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P. Versura
University of Bologna

M. C. Maltarello
University of Bologna

F. Bonvicini
University of Bologna

R. Laschi
University of Bologna

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THE LECTIN-GOLD TECHNIQUE: AN OVERVIEW OF APPLICATIONS TO PATHOLOGICAL PROBLEMS

P. Versura*, M.C. Maltarello, F. Bonvicini, R. Laschi

Institute of Clinical Electron Microscopy, University of Bologna, Italy

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Abstract

Lectins are proteins, mainly of vegetal origin, which recognize glycosidic residues with high specificity; for this property they have been used for many studies of molecular biology. The colloidal gold represents at present the most popular electron dense marker employed in immunocytochemistry, since it offers intrinsic and unique characteristics which are superior to those displayed by the other markers.

The cytochemical method which utilizes the gold-labelled lectins takes advantages from both the two systems, in order to optimize the localization of the glycoconjugates.

The present paper reviews both the technical aspects of the preparation of the lectin-gold complex and its application to some selected pathological problems. In particular, the papers concerning the eye and ear tissues, the urinary, reproductive, nervous and digestive systems and the blood cells are quoted.

Key words : lectin, gold, transmission electron microscopy, scanning electron microscopy, blood cells, cartilage, digestive system, ear, eye apparatus, nervous system, reproductive system, salivary glands, urinary system.

*Address for correspondence:
Piera Versura,
Institute of Clinical Electron Microscopy,
University of Bologna,
Via Massarenti, 9,
I-40138 Bologna, Italy
Phone No.: 051-302874

Introduction

The lectins, particularly those isolated from plants, have been demonstrated to be extremely useful in the study of the social behaviour of the cell, which is mediated through its surface (Kornfeld and Kornfeld, 1978; Lotan, 1979). In fact some complex activities of the cell, like growth regulation, differentiation and maybe also malignant transformation appear to be related to the cell surface and, more in particular, to the carbohydratic chains protruding from it (Spicer et al., 1981). Since the lectins recognize precisely these glycosidic residues as their natural substrate (Debray et al., 1981; Goldstein and Hayes, 1978), they have been successfully applied to investigate quite a large number of biological problems either at the light or at the electron microscope level.

To reference or comment on all the studies relating to the use of lectins as probes is beyond the purpose of this paper. Here, we will give an overview on the applications of the complex named lectins-colloidal gold in which the gold particles have been employed as ultrastructural markers. In particular, the lectin-gold complex preparation, the problems related to specimen processing and the procedures to carry out the cytochemical reactions will be discussed. The application of this technique to pathological problems are manifold and we will focus our attention mainly on tissues and organs of our specific interest.

The Lectins

The discovery of the existence of lectins in cells, especially those in the plant seeds, can be dated around the beginning of this century (Sharon and Lis, 1987), but it is only in the last two decades that these biological molecules reached an increasingly wide popularity (Roth, 1978). The total number of specific publications has increased at least twenty-fold since 1960.

The names used to designate the lectins have changed quite a lot during these 90 years, depending upon the development of knowledge in this field. Historically the oldest names, some of them still in use, were derived from botanical names of the plant source. Later on, the terms phytoagglutinin or, more often, phytohemoagglutinin were introduced, after their erythroagglutinin activity had been discovered. Landsteiner (1936) proposed the term "Normalantikorper" (normal antibodies), although this name was not

generally accepted. In fact remarkable differences exist between the two classes of proteins. Firstly, the lectins are constitutive proteins in the organism and they are not produced as the consequence of an injury; in addition, the range of specificity of the lectins appears to be less broad than that of the antibodies; finally, while the antibodies share a similar structure, the lectins vary a lot in terms of amino acidic composition. Boyd (1954) suggested that: "It would appear a matter of semantics as to whether a substance not produced in response to an antigen should be called an antibody even though it is a protein and combines specifically with certain antigen only. It might be better to have a different word for these substances and the present writer would like to propose the word lectin, from the Latin lectus, the past participle of legere meaning to pick, choose or select". In 1981, the Nomenclature Committee of the International Union of Biochemistry (Dixon, 1981) recommended, with minor modification, the definition given by Goldstein et al. (1980) :"a lectin is a carbohydrate-binding protein (or glycoprotein) of non-immune origin which agglutinates cells and/or precipitates glycoconjugates".

The first classification of the lectins was based on the four configurations at C-3 and C-4 of the pyranose ring (Makela, 1957). At present, the generally accepted classification organizes the lectins in the groups listed in Table 1. Most of the lectins, with few exceptions, interact with the non-reducing, terminal glycosyl groups of polysaccharide and glycoprotein chain-ends. After some controversy regarding the nature of the forces involved in the carbohydrate-lectin interaction (Monsigny et al., 1980), it has been recently proposed (Kronis and Carver, 1985) that both intermolecular hydrogen bonds and Van der Waals forces stabilize the complex. Some lectins appear to be complementary to a single glycosidic unit, but there is evidence that some other lectins can accommodate two to six residues at the same time. As an example, Concanavalin A's (Con A) tetrameric structure possesses four carbohydrate-specific binding sites, while Wheat Germ Agglutinin (WGA) displays two independent sites for N-acetylglucosamine and N-acetylneuraminic acid.

The ability to agglutinate cells, by forming multiple cross-bridges between them, is the most remarkable phenomenon of the biological activity of the lectins. Experiments on agglutination, controlled by blocking with the appropriate inhibitory sugar, provide information on the nature of the receptors at the surface of the cells. This appeared of particular interest in the study of the malignant transformation of the cell, as marked differences in agglutinability between normal and tumor cells were discovered, although the exact mechanism has not yet been clarified. It has been recently proposed for Con A, by means of an elegant technique, that the receptors in the plasma membrane are associated with underlying actin filaments (Katsumoto and Kurimura, 1988).

Many lectins exhibit a mitogenic activity, in particular they are able to stimulate quiescent lymphocytes to a state of growth and proliferation. Moreover, treatment of both human and murine lymphocytes with various lectins, especially Concanavalin A, stimulates the production of suppressor cells, which are able to inhibit T and B cells in vitro. The mediation of killing of target cells by lymphocytes and macrophages, an insulin-like effect on fat cells and the promotion of cell adhesion and spreading are other remarkable biological effects of these substances. Several lectins are toxic to mammalian cells both in vitro and in vivo, through a mechanism still incompletely understood. For a more detailed description of the effects of lectins on the living cells, the reader is referred to Lis and Sharon (1986).

The Markers

In immunocytochemistry, a common problem is the visualization of the sites in which a specific interaction occurs between the ligand and its target. Therefore, the lectins are conjugated with suitable macromolecular complexes, the so-called "markers", displaying intrinsic characteristics which render them visible at the light microscopy (LM) or at the transmission (TEM) and scanning (SEM) electron microscopy level.

Lectins conjugated with fluorescent molecules (fluorescein, FITC; rhodamine, TRITC), visible with LM equipped with an epi-fluorescent condenser, are commercially available. The method of conjugation is quite simple and standardized (Roth et al., 1978). The use of horseradish peroxidase as a marker for detection of lectin-binding sites at the LM level has been widespread until recently. Peroxidase acting as an electron donor is capable of transforming some substrates into insoluble colored products, through the reduction of H_2O_2. The product of the reaction is also visible at the ultrastructural level, since chromogenic molecules, like the 3-3'-diaminobenzidine (DAB), after oxidation, can be osmicated into polymers which are strongly electron-dense. The peroxidase-conjugated lectins (Avrameas, 1979; Geoghegan and Ackerman, 1977), present some disadvantages, related to the marker itself. In fact, it is well known that the product of reaction diffuses from the specific site and, as a consequence, there can be masking of the underlying subcellular structures making it difficult to exactly locate the specific structure of interest. The same consideration is valid for the use of the Avidin-Biotin system in the lectin cytochemistry, which is discussed in more detail in Skutelsky and Bayer (1979), in Hsu and Raina (1982) and in Skutelsky et al., (1987). On the contrary, this problem would not be present in a Streptavidin-gold to Biotin reaction (in which the antibody or the lectin is biotinilated). Ferritin-conjugated lectins provide a better localization of the binding sites (Roth and Binder, 1978). In addition, since the ferritin is a particulate marker, the reaction can be quantified (Nicolson and Singer, 1971; Nicolson, 1978; Hixson et al., 1979). Lectin glycosidic receptors revealed through the use of hemocyanin, iron-dextran and mannan-iron as markers have been demonstrated by Martin and Spicer (1974), Roth and Franz (1975), Brown and Revel (1976), Baccetti and Burrini (1977), and Nemanic (1979).

Colloidal Gold

Among all the particulate markers, colloidal gold, due to its peculiar physico-chemical characteristics, certainly is one which has been used with success in the last several years. The colloidal gold system is in fact reproducible and colloidal gold particles can be quite easily prepared in a wide range of sizes. This makes the system extremely flexible

Table 1: Classification of lectins in groups

LECTIN			
Taxonomic name	Common name	Mol.Wt.	Nominal specificity
	Glucose/Mannose Group		
Canavalia ensiformis	Concanavalin A	102	α-D-man,α-D-glc
Lens culinaris	Lentil	49	α-D-man
Pisum sativum	Pea	49	α-D-man
Vicia faba	Broad bean	50	D-man,D-glc
	N-Acetylglucosamine Group		
Bandeiraea simplicifolia Griffonia			
BS-I, BSI-AB3, BSI-A2B2, BSI-A3B1 ^		114	α-D-gal;α-D-galNAc
BSI-B4 ^^		114	α-D-gal
BSI-A4 *		114	α-D-galNAc
BS-II ^^		113	D-glcNAc
Lycopersicon esculentum	Tomato	71	(D-glcNAc)3
Phytolacca americana	Pokeweed	32	(D-glcNAc)3
Solanum tuberosum	Potato	100	(D-glcNAc)3
Triticum vulgare	Wheat Germ	36	(D-glcNAc)2,NeuNAc
Ulex europeus II	Gorse	170	(D-glcNAc)2
	N-Acetylgalactosamine/galactose group		
Abrus precatorius	Abrin	134	D-gal
Arachis hypogea	Peanut	120	β-D-gal(1-3)-D-galNAC
Dolichos biflorus	Horse gram	140	α-D-galNAc *
Glycine max	Soybean	110	D-galNAc
Helix pomatia	Roman snail	79	D-galNAc *
Phaseolus vulgaris	Red kidney bean	128	Oligosaccharide
Ricinus communis	Castor bean	120	β-D-gal
Vicia villosa	Hairy vetch	139	D-galNAc *
	L-Fucose Group		
Tetragonolobus purpureas	Lotus	120	α-L-fuc
Ulex europeus I	Furze	68	α-L-fuc
	Sialic acid Group		
Limulus polyphemus	Horseshoe crab	400	NeuNAc

Specificity blood group : * A; ^ A,B; ^^ B
Molecular Weight expressed X 10^{-3}

for the performance of double and triple labelling (Horisberger and Vonlanthen, 1979; Goodman et al., 1980; Horisberger, 1981). This marker is largely employed for the observation by TEM and SEM (Horisberger et al., 1975; 1977; Hodges et al., 1987; Eskelinen and Peura, 1988), but also at LM, either directly or through a silver enhancement which will be discussed later.

Gold colloids have been prepared from tetrachloroauric acid ($HAuCl_4.3\ H_2O$) by a number of reducing agents including chemical (like phosphorus, formaldehyde, ethanol, tannic acid, ascorbic acid, sodium citrate) and physical (ultrasonics, UV) processes (Fig. 1). Depending on the agent used and the amount of it, the colloid varies in size (Frens, 1973). The gold particles, when in aqueous solution, exhibit a negatively charged surface. As a consequence, the stability of the colloid is guaranteed by the mutually repulsive forces among the particles in non-ionic or very low ionic conditions. Several factors can affect the stability of the colloid (Horisberger, 1981), leading to its aggregation, which is visually detected by a turning of the color from red to violet and blue. The aggregation can be prevented by the addition of positively charged molecules (as the proteins are) which adsorb on the gold particles surface and render the colloid stable. Virtually any protein can be adsorbed onto the surface of the colloidal gold beads. The mechanism by which the protein adsorption occurs is complex and still not completely understood. Generally adsorption is done at or slightly above the isoelectric point of the protein so that it exhibits no net charge, is least H_2O soluble and will bind by hydrophobic means to the gold. Conversely, charge-

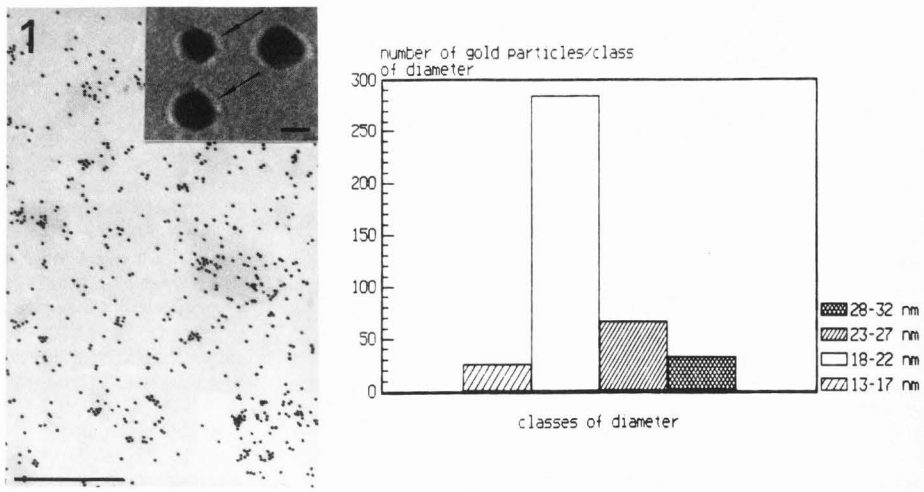


Fig. 1. A routine preparation of colloidal gold particles medium diameter 18-21 nm is shown on the left side. The distribution of the gold beads into a histogram according to different classes of diameter is represented on the right side. In the inset, a negative stained Con A-gold preparation is shown; the arrows indicate the pale halo surrounding the gold particles, which represents the protein coating. Left side: TEM, bar = 500 nm; Inset: TEM, bar = 10 nm

charge (+)protein (-)gold interactions can actually lead to flocculation.

Preparation of the Lectin-Gold Complex

It has been demonstrated that the adsorption of the protein to the gold is a complex phenomenon related to the size of the particles (Horisberger, 1978a), to the presence of salts in the solution and to the isoelectric point (pI) of the protein (Geoghegan and Ackerman, 1977). In particular, it appears that the maximal binding of protein starts at or just on the basic side of this pI value. For the lectins, a list of the pH values is given in Table 2. To exactly assess the working pH value of conjugation, some preliminary phases are needed, which have been introduced and described in Hodges et al., 1984.

The determination of the optimal lectin concentration needed to stabilize the colloidal gold is usually carried out following the "serial dilution method" proposed by Horisberger (1981). Briefly, the method consists of a titration performed by adding aliquots of serially diluted protein to a constant volume of gold sol at a given (previously calculated) pH. An electrolyte (sodium chloride) is then added after one minute. If there is insufficient protein to stabilize the sol, the gold solution aggregates and its color turns from red to blue. If, on the contrary, the sol is stabilized against the aggregating effect of the electrolyte, no color change occurs. Generally, in the preparation of the complex, the lectin is added in about 10% excess of the properly calculated amount. The purification of the complex is carried out by one or more steps of centrifugation, based on the gold size. The pellet is resuspended in a low ionic strength buffer prepared at a suitable pH. The gold probe thus obtained is made more stable by the addition of a stabilizer such as Carbowax 20-M under sterile conditions (Horisberger and Vonlanthen, 1983). Some lectins, like the Wheat Germ Agglutinin (WGA), may have to be pre-conjugated with another molecule such as Bovine serum albumin (BSA), in order to increase its size, which, due to its low molecular weight, is unsuitable for a direct conjugation with the gold. The method is described in Horisberger (1978b) and Roth and Binder (1978).

Visual control of the lectin-labelled gold particles is usually carried out in TEM using a negative staining, in which case a protein monolayer can be observed surrounding the particles (Fig. 1, inset). Biological control is possible with a gold probe bioactivity assay using previously sensitized indicator cells. The presence of the ligands on gold probes is demonstrated by the addition of the appropriate indicator cells followed by checking for agglutination. The technique is fully explained in Hodges et al. (1984).

The storage of the lectin-gold complexes does not present particular problems; provided that they have been stored at 4°C they can be used for 1-2 months without any apparent loss of activity. In this regard, it has been reported that Au50 particles labelled with IgG lost only 40% of activity after 5 years at 4°C (Horisberger, unpublished observation).

TABLE 2: pH values of lectins

Lectin	pH of colloidal gold
Concanavalin A (Con A)	7.2
Helix Pomatia (HPA, HPL)	7.5
Lotus tetragonolobus (LTA)	6.3
Peanut Lectin (PNA)	6.3
Ricinus Communis I and II (RCA)	8.0
Soybean Lectin (SBL)	6.1
Ulex europeus I (UEA)	6.3
Wheat Germ Agglutinin (WGA)	7.0

Pre-embedding Techniques

So-called "pre-embedding" techniques concern the performance of the immunocyto/cytochemical reactions on the cells or the tissue before they are processed for microscopic observation. The reactions are usually performed after only a mild pre-fixation with various solutions, in order to stabilize the distribution of the target sites as in vivo. In this way, the effects of dehydrating agents and embedding media is avoided and may result in a better preservation of the target. Of course, while this is the technique of choice in the case of plasmalemmal targets, it presents several problems for intracellular targets. When the glycosidic residues are located in the cytoplasm, the complex lectin-gold, because of its

dimensions, may have difficulty in reaching and binding to them. In general, the plasma membranes need to be made permeable through either chemical or physical treatments, which can be very damaging for the cell both in terms of morphology and target retention.

In our experience, a protocol for a pre-embedding lectin-gold reaction can be carried out with a direct procedure as follows: 1) rinse specimens briefly in two changes of a washing buffer solution (phosphate buffer saline (PBS), Sorensen buffer, cacodylate) at a suitable molarity and pH. Because of its carbohydratic nature, avoid adding albumin to the buffer; 2) pre-fix the specimens in an appropriate fixative, chosen on the basis of prior tests to assess suitability for retention of both structural and glycosidic receptors integrity. Commonly used fixatives are paraformaldehyde (1-4%) and glutaraldehyde (0.1-0.5%) dissolved in the washing buffer above. Fixation time is usually 10-20 minutes at room temperature; 3) rinse three times (5-10 minutes each) in the washing buffer; 4) 40 microns thick sections are obtained from the tissue block through a Vibratome; 5) rinse 10 minutes in Tris buffer saline 0.01 M (pH according to that of the lectin-gold complex to be applied); 6) incubate for 1-3 hours in the lectin-gold complex at 4°C, gently agitating the sections each 15-20 minutes; the medial concentration of the gold particles, calculated according to Horisberger and Rosset (1977), Horisberger (1978b) and Frens (1973) is 1.06 x 10^{12}/ml for a 18 nm size gold preparation; 7) rinse several times (20-30 minutes in all) in Tris buffer saline; 8) rinse three times in the washing solution; 9) fix for 1-2 hours in 2.5% glutaraldehyde dissolved in the buffer 1, at 4°C; 10) wash overnight, dehydrate, process for TEM by embedding in Epon or Araldite, or process for SEM drying by critical point drying or freeze drying procedures.

The performance of the reaction is relatively simple. Provided that a good tissue penetration of the complex is achieved (in our experience the reagents penetrate the superficial 9-10 micrometers of the section) one has to pay attention mainly to the removal of the excess of the lectin-gold complex after the incubation step. The indirect procedure can be also applied with these same modalities, utilizing appropriate reagents and concentrations. The problem of the control experiments for the direct procedure will be discussed later.

The detection of membrane glycosidic receptors can also be accomplished by applying the fracture-labelling technique (Pinto da Silva et al., 1981a,b; Kan and Nanci, 1988), in which cells and tissues are fixed, impregnated in glycerol, frozen, freeze-fractured, thawed and incubated in the lectin-gold complex. In this way the cross-fractured cytoplasm is also exposed to the label and, therefore, the problems of the penetration of the complex can be overcome. A survey of the use of lectin-gold technique associated with the fracture-label can be found in Pinto da Silva (1984).

Finally, the use of ultrathin frozen sections as an alternative method to provide information on cross-sectioned non-embedded material has been considered (Griffith et al., 1982). It is worthwhile to mention that the ultracryomicrotomy technique offers a much shorter time for tissue processing, which is an advantage when larger series of pathological tissue have to be examined. However, although notably improved from a technical standpoint, cryoultramicrotomy is still, in our opinion, an approach reserved only for targets that are particularly difficult to locate because of their sensitivity to solutions utilized in the processing techniques. This is not the case for most of the glycosidic residues we have considered and, therefore, more conventional techniques have proved successful with lectin-gold cytochemistry.

Post-embedding Technique

Many of the problems related to the pre-embedding method can be overcome by the post-embedding techniques. However, several other difficulties arise due to the embedding medium itself, since it not only represents a mechanical barrier to the penetration of the lectin-gold complex, but it can also affect the physico-chemical integrity of the tissue. For a complete review of these aspects see Causton (1984).

Conventionally formalin-fixed and paraffin-embedded specimens can be utilized (Roth, 1983a, 1987). This may be of particular relevance for the recovery of interesting biopsies from the histological archives of Institutes. However, preliminary tests (our unpublished observations) have demonstrated that the glycosidic residues are resistant to formalin fixation for no more than 3 days; after this period the labelling appears reduced in intensity. Since the fixation time is usually 24 hours or less, most of the specimens can be satisfactorily recovered. Labelling can also be achieved by using specimens fixed with other solutions for LM, e.g., Bouin's and Carnoy's, but a higher non-specific staining has been frequently observed (Roth, 1983a, our unpublished observations). A full description of the effects of various fixatives is given in Allison (1987) and in Byrne (1987).

A silver enhancement is usually required to visualize the lectin-gold complex at the LM level. With this technique (Danscher and Norgaard, 1983; Taatjes et al., 1987) the diameter of the gold particles is increased by the deposition of a shell formed by several layers of metallic silver. This reaction is based upon the ability of the gold to catalyze the reduction of silver ions, adherent to its surface, into metallic silver, through hydroquinone. The gold-silver staining procedure is a method which links light microscopic to ultrastructural observations; it is easily performed, extremely sensitive and permits one to store the glass slide without any appreciable loss in staining intensity. According to our protocol, deparaffinized and rehydrated sections can be treated following a direct procedure as described:

- rinse the sections in Tris buffer saline 0.01M;
- incubate in the lectin-gold complex 1 hour at room temperature in a dark, moist chamber;
- wash thoroughly but gently with 0.01M phosphate buffer saline and wipe away the excess of the buffer around the sections;
- develop in silver nitrate. The developer solution, according to Danscher and Norgaard (1983), is prepared as follows:

A. Arabic gum
B. Citrate buffer, pH 3.5
C. Reducing agent: hydroquinone
0.85 gr in 15 ml H_2O
D. Silver lactate 0.11 gr in 15 ml H_2O

Before use mix the reagents into the following proportion (in a dark room): A: 60 ml; B: 10 ml; C:

15 ml; D: 15 ml. Add D as the last component and mix well; place the final solution in a flask and allow it to reach 25° C in a warm bath. Set several drops of the developer solution on the pre-warmed glass slides;

- wash in running distilled water;
- mount on a glass coverslip with glycerol, to avoid oxidation.

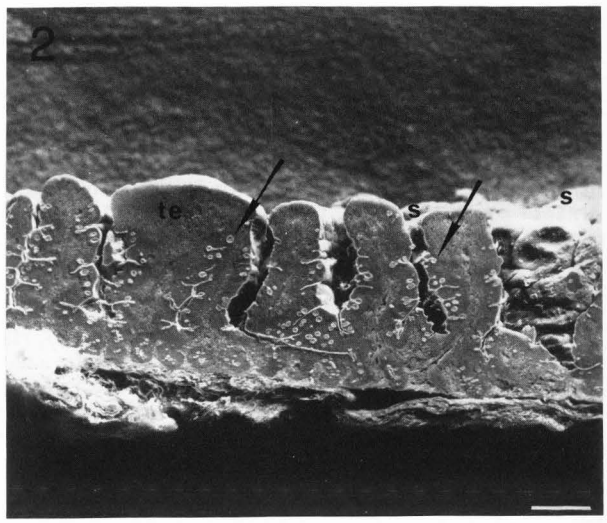
At present, several commercial suppliers offer very carefully prepared and easy to use kits for silver development.

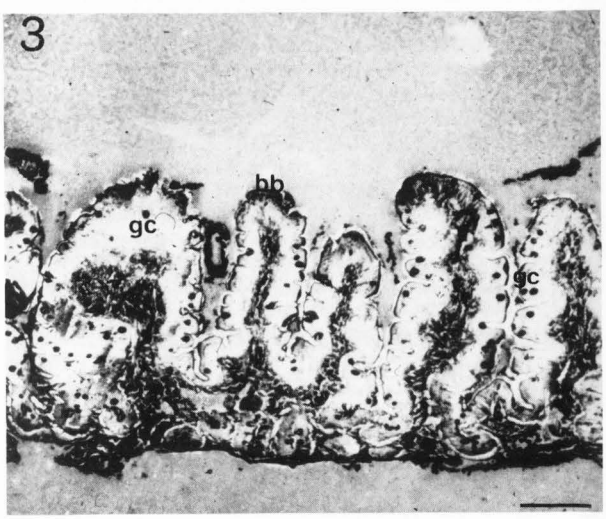
The product of the gold-silver reaction can also be detected in SEM either in the secondary electron (SE) or the backscattered electron (BSE) mode following the method of Pasquinelli et al., 1985.

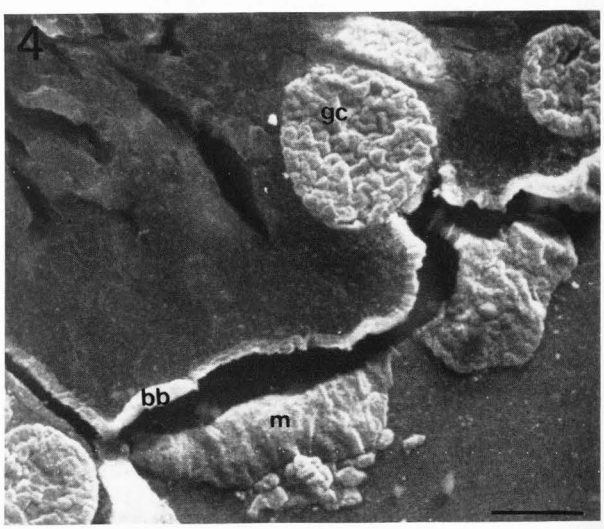
The same protocol has also been applied to bulk specimens recovered from paraffin (Figs. 2, 3, 4) and observed in SE and BSE. In this way a useful correlation between the fine morphology of the transected edge and the surface features of the same specimen can be obtained. It is interesting to also note that the reverse processing can be followed, i.e., recovery from SEM to LM and that the glycosidic residues can still be detected by the lectin-gold complex. The techniques have been explained in Versura et al. (1986a) (Fig. 5).

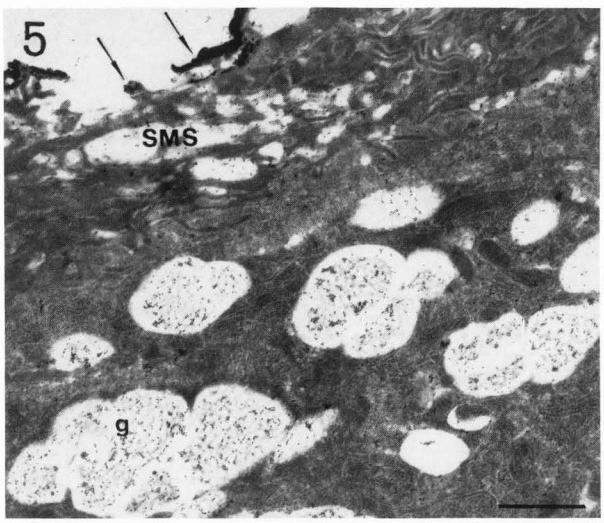
Good results in the localization of the glycosidic residues using TEM have been reported after a low concentration glutaraldehyde fixation followed by embedding in low cross-linked hydrophilic resins (Lowicryl K4M). Fixative solutions prepared with paraformaldehyde alone or in conjunction with glutaraldehyde have also been successfully used. As always in immunocytochemistry the best protocol has to be worked out each time for each new target. Therefore it is not completely correct to apply lectins with different specificities to sections derived from only one type of preparation. The necessity to work with human biopsies, which are often small and, in the majority of the cases, single, has forced us to find a procedure common for all the glycosidic receptors of interest. In our experience the best compromise for retention of morphology, preservation of glycosidic receptors chemical structure, easiness of embedding and sectioning, has been achieved by processing the specimens with 2.5% glutaraldehyde-1.6% paraformaldehyde in 0.1M cacodylate buffer as fixative and with Epon as embedding medium (Versura et al., 1986a). We have found the labelling intensity optimal and the unwanted background staining has always been at acceptable levels (Versura et al., 1986b), although the contrary has been reported by others (Roth, 1983b). Since we use an epoxy resin, semi-thin and ultrathin sections (in post-embedding) necessitate the use of an etching step before the incubation with lectin-gold complex. This is accomplished through a short pre-incubation in 3% NaOH dissolved in absolute ethanol, for a variable period of time according to the thickness of the section. Ultrathin glycol methacrylate (GMA) sections have been also utilized in Gros et al. (1977). The use of LR White as a hydrophilic resin (Herken et al., 1988) has been recently introduced and gives good results, at least comparable to those we have had using Epon (Fig. 6, unpublished results). The schedule of labelling semithin and ultrathin sections from Epon embedded material is given in (Roth, 1983b; Lucocq and Roth, 1984; Versura et al., 1986a).

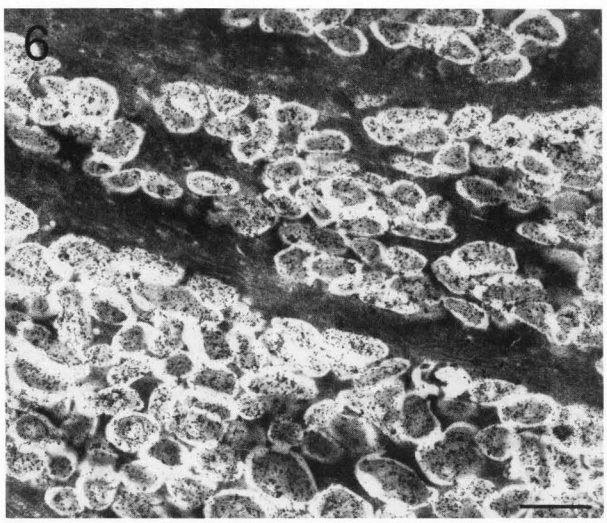
The indirect method has also been successfully











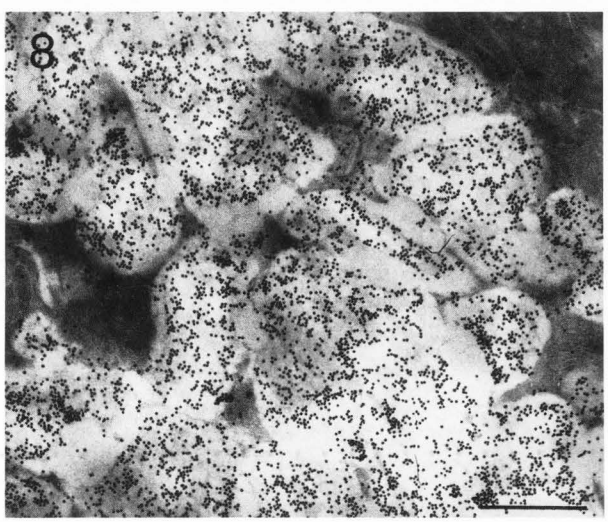


Fig. 2 (on the facing page). Jejunal mucosa biopsy. Specimen recovered from paraffin. WGA-gold-silver complexes mark the brush border of the enterocytes and the goblet cell mucins (gold size = 20 nm); te = transected edge; s = luminal surface; arrows = goblet cells. SEM. Bar = 100 micrometers.

Fig. 3 (on the facing page). Section of jejunal mucosa biopsy. Paraffin section obtained from the same specimen as in Fig. 2. WGA-gold silver complexes are also visible in light microscopy (gold size = 20 nm); bb = brush border; gc = goblet cells. LM. Bar = 100 micrometers.

Fig. 4 (on the facing page). Jejunal mucosa biopsy. Same paraffin section as in Fig. 3 observed in SEM in backscattered mode; bb = brush border; gc = goblet cells; m = mucus. SEM. Bar = 10 micrometers.

Fig. 5. Human tarsal conjunctiva. Also after the recovery of the specimen from SEM, the glycosidic receptors for PNA are detected specifically by the lectin-gold technique (arrows = layer of sputtered gold) (gold size= 17 nm). g = goblet cell granules; SMS = mucus-secreting vesicles belonging to the so-called second mucus system. TEM. Bar = 1 micrometer.

Fig. 6. Human conjunctiva. LR White was used as embedding medium. The granules of a goblet cell are marked by the WGA-gold complex (gold size = 17 nm). TEM. Bar = 1 micrometer.

Fig. 7. Human conjunctiva. Epon was used as embedding medium. The reaction was carried out in a direct procedure, according to the protocol described in Versura et al. (1986a). The WGA-gold complexes mark the goblet cell granules with a density of labelling comparable to that shown in Fig. 6 (LR White embedded specimen) (gold size = 15 nm). TEM. Bar= 500 nm.

Fig. 8. Human conjunctiva. WGA binding sites. Ultrathin section adjacent to that shown in Fig. 7. The reaction was carried out in a three step procedure, with the Protein A-gold complex as the third layer (see text). The density of labelling is comparable to that shown in Fig. 7 (gold size = 15 nm). TEM. Bar = 500 nm.

applied, resulting in an increased density of labelling in comparison to the direct method (Schrevel et al., 1979, Horisberger and Vonlanthen, 1980). This method (two steps or more) involves the use of a labelled antibody directed at the unlabelled lectin used as the first incubation step. As an alternative to the use of antibodies, glycosylated derivatives of horseradish peroxidase and ferritin have been prepared for most common lectins (Kieda et al., 1977). Since lectins are multivalent, they can bind either the glycosidic residues located on the tissue or a marker linked to the sugar specific for the lectin. Therefore, glycosylated markers are actually used as the second step. The value and the limits of these markers are discussed in Schrevel et al. (1979) and Horisberger (1984).

Our personal experience mainly concerns the use of anti-lectin immunoglobulins, followed by the Protein A-gold technique (three step procedure). Briefly, the schedule of labelling ultrathin sections from Epon embedded material is as follows: 1) etch the sections for 10 seconds in 3% NaOH dissolved in absolute ethanol; 2) wash 10 minutes in 0.15M Dulbecco's PBS; 3) incubate in the unlabelled lectin dissolved 0.01-0.05 mg/ml Dulbecco's PBS, for 30 minutes at room temperature in a moist chamber; 4) wash in PBS 10 minutes; 5) incubate the sections in the antiserum anti-lectin (from rabbit) dissolved 1:800/1000 in PBS + 1% ovalbumin, for 30 minutes; 6) wash thoroughly in PBS; 7) incubate the sections in the protein A-gold complex (gold size = 15 nm) prepared according to Roth (1982), and diluted 1:20 in PBS + 1% ovalbumin, for 30 minutes; 8) wash, counterstain with uranyl acetate and lead citrate and observe.

In our experience, the marking of WGA glycosidic receptors in conjunctival goblet cells, both according to the direct (Fig.7) and the indirect (Fig.8) method does not differ significantly.

Control Reactions

Control reactions must be carried out in conjunction with the proper reaction either as a direct or of an indirect procedure. Schematically:

- direct procedure:
  1. by addition of 0.1-0.5 M of the appropriate inhibitory sugar to the lectin-gold complex 1 hour before the incubation;
  2. by incubation of the sections with an excess of the native lectin (1 mg/ml double glass distilled water, 30 minutes) before the application of the lectin-gold complex;
  3. by treatment of sections with 1% periodic acid for 15 minutes before the incubation in the lectin-gold complex.
- indirect procedure
  1. by omission of the lectin incubation step;
  2. by incubation of the sections in the native lectin added with 0.5 M of the specific inhibitory sugar as the first step.

Quantitative Evaluation

It is well established that the electron density displayed by the gold particles as electron microscopic (EM) markers allows useful semi-quantitative determinations of the presence of the specific antigen or sugar residues of interest.

We collect a minimum of 15 micrographs for each specimen of a given experiment (for each lectin receptor site under investigation), taken at an original magnification of 9,500 X. A carbon grating replica with 2160 lines per mm is used for the calibration of the microscope. A magnification of 50,000 X is achieved photographically in the final micrographs used for the quantitative evaluations. An image analyzer (Quantimet 920 from Cambridge Instruments) is used to perform the analysis of the micrographs. The images are detected by a TV-scanner (Plumbicon TV Scanner) on the automatic digitally controlled macroviewer. The blank field is set up by a shading corrector in order to rectify the parts of images where the illuminating light is unequally distributed. The scanner sensitivity is set at 1.02 by using an automatic brightness device and a special interactive filtering procedure is used to separate the gold dots which are occasionally aggregated. The calibration value is set in accord with the final magnification of the micrographs. The area of interest (for most of our studies: single mucus granules of the secreting cells) labelled by the gold dots, which are detected at 63 grey level threshold, are interactively edited, transferred into image memory and processed by a computer program which calculates the number of gold particles related to the granule area. The data are then distributed into histograms in which the frequency of gold particles per square micron of mucus granule is shown.

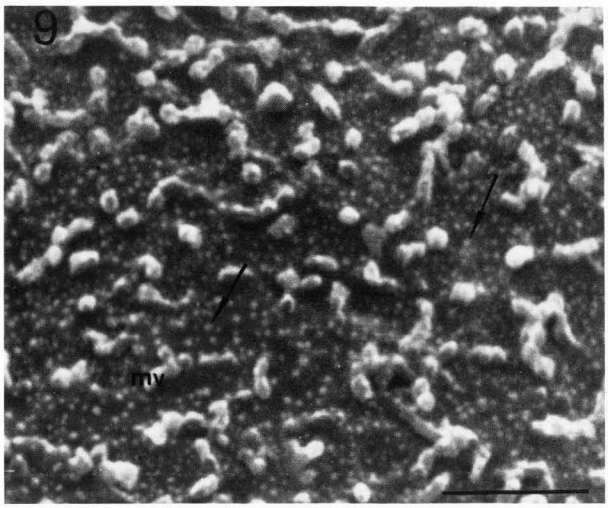
Applications of the Technique to Problems in Pathology

The use of either fluorescein isothiocyanate or peroxidase as markers for the localization of lectin receptors has been widespread in the study of practically all tissues and cells. However, the application of lectin-gold complex procedures have thus far seen a relatively limited application. In fact, most of the tissues and cells that had been previously investigated at LM level have not been reconsidered for a more accurate study with the lectin-gold technique. As we have mentioned, this technique can also provide a semi-quantitative evaluation of the results. This occurs despite the relative ease of the technique; as we have shown in the first part of this paper, the method can be set up and standardized in any EM laboratory.

In Table 3 we have listed the most relevant papers in which the technique has been applied, along with some selected papers in which other markers have been utilized, in order to compare the respective results. Brief comments on the results obtained in these works follows.

The Eye

Lectin receptors in human cornea have been studied, exceptionally, first at the ultrastructural level and only later at LM. In fact, Bonvicini et al. (1982) showed, by EM, the presence of glycosidic receptors for WGA and Con A on the corneal epithelial and endothelial surfaces, and on the conjunctival epithelial cells (Fig. 9). In particular, for WGA, in both corneal and conjunctival epithelium the gold beads were uniformly and continuously distributed on the cell surfaces; a continuous row of gold granules was also observed on the corneal endothelial surfaces facing the anterior chamber (Versura et al., 1984) (Fig. 10). In contrast, a patchy distribution of the Con A receptors was detected in all the three types



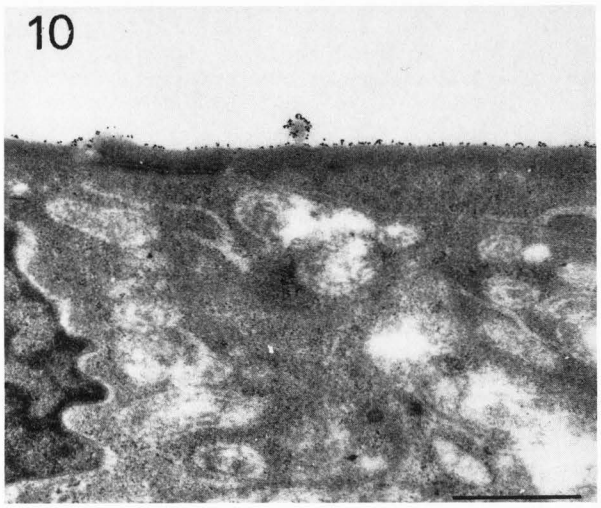


Fig. 9. Human corneal epithelium. Au20-Con A complex. The gold particles (arrows) are distributed quite uniformly on the cell plasma membrane. mv = microvilli. SEM. Bar = 500 nm.

Fig. 10. Human corneal endothelium. WGA binding sites are located as a continuous row on the cell plasma membrane facing the anterior chamber (gold size = 20 nm). TEM. Bar = 1 micrometer.

TABLE 3: List of references on the applications of the lectin-gold complex technique

cell or tissue	reference	cell or tissue	reference
EYE		rat hippocampus	Bonvicini et al., 1987
human corneal epithelium	Bonvicini et al., 1982		
human corneal endothelium	Versura et al., 1984	REPRODUCTIVE SYSTEM	
cornea lattice dystrophy	Panjwani et al., 1987	human cervix	Versura et al., 1988b
human conjunctiva	Versura et al., 1986b,c;1987; 1988c,d Kawano et al., 1984	human spermatozoa	Mortimer et al., 1987
		testicular tissue	Kan and Nanci, 1988
monkey retina and retinal pigment epithelium	Koide et al., 1986	CARTILAGE	
human retina	Shuster et al., 1987	epiphyseal chondrocytes	Velasco et al., 1988
retinal capillaries	Thouron and Pino, 1986	DIGESTIVE SYSTEM	
		incisor enamel	Nanci et al., 1987
URINARY SYSTEM		intestinal goblet cells	Roth, 1983b, c
glomerular podocytes	Roth et al., 1983	human colon	Boland and Roberts, 1988
glomerulus	Brown et al., 1986		
podocyte plasmamembrane	Kunz et al., 1984; Orci et al., 1984	human stomach	Bonvicini et al., 1986
		rat hepatocytes	Horisberger et al., 1978
kidney Henle's and Macula densa	Rielle et al., 1987	BLOOD CELLS	
glomerular capillary	Chevalier et al., 1987	human erythrocytes	Horisberger and Vonlanthen, 1979
rat kidney collecting ducts	Brown et al., 1985		
kidney urinary tubule	Roth and Taatjes, 1985	eosinophilic leukocytes	Lee et al., 1987
		human platelets	Nurden et al., 1980
rat papilla	Weyer et al., 1988	neutrophils and alveolar macrophages	Christiansen and Skubitz, 1988
goat prostate	Tsukise and Yamada, 1987	human macrophages	Kreipe et al., 1986
		murine macrophages	Satoh and Yamazaki, 1987
SALIVARY GLANDS			
mouse submandibular gland	Menghi and Bondi, 1987	EAR	
		organ of Corti	Tachibana et al., 1987a
rat submandibular gland	Versura et al., 1988a		
salivary glands	Nakajima et al., 1988	cochlea	Tachibana et al., 1987b
NERVOUS SYSTEM			
neural cells	Seeley and Field, 1988	middle ear mucosa	Tanimura et al., 1987a,b

of cells under investigation. Panjwani et al.,(1987) subsequently confirmed these results using biotin-labelled lectins and demonstrated the presence of SBA receptors in the corneal epithelium of several mammalian species. In addition, Ricinus communis Agglutinin I (RCA-I), Con A and WGA receptors were found in the normal corneal stroma. The same group (Panjwani et al., 1987), demonstrated later that WGA-, RCA-I- Con A-, peanut agglutinin (PNA)- and soybean agglutinin (SBA)- positive abnormal deposits are present in corneas with lattice dystrophy, a disease which is characterized histologically by the presence of Congo red-positive lesions consisting of amyloid fibrils. Amyloid glycoconjugates with beta-galactose-N-acetyl-galactosamine (recognized by PNA) have never been demonstrated in non-ocular tissues with amyloidosis. It remains therefore to establish if these glycoconjugates detected by PNA are specific just for corneal amyloidosis.

In normal conditions, the ocular surface facing outward is moistened by a tear film that consists of three layers. It is well known that the mucus layer, produced by the conjunctival goblet cells maintains the tear film stability by making the ocular surface wettable. This mucus consists mainly of glycoprotein molecule the nature of which has been investigated especially from a biochemical standpoint by studying samples of extruded mucus. Since there are additional sources of mucus, besides goblet cells (e.g., the lacrimal gland), collecting mucus in such a way permits the evaluation of the whole carbohydrate content of the film and not only of the layer which is specifically devoted to its stability. Lectin cytochemistry was attempted at LM level by Kawano et al.,(1984). The use of FITC-conjugated lectins did not allow recognition of the presence and distribution of glycosidic residues at the level of the so-called second mucus system (SMS) vesicles, also responsible for the production of the mucus layer. The application of the lectin-gold technique, on the contrary, not only differentiates between the two sources (goblet cells and SMS vesicles) but also permits quantitative analysis of the presence of the glycoconjugates in the pathological as compared to normal conditions.

In the goblet cells of normal human conjunctiva (Versura et al., 1986b), WGA, PNA, SBA and Con A glycosidic receptors, were seen to be present in a decreasing density. All the SMS vesicles are positive for WGA and negative for Con A, intermediate results were obtained for SBA and PNA. This means that these two sources of mucus contribute differently to the general pool, probably because the non-goblet epithelial cells are not specifically differentiated for mucus secretion and partially lack in the enzymes for its production.

In patients suffering from Keratoconjunctivitis sicca (KCS) the pattern of glycoconjugates drastically changes (Versura et al., 1986c), in particular WGA, PNA and SBA receptors decrease but Con A receptors increase, while the mucus from the SMS vesicles appears unaffected. This alteration would impair the function of the mucus layer as regards the stability of the film as has been demonstrated for the rheology of other kinds of mucus (Versura et al., 1988b). This means that, even when a normal tear volume is produced, the altered mucus does not permit the aqueous phase to remain and form a three-layered tear film. This series of studies led us to consider that a possible relationship exists between mucus alteration and the onset of a dry-eye condition (Versura et al., 1988d). The pattern of glycoconjugates is not restored by the administration of hydroxy-propyl-methylcellulose, a drug used to soften the sensation of ocular discomfort in dry-eye patients (Versura et al., 1988c).

In the asymptomatic contact lens wearers, all the types of glycosidic receptors under investigation were decreased as compared to the normal subjects (Versura et al., 1987). One of the numerous factors determining the biocompatibility of a contact lens, is the presence of a continuous tear film over the lens. This primarily means the presence of a thin normal mucus layer over the lens surface, on which the other layers can build. Therefore, the same considerations reported above for KCS patients are also true for the asymptomatic CL patients. In addition, an altered mucus adherent to the lens surfaces can affect the more or less complete removal of organic deposits by the usual commercially available washing solutions.

The lectin-gold technique has also been applied to investigate the glycosidic receptors of the cell plasma membranes in the retinal photoreceptors from monkey (Koide et al., 1986) and human (Shuster et al., 1987) eyes. The endothelium of retinal capillaries has been studied by Thouron and Pino (1986), the pattern of distribution of sugar residues in the retinal pigment epithelium has been characterized by Koide et al. (1986).

In the lens, a peculiar organ constituted by a collection of differently aged cells of epithelial origin, we were able to demonstrate that the Con A binding sites display a different distribution on the fiber plasma membrane between the cortical (Fig. 11) and the nuclear (Fig. 11, inset) zone (Versura et al., unpublished observations). The decrease in density of the glycosidic receptors for Con A could be a consequence of the age of the cells, the nuclear fibers being older than the cortical ones. These studies are still in progress and we are waiting for the complete characterization of the lectin-binding sites before final conclusions can be drawn.

Urinary System

The glycocalyx composition of glomerular podocytes has been investigated by Roth et al., (1983). This structure is of particular interest in that it plays an important role as a selective barrier to the passage of macromolecules and defines regional differences in the membrane composition of the glomerulus. Helix pomatia (HPL) binding-sites were found associated only to the podocyte foot process base facing the basement membrane. In contrast, WGA receptors were observed in all regions of the podocyte plasma membrane and in all the three layers of the glomerular basement membrane. Studies performed on isolated basement membrane showed a positivity for WGA only. This supports the idea that HPL receptors are associated with the glycocalyx of the podocyte foot process base rather than with a glyco-component of the basement membrane. In a later study from the same group (Brown et al., 1986), it has been shown that after neuraminidase digestion of thin sections and labelling following a post-embedding procedure, the HPL-binding sites markedly increase on the rest of the podocyte plasma membrane adjacent to the urinary space. An electrophoretic correlative study has demonstrated that these additional sites are associated with the major epithelial

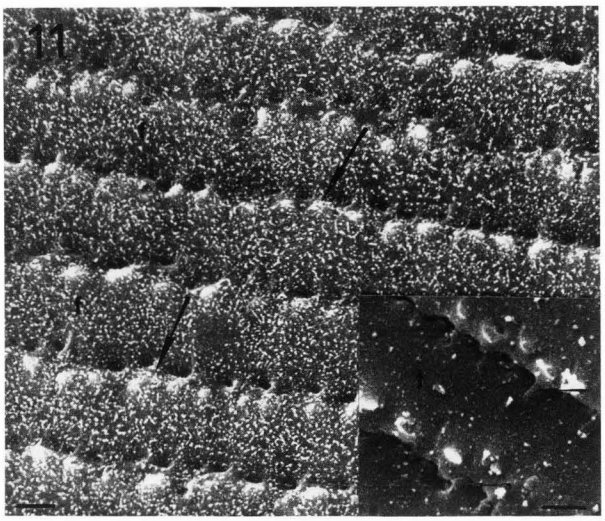


Fig. 11. Rat lens. Cortical zone. The Con A gold complexes display a heavy labelling on the fiber plasma membrane. Pre-embedding procedure (gold size = 20 nm). Inset: rat lens, nuclear zone. The Con A binding sites appear decreased in density. Post-embedding procedure. f = fibers; arrows = interdigitations between adjacent fibers (gold size = 20 nm). Bar = 1 micrometer (SEM and inset).

cell sialoglycoprotein named podocalyxin, while the sites recognized by HPL without any digestion are associated with another glycoconjugate displaying a lower electrophoretic mobility. The presence of HPL binding sites was further demonstrated to be associated with the existence of a normal podocyte architecture (Kunz et al., 1984). In addition they appear considerably reduced in experimental nephrosis in which foot process retraction occurs (Orci et al., 1984). The use of freeze-fracture cytochemistry to gain access to and to label glomerular components in order to study the distribution of WGA receptors and anionic sites was proposed by Chevalier et al. (1987), and has led to the further characterization of the membrane domains in this region.

The distribution of sialic acid residues in the glycocalyx of the epithelial cells in proximal and distal tubules as well as thin limbs of Henle of rat kidney was studied by Roth and Taatjes (1985) using the sialic acid specific Limax flavus lectin. A gross heterogeneity in glycocalyx composition among these three areas was demonstrated and a slight difference was shown between the medullary and cortical portions of the thick ascending limb. Rielle et al. (1987) demonstrated that Helix pomatia binds more to macula densa cells than to adjacent cells of the thick ascending limb, while WGA and Con A do not display any differential pattern of labelling. These differences may be ascribed to the functional diversity exhibited by all these regions. In addition, no relationship between vasopressin and HPL-binding sites on collecting duct epithelial cell plasma membrane has been demonstrated by studying the rat papilla with cytochemical methods (Weyer et al., 1988). Also the intercalated cells located along the collecting ducts showed different Helix pomatia and Dolichos biflorus binding sites, suggesting that these cells represent a heterogeneous population in the different portions of the duct (Brown et al., 1985).

The study of the secretory glycoconjugates of the goat prostate (Tsukise and Yamada, 1987) by means of Con A and WGA confirmed the existence of three different types of cells: mucus-producing cells, protein-secreting cells and cells intermediate between the two. The characterization of these differences appears of particular relevance for the study of some physiological functions, such as the stabilization of spermatozoa.

Salivary Glands

The lectin-gold technique was applied to localize the blood group antigens in human labial salivary and submandibular glands (Nakajima et al., 1988). A, B and H antigens were recognized, respectively, by Dolichos biflorus agglutinin (DBA) or HPA, Griffonia Simplicifolia agglutinin-IB4 and Ulex Europeus agglutinin I in mature secretory granules, but not in the granules present in cells in the early or middle phase of maturation. A mosaic of cellular and subcellular distribution was actually found in relation to the maturation cycle of the secretory cells.

The glycosidic receptors in the normal submandibular glands from rat (Versura et al., 1988a) and mouse (Menghi and Bondi, 1987) were examined. In addition, the effects of the chronic treatment with isoproterenol and reserpine on the mucus composition were analyzed by using WGA, SBA, PNA and Con A as probes (Versura et al., 1988a). It was demonstrated by a quantitative analysis of the labelling that the treatment induces a change in the composition of the glycoconjugate produced by the mucus cells. This could impair the physical characteristics of the mucus. These changes may be similar to those observed in the mucus of patients suffering from cystic fibrosis.

Nervous System

The distribution of "structural" glycoconjugates in the rat hippocampus has been studied by both light and electron microscopy using gold-labelled lectins with different sugar specificities (Con A, WGA, PNA and LTA) (Bonvicini et al., 1987). There are intensely stained, weakly stained and unstained neural cells both in Ammon's horn and in the dentate gyrus, these characteristics being related to the ultrastructural aspect of the cell (Figs. 12, 13). The data from this study led the authors to conclude that these neurons undergo different functional states, which may be related to the synthesis of neurotransmitter precursors or receptors.

The ability to visualize gold particles in both LM and TEM has facilitated the use of stable complexes between WGA and 5/10 nm gold as useful cellular markers in the study of the interactions between the transplant and host in neural grafting (Seeley and Field, 1988). The visualization of ultrastructure at a high level of resolution in this host-transplant interaction was critical in the analysis of the relations between neuronal and glial cells which may be fundamental for the formation of neural connections between transplant and host.

Reproductive System

It is well established that the mucus secreted by the cells of the cervical mucosa is fundamental in controlling sperm and bacterial access to the upper reproductive tract. Several biochemical studies have been carried out on cervical mucus samples taken by aspiration, a method of collection that carries the risk of introducing foreign material to the sample to

be analyzed. The lectin-gold technique was applied to characterize the presence and distribution of some glycosidic receptors during phases in the menstrual cycle of normal young women (Versura et al., 1988b). Each of the lectin-binding sites (WGA, PNA, SBA) appeared to be produced differently during the cycle. These results provide a further support to the hypothesis that the cycle-related alterations of the viscoelasticity of the cervical mucus can be ascribed to the glycoconjugate cyclic variations.

PNA-gold complexes were utilized to study the ultrastructural localization of the glycosidic receptors on spermatozoa, previously detected at LM level by FITC-PNA (Mortimer et al., 1987). It has been confirmed that the lectin binds to the outer acrosomal membrane, thus FITC-PNA labelling may be used to monitor the sperm acrosomal reaction.

The fracture-label cytochemical technique was compared with the BSE mode in SEM to visualize the distribution of RCA-I and HPL binding sites on freeze-fractured preparations of the hamster testis (Kan and Nanci, 1988). It has been shown that both lectins label the seminiferous tubules uniformly, while the spermatids display a regionalization of RCA-I sites on their surface. In particular, the highest concentration of gold particles was observed over the head and the tail portions, while the neck region appeared only weakly labelled.

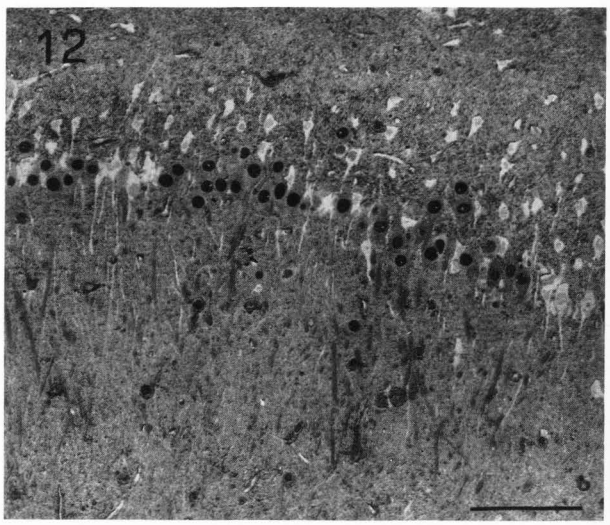
Cartilage

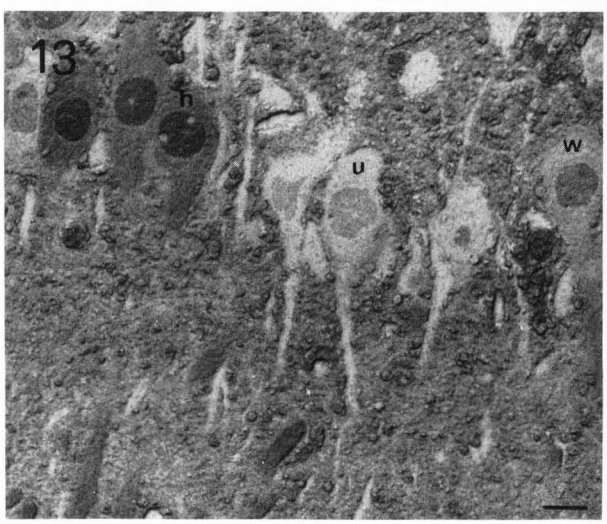
Glycosidic receptors for WGA, DBA, RCA-I and Limax flavus agglutinin have been detected in rat epiphyseal chondrocytes by Velasco et al. (1988). The results suggest that the distribution of products in the Golgi apparatus of chondrocytes differs from that observed in the major part of secreting cells. In particular, the cis and medial faces are involved primarily in the synthesis of oligosaccharides while glycosaminoglycan assembly would occur in the trans face.

Digestive System

The three-dimensional distribution of glycoconjugates in the basal membrane of ameloblasts was examined by applying the lectin-gold technique in SEM (Nanci et al., 1987); the lectin-binding sites were visualized by BSE imaging (BEI). This basal lamina was found to be extremely rich in N-acetyl-D-galactosamine; fucose and mannose were also detected. These sugars would provide the basal lamina with selected properties regulating the access of the material in and out of the enamel layer.

WGA and PNA receptors were detected in human gastric mucosa in normal and diseased patients, by applying the technique to dewaxed paraffin blocks which were then observed with the SEM in the BEI





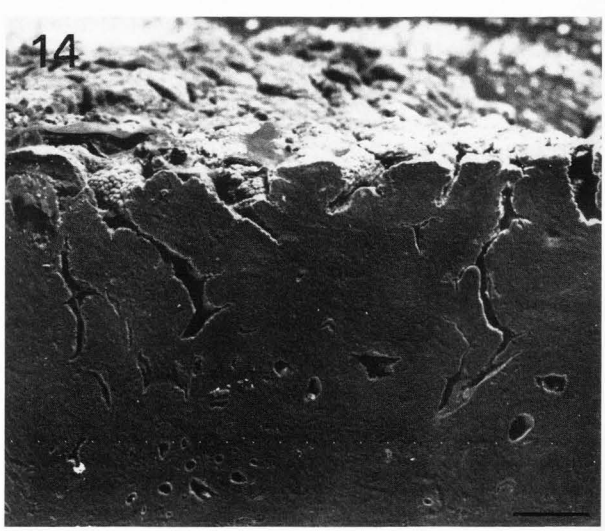


Fig. 12. Rat hippocampus. Semithin section. Pyramidal cells of Ammon's horn displaying different intensity of WGA-gold labelling at SEM in BEI mode in reverse polarity (gold size = 17 nm). SEM. Bar = 100 micrometers.

Fig. 13. Rat hippocampus. Detail of Fig. 12. Heavily labelled (h), weakly labelled (w) and unlabelled (u) neural cells (gold size 20 nm). SEM in BEI mode in reverse polarity. Bar = 10 micrometers.

Fig. 14. Gastric polyp. Paraffin block reprocessed for SEM. The surface epithelial cells diplaying an intermediate appearance between gastric and intestinal cells are mainly labelled by the PNA-gold-silver complexes (gold size = 20 nm). SEM. Bar = 100 micrometers.

mode (Bonvicini et al., 1986). It has been shown that in the case of ulcers, a marked modification in the glycosidic residues content occurs. The presence of an impaired mucus secretion could play a role in the pathogenesis of gastric ulcer. As for polyps and intestinal metaplasia, the necessity of taking into account their cytochemical parameters, given by the lectin binding, in the classification of these lesions has been stressed (Fig. 14).

The pattern of distribution of lectin binding sites at the level of the intestinal goblet cells has been investigated by Roth (1983c) and successively quantitated by Boland and Roberts (1988). Several lectin receptors have been demonstrated on the surface of isolated rat hepatocytes by TEM and SEM (Horisberger et al., 1978), by the use of multiple labelling procedures.

Blood Cells

One of the earliest cellular models on which the lectin-gold cytochemistry was applied is represented by the erythrocytes. Con A, SBA and WGA glycosidic receptors have been localized on the same cell surface membrane at the same time (Horisberger and Vonlanthen, 1979) by a triple labelling procedure. Quantitative binding results indicate that the lectin receptors are spatially separated on the membrane and display a different topographic distribution. The platelet surface membrane was also investigated by the same group (Nurden et al., 1980) in order to study the role of membrane glycoprotein in the physiologic mechanism of platelet aggregation and adhesion.

FITC-bound lectins were used for eosinophilic leukocytes and monocytes collected from venous blood. It is worthwhile to mention that GSA-I and SBA can be considered to be reliable markers for human eosinophils (Lee et al., 1987). Con A, HPA, SBA, UEA, WGA binding patterns were found to be useful for defining certain activation states in monocyte / macrophage differentiation; in contrast, lectin cytochemistry did not clearly discriminate between subpopulations of macrophages (Kreipe et al., 1986). Only recently have the lectin receptors been detected at the ultrastructural level on the surface of human neutrophils and alveolar macrophages (Christiansen and Skubitz, 1988) and of murine macrophages (Satoh and Yamazaki, 1987).

Ear

WGA binding sites were revealed in the organ of Corti (Tachibana et al., 1987a) and in the cochlea (Tachibana et al., 1987b) of experimental animals by means of a post-embedding technique utilizing gold complexes. Since the biochemical techniques currently available do not allow one to separately study the glycosidic residues in the subcellular fractions of the organ of Corti (it is still technically impossible to separate them) lectin-gold cytochemistry in TEM appears the most suitable approach to overcome the problems. WGA glycosidic receptors were found on the plasma membrane, lysosomes and cytoskeletal elements of both hair and supporting cells as well as on the tectorial and basilar membranes. No unique WGA-binding characteristics were observed among the different cells of the organ of Corti.

The same method has been applied to investigate the glycoconjugates present at the level of the glycocalyx (Tanimura et al., 1987a) and of the secretory granules (Tanimura et al., 1987b) of the guinea pig middle ear mucosa.

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Discussion with Reviewers

T.F. Beals: Would you comment on the advantages (and disadvantages) of using gold-antilectin versus gold-lectin techniques?

Authors: The benefits of the gold-antilectin technique are mainly related to the possibility of using only one conjugated antibody or Protein A-gold complex against a variety of anti-lectin antibodies. On the other hand, the method of conjugation of the most utilized lectins is already well standardized, relatively easy to perform, the gold-lectin complexes are stable in the time, only one hour is required for the performance of the reaction. Finally, in our experience, the density of labelling does not differ significantly when comparing the direct versus the indirect method.

T.F. Beals: Your use of gold labeling for quantitative studies takes advantages of the ability to point-count gold (quantitation of enzyme labels is very questionable). However, the number of gold particles conjugated with the lectin in the complex, and the number of lectin molecules which will react with each glycosidic target must be determined. How are these two

variables measured in your system?
Authors: As far as we know from the literature, while the number of sugar binding sites on the lectin structure has been established in most of the cases, no precise data are available as to the number of lectin molecules conjugated to each gold particle. What we actually do in terms of quantization of the labelling is a semi-quantitative analysis which only determines the density of particulate markers. As pointed out also by all previous authors who utilized this approach, the analysis is not intended at all to directly measure the amount of the target molecules present on the tissue section.

T.F. Beals: In which pathological processes do you foresee the greatest interest and advances using the gold-lectin technique?
Authors: Lectin cytochemistry represents a useful complementary technique in the study of tumor pathology (Damianov, 1987). In particular, UEA-I has been recognized as a marker for endothelial cells and tumor cells of vascular origin. Another interesting application could be the study of pre-cancerous lesions of gastrointestinal tract. Basement membrane and extracellular matrix changes in different pathological processes also need further investigation by this technique.

Additional Reference

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