The Cercarial Glycocalyx of *Schistosoma mansoni*

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**ABSTRACT** Cercariae, the freshwater stage of *Schistosoma mansoni* infectious to man, are covered by a single unit membrane and an immunogenic glycocalyx. When cercariae penetrate the host skin, they transform to schistosomula by shedding tails, secreting mucous and enzymes, and forming microvilli over their surface. Here the loss of the glycocalyx from cercariae transforming in vitro was studied morphologically and biochemically. By scanning electron microscopy, the glycocalyx was a dense mesh composed of 15-30 nm fibrils that obscured spines on the cercarial surface. The glycocalyx was absent on organisms fixed without osmium and was partially lost when parasites aggregated in their own secretions before fixation. By transmission electron microscopy, a 1-2-μm thick mesh of 8-15-nm fibrils was seen on parasites incubated with anti-*schistosomal* antibodies or fixed in aldehydes containing tannic acid or ruthenium red. Cercariae transformed to schistosomula when tails were removed mechanically and parasites were incubated in saline. Within 5 min of transformation, organisms synchronously formed microvilli which elongated to 3-5 μm by 20 min and then were shed. However, considerable fibrillar material remained adherent to the double unit membrane surface of schistosomula. For biochemical labeling, parasites were treated with eserine sulfate, which blocked cercarial swimming, secretion, infectivity, and transformation to schistosomula. Material labeled by periodate oxidation and NaB₃H₄ was on the surface as shown by autoradiography and had an apparent molecular weight of >10⁶ by chromatography. Periodate-NaB₃H₄ glycocalyx had an isoelectric point of 5.0 ± 0.4 and was precipitable with anti-*schistosomal* antibodies. More than 60% of the radiolabeled glycocalyx was released into the medium by transforming parasites in 3 h and was recovered as high molecular weight material. Parasites labeled with periodate and fluorescein-thiosemicarbazide and then transformed had a corona of fluorescence containing microvilli, much of which was shed onto the slide. Material on cercariae labeled by iodogen-catalyzed iodination was also of high molecular weight and was antigenic. In conclusion, the cercarial glycocalyx appears to be composed of acidic high molecular weight fibrils which are antigenic and incompletely cleared during transformation.

The helminth parasite *Schistosoma mansoni* undergoes a series of adaptive changes as it moves from its intermediate snail host to man. Cercariae are shed from the snail into fresh water, where they swim until they find a host. Cercariae are composed of a body (CB) and a tail, each ~120 μm long and 25 μm in diameter. The CB contains excretory, nervous, and digestive systems, and large secretory glands, all of which are surrounded by two muscle layers (54). The outermost layer of the parasite is a syncytium called the tegument, which is connected to cell bodies beneath the musculature (20). The cercarial tegument is bounded by a single unit membrane with a surface area of 20,000 μm² over the CB (43), and a thick, periodic acid Schiff-positive glycocalyx (24, 53).

Cercariae that penetrate a host undergo a set of changes called transformation (54). The tail is lost and the secretory glands release both a mucus, which promotes attachment to the skin, and enzymes, which degrade the skin (17, 54). In addition, transient microvilli appear on the surface and a double unit membrane is formed on the tegument (20). At the same time, some of the glycocalyx is lost (20, 53). Transformation is complete in ~3 h. The parasites are called schistosomula. Transformation can also be produced in vitro when parasites penetrate excised skin (56) or when tails are removed mechanically and CBs are incubated in physiological media (6, 38). The surface of cercariae and schistosomula is immunolog-
ically important. The cercarial glyocalyx binds antibodies from humans and laboratory animals infected with *S. mansoni* (25, 26, 51, 55, 59). The surfaces of both cercariae (8) and newly transformed schistosomula (11, 39) activate complement by the alternative pathway. In vitro, schistosomula are killed by human eosinophils and rodent granulocytes in antibody-dependent reactions during the first 24–48 h after transformation (3, 10, 36). Later, the schistosomula become resistant to antibody-mediated killing (10, 32, 39). The composition and amount of antigenic glyocalyx retained after transformation are, therefore, of major interest.

The lack of biochemical data on the cercarial glyocalyx is due to difficulties in handling the parasite. Cercariae swim at a rate of 2 mm/s and so resist centrifugation. Furthermore, the parasites secrete mucus and aggregate, so they cannot be easily separated (24, 38, 55). Therefore, in the labeling studies performed here, cercarial swimming and secretion were inhibited with eserine sulfate (ES1; reference 1). In addition, ES reversibly inhibits transformation.

The cercarial glyocalyx was studied morphologically and biochemically. First, we examined the glyocalyx by scanning microscopy to allow comparison with previous transmission microscopy observations and to follow the structure through transformation. Second, we labeled immobilized cercariae with sodium metaperiodate (Per) and NaB3H4 (15, 43) and determined the distribution of the label by light microscopic autoradiography (LMARG). The molecular weight and isoelectric point of the radiolabeled material were also determined, as was its loss during transformation. Third, we examined transformation by fluorescence microscopy by labeling the glyocalyx first with periodate then with fluorescein-thiosemicarbazide (FITSC; reference 16). Alternatively, the glyocalyx was labeled with fluorescein anti-schistosomal antibodies (4), or the new schistosomal membrane was labeled with fluorescent concanavalin A (FICOnA; reference 45).

**MATERIALS AND METHODS**

Reagents: Artificial pond water (APW) contained 50 mg CaCl2, 120 mg MgSO4, 15 mg FeCl3, and 4 mg K2SO4 in 1 liter distilled water, buffered with 20 mM HEPES, pH 7.23. Phosphate-buffered saline (PBS) contained 0.01 M sodium phosphate, pH 7.2, and 0.14 M NaCl. Hank's balanced salt solution, Earle's minimal essential medium, RPMI-1640, and Earle's lactalbumin were purchased from M.A. Bioproducts (Walkersville, MD), and buffered with 10 mM HEPES, pH 7.2. Per, ES, serotonin, RNAse, DNAse, and EDTA were purchased from Sigma Chemical Co. (St. Louis, MO); Iodogen from Pierce Chemical Co. (Rockford, IL); NaB3H4 (6-10 Ci/mM) and Na125I (15 Ci/mg) from Amersham Corp. (Arlington Heights, IL); FITSC from Molecular Probes with 10 mM HEPES, pH 7.2. Per, ES, serotonin, RNASe, DNASe, and EDTA from Pharmacia Fine Chemicals (Piscataway, NJ); polyacrylamide and Bio-Rad Laboratories (Richmond, CA); and Bio-Fluor from Zwittergent detergent Lyre gels from Bio-Rad Laboratories (Richmond, CA); and Bio-Fluor from Schwartz/Mann (Spring Valley, NY): Zwittergent detergent and newly transformed schistosomula (11, 39) activate complement to schistosomula in 300 s RPMI for 1–3 h, and organisms were separated from the medium by centrifugation. For chromatography, SDS or urea was added directly to the medium.

**Autoradiography:** LMARG and grain counts to localize radioactivity on Per-NaB3H4- and Na125I-labeled parasites were performed by use of NTB2 emulsion as previously described (43).

**SDS PAGE:** Parasites were boiled for 2 min in 1% SDS, 2%/β-mercaptoethanol, and 0.01 M Tris, pH 6.8, and were electrophoresed in 13-cm tubes containing 5% acrylamide in the running gels and 3% acrylamide in the stack (28). One samples were solubilized and electrophoresed on aqua-bisulfate-buffered SDS (60). Gels were sliced and counted in Bio-Fluor as previously described (43). We estimated the efficiency of counting by measuring external standard ratios and comparing with quenched tritium standards.

**Column Chromatography:** Per-NaB3H4-labeled parasites were solubilized in 150 µl of buffer containing 4 M guanidine, 1% Twizztergent, and 0.1 M Tris, pH 7.0. Samples were chromatographed on a 100 × 6.0 cm column of Sepharose CL-2B at a flow rate of 2 ml/min (14). Per-FITSC-labeled organisms were also chromatographed and fractions were measured for fluorescein with a fluorometer.

**Immune Precipitation:** The IgG fraction of serum from a rabbit hyperimmunized to *S. mansoni* (IRS) by repeated injections of schistosome homogenate in Freund's adjuvant was purified and provided by Dr. Donald Hart, Harvard Medical School. Per-NaB3H4- and Iodogen-Na125I-labeled parasites were solubilized in PBS containing 0.1% NP40 and incubated for 60 min at room temperature with protein A-Sepharose, which was precoated with IRS or with normal rabbit IgG (NRS) as a control (27). We washed the Sepharose and eluted the precipitated radioactivity by boiling it in 1% SDS.

**Isoelectric Focusing:** 2,000–10,000 Per-NaB3H4-labeled organisms were incubated in 1% NP40, 50 µg/ml RNAse, and 1 mg/ml DNAse for 10 min at 4°C, spun to remove insoluble material, and 8 M urea, 2% β-mercaptoethanol, and 2% Amphotelys were added (35). 20-µl samples were focused on a 11-cm flatbed isolectric focusing gel containing 8 M urea, 1% NP40, 4% Amphotelys, and 4% Bio-Lyte for 4,000–8,000 V h (37). 1-cm samples were cut out, incubated with 1 ml distilled water, and then counted in a scintillation counter or measured with a pH meter. Alternatively, samples were focused on a 100-ml column containing urea, Amphotelys, NP40, and a gradient of sucrose from 0–20%.

**Fluorescence Microscopy and Photometry:** Parasites were labeled with Per-FITSC, rhodamine-conjugated IRS (4), or FICOnA (45). Fluorescent parasites were observed with a Leitz Orthoplan microscope equipped with a Plom illumination system for fluorescein or rhodamine, and Smith-T interference-contrast optics (E. Leitz, Inc., Rockleigh, NJ). Parasites were photographed with Tri-X film at 1600 ASA. The fluorescence of a 200-µm2 area of ES-treated parasites labeled with FITSC was measured with a Leitz MPV Compact photometer attached to the fluorescence microscope (45).

**Scanning Microscopy:** Parasites were fixed for 20 min on ice with 2% glutaraldehyde and 2% osmium tetroxide in 0.1 M sodium phosphate, pH 7.
7 (19). Alternatively, organisms were fixed for 30 min on ice with 1% formaldehyde and 3% glutaraldehyde in 0.1 M cacodylate, pH 7.4 (22), with or without postfixation in 1% osmium on ice for 60 min. Parasites were then dehydrated in graded alcohols, critical-point dried, and sputter-coated (44).

**Transmission Microscopy:** Parasites were fixed in mixed aldehydes for 30 min on ice (22), postfixed in osmium for 90 min on ice, stained en bloc with uranyl acetate for 2 h at room temperature, dehydrated, and embedded (50). Some parasites were preincubated with 1 mg/ml IRS or NRS for 1 h at room temperature, and washed four times before fixation in mixed aldehydes (4). Alternatively, parasites were fixed in glutaraldehyde containing ruthenium red (30) or tannic acid with or without 0.5 mg/ml saponin (34), and postfixed with osmium. Thin sections were cut with a diamond knife and stained on grid with 4% uranyl acetate for 5 min at room temperature and lead citrate for 1 min (41). Scanning and transmission samples were examined with a JEOL 100C-ASID electron microscope.

**Repetition of Experiments:** Each experiment was repeated at least three times.

**RESULTS**

**Cercarial Transformation**

More than 90% of cercariae synchronously transformed to schistosomula when tails were removed and organisms were placed into PBS, Hank's balanced salt solution, Earle's minimum essential medium, or RPMI, whereas only 10 ± 6% of tailed organisms transformed. CBs were inhibited from transforming by APW, 1 mM ES, or 10 mM EDTA. However, 80 ± 5% of CBs transformed when ES was removed. In 1 mM serotonin, 59 ± 16% of CBs transformed. Therefore, in the studies below, CBs were used to observe the ultrastructure of transformation and parasites were immobilized with ES for biochemical labeling.

**Microscopy of the Cercarial Surface**

The surfaces of whole cercariae and of tailless CB look the same, so they are described together. The surface of cercariae in APW also looked the same when fixed with glutaraldehyde and osmium or with mixed aldehydes and then postfixed with osmium. The glycocalyx appeared as a meshwork of 15-30-nm fibrils that covers the spines (Figs. 1-3) except on the anterior tip of the parasites. When osmium was omitted during fixation, the meshwork was absent, and naked spines and pits 0.2-0.4 μm in diameter were seen on the parasite body (Fig. 4). Parasites that were aggregated by their glandular secretions (Fig. 5) had less glycocalyx than either unaggregated parasites or organisms in which secretion was blocked with serotonin. Cercarial tails had a fibrillar mesh identical to that seen on the body, but the tips of spines were often visible. However, in the absence of osmium, no pits were seen on the tail.

As shown by transmission microscopy, the cercarial glyocalyx is a 1-2-μm-thick fibrillar mesh covering the entire tegument of organisms preincubated in IRS and fixed with aldehydes and osmium (Figs. 6 and 7). Organisms fixed without preincubation in IRS, or after preincubation in NRS, did not have a visible glyocalyx (Fig. 8). The glyocalyx also appeared as a mesh of 8-15-nm fibrils on parasites fixed in aldehydes containing either tannic acid (Fig. 9) or ruthenium red (Fig. 10). The cercarial tegument was covered by a single unit membrane beneath the glyocalyx (Figs. 9 and 10).

**Scanning and Transmission Microscopy of Cercarial Transformation to Schistosomula**

Microvilli covered the entire surface of transforming CBs (Fig. 11). 5 min after the start of transformation (Fig. 12), microvilli barely projected through the dense glyocalyx. After 10 min (Fig. 13) and 20 min (Fig. 14), the microvilli were 1-2 μm and 3-5 μm long, respectively. There were 2.0 ± 1.2 microvilli/μm² surface area, and the density remained constant over time. Fibribs of glyocalyx extended between microvilli, some of which were covered by 15-30-nm spheres (Figs. 13 and 14). After 40-60 min, microvilli were lost from much of the surface of transforming CBs (Fig. 15). However, the glyocalyx still covered spines on the middle and posterior of CBs. 3 h after transformation, spines projected through fibrillar material on the surface of mechanically and skin-prepared schistosomula (Fig. 16). Tails in RPMI did not form microvilli and did not shed their glyocalyx. Transforming parasites fixed without osmium had no glyocalyx on spines and microvilli (Fig. 17).

By transmission microscopy, microvilli were 100-150 nm in diameter, were bound by a single unit membrane, and had a thick coat of glyocalyx (Figs. 18-20). Simultaneous with microvilli formation, multilaminate vesicles entered the tegumental cytoplasm from cell bodies beneath the muscle and discharged onto the parasite surface (Figs. 18 and 19). The tegument was covered in part by a pentalaminar, double unit membrane at 10-30 min (Fig. 21). A double membrane covered the entire surface at 60-120 min (Fig. 22). Residual glyocalyx overlay the double membrane and varied in thickness from <0.1-0.5 μm (Fig. 22).

**Labeling with Periodate and Trinitated Borohydride**

Per-NaB₃H₄-treated cercariae incorporated 25 times the radioactivity of unoxidized controls. The amount of radioactivity on cercariae, 52 ± 15 dpm/organism (mean ± SD), was equivalent to ~3 x 10⁹ NaB₃H₄-reduced molecules/organism or 1.5 x 10⁵ molecules/μm² area of the parasite surface. LMARG showed that on Per-NaB₃H₄-labeled cercariae the surface grain density was 34 times that of the interior (Table I). Surface grains accounted for 92% of the total grains on Per-NaB₃H₄ cercariae. In contrast, on unoxidized organisms, surface grain density approximated that of the interior and accounted for only 21% of the total radioactivity. By scanning and transmission microscopy, there was no alteration in the glyocalyx or the surface membrane after Per-NaB₃H₄ labeling.

On reduced SDS PAGE, most Per-NaB₃H₄-labeled material on cercariae and CBs was retained in the stack (Fig. 23). This high molecular material was not labeled on organisms treated with NaB₃H₄ alone and was precipitated by IRS but not by NRS (Fig. 24). On Sepharose 2B, 70-90% of the radioactivity on cercariae eluted in the void volume (V₀) with a broad shoulder extending to Rₜ 0.5, whereas the remaining label eluted with the total volume (Vₜ; Fig. 25). Label on isolated tails eluted with an Rₜ of 0.2-0.3, which was slightly behind material from CB. On the same column, dextran of 2 x 10⁶ mol wt had a peak with an Rₜ of 0.5-0.6. Radioactivity on unoxidized organisms eluted entirely in Vₜ. Per-NaB₃H₄ material had an isoelectric point of 5.0 ± 0.4 (Fig. 26).

**Loss of Per⁻³H-Labeled Material from Transforming CBs**

CBs lost 43 ± 13 and 63 ± 4% of the Per-NaB₃H₄-labeled material when they transformed for 1 and 3 h, respectively. LMARG of radiolabeled CBs showed that 45% of the surface radioactivity was lost when organisms transform for 1 h (Table
FIGURES 1–5 Scanning micrographs of cercariae and CBs. Glycocalyx covers the surface of a CB so that spines are barely seen on the acetabulum (A) and on the middle body (M, Fig. 1). A ciliated nerve ending (n) projects through the glycocalyx. Over the middle (Fig. 2) and posterior (Fig. 3) CB, the glycocalyx is a fibrillar mesh, composed of 15–30-nm fibrils and spheres (circles). The glycocalyx is absent on a cercaria fixed without osmium (Fig. 4) so that naked spines (s) and pits (p) 0.2 μm in diameter are seen. After aggregation in their secretions (Fig. 5), parasites also lose glycocalyx so that spines are visible. (Fig. 1) × 5,000. (Figs. 2, 4, and 5) × 25,000. (Fig. 3) × 50,000.

There was no increase in radioactivity on the interior of parasites, which suggests that surface material was shed. On SDS PAGE, 77 ± 16% of the radioactivity in the pellet of organisms was retained in the stack, whereas the remaining material ran with the front. Similarly, 97 ± 3% of the radioactivity released into the medium by cultured CBs was retained in the stack of SDS PAGE. On Sepharose 2B, 66 ± 6% of radioactivity eluting at $V_R - R_0$ 0.5 remained with pelleted CBs transforming for 1 h, whereas 34% was found in the medium. After 3 h, 33 ± 6% of the radioactivity at $V_R - R_0$ 0.5 remained with the pellet and 67% was recovered in the medium (Fig. 27). At the same time, radioactivity eluting at $V_i$ accounted for only 16 ± 10% of the total radioactivity (Fig. 27), which suggests that there had been no degradation of
FIGURES 6–10  Transmission micrographs of the surface of cercariae. The cercarial tegument (t) is a 0.2–1.0-μm thick syncytium that overlies extracellular matrix (ecm), muscle (mu), and tegumental cell bodies (tcb) filled with membranous vesicles (Fig. 6). The tegument itself contains spines (s) and mitochondria (mi, Fig. 7). The glycocalyx (g) appears as a 1–2-μm-thick fibrillar mesh when it is stained with IRS (Figs. 6 and 7), tannic acid (Fig. 9), or ruthenium red (Fig. 10). The glycocalyx is poorly visualized when fixed initially with mixed aldehydes alone (Fig. 8). The surface of cercariae and nontransforming CBs is bound by a single unit membrane (arrowheads, Figs. 9 and 10). (Fig. 6) × 21,000. (Figs. 7 and 8) × 58,000. (Figs. 9 and 10) × 100,000.

high molecular weight material. In contrast, 91 ± 5% of the Per-NaB₃H₄-labeled material was retained by CBs incubated in RPMI plus ES, which inhibited transformation. Less than 8% of the radioactivity eluting at $V_{G-R}$ 0.5 was released into the medium, and radioactivity at $V_1$ was ~13% of the total radioactivity.

Labeling with Iodogen-Na¹²⁵I

Cercariae treated with Iodogen-Na¹²⁵I bound 50 times the radioactivity of organisms treated with Na¹²⁵I alone. On LMARG, both surface and internal grain density on Iodogen-Na¹²⁵I organisms were greatly increased as compared with
controls with Na\textsuperscript{125}I alone. More than 80% of the incorpo-
rated radioactivity was extractable with chloroform and meth-
anol (2:1) and was presumably bound to lipid. Most of the re-
mainder Iodogen-Na\textsuperscript{125}I-labeled material was retained in
the stack of reduced SDS PAGE (Fig. 23). No radioactivity
was found in the stack when organisms were treated with
Na\textsuperscript{125}I only. The high molecular weight material was speci-
cally precipitated by IRS (Fig. 24), and 78 ± 3% of this
material was released into the medium by CB transformed for
1 h.

**Light Microscopic Studies**

Cercariae oxidized with periodate and then treated with
FITSC bound 10 ± 3 x 10^7 molecules fluorescein/organism, or
~5 x 10^7 molecules/μm\(^2\) surface area. Unoxidized parasites
bound only 4% of the FITSC bound to Per-treated organisms.
On the Sepharose column, 10--25% of the FITSC eluted with
V\textsubscript{0} and the rest with V\textsubscript{e}. This may indicate instability of
the linkage between the sugar and fluorochrome over time as
seen in other systems (40). Fluorescein was seen diffusely over
the surface of the body and tail of cercariae, and the spines
appeared black against the bright background (Fig. 28). The
amount of fluorescein on the body varied and was greatest
posteriorly (Fig. 28). The anterior body emitted 46 ± 12% the
fluorescence of the posterior body, the middle body 75 ± 12%,
and the tail 55 ± 16%.

CBs lost 46 ± 8% of the FITSC when they transformed for
1 h. On 85 ± 10% of the parasites, a fluorescent corona extended 3--4 μm from the surface (Fig. 29). Within the corona,
microvilli projected perpendicularly from the parasite
surface (Fig. 30). Motile, transforming CBs deposited fluores-
cent patches, that contain material visible by interference-
contrast microscopy (Figs. 31 and 32).

Rhodamine-conjugated IRS-labeled CBs also formed a flu-
orescent corona on their surface during transformation (Fig.
33). FICOn A did not bind to the cercarial glycocalyx but only
to the anterior tip of the parasite, where gland ducts open to
the exterior. When CBs transformed for 1 h, FICOn A bound in
either fashion to CBs, with more fluorescence at the head than at the posterior body (Fig. 34). After 3 h, FICOn A bound to the entire surface of the schistosomula (4).

**DISCUSSION**

The cercarial glycocalyx is a 1--2-μm thick mesh of 15--30 nm
fibrils that envelops the organism. Radiolabeled and detergent
solubilized glycocalyx has a molecular weight in the millions
and an isoelectric point of 5, and is antigenic. The meshwork is
partially removed by cercarial secretions and by shedding
of the cercarial membrane via microvilli during transforma-
tion to schistosomula. However, several hours after transfor-
mation, fibrillar material 0.1--0.5-μm thick overlies the double
membrane surface of schistosomula and organisms retain
>30% of the radiolabeled glycocalyx.

**Intact Cercarial Glycocalyx**

The glycocalyx is a labile structure, which is difficult to
demonstrate by scanning microscopy. Previous studies have
failed to visualize it because of the omission of osmium
together (7, 42, 58), or perhaps because of the extensive
loss of glycocalyx that occurs when cercariae secrete and
aggregate (33). The lability of the coat extends also to trans-
migration microscopy, where we failed to visualize the glyco-
calyx with conventional fixatives. However, others, using
higher aldehyde concentrations, have seen a fibrillar coat
similar to that shown in Figs. 6--10 (20, 24, 48). In addition,
the glycocalyx is consistently demonstrated when stained by
ruthenium red, tannic acid, or antibody (25, 26), and by
techniques that depend upon periodate oxidation (24, 52, 53).
These staining properties suggest that the glycocalyx may be
a complex carbohydrate such as a proteoglycan or a lipopol-
saccharide. The glycocalyx covers the spines, as shown by
transmission microscopy, but the fibrils are 8--15 nm rather
than 15--30 nm as seen by scanning. This difference is prob-
due to the coat of gold-palladium on scanning samples.

The high molecular weight material labeled by Per-NaB\textsubscript{3}H\textsubscript{4}
is most likely the cercarial glycocalyx, First, LMARG localized
radioactivity to the parasite surface. Second, Per-FITSC fluo-
rescence was greater on the posterior body than the anterior
body or tail, which is the same distribution as the fibrillar
mesh seen by scanning microscopy. Third, IRS, which binds
specifically to the glycocalyx, precipitated Per-NaB\textsubscript{3}H\textsubscript{4}
labeled material. Fourth, the mesh, the radiolabel, and FITSC were
all lost during transformation. Finally, Iodogen-Na\textsuperscript{125}I labeled
similar high molecular weight material on cercariae, which
suggests that this is the major moiety on the parasite surface.
The radiolabeled glycocalyx had a molecular weight of >10^6
and an isoelectric point of 5. Because the glycocalyx is labeled
by Per-NaB\textsubscript{3}H\textsubscript{4}, and by iodination, it may contain sialic acid
(15) and tyrosine, histidine, or lipid (21, 31).

**Loss of the Glycocalyx with Transformation**

During transformation, the cercarial membrane is lost through
the formation and shedding of microvilli, as has been
previously suggested (20, 33). Microvilli synchronously arose
and elongated when tails are lost and CBs were placed in
saline. By the time microvilli were shed at 40--60 min, they
reached an average length of ~4 μm. Since the villous diam-
eter was ~0.15 μm and there were 2 villi per μm\(^2\) surface
areas, ~4 μm\(^2\) membrane/μm\(^2\) surface was shed. This suggests
that some new membrane as well as the original cercarial
membrane was incorporated into the microvilli. The single
membrane removed by the loss of the microvilli is replaced by
a double membrane system transported to the tegument in
multilamellar bodies from the syncytial cell bodies beneath
the muscle (20, 61). In the first hour after transformation,
both single and double membranes are present on the tegu-
ment surface (20).

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**Figures 11--17** Scanning micrographs of CB transforming to schistosomula. Within 10 min in RPMI, CBs form microvilli over
their head (H) and middle body (M, Fig. 11). Microvilli (m) are short at 5 min (Fig. 12), and elongate to 2--3-μm at 10 min (Fig. 13)
and 3--5 μm at 20 min (Fig. 14). Microvilli are at first an apex for the attachment glycocalyx fibrils (arrowheads, Figs. 12 and 13)
but appear later to separate the glycocalyx (arrowheads, Fig. 14). Long microvilli are covered by 15--30-nm spheres (circles, Fig.
14). Microvilli are absent after 40--60 min (Fig. 15), although much glycocalyx remains and obscures spines. 3 h after transfor-
mation, schistosomula still have fibrillar material in areas between spines (Fig. 16). Without osmification, the spines (s) and microvilli are
naked (Fig. 17). (Fig. 11) × 6,500. Figs. 12--17 are all of the posterior body; x 25,000.
FIGURES 18–22  Transmission micrographs of transforming CBs. The surface of a CB transforming for 30 min is covered with microvilli (m, Fig. 18). Within the tegument (t) are spines (s) and multilaminate vesicles (mv), which discharge onto the tegumental surface (arrowhead, Fig. 19). Microvilli are 0.1 μm in diameter, covered with a dense coat of glycocalyx (g), and bound by a single unit membrane (Fig. 20). After 30 min, areas of single unit membrane (arrows, Fig. 21) alternate with areas of double unit membrane (arrowheads, Fig. 21) on the tegumental surface. After 60 min, fibrillar glycocalyx is still present on the double unit membrane, which covers the parasite surface (Fig. 22). CBs in Figs. 18, 19, and 22 are stained with antibody, in Figs. 20 and 21 with tannic acid. (Fig. 18) × 50,000. (Fig. 19) × 80,000. (Fig. 20) × 103,000. (Fig. 21) × 140,000. (Fig. 22) × 180,000.
TABLE I

Light Microscopic Autoradiography of Per-labeled Cercariae

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Grain density</th>
<th>S/I</th>
<th>Absolute No. S/I</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB NaB(^{3})H(_{4}) only</td>
<td>1.8 ± 0.4</td>
<td>1.3 ± 0.5</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>CB Per-NaB(^{3})H(_{4}) Intact</td>
<td>1.7 ± 0.4</td>
<td>16.1 ± 5.7</td>
<td>9.6 ± 2.6</td>
</tr>
<tr>
<td>Transformed</td>
<td>1.3 ± 0.3</td>
<td>8.9 ± 2.9</td>
<td>6.5 ± 2.0</td>
</tr>
<tr>
<td>Cercariae Per- NaB(^{3})H(_{4})</td>
<td>1.3 ± 0.3</td>
<td>22.7 ± 7.2</td>
<td>34.0 ± 13</td>
</tr>
</tbody>
</table>

Grain density = No. of grains per 100 \(\mu\)m\(^2\). Values represent the mean ± SD of 30-50 organisms for each condition. \(P\) values show the significance (Student's t-test) of differences between values of Per-NaB\(^{3}\)H\(_{4}\)-labeled organisms and organisms treated with NaB\(^{3}\)H\(_{4}\) alone. CBs were transformed for 1 h. For LMARG, CBs were labeled in the cold and so may have lost some of their glyocalyx when they aggregated in their own secretions. The density of radioactivity on the surface of CBs (16.1) is, therefore, somewhat less than that on cercariae labeled in the presence of ES (22.7).

![Figure 23](link-to-image)

The loss of the glyocalyx is a complex process that appears to involve at least three mechanisms. First, the mesh on the surface of cercariae becomes thinner when parasites in APW aggregate in secreted mucus. This loss is independent of membrane changes, because these cercariae retain a single unit membrane. The loss is apparently caused by glandular secretion, because the loss, secretion, and aggregation are all inhibited by ES. Second, 30-50% of the glyocalyx is lost during the time that microvilli are formed and shed (20). Third, after microvilli have been shed at 1 h, there is a continued loss of the glyocalyx. This loss may be due not only to glandular secretion, but also to surface membrane turnover, which is occurring with a halftime of 10-12 h (43).

The lost glyocalyx can be recovered from the medium and is still of high molecular weight, suggesting that it is sloughed largely intact (43, 45). This conclusion is supported by both LMARG, where no radioactivity is internalized by transforming organisms, and by the observation of fluorescent glyocalyx shed onto the slide.

Residual glyocalyx was present on schistosomula chemically and ultrastructurally, which agrees with previous studies of schistosomula transforming in rodent skin (20, 53). However, the glyocalyx on both skin and mechanically transformed parasites appeared thicker than was previously demonstrated (2, 6, 7). The glyocalyx retained on schistosomula, which is of high molecular weight, and excluded from SDS PAGE, appears to have been labeled on schistosomula by iodination (49) or by NaB\(^{3}\)H\(_{4}\) after oxidation with periodate or galactose oxidase (43). It is unlikely that the glyocalyx or its cleavage products account for the dozen or so proteins...
**Immunological Relevance**

The surface of cercariae is antigenic (24-26, 51, 55, 59) and activates complement by the alternative pathway (8, 11, 39). The high molecular weight acidic material labeled here appears to be the major cercarial surface antigen. It is possible that residual cercarial glyocalyx accounts for a sizable fraction of the antibody and/or complement binding sites on the surface of schistosomula, as well as of cercariae (9, 10, 32, 39, 46). The loss of residual glyocalyx may coincide with a decrease in antibody binding and complement fixation and may account for the increasing resistance of developing schistosomula to immune attack (9, 10, 39, 46). Consistent with this hypothesis, mice injected with irradiated cercariae are better protected against reinfection than are those exposed to irradiated schistosomula (47). However, other smaller antigens have been identified on the surface of schistosomula (12, 13, 18, 57) and recently monoclonal antibodies to two of these antigens have been shown to be protective in rats (13) and mice (18).

The corona of FITSC and rhodamine-conjugated IRS on transforming CBs resembles the cercarienhülle reaktion, a light microscopic assay for schistosomiasis (51, 55, 59). Living cercariae incubated in saline with patient sera with or without complement for 1-5 h form a 3-4-μm-thick surface coat in immune but not in normal sera. We speculate that cercariae transform in saline in a poorly synchronized fashion and that visualization of the cercarienhülle reaktion depends upon the binding of enough antibody and complement to render the shedding glyocalyx refractile. In the same way, antibody and complement appear to stabilize the glyocalyx for scanning microscopy in the absence of osmium (58).

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**FIGURES 28–34** Light micrographs of cercariae and transforming CBs. The surface of a Per-FITSC-labeled cercaria (Fig. 28) is diffusely fluorescent with the posterior body appearing brightest. Areas of the head (H), middle (M), posterior (P) body, and tail (T) measured with the photometer are marked. Spines are seen as black dots and are more dense on the body than on the tail. Cercariae incubated with FITSC alone do not fluoresce (not shown). A CB transforming for 30 min (Fig. 29) has a fluorescent corona (arrowheads), which is thickest on the posterior body (P). Interference-contrast microscopy of the same organism (Fig. 30) shows that microvilli project perpendicular to the parasite surface in regions of the corona (arrowheads). Transforming CBs are motile and shed fluorescent material (Fig. 31), which is fibrillar (arrow) and globular (arrowheads). This material is also visible by interference-contrast microscopy (Fig. 32). A CB incubated with rhodamine-conjugated IRS and transformed for 30 min also has a fluorescent corona (arrowheads, Fig. 33) on its posterior body (P). A CB transformed for 1 h binds FlCon A in a reticular fashion over its posterior body (Fig. 34). Spines appear as bright dots. (Fig. 28) × 700. (Figs. 29–34) × 875.


