GLYCOCONJUGATES IN THE RECTAL GLAND OF SPINY DOGFISH, SQUALUS ACANTHIAS; AS REVEALED BY LECTIN BINDING TO CRYOSTAT SECTIONS

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Plant lectins are useful tools in the study of cells and tissues by their ability to specifically bind to sugar moieties of glycoproteins and glycolipids. The epithelial cells of the rectal gland, like other transporting epithelial cells, display two portions of the cell membrane (plasmalemma), which differ largely by their organization and function, namely the apical cell membrane and the elaborately folded baso-lateral cell membrane. For the characterization of these cell membrane domaines in biochemical and cell biological experiments with rectal glands of Squalus, we searched for potential markers by the application of lectin-fluorochromes to unfixed tissue. Also, information on the chemical nature of glycoconjugates was gathered.

Male dogfish were pithed, the rectal gland was excised and small samples, approximately 0.5 cm^3, of the distal end were mounted on pieces of cardboard and rapidly immersed into thawing isopentane (-80°C). Serial sections (6 to 7 µm) were cut with a cryostat (Frigomat, Leica) and mounted on glass slides. The slides were briefly rinsed in aceton (-25°C, 5 sec.) and stored at -20°C for further study. Before incubating the sections with lectins, the slides were prerinsed with PBS. Various lectins coupled to fluorochromes, such as fluorescein isothiocyanate FITC, and tetramethyl-rhodamin isothiocyanate TRITC, (Vector Laboratories, Burlingame) were applied in varying dilutions, from 1:40 to 1:120 corresponding to concentrations of 1 to 0.3 mg/ml. After three rinses with PBS, the sections were soaked with a small amount of antifading (2.5% diazobicyclooctane DABCO in 40% glycerol in distilled water) and covered with glass slips. The preparations were viewed with an epifluorescence microscope (Olympus BHT) with 25x and 40x Plan-Neofluar lenses (Zeiss).

The lectins used and the localization of lectin binding sites are summarized in the table (see also for abbreviations). ConA, LCA and ECL marked the region of the thin endothelial cells between the tubules (Figure 1). The entire plasmalemma seemed to be distinctly labelled by WGA, s-WGA and DSL (Figure 2). GSL II, in addition to cytoplasmic binding, marked striated structures, presumably the folds of the baso-lateral cell membrane, as was also observed in the case of STC (Figure 3). A strong binding to the apical region of the cell was present after the application of PHA-E (Figure 4), and, to a minor degree with SJA. Binding of lectin-fluorochromes was completely absent in the case of DBA, RCA-1, SBA and UEA-I.

In conclusion, the study of cryostat sections of fresh, unfixed tissue from rectal glands with lectin-histochemistry revealed a fair number of plant lectins as possible candidates for binding to living cells or components derived from cells, such as membrane vesicles. Interestingly, most lectins that showed affinity to the parenchyma cells belonged to the N-acetyl glucosamine group. Lectins of the glucose/mannose group in contrast were detected in the surrounding connective tissue capsule. By the pronounced binding to the apical cell region with PHA-E or the baso-lateral cell region with STL and GSL II, these few lectins may provide markers for the

membrane domains. While the binding to the cell membranes was strongly suggested by these light microscopic observations, the exact localization of the glycoconjugates will be available with information from binding studies at the EM-level, such as the localization of lectin-gold conjugates. The detection of binding sites for lectins with a variety of different nominal sugar specifities augments the biochemical knowledge of the glycoconjugates which are present in the rectal gland of Squalus.

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Table. Binding of lectins on cryosections of the rectal gland of Squalus acanthias, as revealed by lectin fluorochrome conjugates.

Lectin (source), Abbreviation	Nominal specificity*	Labelled structure
I. Glucose/mannose group		
Canavalia ensiformis, ConA	αMan>αGlc>GlcNAc	Connective tissue, endothelial cells
Lens culinaris, LCA	αMan>αGlc=GlcNAc	Connective tissue, endothelial cells
Pisum sativum, PSA	αMan>αGlc>GlcNAc	Connective tissue
II. N-acetyl glucosamine group		
Triticum vulgare (Wheat germ), WGA, s-WGA	GlcNAc>βGlcNAc>NeurAc	Plasmalemma
Datura stramonium, DSL	$GlcNAc(\beta 1,4GlcNAc)_{1-3}$	Plasmalemma
Griffonia simplicifolia, GSL-II	α and β GlcNAc	Baso-lateral cell membrane
Lycopersicon esculentum, LEL	$GlcNAc(\beta 1,4GlcNAc)_{1-3}$	Cytoplasm
Phaseolus vulgaris, PHA-E	GlcNAc-β(1,2) Manα(1,3Man)	Luminal (apical) cell membrane, endothelial cells
Solanum tuberosum, STL	$GlcNAc(\beta 1,4GlcNAc_{\backslash 1-4}$	Baso-lateral cell membrane
III. N-acetyl galactosamine/galactose group		
Dolichos biflorus, DBA	GalNAcα1,3GalNAc	---
Vicia villosa, VVA	βGalNAc	Connective tissue
Griffonia simplicifolia, GSL-I	GalNAc	Connective tissue
Phaseolus vulgaris, PHA-L	Galβ(1,4)GlcNAcβ(1,2)Manα(1,6)	Cytoplasm
Glyine maximus (Soy bean), SBA	α and β GalNAc	---
Sophora japonica, SJA	α and β GalNAc	Cytoplasm
Arachis hypogaia (Peanut), PNA	Galβ(1,4)GalNAc	Cytoplasm
Ricinus communis, RCA-I	βGal>αGal>GalNAc	---
Erythrina cristagalli, ECL	Galβ(1,4)GalNAc	Connective tissue, endothelial cells
Artocarpus integrifolia, Jacalin	Galβ(1,3)GalNAc	Connective tissue
IV. L-fucose group		
Ulex europaeus, UEA-I	α-L-Fuc	---

* After Damjanov, Lab. Invest. 57:5-20 (1987), modified

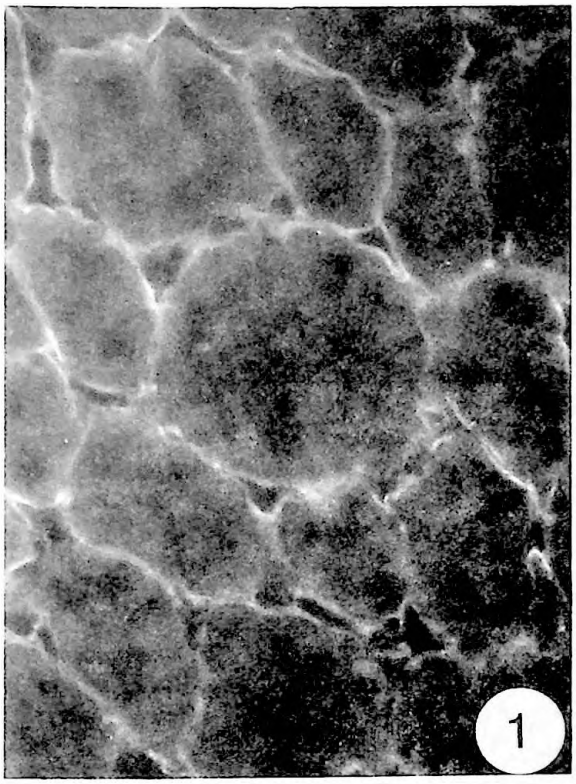
Figures 1-4. Fluorescence micrographs of cryosections of fresh, unfixed rectal glands, after labelling with lectin coupled to fluorochromes. The tubules in the parenchyma are cross sectioned.
x 350

Figure 1. Lens culinaris agglutinin-FITC. Weak to moderate binding is observed only at the endothelium, which encompasses the peritubular capillaries.

Figure 2. Succinylated wheatgerm agglutinin-FITC. Strong labelling is present at the luminal side of the tubules and at the baso-lateral side. As seen in electron micrographs, the baso-lateral cell membrane exhibits extensive folding throughout the cell bodies, resulting in a striated appearance of the cells in the light microscope.

Figure 3. Griffonia simplicifolia lectin II-FITC. Moderate binding to striated structures in the cells, which may represent , like in figure 2, the folded baso-lateral cell membranes. The lectin apparently does not bind to the luminal (apical) cell membrane.

Figure 4. Phaseolus vulgaris agglutinin-E-FITC. The lectin strongly marks the luminal side of the epithelial cells, presumably at the apical cell membrane. In addition, the endothelial cell processes display moderate labelling.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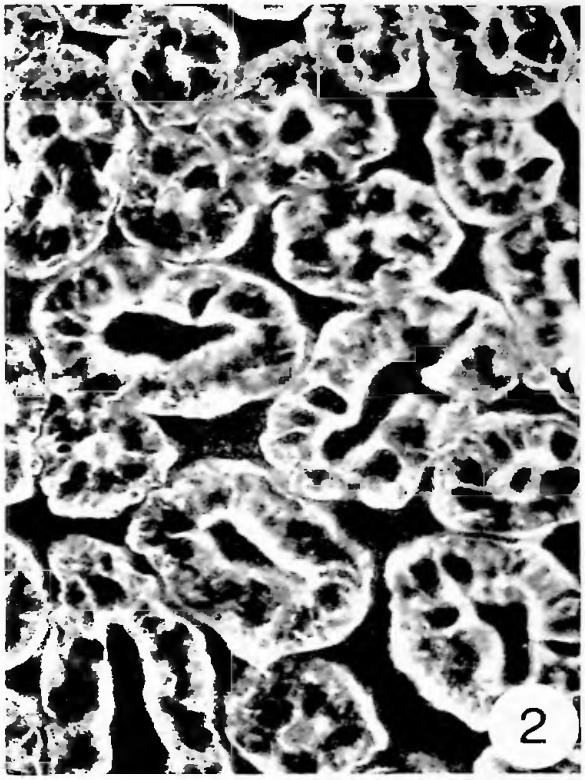
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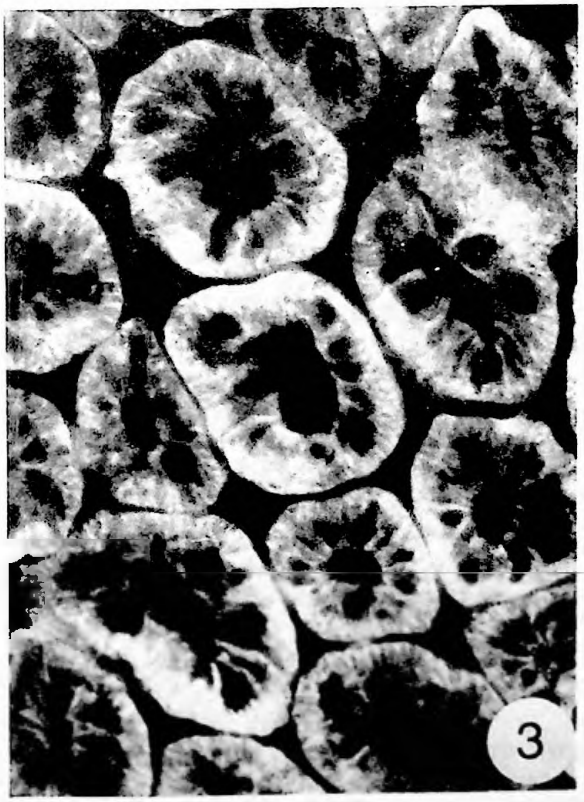
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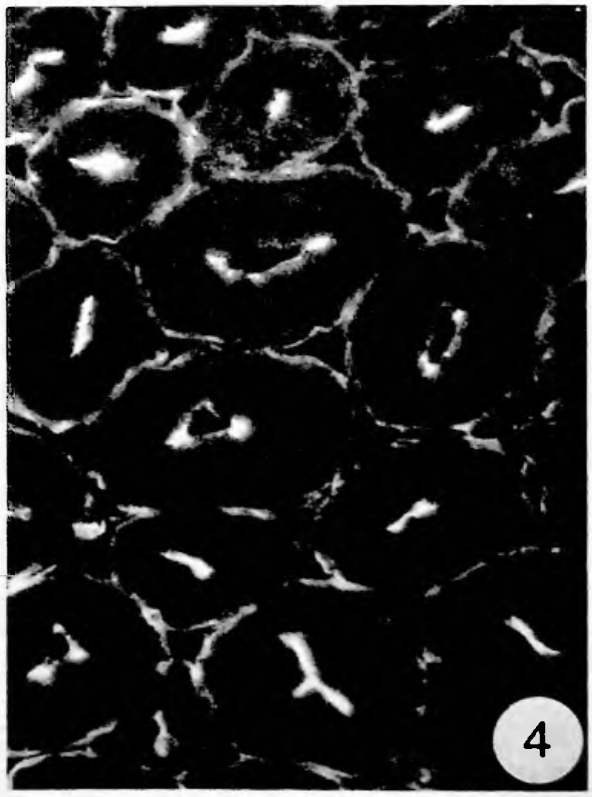
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