LECTIN-BINDING SITES ON THE PLASMA MEMBRANES OF RABBIT SPERMATOZOA

Changes in Surface Receptors during Epididymal Maturation and after Ejaculation

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ABSTRACT

Modifications in rabbit sperm plasma membranes during epididymal passage and after ejaculation were investigated by use of three lectins: concanavalin A (Con A); *Ricinus communis* I (RCA_I); and wheat germ agglutinin (WGA). During sperm passage from caput to cauda epididymidis, agglutination by WGA drastically decreased, and agglutination by RCA_I slightly decreased, although agglutination by Con A remained approximately unchanged. After ejaculation, spermatozoa were agglutinated to a similar degree or slightly less by Con A, WGA, and RCA_I, compared to cauda epididymal spermatozoa. Ultrastructural examination of sperm lectin-binding sites with ferritin-lectin conjugates revealed differences in the densities of lectin receptors in various sperm regions, and changes in the same regions during epididymal passage and after ejaculation. Ferritin-RCA_I showed abrupt changes in lectin site densities between acrosomal and postacrosomal regions of sperm heads. The relative amounts of ferritin-RCA_I bound to heads of caput epididymal spermatozoa were greater than those bound to heads of cauda epididymal or ejaculated spermatozoa. Tail regions were labeled by ferritin-RCA_I almost equally on caput and cauda epididymal spermatozoa, but the middle-piece region of ejaculated spermatozoa was slightly more densely labeled than the principal-piece region, and these two regions on ejaculated spermatozoa were labeled less than on caput and cauda epididymal spermatozoa. Ferritin-WGA densely labeled the acrosomal region of caput epididymal spermatozoa, although labeling of cauda epididymal spermatozoa was relatively sparse except in the apical area of the acrosomal region. Ejaculated spermatozoa bound only a few molecules of ferritin-WGA, even at the highest conjugate concentrations used. Caput epididymal, but not cauda epididymal or ejaculated spermatozoa, bound ferritin-WGA in the tail regions. Dramatic differences in labeling densities during epididymal passage and after ejaculation were not found with ferritin-Con A.

KEY WORDS spermatozoa · membrane · lectin · receptor · fertilization · reproduction

Upon leaving the testis, mammalian spermatozoa are incapable of fertilizing ova. However, they progressively gain the ability to fertilize ova during their subsequent passage through the epididymis (7, 44, 45, 58). This phenomenon is generally referred to as "epididymal maturation" of spermatozoa. The epididymis is anatomically divided into three major regions in order of sperm passage: caput (head), corpus (body), and cauda (tail). Spermatozoa isolated from the caput epididymidis are virtually incapable of fertilizing ova, although those isolated from the cauda epididymidis possess full fertilizing capacity similar to that of ejaculated spermatozoa. In the rabbit, spermatozoa begin to acquire the ability to fertilize as they descend to the middle corpus epididymidis (6, 44).

Functional maturation in the epididymis occurs concomitantly with a variety of morphological and biochemical modifications of the spermatozoa. Among the reported modifications are changes in: (*a*) the size, shape, and internal structure of the acrosome (4, 5, 15); (*b*) cohesiveness between the outer acrosomal membrane and overlying sperm plasma membrane (5, 15); (*c*) reactivity of spermatozoa to nigrosin-eosin stain (12, 19, 46) and to iodoacetamide and disulfide-reducing agents (8, 9); (*d*) light-scattering properties (30); (*e*) lipoprotein content (28); (*f*) electrophoretic mobility of spermatozoa (3); and (*g*) migration or loss of the cytoplasmic droplet in the sperm tail (11, 31). Although epididymal maturation of spermatozoa may involve the above morphological, physiological, and biochemical modifications, changes in the properties of the sperm plasma membrane are likely to be important in epididymal maturation of sperm. Direct evidence for biochemical modification of sperm surfaces during epididymal maturation is that certain anionic surface charges, identified by their binding of positively charged colloidal iron hydroxide (CIH)[1] particles, tend to change as maturation progresses (9, 16, 57).

During their passage through the epididymis, spermatozoa are known to be modified by adsorption of materials secreted by the epididymis and vas deferens (2, 23, 24). Some of these adsorbed materials are tightly bound and are not readily removed by washing in physiological saline or by treatment with common organic solvents (51). In addition, components of seminal plasma bind to the surfaces of spermatozoa during ejaculation. Some of these components are immunogenic and have been called "sperm-coating antigen(s)" (10, 14, 51, 52), and others are known proteins such as lactoferrin, an iron-binding protein found in human seminal plasma (22). The mechanism by which these surface-coating substances or antigens are adsorbed onto the sperm surfaces, and the functional significance of these substances in fertilization remain to be determined.

In an investigation of the surface properties of rabbit spermatozoa, we found that *N*-acetyl-D-glucosamine-like or *N*-acetylneuraminic acid-like residues in oligosaccharides recognized by wheat-germ agglutinin (WGA) (13, 21) were modified after contact of spermatozoa with seminal plasma in a manner such that the spermatozoa became less agglutinable by WGA (41). Apparently, the WGA binding sites on rabbit spermatozoa undergo profound modification as a result of contact with seminal plasma. We found that other lectin-binding oligosaccharides, such as those recognized by concanavalin A (Con A) which binds α-D-mannose-like residues (1, 49), and by *Ricinus communis* agglutinin (RCA_I) which binds to β-D-galactose-like residues (36, 37), also change during epididymal maturation and after ejaculation of spermatozoa. Using ferritin-conjugated lectins as molecular probes (method reviewed in reference 35), we report here on distributions and relative densities of lectin-binding sites on various regions of the plasma membranes or spermatozoa isolated from the caput and cauda epididymidis and from semen.

MATERIALS AND METHODS

Inorganic chemicals were obtained from Mallinckrodt Inc. (St. Louis, Mo.), Fisher Scientific Co. (Pittsburgh, Pa.), and Allied Chemical Corp. (Morristown, N. J.). Tris(hydroxymethyl)aminomethane hydrochloride (Tris·

[1] Abbreviations used in this paper: BSA, bovine serum albumin; Con A, concanavalin A; CIH, colloidal iron hydroxide; ferritin-lectin, ferritin-conjugated lectin; ferritin-Con A, ferritin-conjugated Con A; ferritin-RCA_I, ferritin-conjugated *Ricinus communis* agglutinin of 120,000 mol wt; ferritin-WGA, ferritin-conjugated wheat germ agglutinin; NANA, *N*-acetylneuraminic aid; PBS, 0.15 M sodium chloride-0.01 M sodium phosphate, pH 7.4 (300 mosmol); RCA_I, *Ricinus communis* agglutinin of 120,000 mol wt; Tris·HCl, Tris-(hydroxymethyl) aminomethane hydrochloride; TBS, 0.14 M sodium chloride-0.01 M Tris·HCl, pH 7.4 (300 mosmol); WGA, wheat germ agglutinin.

HCl), lactose, and *N*-acetyl-D-glucosamine were products of Sigma Chemical Co. (St. Louis, Mo.). Crystalline bovine serum albumin (BSA) was obtained from Reheis Division of Armour Pharmaceutical Co. (Chicago, Ill.) and α-methyl-D-mannoside from CalBiochem Corp. (San Diego, Calif.). Glutaraldehyde was obtained as an "ultra-pure" 50% solution from Biodynamics Research Corp. (Rockville, Md.) and paraformaldehyde from Electron Microscopy Sciences (Warren, Pa.). Biogel A-1.5m (200–400 mesh) and Biogel P-300 (200–400 mesh) were obtained from Bio-Rad Laboratories (Richmond, Calif.) and Sephadex G-100 and G-150 (fine) from Pharmacia Fine Chemicals, Div. of Pharmacia Inc. (Piscataway, N. J.).

Ferritin-Lectin Conjugates

Ferritin conjugates were synthesized and purified by a modification of previous procedures (38, 40, 43). Lectin was added to purified ferritin (Immuno-Diagnostics, Solana Beach, Calif.) in a ratio of 1:5 wt/wt in 0.2 M sodium chloride, 0.005 M sodium phosphate buffer, pH 7.2, containing 0.2 M D-galactose or 0.2 M *N*-acetyl-D-glucosamine (RCA_I or WGA), or 0.5 M sodium chloride, 0.01 M sodium phosphate buffer, pH 6.5, containing 0.2 M α-methyl-D-mannoside (Con A). The reaction mixture was split into two equal portions, and 10 μl of 1% glutaraldehyde in distilled water was added to one of the samples. 10 min later and at each successive 10 min interval, 10 μl of 1% glutaraldehyde was added to each reaction mixture. When the sample containing the additional 10 μl of glutaraldehyde displayed a slight turbidity under a strong beam of visible light, the reactions in both samples were terminated by addition of 0.25 vol of 1 M glycine in PBS. The samples were centrifuged at 27,000 *g* for 15 min at 4°C, and the small pellets were discarded. The supernates were pooled and centrifuged at 160,000 *g* for 120 min onto a 0.5-ml cushion of Sepharose 4B beads. After removal of the supernates containing unconjugated lectin, the pellet of ferritin and ferritin-lectin was gently resuspended in PBS and purified by affinity chromatography (40, 43). Ferritin-WGA was immediately dialyzed after conjugation with 0.14 M sodium chloride, 0.01 M Tris-HCl, pH 7.4 (TBS), and chromatographed on a 2 × 100-cm column of Bio-Gel A 1.5 m in TBS. Ferritin-lectin conjugate activities were monitored by agglutination of rabbit erythrocytes (40).

Sperm-Labeling Procedures

Ejaculated spermatozoa and spermatozoa from caput and cauda epididymidis of the rabbit were washed twice by centrifugation in TBS containing 0.1% BSA (25°C). Some of the spermatozoa were fixed with 3% formaldehyde in TBS for 15 min at 25°C and then washed twice in TBS containing 0.1 M glycine.

Unfixed and formaldehyde-fixed spermatozoa were incubated in TBS containing 0.1% BSA at 0°C or 25°C with several dilutions of each lectin conjugate at concentrations varying from 0.1 to 4 mg/ml protein. After 7–14 min in the labeling solution, samples were diluted 10-fold in TBS containing 0.1% BSA and washed twice by centrifugation. In addition, control samples contained α-methyl-D-mannoside (ferritin-Con A), lactose (ferritin-RCA_I), or *N*-acetyl-D-glucosamine (ferritin-WGA) in the incubation and washing solutions at final concentrations of 50 mM. After the last wash the spermatozoa were fixed in 2% buffered glutaraldehyde (375 mosmol) for 1 h at 4°C, washed, and then postfixed in 1% osmium tetroxide in PBS for 1 h at 4°C. The osmium-fixed samples were washed and dehydrated in a graded series of acetone before being embedded in Epon. Thin sections were examined either without staining, or after they had been stained with uranyl acetate.

Sperm Agglutination

Spermatozoa were washed twice in TBS containing 0.1% BSA. The washed sperm suspensions (~ 10^8 spermatozoa/ml) were placed in small, round, glass wells containing an equal volume of lectin solution, and the mixtures were agitated for 15–30 min at 25°C. After agitation, the sperm suspensions were microscopically examined, and the agglutination of the spermatozoa into clumps were visually recorded on a qualitative scale from 0 (no agglutination) to 4+ (complete agglutination into a few large sperm clumps) according to the procedures of Nicolson and Yanagimachi (41).

RESULTS

Lectin-Mediated Sperm Agglutination

When ejaculated spermatozoa or spermatozoa from caput and cauda epididymidis were washed and mixed with high concentrations of the lectins, strong agglutination occurred; however, agglutination was prevented by the appropriate saccharide inhibitors (Table I). When lectin-mediated agglutination of caput epididymal spermatozoa was compared with agglutination of ejaculated spermatozoa or cauda epididymal spermatozoa, the caput spermatozoa were consistently more agglutinable with WGA and RCA_I. The most dramatic difference in agglutinability was found with WGA. Caput epididymal spermatozoa were agglutinated strongly by WGA down to low lectin concentrations, and cauda epididymal and ejaculated spermatozoa were agglutinable only at the highest lectin concentrations tested (≥125 μg/ml). Similar but less pronounced differences were found with RCA_I-mediated agglutination. After ejaculation, spermatozoa were agglutinated to a similar degree by WGA and RCA_I, as compared to cauda epididymal spermatozoa. Only a very small differ-

TABLE I

*Agglutination of Rabbit Spermatozoa by Lectins**

Lectin	Inhibitor (50 mM)	Source of spermatozoa	Concentration of lectin, μg/ml‡							
			250	125	60	30	15	8	4	2
Con A	None	Caput ep.	4+	4+	4+	4+	4+	3+	2+	+
	None	Cauda ep.	4+	4+	4+	4+	3+	3+	2+	+
	None	Ejaculate	4+	4+	4+	4+	3+	2+	±	0
	α-methyl-D-mannoside	Cauda ep.	0	0	0	0	0	0	0	0
WGA	None	Caput ep.	4+	4+	4+	4+	4+	3+	+	0
	None	Cauda ep.	2+	+	0	0	0	0	0	0
	None	Ejaculate	+	±	0	0	0	0	0	0
	N-acetyl-D-glucosamine	Cauda ep.	0	0	0	0	0	0	0	0
RCA_I	None	Caput ep.	4+	4+	4+	4+	4+	3+	2+	+
	None	Cauda ep.	4+	4+	4+	3+	2+	+	0	0
	None	Ejaculate	4+	4+	4+	3+	2+	+	0	0
	Lactose	Cauda ep.	0	0	0	0	0	0	0	0

* Spermatozoa were collected from adult, fertile rabbits. Immediately after ejaculation, each animal was sacrificed to obtain sperm from caput and cauda epididymidis. The data shown here were obtained from one animal, and were repeated twice with other animals with essentially the same results. Spermatozoa were agglutinated as described in Materials and Methods.

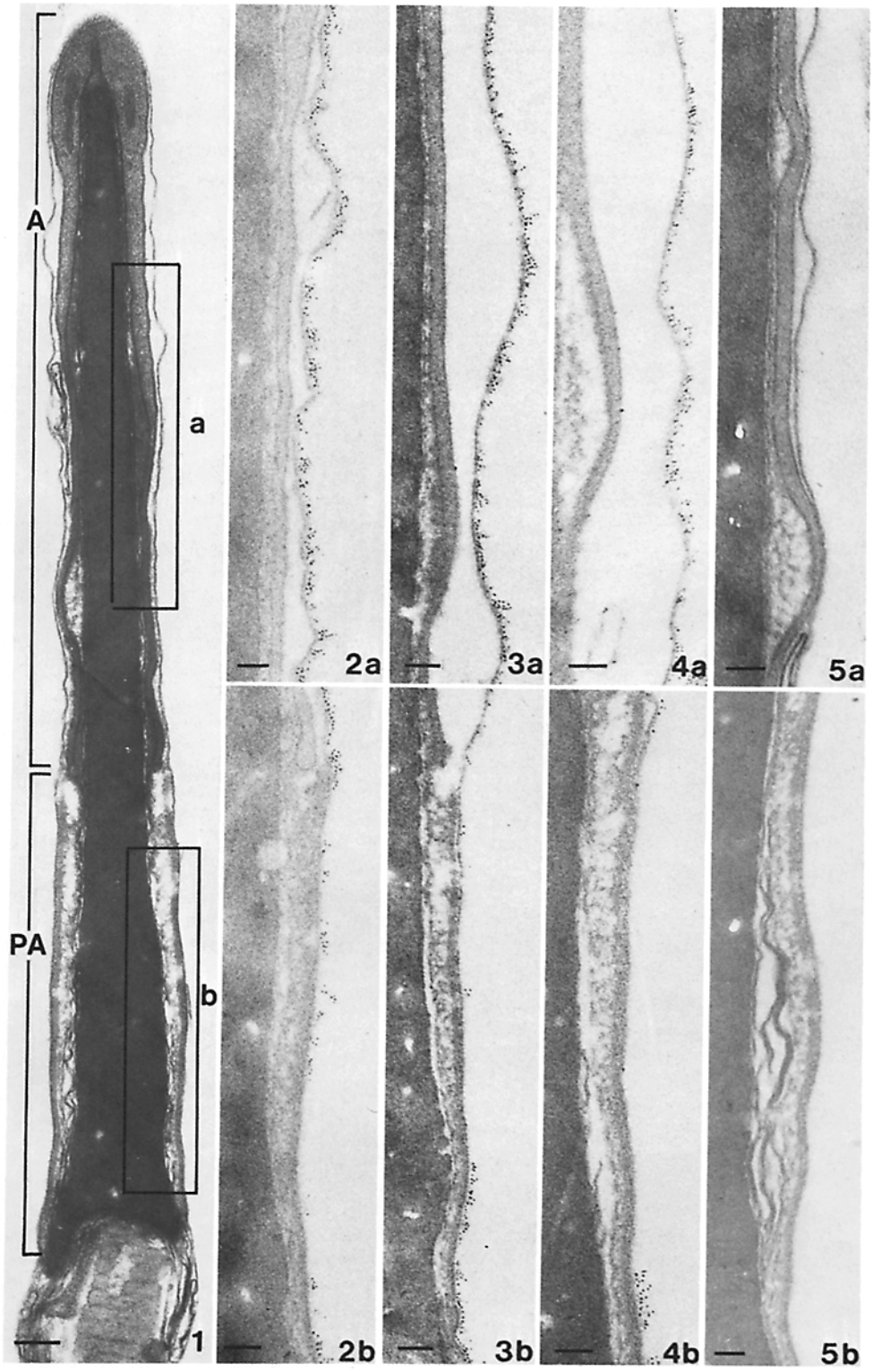
‡ Agglutination score after incubation in lectin solutions for 15 min at 25°C was determined as described (41) on a qualitative scale from 0 (no agglutination; all single spermatozoa) to 4+ (complete agglutination; no single spermatozoa).

ence in Con A-mediated agglutination was found among caput epididymal, cauda epididymal, and ejaculated spermatozoa; the last being slightly less agglutinable compared to epididymal sperm (Table I).

Localization of Lectin-Binding Sites

The rabbit spermatozoon is morphologically divisible into a head (including the acrosomal and postacrosomal regions, cf. Figs. 1, 11) and a tail (including the middle piece and the principal piece, cf. Fig. 6). Labeling of unfixed, washed spermatozoa from the caput epididymidis with ferritin-RCA_I at 25°C resulted in dense, uniform, ferritin-lectin localization in the acrosomal region (Fig. 2*a*), and patchy labeling in the postacrosomal region (Fig. 2*b*). We have previously reported that the patchy distribution of RCA_I receptors in the postacrosomal region of unfixed rabbit spermatozoa labeled at 25°C is probably due to lectin-induced redistribution, and perhaps to some ligand-induced receptor shedding (42). In contrast to these results, unfixed caput epididymal rabbit spermatozoa labeled at 0°C, or formaldehyde-fixed spermatozoa labeled at 25°C, were uniformly labeled with ferritin-RCA_I conjugates (42). Comparisons of the head regions of unfixed spermatozoa isolated from caput epididymidis, cauda epididymidis, and ejaculate (labeled with ferritin-RCA_I under the same conditions at 25°C) revealed distinct differences in ferritin-lectin binding. Head regions of caput epididymal spermatozoa bound relatively more ferritin-lectin than those of cauda epididymal or ejaculated spermatozoa (Figs. 2–4). Ferritin-lectin labeling was specific, because inclusion of an inhibitory saccharide in labeling and wash solutions blocked the binding of the ferritin-lectin conjugate (Fig. 5).

Ferritin-RCA_I also bound to tail regions of unfixed rabbit spermatozoa. On caput and cauda epididymal spermatozoa, both middle piece and principal piece regions were almost equally labeled (Figs. 7, 8), whereas in ejaculated spermatozoa the middle piece region was much more densely labeled than the principal piece region (Fig. 9). Comparisons of spermatozoa from caput epididymidis, cauda epididymidis, and ejaculate showed that binding of ferritin-RCA_I was most prominent in caput epididymal spermatozoa and least prominent in ejaculated spermatozoa (Figs.


A
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7–9), although the differences were not so dramatic as those seen in the head region. Labeling in tail regions was also specific and could be blocked by inhibitory saccharides (Fig. 10).

The relative differences in ferritin-RCA_I binding to unfixed, washed rabbit spermatozoa have been compiled in Table II. The degree of ferritin-lectin labeling with various dilutions of the initial conjugates indicates that the relative differences in ferritin-lectin binding shown in Figs. 2–4 and 7–9 hold for a variety of ferritin-lectin concentrations.

Ferritin-WGA bound well to the acrosomal and postacrosomal regions of caput epididymal spermatozoa (Fig. 12). However, labeling of cauda epididymal spermatozoa was quite sparse (Figs. 13, 14), except for the apical area of the acrosomal region (Fig. 13*c*). Ejaculated spermatozoa bound only a few molecules of ferritin-WGA (Fig. 14). As previously reported, specific binding of ferritin WGA was prevented by inclusion of an inhibitory saccharide, *N*-acetyl-D-glucosamine (Fig. 15). Caput epididymal spermatozoa, but not cauda epididymal or ejaculated spermatozoa, bind some ferritin-WGA in tail regions (Table II).

Ferritin-Con A conjugates bound well to all head and tail regions of the caput and cauda epididymal and ejaculated spermatozoa, which leads us to conclude that modifications in Con A receptors are not drastic during epididymal maturation and after ejaculation of rabbit spermatozoa. The results of several ferritin-Con A labeling experiments have been compiled in Table II.

DISCUSSION

Agglutination by plant lectins has been used to identify saccharide components on the surfaces of mammalian spermatozoa (reviewed in reference 32). Uhlenbruck and Herrmann (50) examined a variety of different lectin-binding sites on ejaculated human spermatozoa before and after treatment with trypsin or neuraminidase. They found that trypsin treatment enhanced *Evonymus europaeus*-mediated agglutination of some sperm samples, and neuraminidase treatment unmasked *Helix pomatia* binding sites on certain specimens. We previously reported that rabbit epididymal spermatozoa were more agglutinable with wheatgerm agglutinin than were ejaculated spermatozoa (41). In the present study, we found that the RCA_I and WGA binding sites on rabbit spermatozoa were modified during the passage of spermatozoa through the epididymis, resulting in decreased lectin agglutinability. Although the total numbers of WGA and Con A binding sites on rabbit cauda epididymal spermatozoa are somewhat similar (39), Con A agglutinates cauda epididymal spermatozoa much more dramatically than WGA (Table I). Discrepancies between the number of lectin-binding sites and the lectin agglutination properties of cells have been documented in a number of diverse systems (32, 34, 35). The factors that control cell agglutination are complex. However, certain properties of lectins and cell surfaces seem to be important in determining the agglutinable state, such as: (*a*) the affinity, valency, size, etc. of the lectin molecules; (*b*) the number, location, density, and mobility of the lectin-binding sites; (*c*) cell-surface properties such as charge density, deformability, and existence of specialized surface structures or components; and (*d*) transmembrane controls exerted on the lectin receptors by peripheral and membrane-associated components (32, 34). We have shown here that the lectin agglutination properties of unfixed, washed rabbit sperma-

FIGURE 1 Morphology of the head regions of a rabbit spermatozoon. *A*, acrosomal region; *PA*, postacrosomal region; *a*, approximate field for Figs. 2*a*–5*a*; *b*, approximate field for Figs. 2*b*–5*b*. Bar equals 0.2 μm; × 33,000.

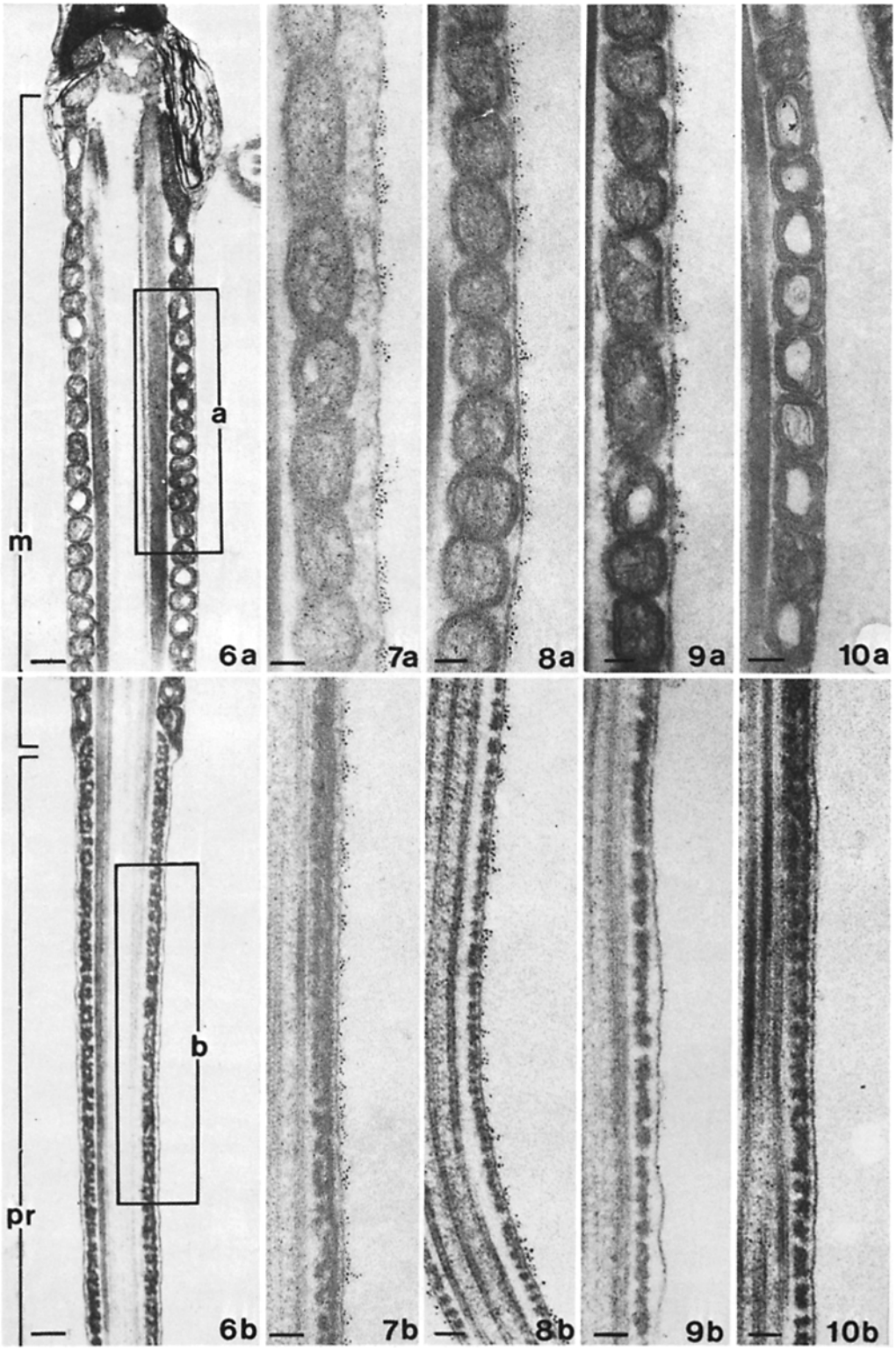
FIGURES 2–5 *a*, acrosomal region; *b*, postacrosomal region. Bars equal 0.1 μm; × 50,000.

FIGURE 2 Unfixed, washed rabbit spermatozoon from caput epididymidis labeled with ferritin-RCA_I at 25°C.

FIGURE 3 Unfixed, washed rabbit spermatozoon from cauda epididymidis labeled with ferritin-RCA_I at 25°C.

FIGURE 4 Unfixed, washed rabbit spermatozoon from ejaculate labeled with ferritin-RCA_I at 25°C.

FIGURE 5 Unfixed, washed rabbit spermatozoon from caput epididymidis labeled with ferritin-RCA_I at 25°C in the presence of 0.1 M lactose.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tozoa generally correlate well with their ability to bind ferritin-lectin molecules, suggesting that a relative loss of certain lectin-binding sites is responsible for diminished agglutinability. The relative losses in sperm lectin reactivity during epididymal passage and after ejaculation seem to be paralleled by increases in surface anionic sites that bind CIH. Bedford et al. (1) found that the binding of CIH at pH 3.0–4.6 to rabbit sperm heads was far greater on spermatozoa isolated from the cauda epididymidis than on those isolated from the caput epididymidis. Yanagimachi et al. (57) noted that CIH binding at pH 1.8 to rabbit sperm tails increased during epididymal passage of spermatozoa, and Flechon (16) has shown that phosphotungstic acid and CIH are bound in more discontinuous distributions on ejaculated sperm compared to sperm isolated from caput or corpus epididymidis. The decreases in RCA_I and WGA binding sites, and increases in low pKa anionic sites on sperm surfaces (1, 57) suggest that residues such as sialic acid or its derivatives might be added to terminal D-galactose or *N*-acetyl-D-glucosamine-like residues, perhaps by glycosyltransferases present in epididymal secretions. In this regard, Fouquet (17) has found that vesicular secretions in the hamster contain high concentrations of sialic acid plus two unidentified sialic acid derivatives. If these derivatives are nucleotide sugars, they might serve as donors in glycosyltransferase reactions (47, 48). Slight changes in the Con A agglutination properties and labeling densities of ferritin-Con A bound to spermatozoa during epididymal passage and after ejaculation were seen, but these were not dramatic. In contrast to our results, Gordon et al. (20) found that the surfaces of washed rabbit caput spermatozoa were not labeled by an indirect Con A-peroxidase technique to visualize Con A-binding sites. The reason for this discrepancy is not clear; it could reflect differences in the washing procedures, labeling techniques, etc. We found that washed caput sperm were highly agglutinable by Con A, indicating the presence of Con A binding sites which were clearly and specifically identified with ferritin-Con A.

Several explanations for the apparent decreases in RCA_I and WGA receptors during epididymal passage and after ejaculation of spermatozoa are possible. For example, degradation of sperm surface glycoproteins and adsorption of coating substance(s) could be involved. Lavon et al. (29) used extraction and gel electrophoresis procedures, and found that several proteins are present in higher quantities in bull caput epididymal spermatozoa than in cauda epididymal spermatozoa. They also noticed that "new," smaller components were products of protein degradation which took place during epididymal passage of the spermatozoa. The most obvious change was noted in sodium hydroxide-extractable proteins which might have been lipoprotein complexes removed from sperm membranes (29). Although the location of the components undergoing epididymal modification was not determined, there is little reason to suspect that they might not be cell-surface components. Antigens present in seminal plasma are known to be adsorbed to rabbit and human spermatozoa (51, 53, 54), and they are not found on testicular and epididymal spermatozoa (51, 55). Baker and Amann (2) were able to detect sperm-coating substances of epididymal origin on ejacu-

FIGURE 6 Morphology of the tail regions of a rabbit spermatozoon. *m*, middle piece; *pr*, principal piece. *a*, section of spermatozoon tail middle piece region; [*a*], approximate field for Figs. 7*a*–10*a*. Bar equals 0.2 μm; × 27,000. *b*, section of a spermatozoon tail principal piece region; [*b*], approximate field for Figs. 7*b*–10*b*. Bar equals 0.2 μm; × 25,000.

FIGURES 7–10 *a*, middle piece region; *b*, principal piece region. Bars equal 0.1 μm; × 50,000.

FIGURE 7 Unfixed, washed rabbit spermatozoon from caput epididymidis labeled with ferritin-RCA_I at 25°C.

FIGURE 8 Unfixed, washed rabbit spermatozoon from cauda epididymidis labeled with ferritin-RCA_I at 25°C.

FIGURE 9 Unfixed, washed rabbit spermatozoon from ejaculate labeled with ferritin-RCA_I at 25°C.

FIGURE 10 Unfixed, washed rabbit spermatozoon from caput epididymidis labeled with ferritin-RCA_I at 25°C in the presence of 0.1 M lactose.

TABLE II

Binding of Ferritin-Conjugated Lectins to the Plasma Membrane of Various Segments of the Rabbit Spermatozoon

Ferritin-conjugated lectin (dilution)	Source of spermatozoa	Binding density of ferritin-lectin to sperm plasma membrane*			
		Head regions		Tail regions	
		Acrosomal	Postacrosomal	Middle piece	Principal piece
RCA_I					
1:2	Caput ep.	++	+	+	+
1:2	Cauda ep.	+ to ++	±	+	+
1:2	Ejaculate	+	±	±	−
1:8	Caput ep.	++	±	+	+
1:8	Cauda ep.	+	±	+	±
1:8	Ejaculate	+	±	±	−
1:16	Caput ep.	++	±	+	+
1:16	Cauda ep.	+	±	±	−
1:16	Ejaculate	±	−	−	−
Con A					
1:2	Caput ep.	++	++	++	++
1:2	Cauda ep.	++	++	+ to ++	±
1:2	Ejaculate	++	++	+	−
1:4	Caput ep.	++	++	+	+
1:4	Cauda ep.	++	++	+	±
1:4	Ejaculate	++	++	+	−
WGA					
1:2	Caput ep.	+ to ++	− to +	+	+
1:2	Cauda ep.	+‡	−	− to +	−
1:2	Ejaculate	±‡	−	−	−
1:8	Caput ep.	+	−	−	+
1:8	Cauda ep.	+‡	−	−	−
1:8	Ejaculate	±‡	−	−	−

* Binding densities of ferritin-lectin conjugates were estimated on a qualitative scale as follows: −, no ferritin-lectin molecules bound (see Fig. 10*a*); ±, sparsely bound ferritin-lectin molecules (see Fig. 13*a*); +, some ferritin-lectin molecules bound (see Fig. 2*b*); ++, dense binding of ferritin-lectin molecules (see Fig. 2*a*). The data shown here were collected from four different males. Sagittal and semisagittal sections of at least 10 spermatozoa were examined from each sample.

‡ Labeled unevenly: relatively dense in the apical area on the acrosomal region.

FIGURE 11 Morphology of the head regions of a rabbit spermatozoon. *A*, acrosomal region; *PA*, postacrosomal region; *a*, approximate field for Figs. 12*a*–15*a*; *b*, approximate field for Figs. 12*b*–15*b*; *c*, approximate field for Fig. 13*c*. Bar equals 0.2 μm; × 33,000.

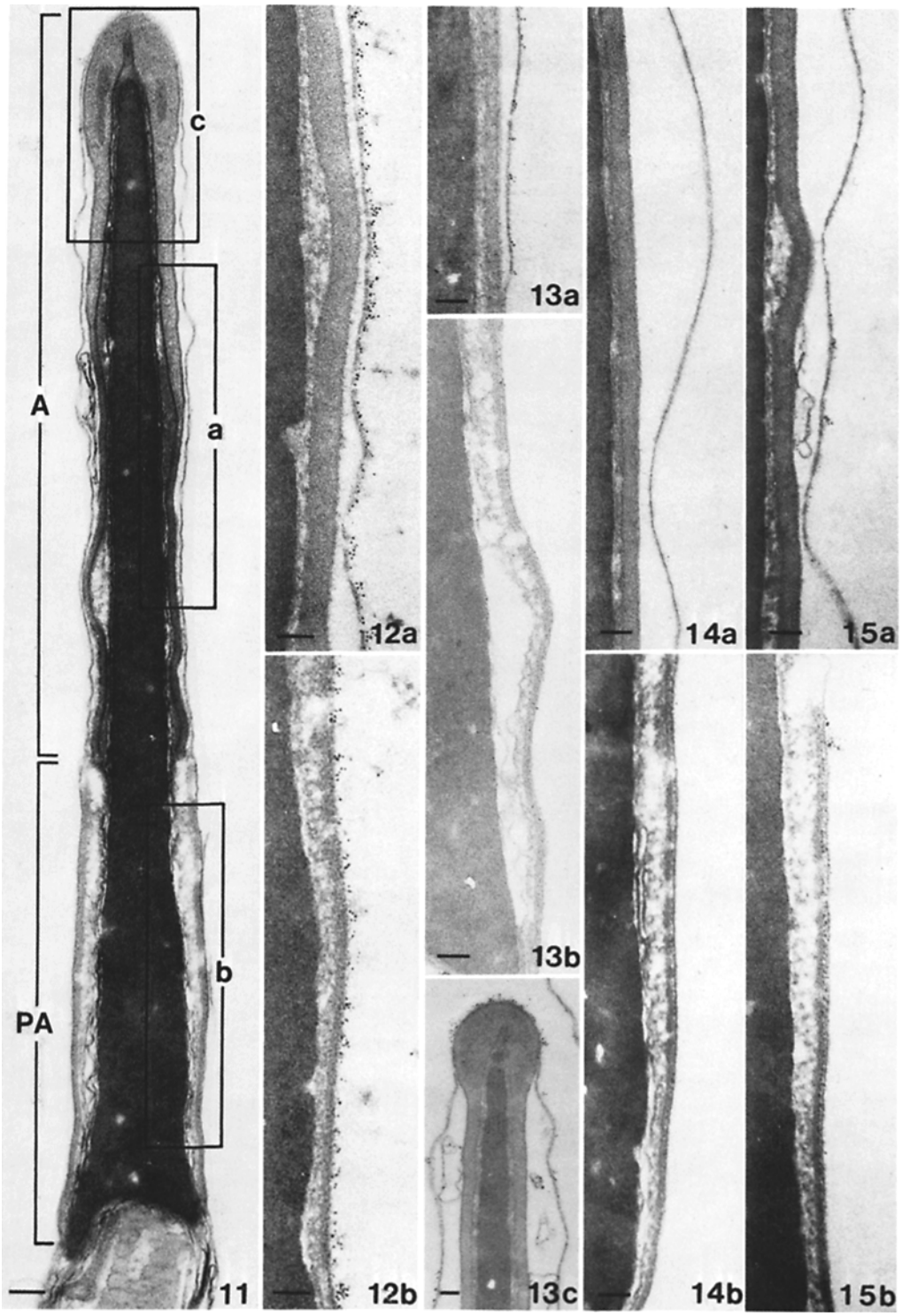
FIGURES 12–15 *a*, acrosomal region; *b*, postacrosomal region; *c*, apical area of acrosomal region. Bars equal 0.1 μm; × 50,000 except for Fig. 13*c* (× 28,000).

FIGURE 12 Unfixed, washed rabbit spermatozoon from caput epididymidis labeled with ferritin-WGA at 25°C.

FIGURE 13 Unfixed, washed rabbit spermatozoon from cauda epididymidis labeled with ferritin-WGA at 25°C.

FIGURE 14 Unfixed, washed rabbit spermatozoon from ejaculate labeled with ferritin-WGA at 25°C.

FIGURE 15 Unfixed, washed rabbit spermatozoon from caput epididymidis labeled with ferritin-WGA at 25°C in the presence of 0.1 M *N*-acetyl-D-glucosamine.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lated bull spermatozoa by using immunodiffusion techniques. Therefore, the possibility exists that such sperm-coating substances could mask lectin-binding sites on sperm surfaces.

Finally, the distributions of certain lectin-binding sites on sperm surfaces were determined. Specific regions of the same sperm head were often labeled differently with the various ferritin-lectin conjugates. For example, ferritin-RCA_I labeling showed abrupt changes in RCA_I-receptor densities and distributions between acrosomal and postacrosomal regions of the sperm head. Also, the plasma membrane surrounding the acrosomal region seems to possess RCA_I binding sites that are relatively less mobile than RCA_I sites in the postacrosomal region (42). The apparent lower mobility of lectin receptors in the acrosomal region of rabbit sperm heads may result from a more rigid plasma membrane in this region due to associative interactions of integral membrane components, or to transmembrane peripheral linkages which can restrain in lateral mobility of membrane components (33). It is interesting that Friend et al. (18) noted that the freeze-fractured plasma membrane in the acrosomal region of guinea pig sperm head exhibits an ordered, lattice-like array of intramembranous particles, and in the postacrosomal region the particles are randomly dispersed. These findings, taken together with our results, suggest that the topographic display and mobility of membrane components are differentially regulated in specific spermatozoa plasma membrane domains. The differential mobility and display of lectin (Results; References 20, 42) and antigenic (25–27) sites could be important in determining the location of sperm-egg interactions and subsequent cell fusion in the postacrosomal sperm region (56).

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