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Cell surface chemistry of arterial endothelium and blood monocytes in the normolipidemic rabbit

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SUMMARY - Chemical mapping of the luminal surface of normal rabbit aortic and coronary endothelium was investigated cytochemically to establish a baseline for further comparison with the biochemical changes possibly induced by the experimental hypercholesterolemia. Morphometric analysis showed that in the aortic endothelium the plasma membrane exposes a large number of uniformly-distributed positively-charged groups of high pK_a, and a heterogeneous pattern of dense anionic groups of low pK_a. Among the latter, only a third was represented by neuraminidase-cleavable sialic acids. These are constituted by various classes of *N*-, and *O*-substituted sialyl residues in glycoconjugates, most frequent being those non-*O*-acetylated at C_8 or C_9. Among the oligosaccharides detected with lectins, very abundant were the glycoconjugates containing mannosyl and subterminal galactosyl, whereas *N*-acetyl-glucosamine, terminal galactosyl and *N*-acetyl-galactosaminyl moieties were rather poorly represented. The density of the latter two markedly increased after its unmasking by neuraminidase treatment. Coated pits contained both anionic and cationic sites, but only few sialic acids and saccharide residues in significantly lower amounts than plasma membrane. The membrane of plasmalemmal vesicles displayed a high number of cationic sites and mannosyl residues, but very few anionic groups, sialyl residues, and galactosyl and *N*-acetyl-galactosaminyl moieties. Coronary endothelium displayed a chemical pattern similar to aorta, with some differences, especially in the frequency of some oligosaccharides. Vena cava was low in acidic groups but rather rich in galactose. Plasmalemmal vesicles were only occasionally labeled by the probes used. Monocyte surface exhibited a high density of anionic sites, and binding sites for wheat germ agglutinin and Ricinus communis agglutinin. No mononuclear cells were observed adhering to endothelial surface.

Key Words: *cell surface - arterial endothelium - monocytes - surface charge - sialic acid - oligosaccharides*

INTRODUCTION

In the vascular regions known to have a predilection to atherosclerosis, the subtle changes in the endothelial cell surface (ECS) chemistry which may be associated with the initiation of plaque formation are poorly understood. The interest on this topic is further justified by the observations that the earliest cellular event, so far detected, is the focal adherence of mononuclear cells to endothelium and their subsequent diapedesis into the intima (Gerrity, 1981; Joris *et al.*, 1983, 1984). In a recent work we have reported that even before monocyte adherence, in lesion-prone areas such as the inner lesser curvature of the aortic arch, the hyperlipidemic rabbit develops within the subendothelium a progressive deposition of extracellular phospholipid lamellae rich in unesterified cholesterol and associated with apoprotein B (Mora *et al.*, 1986). We tentatively called these features extracellular liposomes (Simionescu *et al.*, 1985; Simionescu *et al.*, 1986).

In continuation of these studies, we have conducted experiments intended to identify biochemical alterations in the chemistry of ECS which in the prelesional stage appear in an area recognized to be a consistent site of atheromas in rabbits fed a cholesterol-rich diet. As previously described, we focused our studies on the aortic arch, which in hyperlipidemic rabbits proved to develop fatty streaks in more than 95% of the cases (Simionescu *et al.*, 1986). Additional specimens were collected from thoracic aorta, coronary arteries, and, for comparison, vena cava known not to be affected by atherosclerosis (Strong *et al.*, 1978). In parallel, the inquiry was addressed to the surface of blood cells, especially monocytes.

In order to be able to evaluate the changes induced by hypercholesterolemia in the cell surface chemistry of the two partners, the arterial endothelium and the circulating

mononuclear cells, we first addressed our inquiry to normal animals with a special emphasis on the aortic arch (Simionescu *et al.*, 1986). The investigation was based on ultrastructural cytochemistry with probes for surface charged groups, sialoconjugates, and oligosaccharide moieties. Some probes were applied to hyperlipidemic animals, the findings being reported in the accompanying paper.

MATERIALS AND METHODS

Animals

The experiments were conducted on sixteen male adult Chincilla rabbits 2-3 kg body weight, fed a normal diet of pelleted rabbit chow and maintained in standard housing conditions. Before sacrifice, animals were fasted overnight with water given ad libitum.

Reagents

Microperoxidase M-11, thereafter referred to as hemeundecapeptide, 3,3' diaminobenzidine tetrahydrochloride (DAB), galactose oxidase type V from Dactylium dendroides, neuraminidase type VI from Clostridium pertringens, 1-ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride, peroxidase from horseradish type VI (HRP), wheat germ agglutinin, mannose, methyl-D-mannopyranoside, methyl-D-galactopyranoside, *N*-acetyl-D-glucosamine, tetrachloroauric acid ($HAuCl_4$), and mucin from bovine submaxillary gland type I were purchased from Sigma Chemical Co., St. Louis, MO, USA. Cationized ferritin, ferritin 6x cryst. cadmium-free, peanut agglutinin and Ricinus communis agglutinin 120 were from Miles-Yeda, Rehovot, Israel, a conjugate of wheat germ agglutinin with ferritin, from E-Y Laboratories, San Mateo, CA, USA, Concanavalin A from BDH Chemicals Ltd., Poole, England, and crystallized bovine serum albumin (BSA) from Mann Research Laboratories, New York, NY, and from GIBCO, Grand Island, New York, NY, USA. Lactose was obtained from Merck, Darmstadt, F.R. Germany.

Probes used

a) *Cationized ferritin (CF)*, M_r 480,000, pI 8.4 (as determined in our laboratory);
b) *Hemeundecapeptide (HUP)*, M_r 1,879, pI 4.85 and molecular diameter 1.66 nm (both estimated as previously indicated (Ghinea and Simionescu, 1985)), optimum pH for peroxidatic reaction (with DAB as H^+ donor) 9.0 (Ghinea and Simionescu, 1985).
c) *Ferritin hydrazide (FH)* was synthesized by coupling adipic acid dihydrazide to ferritin via 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (Rolfman *et al.*, 1980).
d) *Horseradish peroxidase-gold conjugate (HRP-Au)*. Colloidal gold particles of ~5 nm diameter were prepared by reducing $HAuCl_4$ with white phosphorus (Horisberger and Rosset, 1977; Slot and Geuze, 1981). To stabilize 1 ml of colloidal gold solution, 26.4 μg of HRP were used according to Ackerman and Freeman, 1979 (slightly modified).
e) *Mucin-gold conjugate (M-Au)* was obtained by adding 22.7 μg of mucin from bovine submaxillary gland to 1 ml of 5 nm colloidal gold particles prepared as described in *d*);
f) *Wheat germ agglutinin (WGA)*;
g) *Concanavalin A (Con A)*;
h) *Ricinus communis agglutinin (RCA)*;
i) *Peanut agglutinin (PNA)*;
j) *Lactosaminated bovine serum albumin-gold conjugate (LacN-BSA-Au)* was prepared by stabilizing 1 ml colloidal gold solution with 55 μg lactosaminated BSA. The latter was obtained from lactose and BSA by the reductive amination method (Gray, 1978).

Plasma lipid analysis

In individual rabbit serum, cholesterol and triglyceride contents were determined enzymatically with the Sigma reagent Kit. Under rather large individual variations, the average values were 40 mg/dl.

General experimental design

The rationale for using the probes mentioned above, in various technical procedures, was based on their reported capability to bind, with different degrees of specificity, to some residues of the ectodomains of some plasma membrane constituents exposed on the luminal endothelial cell surface (Fig. 1, Tables 1 and 2). Most of the data on the ECS chemistry were obtained, so far, on microvessels (see for reviews Simionescu *et al.*, 1982; Simionescu, 1983; Simionescu and Simionescu, 1986), and much less information is available on the arterial endothelium.

Cell surface charge. Anionic sites were visualized with *cationized ferritin* pI 8.4, capable to bind electrostatically to strong negatively-charged residues of low pK_a values, such as the sulfate groups of glycosaminoglycans, the carboxyl groups of sialoconjugates and hyaluronic acid, the β-carboxyl groups of Asp, and the γ-carboxyl groups of Glu in proteins and glycoproteins (Simionescu *et al.*, 1982; Simionescu, 1983; Ghinea and Simionescu, 1985; Simionescu and Simionescu, 1986).
Cationic sites were detected with *hemeundecapeptide* pI 4.85 (Ghinea and Simionescu, 1985), that can mark positively-charged groups of high pK_a values, virtually amino groups in proteins and glycoproteins (e.g. primary amino groups (pK 10.6) of Lys and HO-Lys, primary amino groups (pK 12) of guanidinium in Arg, tertiary amino groups of histidinium (pK 8), and quaternary amino groups (pK >12) in phosphatidylcholines (Simionescu *et al.*, 1982; Simionescu, 1983; Ghinea and Simionescu, 1985; Simionescu and Simionescu, 1986) (Fig. 1 and Table 1)).

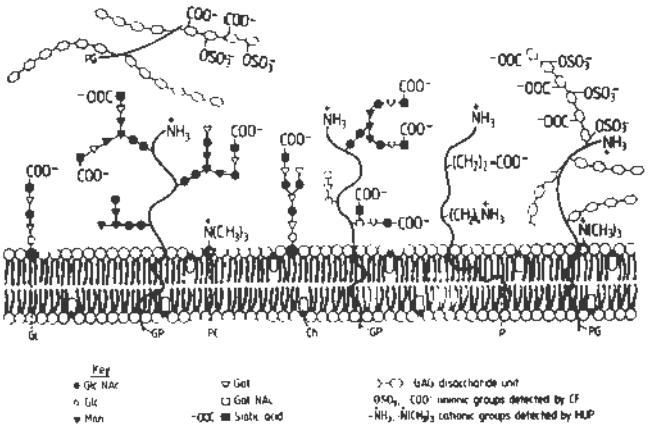


FIGURE 1 Diagramatic representation of the cell surface components bearing charged groups and oligosaccharide moieties detectable by the tracers used in the present study, components which proved to occur on the cell coat of arterial endothelium and blood monocytes. PG: proteoglycans; GP: glycoproteins; GL: glycolipids; P: proteins; PC: phosphatidylcholines; C: cholesterol.

TABLE 1

Labeling properties of the probes used for detecting the cell surface charge distribution

Probe	Specificity	Detected sites	Charged groups	Components bearing charged groups	Main references
CF, pI 8.4	electrostatic interaction	anionic of low pK_a	sulfate carboxyl β-carboxyl γ-carboxyl	HS, HP, CS-4, CS-6, DS, KS sialoglycoconjugates, HA Asp of P and GP Glu of P and GP	Skutelsky and Danon, 1976; Simionescu *et al.*, 1981*b*; Ghinea and Simionescu, 1985
HUP pI 4.85	electrostatic interaction	cationic of high pK_a	guanidinium terminal α-amino ε-amino tertiary amino quaternary amino	Arg of P and GP amino acids Lys, HO-Lys of P and GP Hys of P and GP phosphatidylcholines	Ghinea and Simionescu, 1985

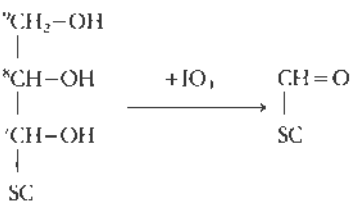
Abbreviations - CF: cationized ferritin; CS: chondroitin sulfate; DS: dermatan sulfate; GP: glycoproteins; HUP: hemeundecapeptide; HS: heparan sulfate; HP: heparin; HA: hyaluronic acid; KS: keratan sulfate; P: proteins.

The specificity of these tracers, the charged groups detected, and the presumed cell surface components bearing these charged groups were depicted in Table 1 and Fig. 1.

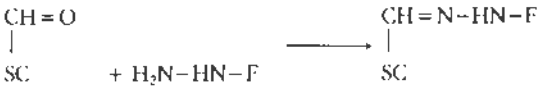
Sialoconjugates - In the cell surface of mammalian cells, the glycoconjugates have been shown to contain several forms of sialic acids which differ in the number and position of their acetyl (or glycolyl) groups (Schauer, 1978; Bhavanandan and Katlic, 1979; Pfannschmidt and Schauer, 1980; Heppelmann and Rahmann, 1981; Schauer, 1985). Some of these can be detected by ultrastructural cytochemistry (Roffman *et al.*, 1980; Schulte and Spicer, 1985) (Fig. 1, Table 2).

Sialyl residues not-O-acetylated at C_8 or C_9. These residues were specifically oxidized with low concentrations of *sodium periodate* (Gahmberg and Andersson, 1977), and the aldehyde groups thus generated at C_7 or C_8 were detected by interaction with *ferritin hydrazide* (Roffman *et al.*, 1980). Since periodate oxidation could not discern *N*-acetyl-, from *N*-glycolyl-derivatives, both sialyl residues were visualized by this technique. The two steps of the reaction occurred on the lateral chain of the sialic acid with sialyl core (SC):

I) sodium periodate oxidation; generation of aldehyde groups:



II) binding of ferritin hydrazide (H_2N-HN-F):



Sialyl residues O-acetylated at the carbon atoms of their side chain, especially C_8 and C_9, are reportedly very resistant to oxidation (Schauer, 1978; Sarris and Palade, 1979). After deacetylation with an alcoholic *potassium hydroxide* solution, these residues were oxidized with *sodium periodate* and labeled with *ferritin hydrazide* (Heppelmann and Rahmann, 1981; Schulte and Spicer, 1985).

N-acetyl-neuraminic acid and N-acetyl-D-glucosamine were simultaneously detected with *wheat germ agglutinin*. This lectin recognizes the *N*-acetylated sialic acids which are not *O*-acetylated at C_4 but does not bind to *N*-glycolyl-neuraminic acid or sialic acids substituted at C_4 (Bhavanandan and Katlic, 1979; Peters *et al.*, 1979). Since in our experimental conditions most of WGA binding sites were removed by tissue treatment with neuraminidase, one can presume that on normal aortic endothelium WGA detected primarily the *N*-acetyl-neuraminic acid. This was visualized by a two-step technique in which *WGA* was followed by a sialoconjugate (*mucin*), adsorbed on gold particles (*M-Au*).

Neuraminidase-cleavable sialyl residues were indirectly estimated either by the reduction in *CF* or *FH* labeling density after endothelial cell surface was exposed to sialydase, or by the increase in FH binding to the *subterminal galactose* and *N-acetyl-galactosamine*. These were unmasked by neuraminidase treatment and rendered *FH-reactive* by oxidation with *galactose oxidase* (Skutelsky and Bayer, 1983). The same procedure, except the sialydase step, was used to detect terminal galactosyl and *N*-acetyl-galactosaminyl residues of ECS glycoproteins (see *Oligosaccharide moieties*).

Oligosaccharide moieties - In the detection of the terminal saccharide moieties, the preference for the two-step techniques (a lectin followed by a glycoconjugate) was based on several advantages: a higher sensitivity (Horisberger, 1984), absence of steric or electric hindrance in the interaction of cell surface with the native lectin as it is the case with the lectins tagged with either HRP, ferritin or gold (Horisberger, 1984; Pino, 1984), and the possibility to quantify labeling.

Mannose-containing glycoconjugates were detected with *Con A*, followed by either *HRP* alone (Wang *et al.*, 1983) or an *HRP-Au* complex (Roth, 1983). Con A has four binding sites for α-D-mannose and α-D-glucose

TABLE 2

Specificity of the probes used for detecting cell surface glycoconjugates

Probe procedure	Specificity	Sequence(s) recognized	Compound detected on ECS	Main references
FH (after oxidation with Na periodate)*	sialyl residues not O-acetylated at C_8 or C_9		sialoconjugates	Roffman *et al.*, 1980; Muresan and Constantinescu, 1985
WGA	NeuNAc (not O-acetylated at C_4), GlcNAc		N-acetyl-sialoconjugates, (not N-glycolyl-) N-acetylglucosamine-terminated glycoconjugates	Bhavanandan and Katlic, 1979; Peters *et al.*, 1979
FH (after oxidation with galactose oxidase)	β-D-galactose, N-acetyl-galactosamine		galactose-, and N-acetylgalactosamine terminated glycoconjugates	Skutelsky and Bayer, 1983
Con A	α-D-mannose> >α-D-glucose	-Manα1 ↘ $^{6}_{3}$Man- -Manα1 ↗	mannose-containing (high mannose, hybrid, and complex) glycoconjugates	Debray *et al.*, 1981; Carver *et al.*, 1985
RCA 120	β-D-galactose	Galβ1→4GlcNAc> >NeuNAcα2→ →6Galβ1→4GlcNAc> >Galβ1→3GalNAc	galactose-terminated and subterminal galactose (O-substituted to C_6 by sialic acids)-containing glycoconjugates	Baenziger and Fiete, 1979; Debray *et al.*, 1981
PNA	β-D-galactose	Galβ1→3GalNAc> >Galβ1→4GlcNAc	galactose-terminated glycoconjugates	Wu, 1984

* By treatment with alcoholic KOH, sialic acids can be de-O-acetylated, rendered oxidizable with Na periodate and labelable with FH.

(Goldstein and Hayes, 1978; Debray *et al.*, 1981; Carver *et al.*, 1985). Cell surface glycoconjugates, except gangliosides, seem to have very little or lack glucose (Dawson, 1978; Kornfeld and Kornfeld, 1978; Berger *et al.*, 1982). The possible Con A binding to the glucose moieties of membrane gangliosides is unlikely, because in these compounds the sugar is substituted at the -OH of C_1 (Fishman and Brady, 1976); this substitution results in a very low binding affinity (Goldstein and Hayes, 1978). As such, we consider that in our system Con A detects primarily mannose-containing glycoconjugates. These are *N*-glycosidic glycoproteins of a high mannose, hybrid or complex type (Hubbard and Ivatt, 1981; Berger *et al.*, 1982), which contain a trimannosidic core (Manα1→6[Manα1→3]Man) that has or lacks a terminal glycosylation (Debray *et al.*, 1981; Hubbard and Ivatt, 1981; Berger *et al.*, 1981; Carver *et al.*, 1985) (Fig. 1 and Table 2). Since HRP is very rich in mannosyl residues, it labeled strongly to Con A binding sites. Gold particles allowed quantitation of binding.

Terminal galactosyl and N-acetyl-galactosaminyl residues, oxidized with *galactose oxidase*, were visualized with *ferritin hydrazide* added simultaneously with the enzyme (Skutelsky and Bayer, 1983).

Subterminal galactosyl and N-acetyl galactosaminyl residues were unmasked by neuraminidase treatment, then oxidized with galactose oxidase making them capable to bind ferritin hydrazide.

For *N-acetyl-glucosaminyl residues* and *N-acetyl neuraminic acid* see *Sialoconjugates*. In a control experiment, the one-step technique WGA-ferritin led to qualitative and quantitative results similar to those obtained by the two-step method using WGA followed by M-Au.

β-D-galactosyl residues were detected with two lectins: Ricinus communis agglutinin (RCA-120) and Peanut agglutinin. *RCA 120* reportedly recognizes galactosyl in either nonsialylated *N*-glycosidic glycoproteins and glycolipids bearing a sequence Galβ1→4GlcNAc or in sialylated forms yielding a NeuNAcα2→6Galβ1→4GlcNAc sequence. To a lesser extent, this lectin binds to *O*-glycosidic glycoproteins containing a Galβ1→3GalNAc sequence (Baenziger and Fiete, 1979; Debray *et al.*, 1981).

PNA recognizes *galactose-terminated glycoproteins or glycolipids* exposing Galβ1→3GalNAc sequences. To a lesser extent the lectin also binds to glycoconjugates containing Galβ1→4GlcNAc (Wu, 1984) (Fig. 1 and Table 2). In our experiments, after each of these two lectins, the specimens were incubated in lactosaminated BSA-Au (LacN-BSA-Au).

General experimental protocol

Preparation of blood cells - Fresh blood from normal rabbits collected in citric acid-Na citrate-dextrose was separated by 1xg sedimentation in 1% Dextran T-500 (Bertino *et al.*, 1963). The supernatant was centrifuged at 500xg for 15 min and the pellet washed with phosphate-buffered saline (PBS) pH 7.4. The remaining erythrocytes were lysed by exposure to deionized water for 10 sec followed by restoration of the osmotic balance with ten-fold concentrated PBS. Cells were again washed with PBS, prefixed with 2.5% glutaraldehyde in PBS for 60 min at 20°C followed by quenching with 1.5 mg/ml $NaBH_4$ in PBS pH 7.4, for 30 min at 4°C. Blood cells were labeled for 60 min at 20°C with 250 μg/ml lectin (wheat germ agglutinin, Concanavalin A or Ricinus communis agglutinin) followed by 30 min exposure to the appropriate electron opaque tracer (mucin-gold for WGA, HRP-Au for Con A and lactosaminated albumin-gold for RCA) in PBS supplemented with 0.25 mM $MnCl_2$. After 60 min fixation with 2.5% glutaraldehyde in 0.1 M HCl-Na cacodylate buffer pH 7.4, the cells were pelleted on 0.22 μm pore size Millipore filter (Millipore Corp.,

Bedford, MA). The following steps were similar to those used for the tissue processing for electron microscopy (see below). Controls consisted in either *a)* competitive inhibition with 0.2 M appropriate monosaccharide or *b)* 30 min incubation with the electron opaque tracer without previous exposure to lectin.

Labeling with cationized ferritin was carried out on unfixed blood cells. The procedures for periodate/ferritin hydrazide and galactose oxidase/ferritin hydrazide are described below.

Preparation of blood-free vasculature - Under general anesthesia with 10% chloralhydrate (3 ml/kg body weight) given intraperitoneally, the abdominal and thoracic cavities were opened. The perfusion was carried out in an open circuit using either the catheterized thoracic aorta as inlet and the cut left atrium as outlet or a catheterized thoracic vena cava as inlet and a cut abdominal vena cava as outlet. The vasculature was washed free of blood by perfusion with PBS pH 7.2 supplemented with 14 mM glucose and gassed with 95% O_2 and 5% CO_2, warmed at 37°C and given under a constant pressure of 110 mm Hg at a flow rate of 10 ml/min for 10-15 min.

Administration of probes - In experiments with CF and HUP, the tracer was added to the perfusate (10 mg tracer/10 ml PBS) and maintained in the circulation for 2 min. The excess unbound probe was washed out by perfusion with PBS in the same conditions as above, then the vasculature was fixed by perfusion for 15 min with 3% glutaraldehyde and 5% formaldehyde in 0.1 M HCl-Na cacodylate buffer pH 7.3 at 37°C. To check whether the interaction of CF with viable ECS at 37°C could induce a redistribution of anionic sites, in control experiments CF was perfused after the endothelium was fixed *in situ* for 10 min with 2% buffered glutaraldehyde.

For the probes detecting sialoconjugates and oligosaccharide residues, after washing out the blood, the vasculature was slightly prefixed *in situ* by perfusing 1% glutaraldehyde in PBS at 37°C for 10-15 min under a pressure of 110 mm Hg. Then, the aorta, particularly the arch lesser inner curvature (Simionescu *et al.*, 1986) and coronary arteries were collected and immersed for additional 15-20 min in the same fixative as above at 20°C, followed by quenching for 30 min at 4°C with 1.5 mg/ml sodium borohydride in PBS pH 7.2, and washing for 30 min with PBS at 20°C. Specimens were further processed according to the specific procedures for ultrastructural cytochemistry.

In control experiments in which WGA-ferritin and Con A-ferritin were applied on unfixed aortic endothelium, the decoration pattern of ECS was comparable with that obtained with same tracers applied on slightly fixed endothelium. The labeling was almost identical especially when specimens were quenched after fixation.

Tissue processing for ultrastructural cytochemistry

Cell surface charge - Cationized ferritin. The aldehyde fixation was completed by immersing the specimens in the same fixative for 90 min, followed by postfixation with 1% OsO_4 in 0.1 M HCl-Na cacodylate buffer pH 7.2, for 90 min at 4°C, staining in block with 0.5% uranyl acetate for 30 min, dehydration in graded ethanol and embedding in Epon 812.

Hemeundecapeptide. After aldehyde fixation, these specimens were incubated for peroxidatic reaction, with 0.15% DAB and 0.02% H_2O_2 in 0.05 M Tris-HCl buffer pH 9.0 for 60 min at 37°C (Simionescu *et al.*, 1975; Ghinea and Simionescu, 1985). Samples were then processed for standard electron microscopy as described above for CF experiments.

Sialoconjugates - Prefixed tissue blocks were used for one of the following procedures, described in their major steps.

Sodium periodate/Ferritin hydrazide (for non-O-acetylated sialyl residues). Tissue samples were incubated with 1 mM sodium periodate for 15 min on ice, followed by exposure to ferritin hydrazide (1 mg/ml) for 60 min at 37°C (Roffman *et al.*, 1980). In control experiments, the first step was omitted.

Potassium hydroxyde/Sodium periodate/Ferritin hydrazide (for O-acetylated sialyl residues). After 15 min incubation with 1 mM sodium periodate on ice, and quenching with 1.5 mg/ml sodium borohydride for 15 min on ice, specimens were treated with 1% KOH in 70% ethanol for 15 min at 23°C. A second oxidation with 1 mM sodium periodate for 15 min on ice was followed by exposure to ferritin hydrazide (1 mg/ml) for 60 min at 37°C. In control experiments, the second oxidation with sodium periodate was omitted (Schulte and Spicer, 1985).

Detection of neuraminidase-sensitive sialyl residues was carried out by treating the tissue samples with 0.4 U neuraminidase/ml PBS for 1 h at 37°C followed either by cationized ferritin, or by sodium periodate (1 mM, 15 min on ice) and ferritin hydrazide (1 mg/ml, 60 min at 37°C), or by galactose oxidase (25 U/ml) together with ferritin hydrazide (1 mg/ml) for 60 min at 37°C (Skutelsky and Bayer, 1983).

Oligosaccharide moieties - Con A/HRP-Au (for mannose-containing glycoconjugates). Specimens were incubated with Con A (250 µg/ml) in PBS supplemented with 0.25 mM $MnCl_2$, 60 min at 20°C, then for 30 min with horseradish peroxidase-gold conjugate (HRP-Au) (Roth, 1983).

Galactose oxidase/Ferritin hydrazide (for terminal galactosyl and N-acetyl-galactosaminyl residues), and *neuraminidase/galactose oxidase/ferritin hydrazide* (for subterminal galactosyl and N-acetyl-galactosaminyl residues), as described for the detection of neuraminidase sensitive sialyl residues.

WGA/M-Au (for *N*-acetyl-glucosaminyl residues and *N*-acetyl-neuraminic acid). Tissue samples were incubated in WGA (250 µg/ml) for 60 min at 20°C, followed by mucin-gold complex (M-Au) for 30 min.

PNA/LacN BSA Au (for terminal galactosyl residues). After incubation with peanut agglutinin (250 µg/ml) for 60 min at 20°C, specimens were immersed in a solution of lactosaminated albumin-gold in PBS supplemented with 0.25 mM $MnCl_2$ for 30 min at 20°C. A similar procedure was used for *RCA 120* (for terminal and subterminal galactosyl residues).

For the lectin experiments, two kinds of controls were conducted: *a)* 30 min labeling with the second tracer (HRP Au, LacN-BSA-Au or M-Au) without previous incubation with lectin; *b)* competition with 0.2 M of the hapten sugar (mannose for Con A, *N*-acetyl-glucosamine for WGA, and galactose for RCA 120 and PNA).

After all these procedures, specimens were fixed for 60 min with 2% glutaraldehyde in 0.1 M HCl-Na cacodylate buffer pH 7.2, at 20°C, postfixed for 90 min at 4°C with 1% OsO_4 in the same buffer and processed for electron microscopy. Thin sections cut with a OmU Reichert Ultramicrotome, were stained with uranyl acetate and lead citrate and examined with Philips 400 HM and 201 C electron microscopes operated at 80 kV.

Morphometry and statistical analysis

From each animal, 3 to 5 blocks were randomly collected from the aortic wall and sections about 70 nm thick were cut (7-15 sections per grid); the pictures were taken at the same magnification for each given tracer. The microphotographs were enlarged at 84,000 or 95,000 ×, and the labeling of endothelial surface was quantitated by counting the number of tracer particles (ferritin or gold) in areas where the trilaminar appearance of plasma membrane was clearly visible. For each experiment, the measurements were made on at least 10 µm of endothelial cell plasma membrane. The sample mean ± standard deviation was expressed as number of tracer particles per μm^2 membrane surface. The latter was calculated from endothelial cell (EC) membrane length and the figures were transformed in values for surface unit, considering a section thickness of 70 nm. The statistical significance between data was appreciated by variance analysis (F test).

RESULTS

Unlike the experiments aimed at detecting the surface charge, conducted on unfixed endothelium, those on sialoconjugates and oligosaccharides, as required by the techniques used, were carried out on a tissue slightly prefixed (10-15 min) with 1% glutaraldehyde before probe administration. To check whether the labeling pattern obtained with the tracers used was similar on fixed or unfixed vessels, for some probes which allow application on fresh tissues (e.g. CF, WGA-Ferritin and Con A-Ferritin), we carried out parallel procedures. The findings revealed that the light prefixation did not significantly affect the labeling patterns. The results of the competition experiments done in the same conditions support these observations. Moreover, previous studies have demonstrated that there is no dynamic redistribution of anionic residues due to CF interaction, the CF binding reflecting the normal localization pattern of anionic determinants (Ottosen *et al.*, 1980; Simionescu *et al.*, 1981*a*; Clough, 1982; Simionescu and Simionescu, 1983). Similar observations have been also reported for ferritin hydrazide used in the same conditions as in our present experiments (Muresan and Constantinescu, 1985).

Endothelium

a) *Cell surface charge*

Anionic sites - The labeling of plasma membrane varied from a nearly continuous layer of one or two rows of particles (the prevailing pattern) to areas of scattered individual particles or small CF aggregates (Fig. 2). All coated pits associated with the luminal membrane were decorated by the adsorbed marker (Table 3). Similarly, 47-55% of coated vesicles appeared to have taken up a large number of CF particles (>50 per vesicle profile) by adsorptive endocytosis (Fig. 2, inset). Relatively large polymorphic uncoated vacuoles (100-150 nm diameter), tentatively identified as endosomes, contained CF particles, most of them apparently in a fluid phase uptake. The probe was also detected in about 10% of the multivesicular bodies encountered. The great majority of plasmalemmal vesicles (up to 97%) associated with the luminal surface of EC were labeled by CF: in ~20% of them the particles seemed adsorbed on vesicle membrane, and in ~70% the tracer apparently occurred in fluid phase (Fig. 2, inset). Because of the short time (2 min) of the endothelial exposure to the probe, no completed transcytosis (Simionescu, 1981; Simionescu *et al.*, 1982) was observed. However, in extremely rare cases, vesicles open on the abluminal front were seen discharging their CF particles into the subendothelial space. No CF particle was seen penetrating the endothelial junctions.

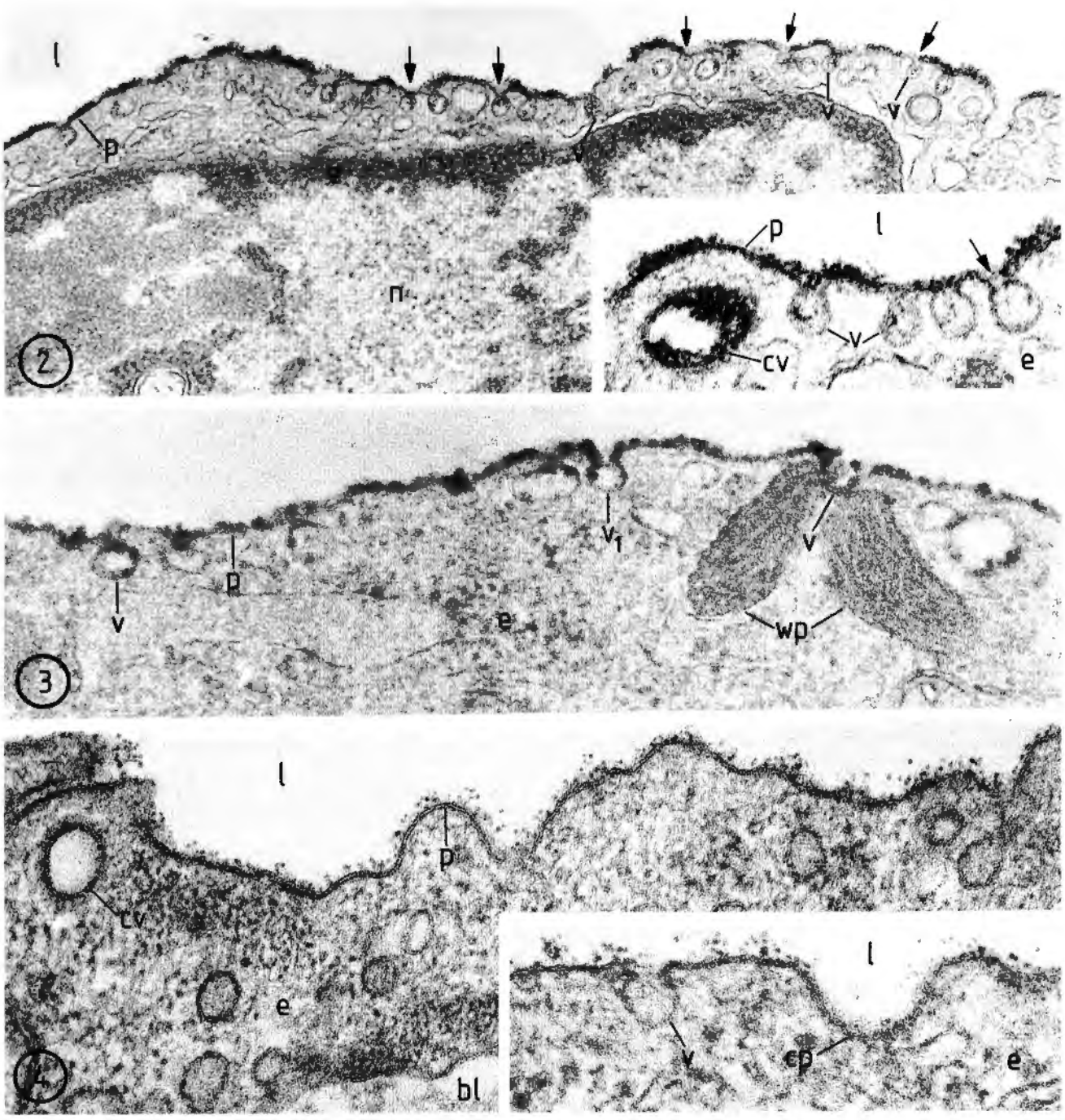
Cationic sites - HUP reaction product decorated as a continuous 20-30 nm thick layer, the plasma membrane, coated pits, and plasmalemmal vesicles associated (open or apparently internalized) with the cell surface (Fig. 3). Occasionally, some coated vesicles and endosome-like structures were marked by the reaction product of HUP peroxidatic activity.

b) *Sialoconjugates*

Sialyl residues not-O-acetylated at C_8 or C_9 - As revealed by the labeling of ferritin hydrazide after ECS treatment with sodium periodate, this form of sialyl residues had a relatively continuous and uniform distribution on plasma membrane (Fig. 4). The average labeling density was 885 ± 136 FH particles/μm^2 membrane area (Table 4). Only few coated pits were marked and usually with a reduced number of particles (Fig. 4, inset). Plasmalemmal vesicles open on the luminal ECS were only to the extent of ~20% labeled by 1 or 2 FH particles, both in aortic and coronary endothelium, whereas in the vena cava the average labeling was ~12% only. Because of prefixation, it was difficult to assess whether this low labeling density of vesicles was real or at least in part the effect of fixation on the vesicle neck glycocalyx. The fact that a certain number of vesicles always contained the tracer would support the assumption that the light fixation used did not prevent FH access to open vesicles. As expected, no image of completed endocytosis or transcytosis was observed in these preparations.

Sialyl residues O-acetylated at C_8 or C_9 - Tissue treatment with alcoholic potassium hydroxide rendered these sialyl residues oxidizable with sodium periodate and subsequently reactive with FH. This procedure revealed that about 40% of the total sialic acid content of the luminal aortic ECS belonged to the O-acetylated form (Table 4). The labeling density on plasma membrane had an average value of 621 ± 187 FH particles/μm^2 membrane surface (Table 4). We are less confident on these values because sometimes the alcoholic potassium hydroxide treatment visibly damaged the plasma membrane. Moreover, while in the other experiments the nonspecific binding did not exceed 5-10%, after this procedure the background level was ~40%.

Neuraminidase-cleavable sialyl residues - Two procedures were used to detect the neuraminidase-sensitive sialic acids. When the enzyme treatment was followed by cationized ferritin, the labeling values decreased from 2531 ± 54 CF/μm^2 endothelial surface to 1677 ± 263 (Table 4). This may indirectly indicate that among the strong acidic resi-



Figures 2 to 7 Represent aortic endothelium from normal rabbits fed a standard diet. General abbreviations - bl: *basal lamina;* cp: *coated pit;* cv: *coated vesicle;* e: *endothelial cell;* l: *lumen;* v: *plasmalemmal vesicle;* p: *plasma membrane.*

FIGURE 2 Cationized ferritin labels rather homogeneously plasma membrane (p); the rows of particles usually stop at the level of the neck of plasmalemmal vesicles (arrows), the great majority of these vesicles containing the tracer apparently in fluid phase (v). Inset: most coated pits and coated vesicles (cv) are marked by CF. n: nucleus. × 43,000; inset: × 95,000.

FIGURE 3 Hemeundecapeptide decorates as a uniform layer of reaction product adsorbed on plasma membrane (p), and vesicles open on cell surface (v). WP: Weibel-Palade body. In v one can see the continuity of the reaction product from plasma membrane to vesicle membrane. × 68,000.

FIGURE 4 To the endothelium oxidized by sodium periodate, ferritin hydrazide binds on high density on plasma membrane (p) but only occasionally decorates coated pits and coated vesicles (cv). Inset: detail showing that FH does not decorate membrane of plasmalemmal vesicles (v), while few particles are bound to a coated pit (cp). × 110,000; inset: × 110,000.

TABLE 3

The extent of cationic ferritin labeling of various microdomains of the luminal surface of arterial endothelium

Endothelium	Plasma membrane (CF particles per μm^2)	Coated pits	Coated vesicles	Multivesicular bodies	Plasmalemmal vesicles		
					open on lumen	closely associated with lumen	apparently free in cytoplasm
		%	%	%	%	%	%
aortic	1766 ± 286	100	47	3	96	77	24
coronary	1970 ± 115	100	55	2	97	71	17

For each type of endothelium 25 µm length of plasmalemma proper profile was counted; ** the number of structures examined was: 300 coated pits, 100 coated vesicles, 65 multivesicular bodies and 1,000 plasmalemmal vesicles.

TABLE 4

Labeling density of the probes used for characterization of cell surface sialoconjugates of aortic endothelium (ferritin particles/μm^2 membrane area)

Cytochemical procedures used					
Sodium periodate — FH	Potassium* hydroxide — Sodium periodate — FH	Galactose oxidase — FH	Neuraminidase — Galactose oxidase — FH	Cationized ferritin	Neuraminidase — Cationized ferritin
885 ± 136	621 ± 187	36 ± 14	314 ± 156	2531 ± 54	1677 ± 263

FH: ferritin hydrazide; * for the complete sequence of this procedure, see Tissue Processing for Ultrastructural Cytochemistry; ± standard deviation of the mean.

dues labelable with CF only a third (34%) were represented by neuraminidase-sensitive sialoconjugates.

The amount of subterminal galactose and *N*-acetyl-galactosamine moieties of the sialoglycoconjugates, that were unmasked by neuraminidase treatment, appeared to be relatively high as indicated by the FH labeling values 7-8 times higher than in specimens treated only with galactose oxidase and FH (Table 4).

c) *Oligosaccharide moieties*

Mannose-containing glycoconjugates - On the luminal surface of aortic endothelium, plasma membrane was almost uniformly decorated by gold particles (Con A/HRP-Au procedure) (Fig. 5). The label density was slightly higher on aortic (1988 ± 499 particles/μm^2 endothelial surface) than on coronary endothelium (Table 5). A large fraction of coated pits (~67%) were marked by the ligand (Table 5 and Fig. 5). A remarkable difference in the labeling of plasmalemmal vesicles was observed between aortic (~68%) and coronary endothelium (only ~16%). In vena cava, the labeling of plasma membrane was heterogeneous and usually at values 30-40% lower than in arteries, while the relatively rare plasmalemmal vesicles were practically unmarked (Table 5).

N-acetyl-glucosamine and N-acetyl-neuraminic acid - By the WGA/M-Au procedure, the number of gold particles per μm^2 of endothelial luminal surface was low: ~69 for aortic and ~317 for coronary endothelium (Fig. 6 and Table 5). The latter figure represented a 4.5 higher value ($p<0.01$) than that counted for the aorta. The binding density on the plasmalemma proper of venous endothelium was comparable to the values recorded for the aortic and coronary endothelium. Coated pits were frequently marked by 1 or 2 particles in coronary, but less frequent in aortic endothelium. Though at low values, the labeling of plasmalemmal vesicles was almost double for the coronary (~18%) than

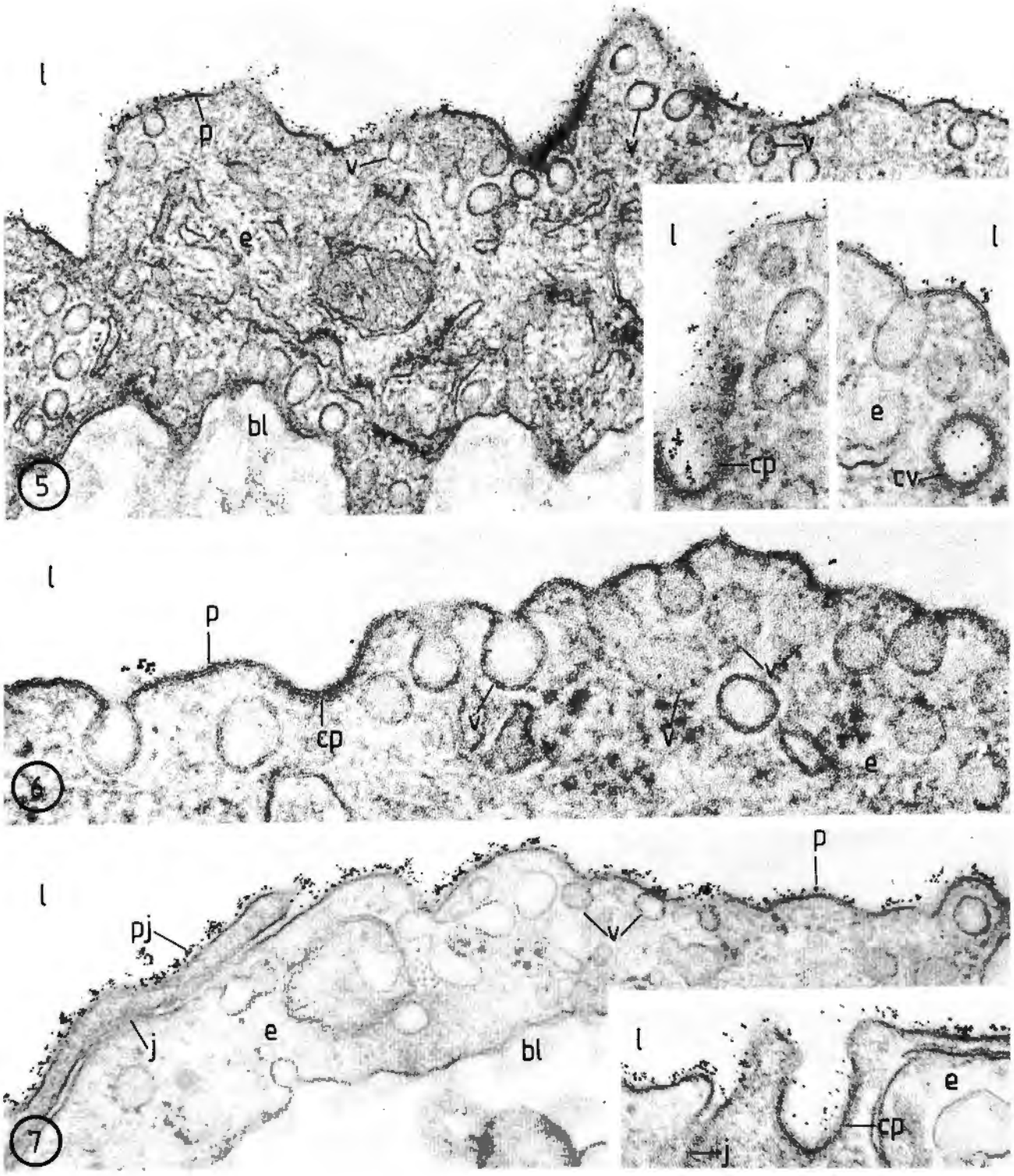


FIGURE 5 The residues detected with Con A/HRP-Au are almost homogeneously distributed but well represented on plasma membrane (p), while plasmalemmal vesicles only occasionally display such tracer particles (v). Inset: most coated pits (cp) and coated vesicles (cv) are marked by this probe. × 35,000; inset: × 110,000.

FIGURE 6 The moieties detectable by WGA/M-Au are very scarcely present on plasma membrane (p), coated pits (cp), and plasmalemmal vesicles (v). × 135,000.

FIGURE 7 Binding sites for RCA followed by LacN-BSA-Au are numerous on the luminal plasma membrane (p) but in a rather patchy pattern. Commonly, plasmalemmal vesicles (v) are not labeled. j: endothelial junction. Note the high density of RCA-binding sites on the plasma membrane of the parajunctional zone (pz). Inset: coated pits show a relative scarce labeling (cp). Junctions were never penetrated by the probes. × 45,000; inset: × 84,000.

TABLE 5

Binding density of the lectin probes used for detecting saccharide residues on cell surface of aortic, coronary and vena cava endothelium

Endothelium	Lectin/second tracer*			
	Con A — HRP-Au	WGA — Mucin-Au	RCA — LacN-BSA-Au	PNA — LacN-BSA-Au
Aorta				
plasma membrane**	1988 ± 499	69 ± 39	925 ± 257	192 ± 39
coated pits°	67.4	18.2	ND	ND
plasmalemmal vesicles°	68.6	9.2	3.1	13.4
Coronary				
plasma membrane**	1397 ± 394	317 ± 64	696 ± 170	
coated pits°	ND	75.0	ND	
plasmalemmal vesicles°	16.7	18.2	5.2	
Vena cava				
plasma membrane**	906 ± 206	301 ± 81	1164 ± 228	
coated pits°	ND	ND	ND	
plasmalemmal vesicles°	0	4.5	0	

* Particulate electron opaque marker (Au); ** number of gold particles/μm² plasma membrane ± standard deviation of the mean; ° percentage of labeled structures; features counted = 2003 plasmalemmal vesicles, and 66 coated pits; ND: not determined (usually due to non significant sampling).

for the aortic endothelium (~9%), while in the venous endothelium the vesicles practically lacked any decoration.

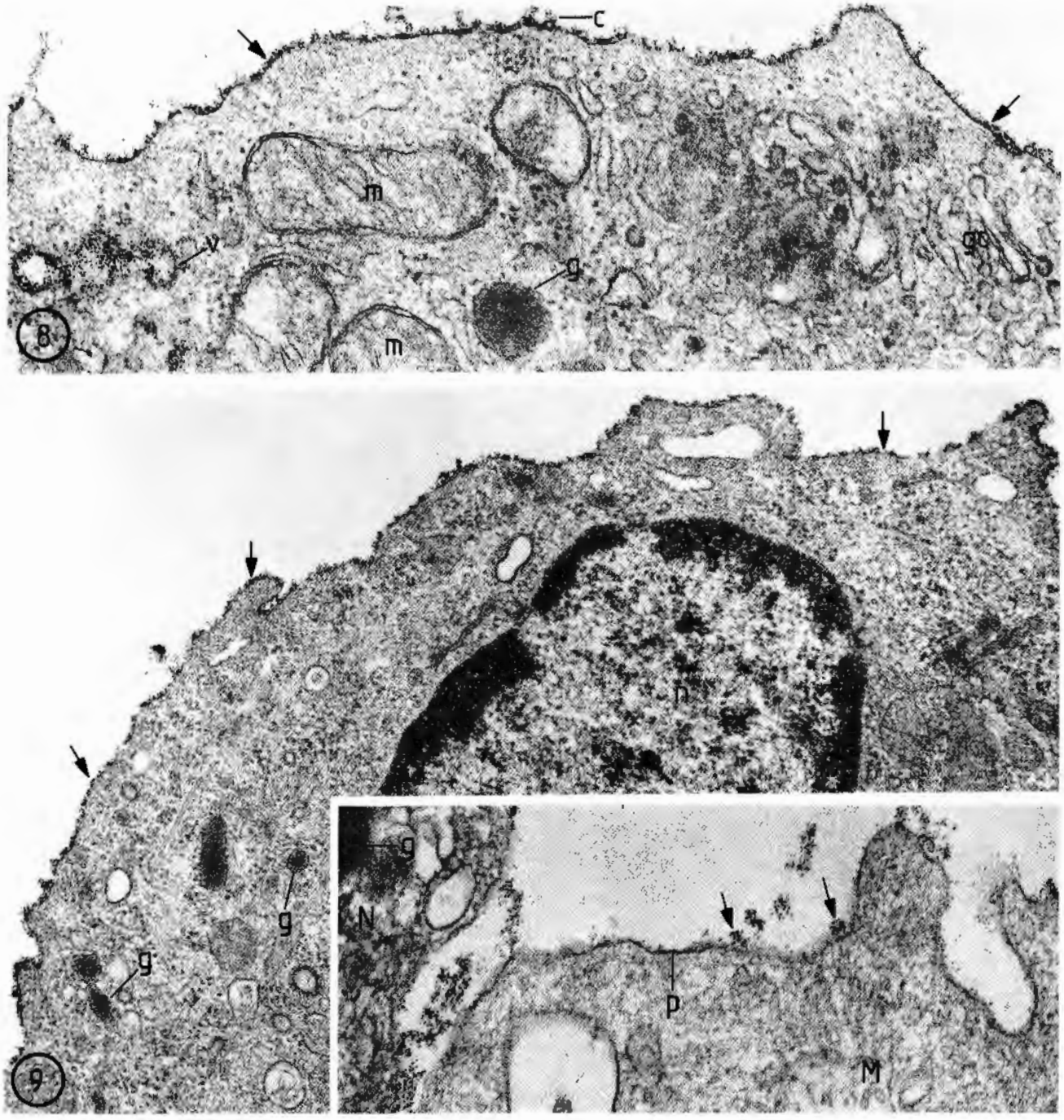
Subterminal and terminal galactosyl residues - As detected with RCA 120 followed by LacN-BSA-Au, these residues of *N*-glycosidic and *O*-glycosidic glycoconjugates (Table 2) were relatively frequent on the luminal plasmalemma of arterial endothelium though plasmalemmal vesicles rarely displayed particles (Table 5 and Fig. 7). The numerical density of gold particles on plasma membrane was almost similar on the endothelium of vena cava (Table 5).

Galactose-terminated glycoconjugates - By the PNA/LacN-BSA-Au technique the binding density of these residues on plasmalemma proper was significantly lower (~192) on aortic endothelium with ~13% decoration of plasmalemmal vesicles (Table 5).

Terminal galactose and N-acetyl-galactosamine - After oxidation with galactose oxidase and treatment with ferritin hydrazide, the luminal surface of aortic endothelium exposed very few terminal Gal and GalNAc residues (Table 4). In contradistinction, the *subterminal* moieties of this kind, unmasked after sialic acid digestion with neuraminidase, were in much higher number (Table 4).

Monocytes

Among the blood mononuclear cells, the monocytes were identified based on their large size (12-18 μm diameter, about twice larger than lymphocytes), the oblong, slightly flattened or indented centrally located nucleus provided with a fine network of chromatin, and the relatively abundant cytoplasm containing a moderate number of lysosomes, prominent Golgi complex, rough endoplasmic reticulum and centrosome. The fine granules (~40 nm diameter) had a homogeneous content and appeared usually in clusters of 10-15 per section. The monocyte cell surface exhibited a uniform high density of CF-detectable anionic sites. Tracer particles occurred in 1-3 rows with a density commonly higher on coated pits than on the plasma membrane. The frequency and distribution of the strong negatively-charged groups were comparable for monocytes (Fig. 8), lymphocytes and eosinophils, and occurred at markedly lower values on neutrophils and erythrocytes. The sialyl residues visualized with FH were rather uniformly distributed on monocyte surface, usually revealed by a single row of ferritin particles (Fig. 9). In contrast, the terminal galactosyl, and *N*-acetyl-galactosaminyl residues, rendered reactive to FH after galactose oxidase treatment, were rather scarce and inhomogeneous (Fig. 9, inset). As detected by Con A, the mannosyl-containing glycoconjugates were a constant component of monocyte glycocalyx (Fig. 10). WGA-detectable sialic acids and *N*-acetyl-glucosamine-terminated glycoconjugates, although heterogeneous, were well represented on monocyte surface (Fig. 11), as well as eosinophil and red blood cell, but were less frequent on lymphocyte and neutrophil (Table 6). The subterminal galactosyl residues (revealed by RCA) frequent on monocyte (Fig. 12), lymphocyte and eosinophil were in an even higher density (almost double values) on the glycocalyx of neutrophils and erythrocytes (Table 6). In the material examined, no monocytes were found attached to the endothelium or undergoing diapedesis.



Figures 8 to 12 Illustrate the cell surface of circulating mononuclear cells with ultrastructural features characteristic for monocytes (normal rabbits fed a standard diet)

FIGURE 8 Cationized ferritin is bound in a dense and rather homogeneous layer of 1-3 particles (arrows): the clusters (c) are probably artifactual. Tracer particles are internalized in vacuoles (vc), presumably part of the endosomal compartment. g: granule; m: mitochondria; gc: Golgi complex. × 49,000.

FIGURE 9 The monocyte plasma membrane oxidized by treatment with Na periodate binds usually in a uniform layer of particles the ferritin hydrazide (arrows). g: granules; n: nucleus. Inset: terminal galactosyl and *N*-acetyl-galactosaminyl residues, after oxidation with galactose oxidase become labelable by ferritin hydrazide. The binding sites (arrows) are relatively rare and inhomogeneously scattered on monocyte plasma membrane (p). g: granule; M: monocyte; N: neutrophil. × 32,000; inset: × 41,000.

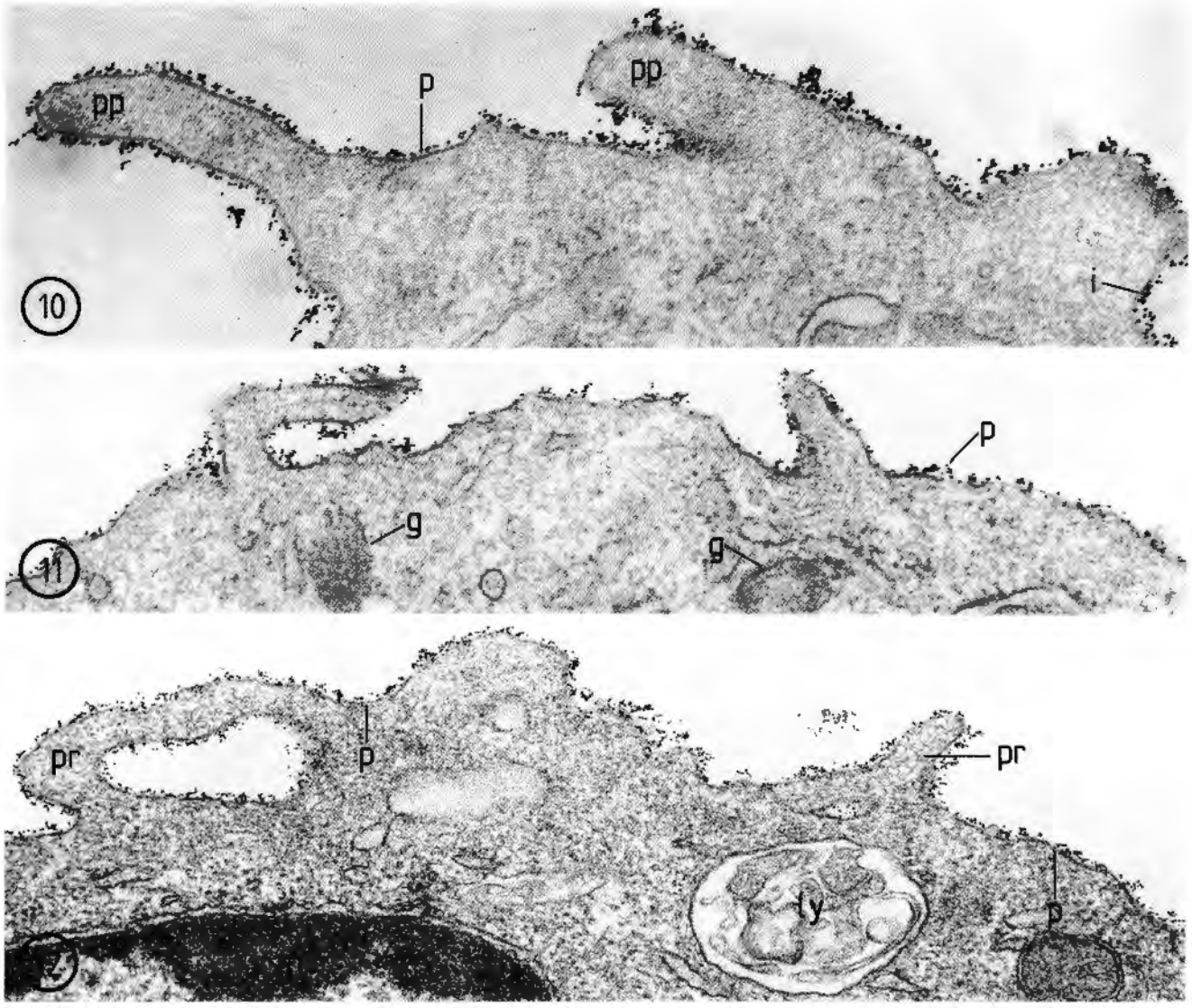


FIGURE 10 The residues visualized by Con A binding are well represented, appearing usually as a single layer of gold particles on plasma membrane (p) and its invaginations (i) and pseudopodes (pp). × 60,000.

FIGURE 11 The sites detected by WGA though relatively frequent on plasma membrane (p), are randomly distributed. g: granules. × 49,000.

FIGURE 12 The RCA-detectable galactosyl residues are well represented on the monocyte plasma membrane (p) and cytoplasmic protrusions (pr). ly: lysosome; n: nucleus. × 43,000.

TABLE 6

Lectin binding on blood cell surface in normal rabbits (number of gold particles/μm^2 plasma membrane surface ± SD)

Cell type	WGA/mucin-Au	RCA/Lac N-BSA-Au	Con A/HRP-Au
monocyte	1108 ± 61	1488 ± 97	1082 ± 139
lymphocyte	487 ± 110	1508 ± 105	925 ± 41
neutrophil	554 ± 18	2235 ± 35	891 ± 118
eosinophil	837*	1491 ± 15	ND
erythrocyte	1048 ± 74	2099 ± 347	897 ± 8

* One datum available; ND: not determined.

DISCUSSION

The endothelial cell surface (ECS) contains the ectodomains of membrane proteins, glycoproteins and glycolipids (including sialoconjugates), and proteoglycans. *In vivo*, some plasma proteins are attached to ECS where they perform their activities (e.g. lipoprotein lipase, molecules of the coagulation system). The molecules of ECS mediate endothelial interactions with both blood cells and permeant molecules (Simionescu and Simionescu, 1986). For the latter, in both normal and pathologic conditions, the EC membrane components function either as a sieving meshwork, a locally differentiated charge barrier (Simionescu, 1983), or as binding sites and receptors (Ghitescu *et al.*, 1986) for molecules to be endocytosed or transcytosed (Simionescu, 1983; Simionescu and Simionescu, 1986).

The so far unsuccessful attempts to obtain a pure fraction of endothelial plasma membrane explain the lack of detailed data on its biochemistry approached mostly indirectly by using membrane markers. The current information on the chemistry of ECS in arteries is limited to reports on its staining with cationic ferritin (Skutelsky and Danon, 1976; Lewis *et al.*, 1982) ruthenium red (Gerrity *et al.*, 1979), Concanavalin A (Weber *et al.*, 1973), anti-podocalyxin-gold conjugate (Horwath *et al.*, 1986), its high content in sialic acids (Born and Palinski, 1985) and the effects of their removal on endothelial interaction with platelets (Görög *et al.*, 1982) and low density lipoproteins (Görög and Born, 1982). Under the limitations of the in culture conditions, several data have been reported on ECS components expressed *in vitro*, such as proteoglycans, enzymes, factors related to hemostasis, recognition sites, and antigenic determinants (for review, see Catravas and Watkins, 1985; Hormia, 1985; Muller *et al.*, 1985; Nees *et al.*, 1985; Novak *et al.*, 1985; Simionescu and Simionescu, 1986).

The ECS moieties detected *in situ* mostly on microvascular endothelium have a heterogeneous composition and a non-uniform distribution along the vascular tree (Simionescu and Simionescu, 1978; Simionescu, 1981; Simionescu *et al.*, 1981*a*; Simionescu *et al.*, 1981*b*; Simionescu *et al.*, 1985). This may explain at least in part the regional differences in the functions and response of vascular endothelium to various pathogenic factors.

Chemical mapping of ECS in arteries is a prerequisite for understanding the subtle molecular events at the blood-vessel wall interface which may initiate or be associated with atherosclerosis.

Although it included rabbit thoracic aorta, coronary arteries and to a certain extent vena cava (as a vessel not affected by atherosclerosis), our studies were focused on the aortic arch which, in our experimental conditions, proved to be consistently a lesion-prone area, being affected by hypercholesterolemia in more than 95% of cases (Simionescu *et al.*, 1985; Simionescu *et al.*, 1986). The observations reported in this paper represent the baseline for the interpretation of the biochemical alterations induced in the arterial ECS by hypercholesterolemia (see accompanying paper).

The ECS of normal arterial endothelium displays a rather uniform and continuous layer of basic residues, presumably constituted by amino groups. As visualized with HUP, these moieties occur equally on plasma membrane, coated pits, and plasmalemmal vesicles open on the luminal surface. Within this rather homogeneous coat of cationic groups, there is a inhomogeneous distribution of anionic sites. These are present in various amounts on plasma membrane and are consistently a part of the coated pit glycocalyx (Fig. 13). Although more than 95% of plasmalemmal vesicles contain CF particles, only a small fraction of these (~20%) appears to bind them by electrostatic adsorption. This indicates that the great majority of plasmalemmal vesicles (~80%) in arterial endothelium are devoid of strong anionic sites, a situation comparable to that found in other continuous endothelia (Simionescu *et al.*, 1985). In

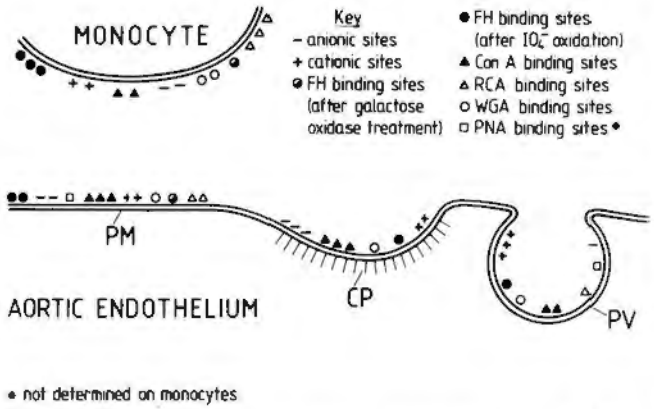


FIGURE 13 Diagram representing the distribution on the cell surface of aortic endothelium and blood monocytes of anionic sites (–), cationic sites (+) and glycoconjugates detectable by FH after sodium periodate (●), FH after galactose oxidase (◕), Con A (▲), RCA (△), PNA (□) and WGA (○).
3 symbols=abundant decoration (range: 1,000-2,000 particles/μm^2); 2 symbols=moderate decoration (range: 500-1,000 particles/μm^2); 1 symbol=poor decoration (fewer than 500 particles/μm^2).

terms of charged domains, there is no significant difference between aortic and coronary endothelia (Table 3). As reported by Simionescu *et al.* (1985), the CF binding density on venous endothelium is comparable or lower than that of venular endothelium, in old animals, and commonly lower than in arterial and capillary endothelia.

As indicated by neuraminidase digestion followed by CF, the high negative charge of ECS is only in part (30-40%) constituted by periodate-sensitive or neuraminidase-hydrolysable sialyl residues. This finding suggests that other components such as proteoglycans and, to a lesser extent, phospholipids may substantially contribute to the net ECS negative charge (Simionescu *et al.*, 1981*b*). Coated pits are usually low in or devoid of sialyl residues, a situation found also in capillary endothelium (Muresan and Constantinescu, 1985).

The aortic ECS contains various forms of *N*-, and *O*-substituted sialic acids (Fig. 13). Part of the sialic acid detected with FH after mild periodate oxidation of ECS may represent *N*-glycolyl-neuraminic acid and the form *O*-acetylated at C_4, that is nonhydrolysable with the used sialydase and unreactive with WGA. A significant fraction of the sialyl residues appears to be *O*-acetylated at C_8 or C_9. The sialic acids appear to be relatively homogeneously distributed on plasmalemma masking most of the galactosyl and *N*-acetyl-galactosaminyl residues of membrane glycoconjugates. After neuraminidase treatment, these residues are exposed and detectable with PNA/LacN-BSA-Au or with galactose oxidase and FH. But even after such treatment, the amount of subterminal Gal and GalNAc moieties visualized by these techniques is relatively low. This is in agreement with the low binding values observed with WGA/M-Au or WGA-F, probably reflecting a reduced density of accessible *N*-acetyl-neuraminic acid. Binding of WGA to aortic endothelium was reported to be low in rat (Hirano and Kawakami, 1984) and scarce in rabbit (Baldwin and Winlove,1984; Baldwin and Chien, 1985). This may be due to some variations in the mechanism and degree of acetylation during sialic acid biosynthesis in different species.

The mannose-containing glycoconjugates are well represented on aortic endothelium both on plasma membrane (coated pits included) and plasmalemmal vesicles, and to slightly lower values on coronary endothelium. The venous endothelium is relatively poor in these components. A similar difference in the Con A binding density was recently reported for the endothelium of arterioles versus venules (Vorobrodt *et al.*, 1986). The RCA 120-detectable glycoconjugates appear to occur in comparable amounts on both arterial and venous endothelium, with rather scarce representation in plasmalemmal vesicles in both types of vessels. Our findings reveal that galactose-terminated glycoconjugates detectable with PNA are poorly represented on the ECS of the aorta, situation similar to that reported by others on rat aorta (Hirano and Kawakami, 1984).

These observations show qualitative and quantitative differences in the amount and distribution of some ECS charged and uncharged glycoconjugates between arterial and venous endothelium, and to a lesser extent between the aortic and coronary ECS. Future studies are required to determine the physiological significance of these regional variations.

Like other blood cells, the cell surface of monocytes display a high net negative charge which in normal conditions secures their electrostatic repulsion from endothelial surface also bearing a strong anionic shield (Fig. 13). Although some differences in the frequency of WGA and RCA binding sites were revealed between monocytes and other blood elements, one cannot speak of a characteristic pattern in the making of their glycocalyx. However, hypercholesterolemia affects more extensively certain components of monocyte surface (see accompanying paper). It was shown that in the human and guinea pig bone marrow, the intensity of Con A binding parallels the maturation in the monocytic cell series being the lowest in premonocytes and highest in macrophages (Ackerman and Freeman, 1979). Based on this, one should expect that the monocyte-derived macrophages of the early intimal lesions would express a higher amount of mannosyl residues than their circulating precursors.

The surface carbohydrates detected on normal arterial endothelium and monocytes may have a critical role in cell metaholism, membrane transport, growth, specific receptor sites, and other cellular and molecular interactions between

vascular endothelium and blood. The extent to which these chemical constituents are affected by experimental hypercholesterolemia is described in the accompanying paper (Ghinea *et al.*, 1987).

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