Con A-HRP·CG). Colloidal gold particles are seen as black circles to which a white cone of shadow is associated (arrowheads, right). (Figs. 1*a* and 2*a*) Thin-section fracture-label; label is mostly associated with euchromatin (*Ec*; cross-fractured areas inside frame are shown at higher magnification, at right. (Figs. 1*b* and 2*b*) CPD-FL; face view of cross-fractured nucleus shows euchromatin as rough textured areas that contrast with the relatively smooth appearance of heterochromatin (*Hc*) and nucleolus (*Nu*). Most gold particles are associated with euchromatin (high magnification of cross-fractured areas inside frame is shown at right). Bars: *a* and *b*, 1 μm; *insets*, 0.5 μm.

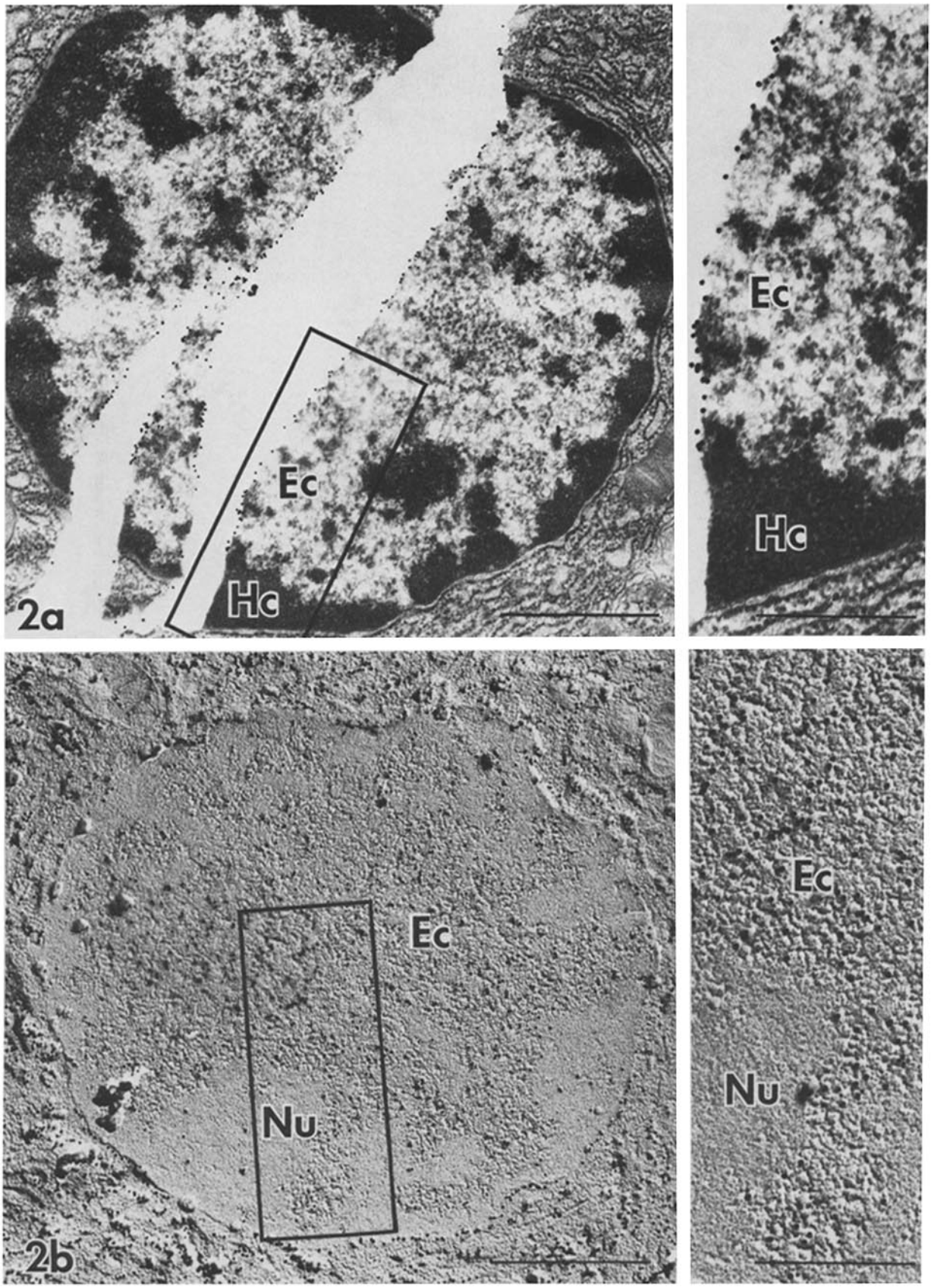


Figure 2.

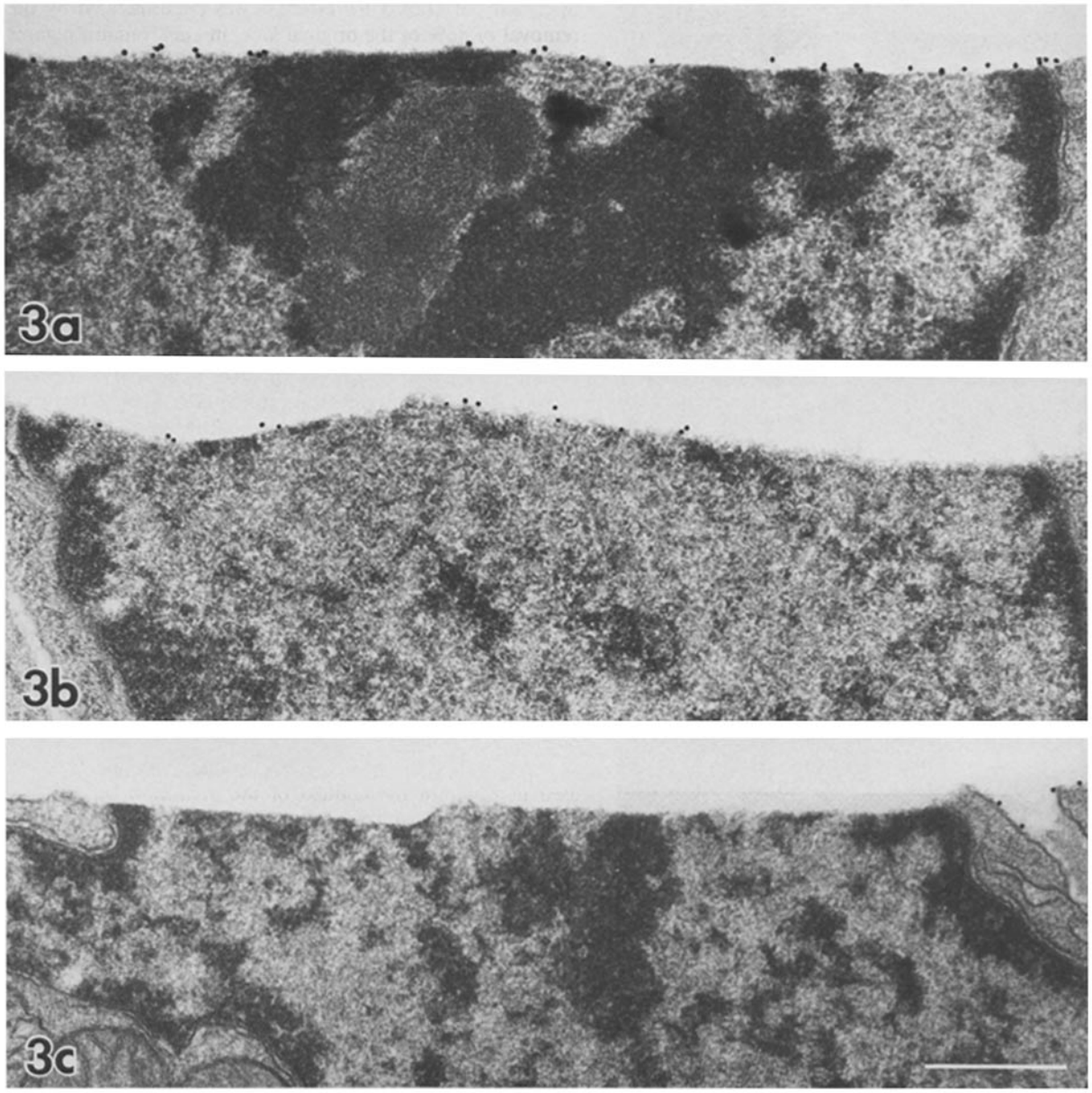


Figure 3. Thin-section fracture-label preparations of duodenal columnar cells labeled directly by UEA I·CG. (Fig. 3*a*) Cross-fractured nucleus showing high density of colloidal gold particles over euchromatin regions (dispersed); heterochromatin regions (condensed) are sparsely labeled. (Fig. 3*b*) Cross-fractured nucleus labeled by UEA I·CG in presence of L-fucose. (Fig. 3*c*) After trypsin treatment, the nucleoplasm is virtually devoid of label. Bar (for *a–c*), 0.5 μm.

within euchromatin regions.[2] When fractured tissues were incubated directly in HRP·CG without prior labeling with Con A, HRP·CG alone significantly labeled regions of the euchromatin (Fig. 4*c*; in the same experiment the labeling intensity of HRP·CG alone in euchromatin was 25 ± 6 gold particles/μm^2 as compared with 58 ± 4 gold particles/μm^2 labeled by Con A-HRP·CG; see Fig. 8*a* for histogram and data). If, however, cross-fractured nuclei were labeled directly by Con A·CG in the presence of methyl-α-D-mannopyranoside (Fig. 5), labeling of both euchromatin and heterochromatin was drastically reduced (78% and 76% reduction in euchromatin and heterochromatin, respectively; see histogram and data in Fig. 8*b*).

To determine the nature of receptors for Con A and for HRP, we labeled trypsinized, fractured tissues with Con A-HRP·CG (Fig. 6). CPD-FL preparations of samples treated with trypsin showed only few gold particles scattered over the nucleoplasm (reduction of 95%, 96%, and 98%, respectively, in euchromatin, heterochromatin, and nucleolus; see Fig. 8*c* for histogram and data).

Similar results were obtained in exocrine pancreatic cells (see Fig. 2 and Fig. 9, *a* and *b* for comparative density of

[2] A previous study showed the binding of Con A·HRP complex to nuclei of adult myocardium and this staining was not prevented by α-methyl-D-mannoside (26). More recently, mannosyl-ferritin markers alone were also found to bind to chromatin in ovarian follicular cell nuclei (56). Since horseradish peroxidase is a glycoprotein that contains many sugar residues including mannose (57), interactions between horseradish peroxidase and certain endogenous lectin-like substances may, therefore, partially account for the Con A·HRP binding (56).

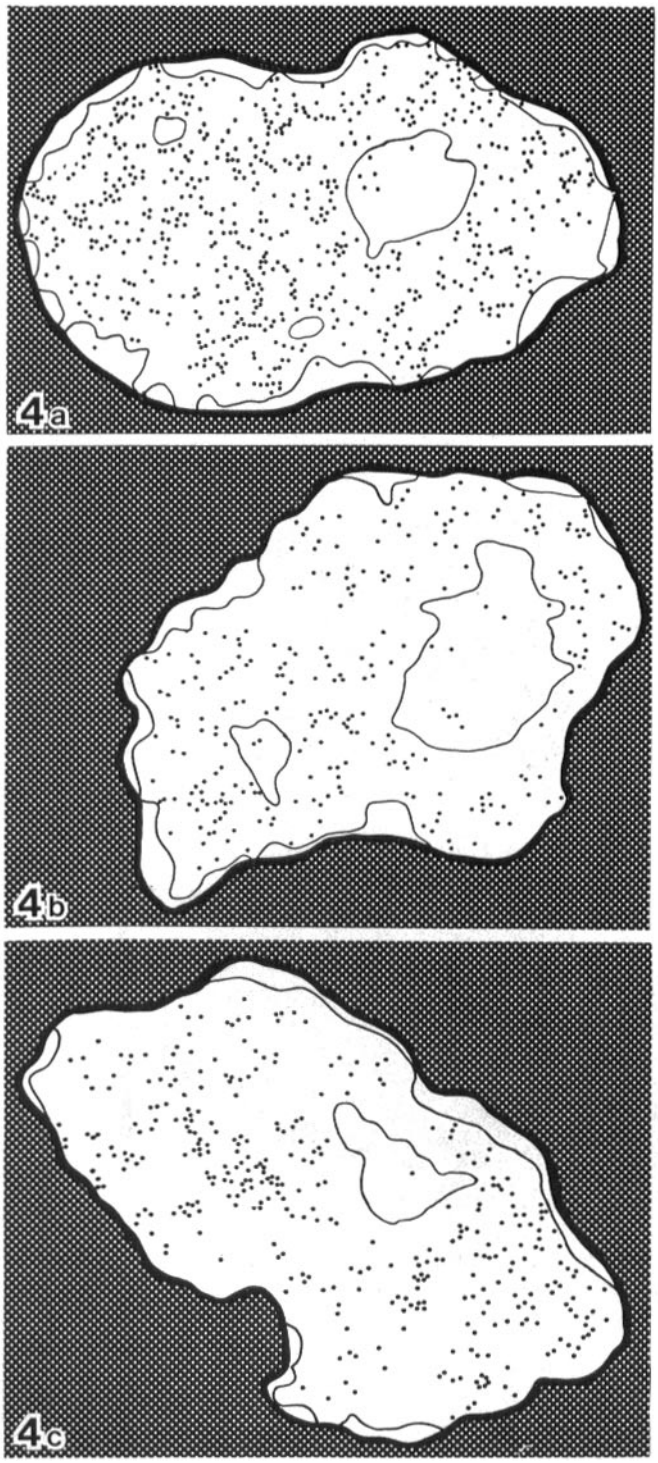


Figure 4. Tracings of cross-fractured nuclei from replicas of duodenal columnar cells after CPD-FL. Labeling by Con A-HRP·CG: (*a*) experimental; (*b*) methyl-α-D-mannopyranoside, control; and (*c*) HRP·CG, control.

labeling by both Con A-HRP·CG and direct Con A·CG conjugates in the presence and absence of methyl-α-D-mannopyranoside).

UEA I. Labeling of cross-fractured nuclei by UEA I·CG showed intense labeling of the euchromatin regions with sparse labeling of heterochromatin and nucleoli in duodenal columnar cells (Figs. 3*a* and 7*a*; see Fig. 10 for histogram). Specificity of UEA I for L-fucose was demonstrated by the removal of 86% of the original label in euchromatin regions and 88% in heterochromatin regions when fractured samples were incubated in UEA I·CG in presence of L-fucose (Figs. 3*b* and 7*b*; see Fig. 10 for histogram). Trypsin digestion of the samples before their incubation in UEA I abolished 99% of the original label in euchromatin, 95% in heterochromatin, and 100% over the nucleolus (Figs. 3*c* and 7*b*; see Fig. 10 for histogram and data).

Discussion

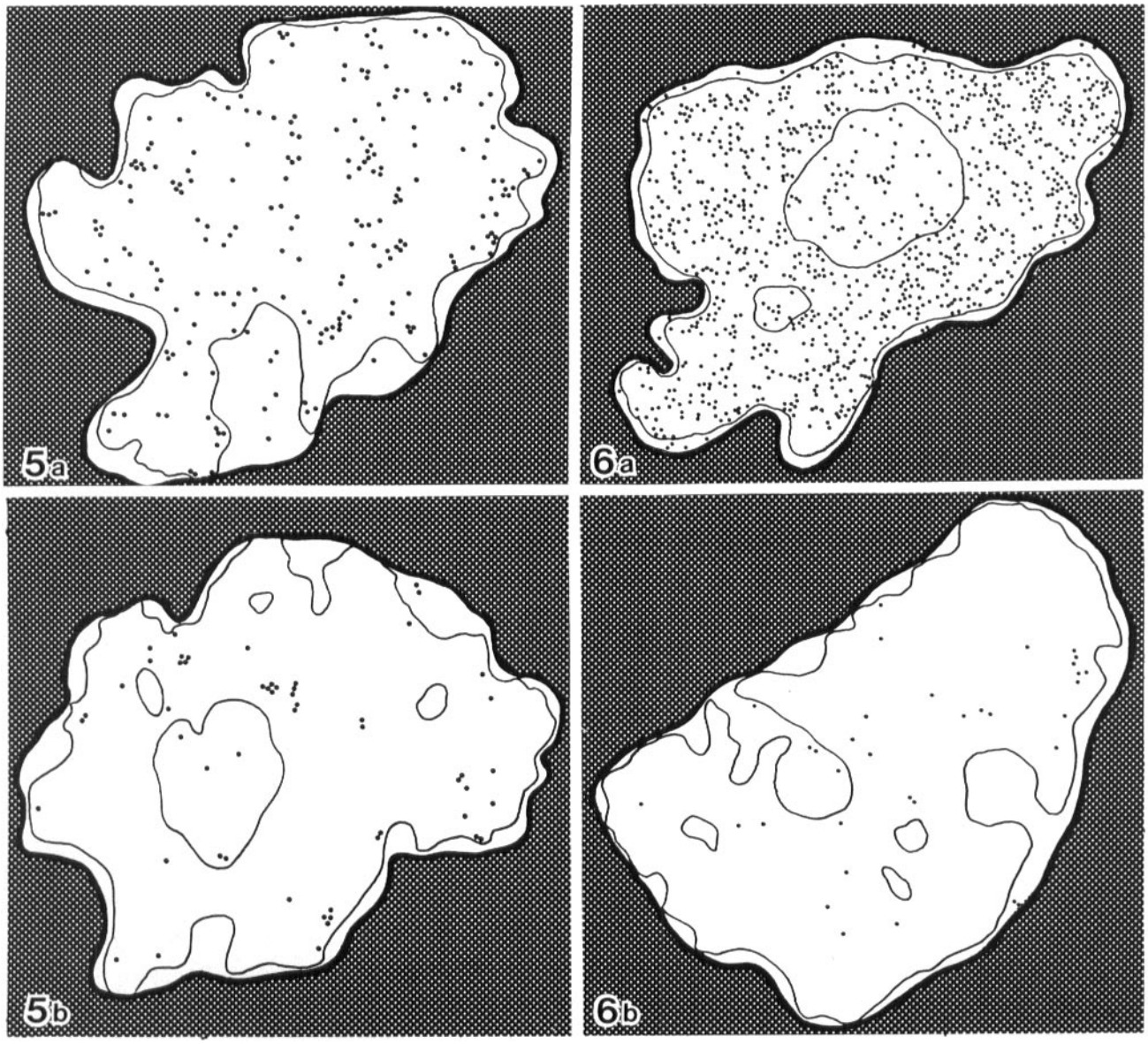
We used fracture-label techniques to localize Con A and UEA I receptors in the nucleoplasm of the interphase nucleus. The receptors are preferentially associated to euchromatin regions. Our results are the first attempt to a quantitative, ultrastructural localization of glycoconjugates within nuclei.

Fracture-Label and Cytochemical Mapping of Nonmembranous Components

The combination of freeze-fracture and cytochemical labeling was first applied to the direct labeling of freeze-fractured plasma and intracellular membranes (1–3, 6, 11, 30, 43, 44, 56, 60, 61). Here, we used thin sections and critical-point-dried replicas of freeze-fractured, cytochemically labeled tissues to provide ultrastructural views of the localization of glycoconjugates within the nucleoplasm of cross-fractured cells.

The terms euchromatin, heterochromatin, and nucleoli are used here within the context of the definition by electron microscopy of the ultrastructure of nuclei in intact cells. In thin-section fracture-label preparations, these three intranuclear regions can be clearly identified. In CPD-FL preparations, euchromatin regions appear less compact (12) and display a coarse texture; the more compact heterochromatin (12) is seen as relatively smooth; the nucleoli are generally distinguished from euchromatin regions by their compact appearance (similar to that of heterochromatin) and from heterochromatin regions by their shape and position (this is not always possible, see below). Overall, the images obtained with Pt/C replicas of these critical-point-dried preparations parallels those of the interphase nucleus observed in thin-sections. Colloidal gold particles bound on the face of fracture are not removed from the replicas by digestion in sodium hypochlorite and are detected as opaque, black spheres with a white cone of shadow (48, 49).

The direct labeling of euchromatin regions by Con A·CG complex could be successfully competed out by methyl-α-D-mannopyranoside. However, methyl-α-D-mannopyranoside was only partially successful when specimens were first treated with Con A and then labeled by HRP·CG. This suggested the existence of two classes of binding sites: one specific for Con A, the other specific for HRP. The presence of HRP receptors was demonstrated by the direct binding of HRP·CG to euchromatin regions. As trypsin treatment of fractured specimens abolished labeling by Con A-HRP·CG or by HRP·CG, we concluded that both receptors are proteins. The receptor sites for UEA I were successfully competed by L-fucose and were susceptible to trypsin digestion. Therefore, UEA I receptor molecules are glycoproteins located within the euchromatin regions of the nucleoplasm.



Figures 5 and 6. Tracings of cross-fractured nuclei from replicas of duodenal columnar cells after CPD-FL. (Fig. 5) Direct labeling by Con A-CG: (*a*) experimental; (*b*) methyl-α-D-mannopyranoside, control. (Fig. 6) Effect of trypsin on labeling by Con A-HRP-CG: (*a*) experimental; (*b*) trypsin pretreatment.

Accessibility of Glycoproteins to Colloidal Gold Markers

It can be argued that euchromatin regions are more heavily labeled because, being less compact, they are more accessible to the lectin. We believe that this is not likely because (*a*) thin-section fracture-label showed that colloidal gold was always on the fracture face of cross-fractured nuclei and never penetrated into the nucleoplasm; (*b*) Pt/C replicas are formed on the fracture faces of fractured, labeled, and critical-point-dried tissues; therefore, any gold particle that might have penetrated into inter-matrix spaces of euchromatin regions cannot be shadow-cast and will be washed off during chemical digestion of the tissue. We concluded therefore that any gold particle seen on Pt/C replicas must represent labeling of glycocomponents at the fracture face of cross-fractured nuclei. On the other hand, although fracture is a random process that in principle will expose representative areas of cross-fractured nucleoplasm, it is possible, but in our opinion, less likely that the physical conformations of glycoprotein in heterochromatin and nucleoli are such that their glycocomponents are not exposed (or exposed less often) by freeze-fracture.

Whereas the nucleolus always appeared as a large, oval, or spherical body that could be easily recognized due to its size and position, there were occasionally nuclei in which the nucleolus was smaller and less conspicuous, and where heterochromatin was prominent. In this case, small patches of heterochromatin may have been included in our quantitation of nucleoli or vice versa.[3] However, this inevitable error does

[3] Not to be confused with the sparse labeling found in peripheral heterochromatin regions are those colloidal gold particles that are localized at the outermost boundary of the nuclei shown on CPD-FL replicas. This labeling (Con A as well as UEA I) is associated with cross-fractured nuclear envelope membranes (46, 48; Kan, F. W. K., and P. Pinto da Silva, manuscript in preparation) which should not be taken as part of the peripheral heterochromatin.

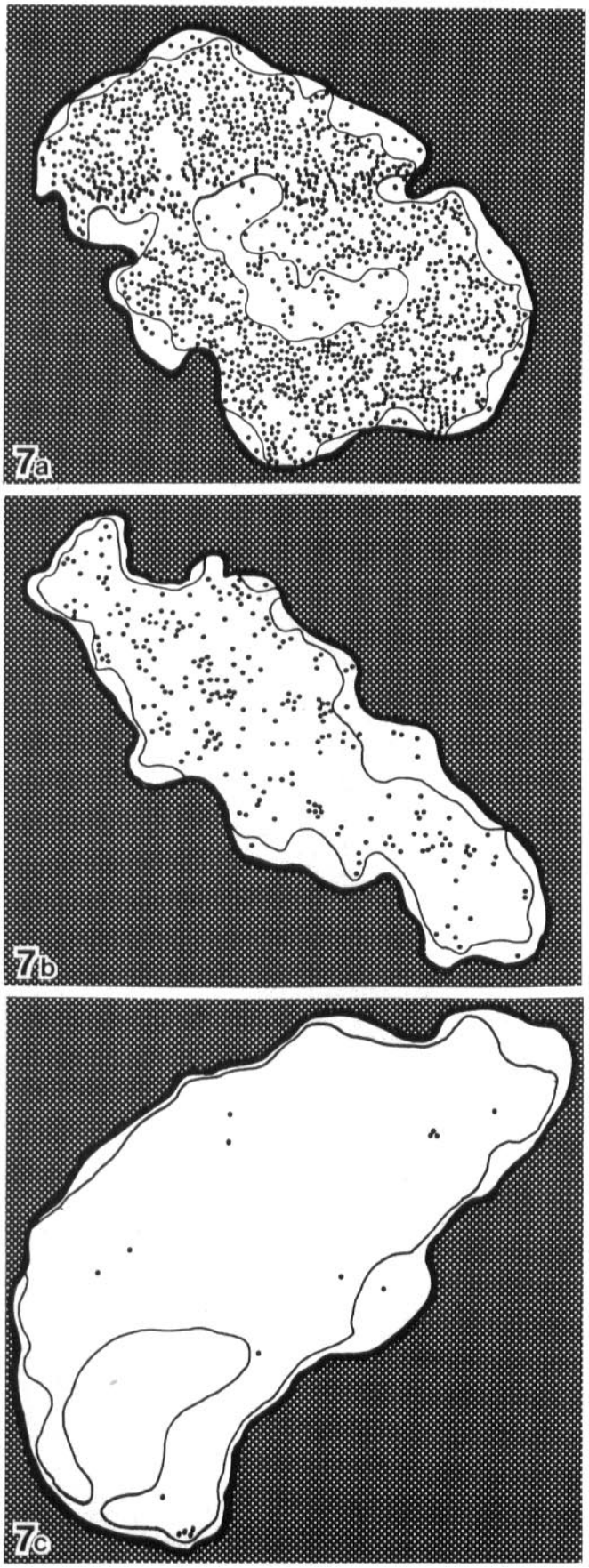


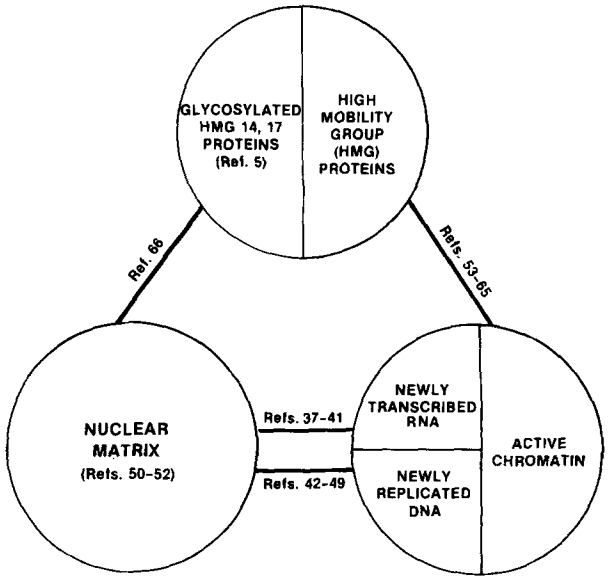
Figure 7. Tracings of cross-fractured nuclei from replicas of duodenal columnar cells after CPD-FL. Labeling by UEA I-CG: (*a*) experimental; (*b*) L-fucose, control; (*c*) trypsin pretreatment.

not affect our conclusions on the preferential association of lectin receptor sites to euchromatin regions. Similarly, due to the intimate intermixing of euchromatin and heterochromatin over many areas of cross-fractured nuclei, we may have included in our quantitation small patches of heterochromatin in euchromatin regions and vice versa. However, these errors could only attenuate and never reinforce our results because: (*a*) small patches of heterochromatin in euchromatin regions would result in lowering the density of labeling for euchromatin; and (*b*) patches of euchromatin in heterochromatin would raise the density of labeling for heterochromatin.

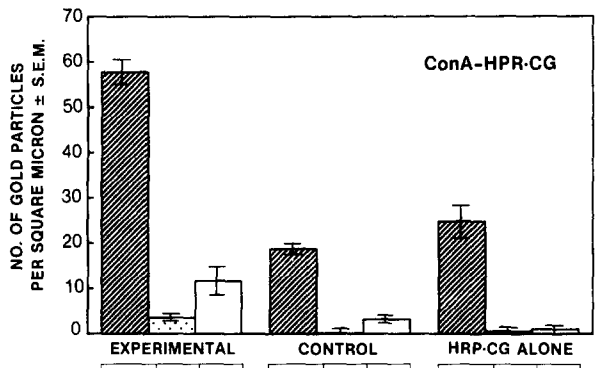
Glycoproteins in the Nucleoplasm

Labeling of euchromatin regions is of particular interest because, in contrast to the inactive heterochromatin, euchromatin has been characterized as transcriptionally active and shown to display DNA template activity (27). Recent work shows that nuclear glycoproteins, nucleic acids, and the proteins of the nuclear matrix do interact: (*a*) newly transcribed RNA (29, 36, 38, 62) and newly replicated DNA (8, 10, 14, 28, 37, 41, 42, 53) are associated with the nuclear matrix (9, 11; for review see reference 7); (*b*) in regenerating liver, newly replicated DNA loops are attached to specific sites of the nuclear matrix (8); (*c*) in calf thymus, the high mobility group (HMG) proteins (which contain sugar residues, including mannose and fucose [51]) are associated with active chromatin (5, 15, 20, 21, 24, 25, 33–35, 63–66); (*d*) HMG 14 and HMG 17 are glycoproteins linked to the nuclear matrix by their glycosylated segments (50). Scheme I illustrates these interactions.

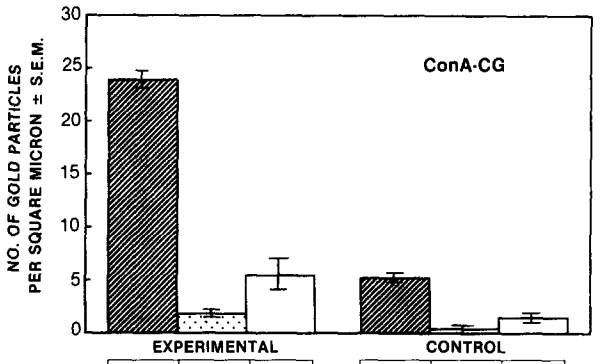
The role of nuclear glycoproteins remains unclear. Glycosylation of protein molecules was seen as a potential source for variability and specificity leading to a functional role in the overall architecture of chromatin (50). Recently, a glycoprotein was found to be associated with the chromatin of Novikoff hepatoma and of Walker 256 carcinosarcoma cells, but it was not detected in the nuclei of normal liver, kidney, and heart tissues (67). The nuclei of regenerating liver cells, which lack this glycoprotein (67), show increased levels of a sulfated glycoprotein (19). Future studies will determine po-



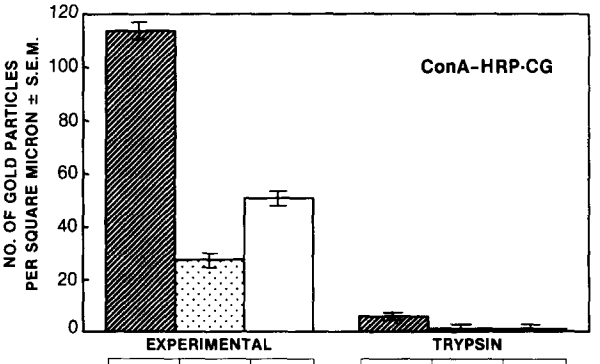
Scheme I. Interactive nature of major components within active chromatin regions.



No. of Nuclei	7	7	5	5	5	5	6	6	4
Total Area (μm^2)	98.0	16.4	5.2	58.2	8.0	6.6	54.2	7.8	3.6
No. of Particles	5,654	63	56	1,064	2	24	1,437	5	4

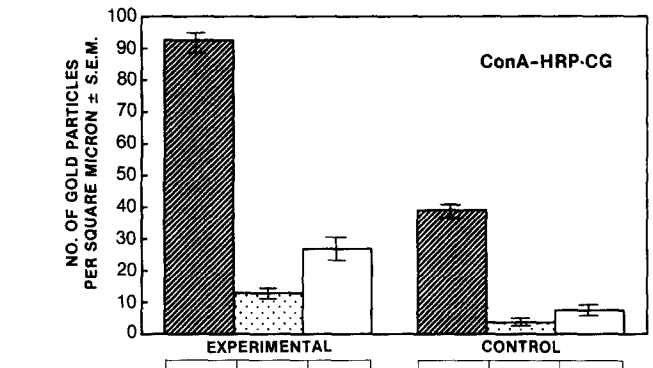


No. of Nuclei	11	11	8	10	10	7
Total Area (μm^2)	104.2	15.6	7.3	73.1	15.1	7.6
No. of Particles	2,483	30	52	390	7	14

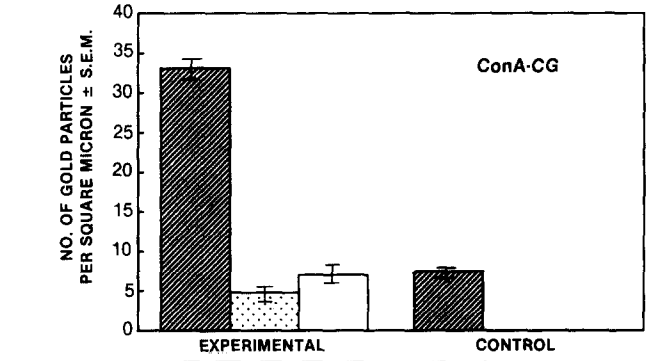


No. of Nuclei	10	10	7	6	6	4
Total Area (μm^2)	74.6	11.7	7.5	63.6	9.7	13.3
No. of Particles	8,312	338	360	356	10	4

Figure 8.

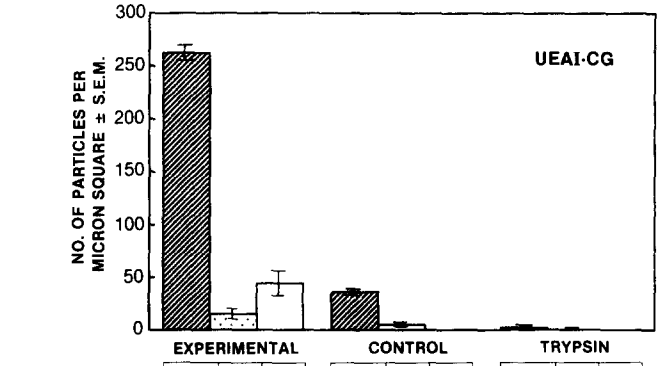


No. of Nuclei	7	7	5	6	6	4
Total Area (μm^2)	63.4	11.3	8.2	66.5	10.9	6.4
No. of Particles	5,742	148	219	2,566	42	53



No. of Nuclei	4	4	4	4	4	4
Total Area (μm^2)	33.9	6.5	9.3	35.9	7.1	—
No. of Particles	1,149	33	66	269	0	—

Figure 9.



No. of Nuclei	12	12	6	8	8	—	5	5	3
Total Area (μm^2)	70.2	14.3	4.2	53.6	11.8	—	44.2	7.5	3.5
No. of Particles	18,407	199	180	1,936	59	—	112	5	0

Figure 10.

Figures 8–10. Bar histograms and data retrieved from examination of cross-fractured nuclei labeled by Con A and UEA I. Areas of euchromatin (▨), heterochromatin (▩), and nucleolus (□) in cross-fractured nuclei were traced and their surface areas measured. Colloidal gold particles were counted over each intranuclear compartment. Labeling intensities (expressed as number of gold particles per square micrometer ± SEM) are depicted in bar histograms. Data relative to each bar is given in the tables. (Fig. 8) Con A labeling of cross-fractured nuclei of duodenal columnar cells. (Fig. 9) Con A labeling of cross-fractured nuclei of exocrine pancreatic acinar cells. (Fig. 10) UEA I labeling of cross-fractured nuclei of duodenal columnar cells.

tential roles for nuclear glycoproteins along two main lines: (*a*) modulation of the physico-chemical environment of the nucleoplasm; and/or (*b*) direct participation in localized molecular interactions at specific sites of the genome.

In summary, our results demonstrate that, within the nucleoplasm, mannose and fucose residues are mainly associated with glycoproteins with euchromatin regions, i.e., the nucleoplasm domains where transcription and replication take place. Fracture-label appears to be a cytochemical approach that, combined with biochemical characterization, can be used to investigate the possible role of these nuclear glycoproteins.

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Note Added in Proof: The presence of glycoproteins in the nucleoplasm poses questions relative to their mode of synthesis and/or intracellular transport. Secretory and membrane glycoproteins are synthesized and glycosylated in the membranes of the endoplasmic reticulum and the Golgi apparatus. Secretory glycoproteins are stored within exoplasmic (i.e., vacuolar) spaces of the cell, and the glycosylated segments of membrane proteins are always exposed to exoplasmic spaces. Therefore, the presence of glycoproteins in the nucleoplasm (a cytoplasmic space) suggests the existence of alternative mechanisms biochemically and topologically distinct for synthesis and/or intracellular transport.

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