BIOLOGY OF REPRODUCTION 19, 26–35 (1978)

Characterization of the Surface Glycoproteins of Rat Spermatozoa[1]

GARY E. OLSON[2] and DAVID W. HAMILTON[3]

Department of Anatomy, Vanderbilt University[2],
Nashville, Tennessee 37232
and
Department of Anatomy, University of Minnesota[3],
Minneapolis, Minnesota 55455

ABSTRACT

The present study investigates the macromolecular composition of a membrance fraction isolated from rat spermatozoa and uses specific biochemical probes to study the externally oriented plasma membrane glycoproteins of caput and cauda epididymal spermatozoa. A highly purified membrane fraction was isolated from rat cauda epididymal spermatozoa using sonication and differential centrifugation. SDS polyacrylamide gels of the isolated membrane fraction revealed a large number of Coomassie Blue staining bands (>25) and 5 PAS positive bands. The galactose oxidase-[^{3}H] sodium borohydride technique was employed on intact spermatozoa to radioactively label externally oriented plasma membrane glycoproteins possessing terminal galactose or galactosamine residues on their oligosaccharide chains. Cauda epididymal spermatozoa possess a 37,000 dalton glycoprotein on the cell surface which is labelled by this technique, but no such component is detected on caput epididymal spermatozoa. Similarly, radioactive labelling of surface sialo-glycoproteins by sequential treatment of spermatozoa with sodium metaperiodate and [^{3}H] sodium borohydride revealed that the 37,000 dalton glycoprotein could be labeled on cauda epididymal spermatozoa, but is not detected on caput epididymal spermatozoa. The significance of these results with respect to maturation of spermatozoa in the epididymis is discussed.

INTRODUCTION

Mammalian spermatozoa pass through much of the length of the epididymis and undergo a maturing process before they acquire the capacity for forward motility and the ability to fertilize an ovum (for reviews see Orgebin-Crist, et al., 1975; Bedford, 1975). A number of studies indicate that changes in both the structure and macromolecular composition of the spermatozoan plasma membrane occur during the maturation process. Ultrastructural investigations have revealed that, in some species, the appearance and topographical configuration of the spermatozoan plasma membrane are altered during epididymal transit (Bedford, 1965; Bedford and Nicander, 1971; Bedford et al., 1972; Fawcett and Phillips, 1969; Olson and Hamilton, 1976). Several different approaches have yielded information on chemical changes in the sperm plasma membrane during epididymal maturation. For example, immunological studies have demonstrated changes in the antigenic properties of the spermatozoan surface (Hunter, 1969; Johnson and Hunter, 1972; Barker and Amann, 1971; Killian and Amann, 1973). Usually these immunologically detected changes are attributed either to loss of specific sperm-coating antigens or to acquisition of new coating antigens secreted from different regions of the epididymis. Additional evidence on maturational changes in the chemical composition of the sperm surface has been obtained using molecular probes which bind to specific externally disposed plasma membrane carbohydrate residues and can be visualized at the electron microscopic level. Ultrastructural studies indicate changes in the abundance and distribution of lectin binding sites on rabbit spermatozoa during epididymal maturation (Gordon et al., 1974, 1975; Nicolson et al., 1977). The utltrastructural data are further supported by chemical analyses which demonstrate an increase in the abundance of concanavalin-A receptors on the surface of rat spermatozoa during epididymal maturation (Fournier-Delpech et al., 1977). Whole cell electrophoresis studies have demonstrated that intact

Accepted December 20, 1977.
Received October 21, 1977.
[1] Research supported by USPHS grants HD-04290 (DWH), HD-05797 and Biomedical Research Grant RR-05424 to Vanderbilt University.

spermatozoa possess a net negative surface charge (Mudd and Mudd, 1929; Nevo et al., 1961) and that there is a marked increase in negative surface charge as sperm mature (Bedford, 1963). Several ultrastructural investigations have employed the technique of Gasic et al. (1968), in which the binding of positively charged colloidal iron particles to the plasma membrane is employed to assess the abundance of negatively charged residues over the sperm surface. With this technique it has been demonstrated in the rabbit, hamster and human that an increase in the density of plasma membrane anionic residues occurs as sperm pass through the epididymis (Cooper and Bedford, 1971; Bedford et al., 1972; Yanagimachi et al., 1972; Flechon, 1975).

The above studies clearly demonstrate qualitative changes in the composition of the spermatozoan plasma membrane during epididymal maturation. However, relatively little work has been done to identify biochemically specific membrane macromolecules which appear or are altered during sperm maturation. Biochemical procedures which radioactively label specific saccharide residues of externally disposed membrane glycoproteins have been used to investigate membrane architecture in several cell types. The galactose oxidase-tritiated sodium borohydride ($[^3H]NaBH_4$) technique, which relies on the enzymatic oxidation of galactose residues followed by their reduction with $[^3H]NaBH_4$ has been used to identify and characterize externally oriented membrane glycoproteins which possess terminal galactose or galactosamine residues on their carbohydrate moieties (Gahmberg and Hakomori, 1973; Steck and Dawson, 1974). A second probe used on intact cells to characterize membrane sialo-glycoproteins employs sodium metaperiodate to oxidize sialic acid residues and subsequent reduction with $[^3H]NaBH_4$ to radioactively label the oxidized residues (Van Lenten and Ashwell, 1971; Liao et al., 1973, Steck and Dawson, 1974; Gahmberg et al., 1976).

In view of the probable importance which externally oriented components of the spermatozoan plasma membrane play in the fertilization process (for reviews see Austin, 1975; Johnson, 1975), this study was undertaken to characterize the macromolecular composition of the plasma membrane of rat spermatozoa and using specific biochemical probes to characterize the externally oriented membrane glycoproteins of caput and cauda epididymal spermatozoa.

MATERIALS AND METHODS

Mature male albino rats (Charles River Breeding Co., Wilmington, MA) were used in this study. Animals were anesthesized with nembutal or ether. Blood was cleared from the epididymis by perfusing warm phosphate buffered saline (PBS 0.14M NaCl, 0.01M Na_2HPO_4/NaH_2PO_4; pH 7.0) via the spermatic artery. The epididymis was then divided into distinct segments; in the present study only the proximal caput and distal cauda were used. Sperm were obtained by gently mincing the epididymal tubule with a razor blade and squeezing the sperm into the PBS. Sperm were then washed once by centrifugation. (500 × g; 5 min) followed by resuspension in PBS. These sperm were immediately used in the labelling studies or fractionated according to the membrane isolation protocol described below.

Membrane Isolation

Washed cauda epididymal spermatozoa were pelleted by centrifugation for 10 min at 2000 × g. The pellet was resuspended into 10mM pipes buffer [Piperazine-N-N′-bis 2-ethane sulfonic acid, Sigma, St. Louis, Mo] (pH 7.0, 4°C) and sonicated for 3, 5 second bursts at 100 W with a Braun sonicator. The sonicated suspension was centrifuged at 500 × g for 5 min. The supernatant was then centrifuged at 100,000 × g for 30 min. The resulting pellet was either used as a crude membrane fraction or, alternatively, was resuspended in 100mM NaCl, 10mM Tris-HCl; pH 7.0 and layered over a cushion of 55% sucrose, 100mM NaCl, 10mM Tris-HCl; pH 7.0 and centrifuged at 100,000 × g for 30 min. The band at the interface was removed with a pasteur pipette, diluted with buffer (100mM NaCl, 10mM Tris-HCl; pH 7.0) and pelleted at 100,000 × *g* for 60 min. The membrane pellets either were used in one of the labelling protocols or were prepared for electron microscopy.

Labelling of Membrane Proteins

Labelling of sperm membrane proteins with galactose oxidase-$[^3H]NaBH_4$ was basically the procedure described by Gahmberg and Hakomori (1973) and Steck and Dawson (1974). Five to 40 units of galactose oxidase, 1U/ul (Worthington Biochemicals, Freehold, NJ) were added to 1 ml aliquots of the sperm suspension in PBS. When caput and cauda sperm were being compared for reactivity with the surface probes, identical sperm concentrations, as determined by hemacytometer counting, were employed. The galactose oxidase-sperm suspensions were incubated for 30 min at 34°C. In some experiments 1U neuraminidase (Sigma) was added to the reaction mixture. After 30 min, 0.5 mCi $[^3H]NaBH_4$ was added and the sperm suspension was further incubated for 20 min at 34°C. The sperm were then pelleted at 1000 × g for 5 min and the pellet washed 2 times by resuspension in 5 ml PBS followed by centrifugation (1000 × g). The membrane fraction of the sperm pellet was solubilized by detergent

extraction as described below.

Isolated sperm membrane fractions were subjected to the galactose oxidase labelling using the protocol described above. After completion of the labelling protocol, the samples were dialyzed exhaustively against several changes of distilled water at 4°C and freeze-dried.

To label sperm membrane sialic acid residues the procedure of van Lenten and Ashwell (1971) as modified by Gahmberg, et al. (1976) was employed. One ml sperm aliquots were incubated with 20 μl of 0.1M $NaIO_4$ for 15 min at 34°C. The sperm were then pelleted at 500 × g for 5 min and resuspended in 1 ml PBS containing 0.5 mCi [^{3}H] $NaBH_4$ for 20 min at 34°C. The sperm were then pelleted at 1000 × g for 5 min and washed twice by resuspension in 5 ml PBS and centrifugation at 1000 × *g* for 5 min. The membrane fraction of the spermatozoa was solubilized by detergent extraction with either Triton X-100 or sodium dodecyl sulfate (SDS).

For Triton X-100 extraction, spermatozoa were suspended in a solution composed of 1% Triton X-100 (Sigma), 1mM EDTA and 10mM Tris-HC1 pH 8.0. Sperm were extracted at 4°C for 1 h. The suspension was centrifuged at 2000 × g for 10 min and the supernatant used for electrophoresis. In some experiments, cauda epididymal spermatozoa were extracted in an SDS solution composed of 1% SDS, 10% sucrose, 1mM EDTA and 10mM Tris-HC1 (pH 8.0). After extraction for 1 h at room temperature, the suspension was centrifuged at 2000 × g for 10 min to remove nonsolubilized sperm organelles and the supernatant used for electrophoresis.

Affinity Chromatography

Aliquots of the galactose oxidase labeled sperm membrane solubilized in 1% Triton, 100mM NaCl and 10mM Tris HCl pH 7.5 were applied to a 0.7 cm × 14 cm column of agarose-*Ricinus communis* 120 (Miles-Yeda, Elkhart, IN). Elution was begun with a solution of 0.1% Triton, 100mM NaCl, 10mM Tris HCl pH 7.5 and, secondarily, with the same buffer containing 0.1M D-galactose. Two tenths ml fractions of the eluate were collected and monitored for radioactivity.

SDS Polyacrylamide Gel Electrophoresis

SDS polyacrylamide gel elcetrophoresis was performed according to the method of Fairbanks, et al. (1971) with 2 modifications: first, both gel and running buffer contained 0.1% SDS and second, N-N′diallyltartardiamide (BioRad, Richmond, CA) was substituted as the cross linker in equal molar concentrations for N,N′-Methylene-bis-acrylamide. Samples for electrophoresis were handled as follows: membrane pellets or freeze-dried samples were suspended in buffer composed of 1% SDS, 1mM EDTA, 10% sucrose and 10mM Tris-HCl (pH 8.0). Triton extracts of spermatozoa were made 1% in SDS; SDS extracts of spermatozoa were used directly. All samples were made 40mM in dithiothreitol and heated to 100°C for 2 min prior to electrophoresis. Five mm × 13 cm 5.6% acrylamide gels were routinely used and run at 100 V for approximately 2 h. Gels were fixed and stained for protein with Coomassie Blue or, for glycoprotein, with PAS according to the method of Fairbanks, et al. (1971). Gels for scintillation counting were not fixed, but instead were immediately frozen on dry ice and sliced into 1 or 2 mm segments which were then dissolved with 0.5 ml of 2% periodic acid. Molecular weights of the membrane polypeptides were determined by comparing their mobilities to the mobilities of protein standards of known molecular weight (Weber and Osborne, 1969). The presence of Triton X-100 in the samples did not appear to affect the rf values of either membrane proteins or the molecular weight standards.

Electron Microscopy

Sperm and sperm fractions were fixed in 5% glutarldehyde buffered with 0.2 M collidine, pH 7.1, for 2 h at 4°C. Tissue was postfixed in 1% OsO_4 buffered with collidine for 1 h at 4°C. The tissue was then washed 2 times in collidine buffer, dehydrated through an ethanol series and propylene oxide and embedded in Epon 812. Thin sections cut on an LKB-Huxley ultramicrotome were stained with aqueous uranyl acetate and lead citrate and examined in a JEOL 100S electron microscope.

RESULTS

Protein Composition of Sperm Membrane Fractions

The membrane pellets isolated by differential centrifugation from sonicated cauda epididymal spermatozoa are composed of tightly packed, rounded and flattened vesicles (Fig. 1). Very little contamination by other spermatozoon organelles was noted in the membrane pellet. Since no attempt was made to fractionate further this membrane preparation, it is likely that it contains both plasma and acrosomal membranes.

SDS polyacrylamide gels of isolated sperm membranes, stained for protein with Coomassie blue, reveal a number of polypeptide bands; at least 25 bands were easily counted and on overloaded gels, more minor components became visible (Fig. 2). Most of the Coomassie blue stained bands ranged in molecular weight from 11,000–157,000 daltons. A single band is present near the top of the gel and displays a relative mobility slower than the flagellar dyneins which are known to have subunit molecular weights in the 325,000–400,000 dalton range (Gibbons et al., 1976). The predominant membrane polypeptides have molecular weights of 105,000, 77,000, 72,000, 54,000, 43,000, 38,000 and 28,000 daltons (Fig. 2). In PAS stained SDS gels of isolated membrane preparations, 5 bands are noted. The high molecular weight band near the origin of the gel is PAS positive as well as bands of apparent molecular weights of 77,000,

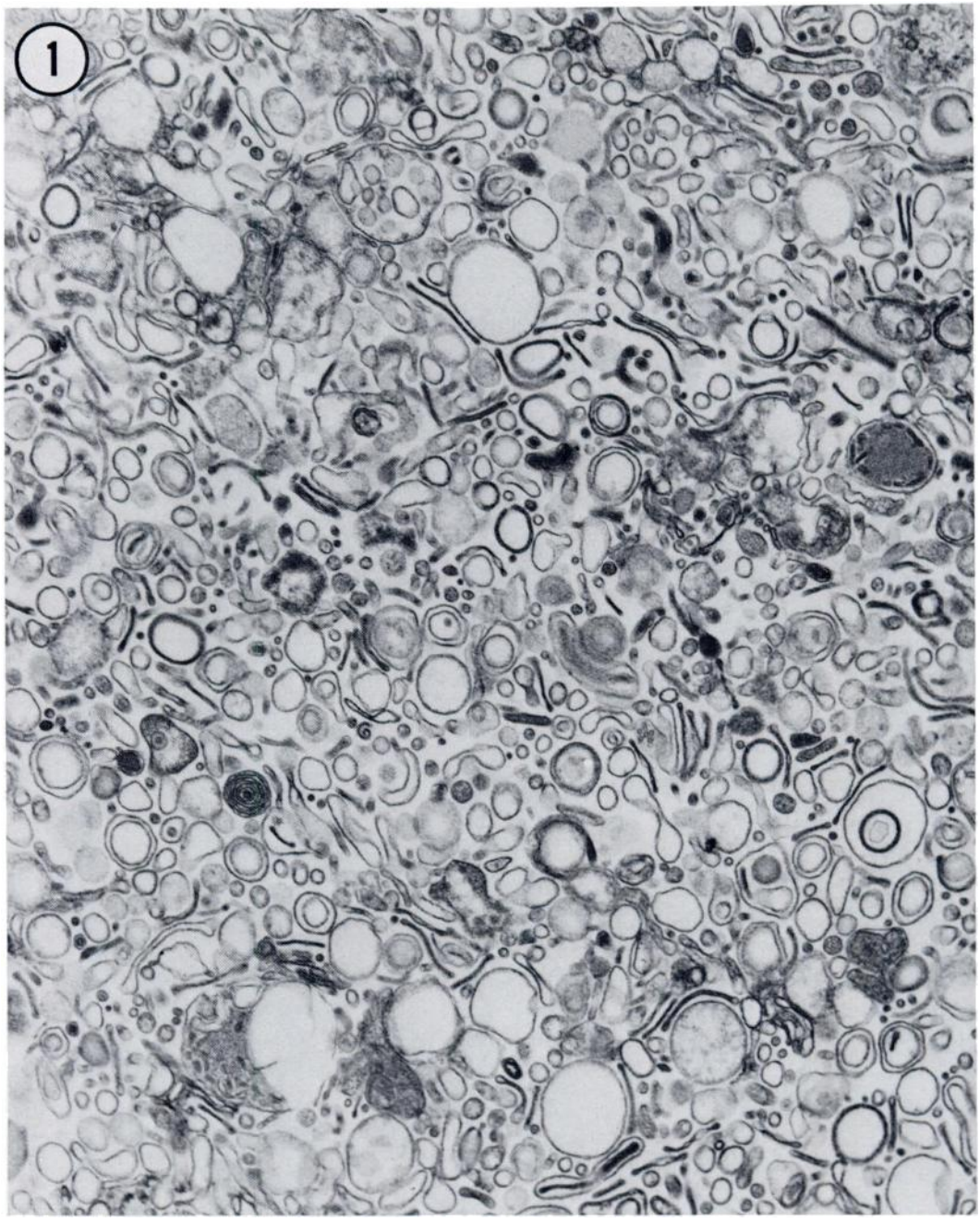

FIG. 1. Electron micrograph of pellet of purified sperm membranes prepared from sonicated cauda epididymal spermatozoa by differential centrifugation. The membranes typically form rounded or flattened vesicles. Little contamination by other spermatozoon organelles is noted.

37,000, 13,000 and 11,000. The most prominent band is the 37,000 dalton component (Fig. 2).

Both Triton X-100 and SDS have been shown to effectively demembranate mammalian spermatozoa (Calvin et al. 1971; Wooding, 1973) and in the current study, we observed that both detergents effectively demembran-

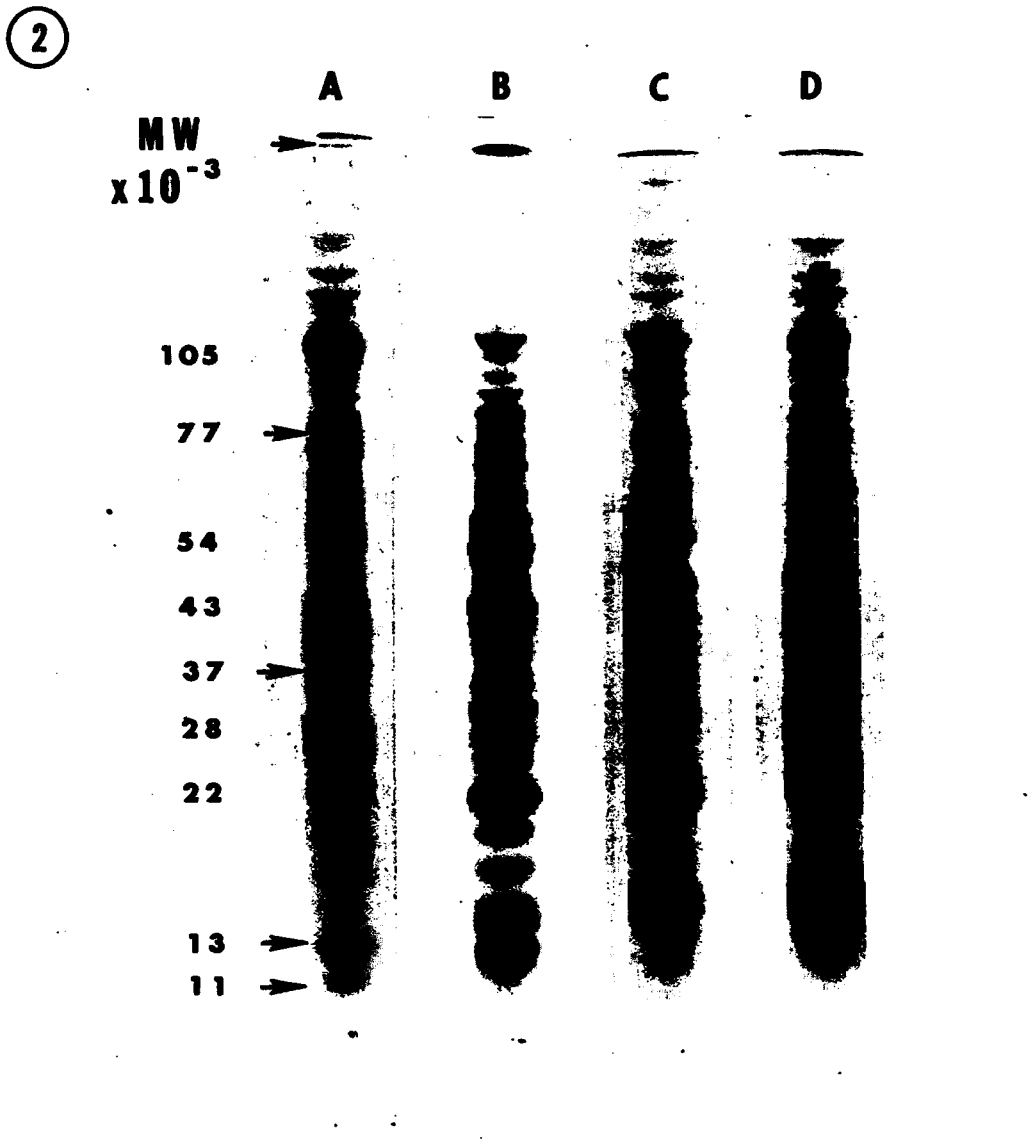

FIG. 2. A series of 5.6% SDS polyacrylamide gels of various membrane functions; in all cases the origin of the gel is toward the top of the page and the tracking dye is toward the bottom.

A. SDS polyacrylamide gel of isolated sperm membrane fraction similar to that shown in Figure 1. A number of Coomassie blue staining bands are present and the molecular weight range of the polypeptides is indicated. The arrows indicate bands which also display PAS stainability.

B. SDS polyacrylamide gel of SDS soluble proteins from cauda epididymal spermatozoa. More bands are present than in gel A, but a number of bands comigrate with those noted in gel A including all PAS positive bands. The extra bands in this gel presumably arise from the solubilization of nonmembrane spermatozoon organelles by SDS.

C. SDS polyacrylamide gel of Triton-100 soluble proteins of cauda epididymal spermatozoa. Many of the bands comigrate with those present in gels of isolated membranes, including the PAS staining components.

D. SDS polyacrylamide gel of Triton X-100 soluble proteins of caput epididymal spermatozoa. Only slight differences in the Coomassie blue banding pattern are noted between this gel and the Triton X-100 extract of cauda epididymal spermaotozoa (gel C).

ated rat spermatozoa. Only Triton X-100 was used to demembranate rat caput epididymal spermatozoa because SDS dissolves most organelles of these spermatozoa (Calvin and Bedford, 1971) and the consequent release of DNA from the sperm nuclei results in a viscous solution which is difficult to utilize for electrophoresis. SDS polyacrylamide gels of the detergent solubule macromolecules from epididymal spermatozoa reveal a number of bands which show relative mobilities exactly similar to the bands observed on gels of isolated membranes including the major membrane polypeptides (Fig. 2). Gels of cauda epididymal SDS-soluble proteins contain several additional bands as compared to gels of cauda spermatozoa Triton X-100 soluble proteins (Fig. 2); these additional bands, of which tubulin from axonemal micutubules is one of the most prominent, are presumably derived from additional organelles solubilized by SDS. The Triton X-100 soluble proteins of caput and cauda epididymal spermatozoa show only minor differences in the electrophoretic distribtion and abundance of the Coomassie blue staining polypeptide bands (Fig. 2).

Radioactive Labelling of Membrane Proteins

Enzymatic labelling of the membrane fraction isolated from cauda epididymal spermatozoa by sequential treatment with galactose oxidase and [^{3}H] $NaBH_4$ resulted in a substantial incorporation of radioactivity into the membrane pellet. SDS polyacrylamide gels of labelled membranes revealed a peak of radioactivity with an apparent molecular weight of 37,000 daltons (Fig. 3). A lesser amount of radioactivity was found migrating close to the tracking dye; the material migrating near the tracking dye is presumed to represent labelled glycolipid since this component partitions into the chloroform phase of a chloroform:methanol (2:1) extraction (unpublished observations). This interpretation is also consistent with previous work on the red cell membrane where this technique leads to enzymatic incorporation of radioactivity into a glycolipid fraction (Gahmberg and Hakomori, 1973; Steck and Dawson, 1974). When galactose oxidase is omitted from the labelling protocol, no incorporation of radioactivity into the 37,000 dalton glycoprotein was noted (Fig. 3).

Galactose oxidase-[^{3}H] $NaBH_4$ labelling of intact spermatozoa also resulted in incorporation of radioactivity into plasma membrane components. At the sperm concentrations used in the current study, the amount of radioactivity incorporated varied linearly with sperm concentration. SDS polyacrylamide gels of the detergent extracts of galactose oxidase labelled spermatozoa revealed the same profile of radioactivity noted in galactose oxidase labelled sperm membranes, in that a single macromolecular peak of 37,000 daltons is present as well as a smaller peak near the tracking dye front (Fig. 4). Concurrent incubation of spermatozoa with galactose oxidase and neuraminidase resulted in stimulated incorporation of radioactivity into the 37,000 dalton peak; at the sperm and enzyme concentrations used, the stimulation was frequently 100% that of controls lacking neuraminidase (Fig. 4). The relative mobility on polyacrylamide gels of the galactose oxidase labelled glycoprotein was slightly retarded in fractions obtained from neuraminidase treated as compared to nonneuraminidase treated spermatozoa (Fig. 4).

Affinity chromatography of the Triton X-100 soluble components of galactose oxidase labelled cauda epididymal spermatozoa on *Ricinus communis* 120-agarose columns revealed a peak of radioactivity which could be eluted with 0.1M D-galactose (Fig. 5). Further analysis of the specifically eluted peak

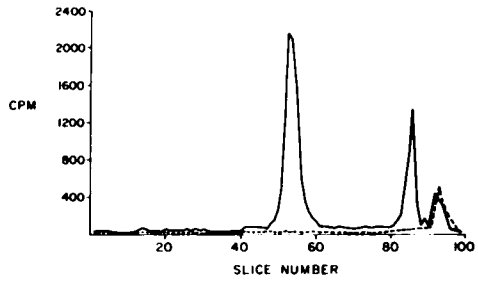

FIG. 3. Graph showing the pattern of radioactivity in sliced SDS polyacrylamide gels of isolated cauda epididymal spermatozoa membranes labeled with galactose oxidase and (^{3}H)$NaBH_4$. The large peak of radioactivity near the center of the gel has apparent molecular weight of 37,000 daltons and corresponds in rf value to the PAS positive component of 37,000 daltons in Fig. 2A. A second peak of radioactivity is present near the tracking dye and presumably represents label incorporation into glycolipid. The dotted line shows the incorporation of radioactivity into a parallel membrane sample incubated without the enzyme galactose oxidase, thereby demonstrating that the incorporation of radioactivity into the major peaks is enzyme dependent.

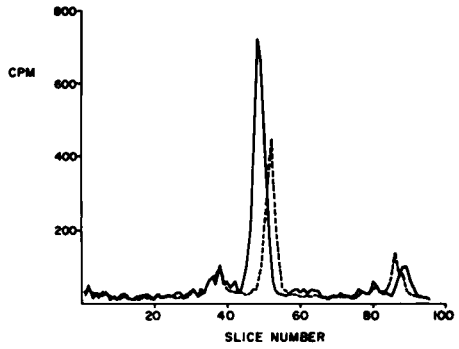

FIG. 4. Graph showing the profile of radioactivity in sliced SDS polyacrylamide gels of galactose oxidase labeled cauda epididymal spermatozoa. Both lines represent equivalent sperm numbers. In the case of the solid line 1 unit of neuraminidase was added to 1 ml of the galactose oxidase-sperm suspension during the course of the labeling protocol, whereas the dotted line is from a parallel incubation lacking neuroaminidase. It is evident that neuraminidase treatment stimulates galactose oxidase dependent incorporation of radioactivity into the 37,000 dalton glycoprotein and also that after neuraminidase treatment this polypeptide shows retarded mobility on SDS polyacrylamide gels.

on SDS polyacrylamide gels revealed it consisted entirely of the 37,000 dalton glycoprotein (Fig. 5).

A comparison of the pattern of radioactivity of SDS gels of detergent extracts of galactose oxidase labelled caput and cauda spermatozoa revealed a striking difference. Whereas cauda epididymal spermatozoa revealed a peak of radioactivity at 37,000 daltons, as noted above, no such peak was present in detergent extracts of caput epididymal spermatozoa (Fig. 6).

Labelling of membrane sialo-glycoproteins was accomplished by sequential treatment of intact spermatozoa with sodium metaperiodate and [^{3}H] $NaBH_4$. SDS gels of the detergent extracts of cauda epididymal spermatozoa revealed a pattern of radioactivity similar to the galactose oxidase labelled cauda spermatozoa. A labelled peak of 37,000 daltons is present, as well as a somewhat larger peak which migrates near the tracking dye. (Fig. 7). Caput spermatozoa labelled with $NaIO_4$ and [^{3}H] $NaBH_4$ display no radioactive peak of 37,000 daltons (Fig. 7).

DISCUSSION

Previous histochemical and immunocytochemical studies have identified qualitative

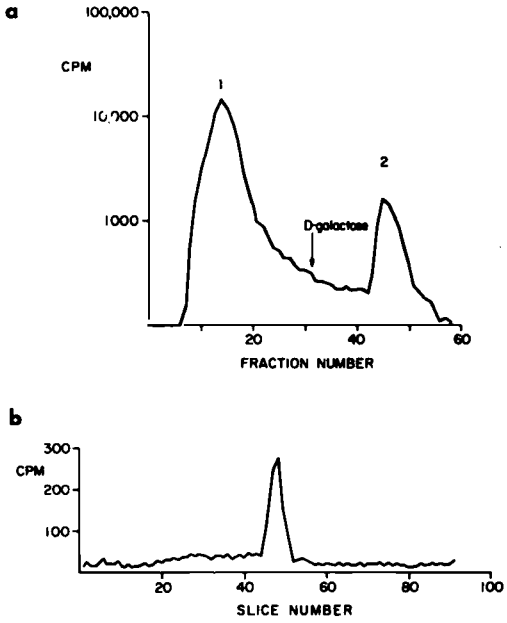

FIG. 5A. Profile of radioactivity eluted from *Ricinus communis* 120-agarose affinity column. Triton soluble proteins of galactose oxidase labeled cauda epididymal spermatozoa were applied to the column and elution was begun with 0.1% Triton X-100, 100mM NaCl, 10mM Tris-HCl, pH 7.5. At fraction 31 elution was begun with the same buffer containing 0.1M D-galactose. Peak 1 consists of free borohydride and glycolipid. The peak eluted in the presence of galactose was run on SDS polyacrylamide gels.

B. SDS polyacrylamide gel showing profile of radioactivity in peak 2 of the affinity column in 5A. All the radioactivity is present in the 37,000 dalton peak.

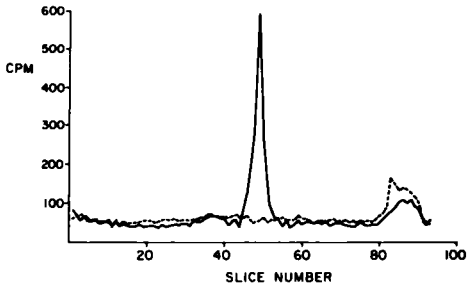

FIG. 6. SDS polyacrylamide gels showing the pattern of radioactivity in galactose oxidase labeled cauda epididymal spermatozoa (solid line) and caput epididymal spermatozoa (dotted line). Detergent extracts from equal numbers of spermatozoa were added to both gels and the obvious difference is the presence of the 37,000 dalton labeled glycoprotein in cauda epididymal spermatozoa and its absence in caput epididymal spermatozoa.

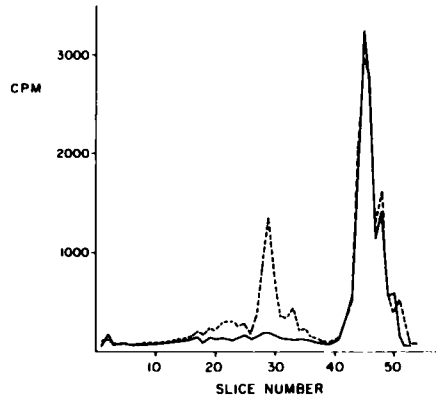

FIG. 7. SDS polyacrylamide gels showing the pattern of radioactivity incorporation into sodium metaperiodate-(^{3}H)$NaBH_4$ labeled cauda epididymal spermatozoa (dotted line) and caput epididymal spermatozoa (solid line). Detergent extracts obtained from equal numbers of caput and cauda spermatozoa were added to both gels. The 37,000 dalton glycoprotein is labeled in cauda spermatozoa, but is not detectable in caput epididymal spermatozoa.

changes in the composition of the spermatozoan plasma membrane which occur during epididymal maturation (for reviews see Bedford, 1975; Johnson, 1975). The present study shows that biochemical probes of known substrate specificity can be used to identify maturational changes in the macromolecular architecture of the rat spermatozoan plasma membrane. The enzymatic probe galactose oxidase has been used in earlier studies to determine the topography of the plasma membrane of erythrocytes and other cell types (Gahmberg and Hakomori, 1973; Steck and Dawson, 1974; Gahmberg et al., 1976). The enzyme is impermeable to intact cells and thus only reacts with the plasma membrane glycoproteins or glycolipids which are exposed to the external environments. Moreover, it reacts specifically with molecules possessing terminal galactose or galactosamine residues (Morell et al., 1966; Steck and Dawson, 1974). The results presented in this study show that cauda epididymal rat spermatozoa possess an externally disposed membrane glycoprotein of 37,000 daltons which is labeled by galactose oxidase-[^{3}H] $NaBH_4$ treatment, while no such reactive component is found on the surface of caput epididymal spermatozoa. However, both cauda and caput sperm appear to contain membrane glycolipid residues which are substrates for the galactose oxidase probe.

The second surface probe we employed in this study has been shown in earlier work to preferentially label sialic acid residues of glycoproteins and glycolipids (Liao et al., 1973; van Lenten and Ashwell, 1971). In this study cauda epididymal rat spermatozoa are shown to contain one externally oriented surface glycoprotein of 37,000 daltons which is labelled by sodium metaperiodate-[^{3}H] $NaBH_4$ and, as with the galactose oxidase probe, this component was not detected on caput epididymal spermatozoa.

The galactose oxidase reactive glycoprotein and the sodium metaperiodate reactive glycoprotein of cauda epididymal spermatozoa appear to be the same glycoprotein. Both peaks comigrate on SDS polyacrylamide gels, having the same apparent molecular weight of 37,000 daltons. The absolute molecular weight of the reactive glycoprotein remains to be determined, since it is known that heavily glycosylated polypeptides may show anomalous rf values on SDS gels (Segrest et al., 1971).

Seondly, neuraminidase treatment, which removes terminal sialic acid residues from glycoproteins, enhances the galactose oxidase dependent labelling of the 37,000 dalton surface glycoprotein of cauda epididymal spermatozoa suggesting that this glycoprotein also possesses terminal sialic acid residues on its oligo-saccharide chains. Similar enhancement of galactose oxidase reactivity by neuraminidase has been reported for the glycoproteins of erythrocyte and lymphoid cell membranes as well as soluble glycoproteins. The apparent explanation for this is that removal of terminal sialic acid residues exposes penultimate galactose moieties which can then serve as substrates for the galactose oxidase probe (Morell et al., 1966; Gahmberg and Hakomori, 1973; Gahmberg et al., 1976).

Finally, the finding that neuraminidase affects the relative mobility on SDS gels of the galactose oxidase labelled glycoprotein indicates that this glycoprotein also possesses sialic acid residues. If the 37,000 dalton sperm surface glycoprotein, which labels with both galactose oxidase and sodium metaperiodate, is in fact a homogeneous component rather than being composed of 2 or more glycoproteins which comigrate on SDS gels, then the above data indicate that either more than 1 oligosaccharide chain or a branched oligosaccharide chain is attached to the polypeptide backbone.

The source of the newly appearing surface component as well as its localization over the sperm surface are important unanswered problems. Cytochemical studies have shown that the sperm surface is a mosaic with respect to the topographical distribution of specific saccharide residues (Cooper and Bedford, 1971; Bedford et al., 1972; Gordon et al., 1974, 1975; Kinsey and Koehler, 1976; Nicolson et al., 1977; Yanagimachi et al., 1972). It is possible, therefore, that the 37,000 dalton glycoprotein on rat cauda epididymal spermatozoa is localized to a restricted region of the sperm surface. Obviously, this surface glycoprotein could arise from several potential sources and we will mention those that appear most likley. First, the protein could be secreted by the epididymal epithelium and subsequently bind to the sperm surface, becoming in effect a coating protein. This suggestion would be compatible with numerous immunological studies which suggest the occurance of this phenomenon (Hunter, 1969; Johnson and Hunter, 1972; Barker and Amann, 1971; Killian and Amann, 1973). A second possibility to explain the appearance of the externally disposed sperm surface protein is that some unmasking or modification event (e.g. glycosylation) occurs to make a membrane component accessible or reactive to the probes used in this study. A third possibility is that the spermatozoa can synthesize new membrane proteins as they proceed through the epididymis, but in view of the low rates of protein synthesis exhibited by mammalian spermatozoa, this is somewhat unlikely. Another possibility we suggest is that the protein is stored within the spermatozoon and that at some point during maturation a specific triggering event causes it to be inserted into the lipid bilayer so that its sugar containing residues are exposed to the extracellular space. Experiments are in progress to discriminate among these different possibilities.

The functional role of this membrane glycoprotein remains to be demonstrated. Its presence correlates with the completion of the maturation process, since it is in the proximal cauda epididymidis that rat spermatozoa attain their maximum level of fertilizing capacity (Dyson and Orgebin-Crist, 1973). It may be that these new membrane proteins play a role in the development of fertilizing capacity and/or (depending on its distribution over the sperm surface) play a role in determining the functional specificity of restricted regions of the sperm surface.

REFERENCES

Austin, C. R. (1975). Membrane fusion events in fertilization. J. Reprod. Fert. 44, 155–166.

Barker, L. D. S. and Amann, R. P. (1971). Epididymal physiology II. Immunofluorescent analyses of epithelial secretion and absorption and of bovine sperm maturation. J. Reprod. Fert. 26, 319–332.

Bedford, J. M. (1963). Changes in the electrophoretic properties of the rabbit spermatozoa during passage through the epididymis. Nature 200, 1178–1180.

Bedford, J. M. (1965). Changes in the fine structure of the rabbit sperm head during passage through the epididymis. J. Anat. 99, 891–906.

Bedford, J. M. and Nicander, L. (1971). Ultrastructural changes in the acrosome and sperm membranes during maturation of spermatozoa in the testis and epididymis of the rabbit and monkey. J. Anat. 108, 527–543.

Bedford, J. M., Cooper, G. W. and Calvin, H. I. (1972). Post-meiotic changes in the nucleus and membranes of mammalian spermatozoa. In: Proc. Int. Symp. The Genetics of the Spermatozoan. (R. A. Beatty and S. Gluecksohn-Waelsch, eds.). pp. 69–89.

Bedford, J. M. (1975). Maturation, transport and fate of spermatozoa in the epididymis. In: Handbook of Physiology, Sec. 7: Endocrinology, Vol. V: Male reproductive system. (D. W. Hamilton and R. O. Greep, eds.). American Physiological Society, Bethesda, MD. 1975. pp. 303–317.

Calvin, H. I. and Bedford, J. M. (1971). Formation of disulfide bonds in the nucleus and accessory structures of mammalian spermatozoa during maturation in the epididymis. J. Reprod. Fert. Suppl. 13, 65–76.

Cooper, G. W. and Bedford, J. M. (1971). Acquisition of surface charge by the plasma membrane of mammalian spermatozoa during epididymal maturation. Anat. Rec. 169, 300–301.

Dyson, A. L. M. B. and Orgebin-Crist, M. C. (1973). Effect of hypophysectomy, castration, and androgen replacement upon the fertilizing ability of rat epididymal spermatozoa. Endocrinology 93, 391–402.

Fairbanks, G., Steck, T. L. and Wallach, D. F. H. (1971). Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry 10, 2606–2617.

Fawcett, D. W. and Phillips, D. M. (1969). Observations on the release of spermatozoa and on changes in the head during passage through the epididymis. J. Reprod. Fert. Suppl 6, 405–418.

Flechon, J. E. (1975). Ultrastructural and cytochemical modifications of rabbit spermatozoa during epididymal transport. In: The Biology of Spermatozoa. Transport, Survival, and Fertilizing Ability. (E. E. E. Hafez and C. G. Thibault, eds.). Karger, Basel. pp. 36–45.

Fournier-Delpech, S., Danzo, B. J. and Orgebin-Crist, M. C. (1977). Extraction of Concanavalin-A affinity material from rat testicular and epididy-

mal spermatozoa. Ann. Biol. Anim. Bioch. Biophys. 17, 207–213.

Gahmberg, C. G. and Hakimori, S. (1973). External labelling of cell surface galactose and galactosamine in glycolipid and glycoprotein of human erythrocytes. J. Biol. Chem. 248, 4311–4317.

Gahmberg, C. G., Hayry, P. and Andersson, L. C. (1976). Characterization of surface glycoproteins of mouse lymphoid cells. J. Cell. Biol. 68, 642–653.

Gasic, G. J., Berwick, L. and Sorrentino, M. (1968). Positive and negative colloidal iron as cell surface electron stains. Lab. Invest. 18, 63–71.

Gibbons, I. R., Frank, E., Gibbons, B. H. and Ogawa, K. (1976). Multiple forms of dynein in sea urchin sperm flagella. Cold Spring Harbor Conferences on Cell Proliferation, V. 3. Cell Motility Book C Microtubules and Related Proteins. (R. Goldman, T. Pollard and J. Rosenbaum, eds.). pp. 915–932.

Gordon, M., Dandekar, P. V. and Bartoszewicz, W. (1974). Ultrastructural localization of surface receptors for Concanavalin-A on rabbit spermatozoa. J. Reprod. Fert. 36, 211–214.

Gordon, M., Dandekar, P. V. and Bartoszewicz, W. (1975). The surface coat of epididymal, ejaculated and capacitated sperm. J. Ultrastruct. Res. 50, 199–207.

Hunter, A. G. (1969). Differentiation of rabbit sperm antigens from those of seminal plasma. J. Reprod. Fert. 20, 413–418.

Johnson, M. H. (1975). The macromolecular organization of membranes and its bearing on events leading up to fertilization. J. Reprod. Fert. 44, 167–184.

Johnson, W. L. and Hunter, A. G. (1972). Immunofluorescent evaluation of the male rabbit reproductive tract for sites of secretion and absorption of seminal antigens. Biol. Reprod. 6, 13–22.

Killian, G. J. and Amann, R. P. (1973). Immunoelectrophoretic characterization of fluid and sperm entering and leaving the bovine epididymis. Biol. Reprod. 9, 489–499.

Kinsey, W. H. and Koehler, J. K. (1976). Fine structural localization of Concanavalin-A binding sites on hamster spermatozoa. J. Supramol. Struct. 5, 185–198.

Liao, T. H., Gallop, P. M. and Blumenfeld, O. O. (1973). Modification of sialyl residues of sialoglycoprotein(s) of the human erythrocyte membrane. J. Biol. Chem. 248, 8247–8253.

Morell, A. G., Van Den Hamer, C. J. A. and Scheinberg, I. H. (1966). Physical and chemical studies on ceruloplasmin. IV. Preparation of radioactive, sialic acid-free ceruloplasmin labeled with tritium on terminal d-galactose residues. J. Biol. Chem. 241, 3745–3749.

Mudd, S. and Mudd, E. B. H. (1929). The specificity of mammalian spermatozoa with especial reference to electrophoresis of serological differentiation. J. Immunol. 17, 39–52.

Nevo, A. C., Michaeli, I. and Schindler, H. (1961). Electrophoretic properties of bull and rabbit spermatozoa. Exp. Cell Res. 23, 69–83.

Nicolson, G. L., Usui, N., Yanagimachi, R., Yanagimachi, H. and Smith, J. R. (1977). Lectin-binding sites on the plasma membranes of rabbit spermatozoa. Changes in surface receptors during epididymal maturation and after ejaculation. J. Cell Biol. 74, 950–962.

Olson, G. E. and Hamilton, D. W. (1976). Morphological changes in the midpiece of wooly opossom spermatozoa during epididymal transit. Anat. Rec., 186, 387–404.

Orgebin-Crist, M. C., Danzo, B. J. and Davies, J. (1975). Endocrine control of the development and maintenance of sperm fertilizing ability in the epididymis. In: Handbook of Physiology, Sect. 7: Endocrinology, Vol. V: Male Reproductive System. (D. W. Hamilton and R. O. Greep, eds.). American Physiological Society, Bethesda, MD. pp. 319–333.

Segrest, J. P., Jackson, R. L. Andreus, E. P. and Marchesi, V. T. (1971). Human erythrocyte membrane glycoprotein: a re-evaluation of the molecular weight as determined by SDS polyacrylamide gel electrophoresis. Biochem. Biophys. Res. Comm. 44, 390–395.

Steck, T. L. and Dawson, G. (1974). Topographical distribution of complex carbohydrates in the erythrocyte membrane. J. Biol. Chem. 249, 2135–2142.

Van Lenten, L., and Ashwell, G. (1971). Studies on the chemical and enzymatic modification of glycoproteins. J. Biol. Chem. 246, 1889–1894.

Weber, K. and Osborn, M. (1969). The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244, 4406–4412.

Wooding, F. B. P. (1973). The effect of Triton X-100 on the ultrastructure of ejaculated bovine sperm. J. Ultrastruct. Res. 42, 502–516.

Yanagimachi, R., Noda, Y. D., Fujimoto, M. and Nicolson, G. L. (1972). The distribution of negative surface charges on mammalian spermatozoa. Am. J. Anat. 135, 497–520.