ULTRASTRUCTURAL CYTOCHEMISTRY OF THE TEGUMENTAL SURFACE MEMBRANE OF *PARAGONIMUS KELLICOTTI*

by Francis M. Gress and Richard D. Lumsden

ABSTRACT

The body surface of adult *Paragonimus kellicotti* is a syncytial epithelium bounded on its free surface by a trilaminar plasmalemma. The outer layer of this membrane is invested with a hirsute coat which, on the basis of its cytochemical staining properties with concanavalin A, bismuth subnitrate, ruthenium red, polycationic ferritin, and colloidal iron, appears to be rich in acidic carbohydrate. Morphological association and common cytochemical reactivity between Golgi complexes of tegumentary cytons and membranous granular inclusions provide evidence for the incorporation of carbohydrate into the granule matrix within the Golgi apparatus. The observed fusion of such inclusions with the body surface suggests a mechanism whereby the contents of cytoplasmic granules contribute to the maintenance and/or renewal of the carbohydrate-rich surface coat and indicates that the glycocalyx of the *P. kellicotti* tegument is not simply a layer of adsorbed host mucin. Although the binding of extrinsic (i.e., host) glycan to the body surface is not excluded by these observations, it appears that the cytochemically demonstrable glycocalyx that invests this trematode consists, at least in part, of the carbohydrate moieties of membrane macromolecules. Ionized acidic moieties associated with the carbohydrate-rich surface coat contribute substantially to the body surface electronegative charge and may partially account for the ability of the flukes to survive in the immunocompetent host.

Francis Gress is N.I.H. Predoctoral Fellow at Tulane University. Richard Lumsden is Professor of Biology and Dean of the Graduate School at Tulane University.

INTRODUCTION

Consideration of the molecular composition, physico-chemical properties, and physiological activities of cell surface membranes has provided investigators with new insight into the probable organization of these supramolecular complexes (Stoeckenius and Engelman, 1969; Dewey and Barr, 1970; Singer and Nicolson, 1972; Oseroff et al. 1973; Singer, 1974). A recent advance of major significance has been the realization that carbohydrate-containing molecules are important constituents of cell surface membranes (Cook, 1968). The results of cytochemical, biochemical, and immunological studies, when considered collectively, indicate that much of the carbohydrate associated with the cell surface is a constituent of the membrane proper and as such does not constitute a superficial and extraneous coat of adsorbed extrinsic polysaccharide (Cook, 1968; Martinez-Palomo, 1970; Winzler, 1970; Kraemer, 1971; Cook and Stoddart, 1973). Sugar moieties present are covalently bonded to membrane proteins and lipids (Cook, 1968; Winzler, 1972; Hughes, 1973) and constitute a histochemically demonstrable glycocalyx (Bennett, 1963; Rambourg, 1971).

Numerous functions have been attributed to the chemical constituents of the glycocalyx. Membrane-associated carbohydrates are known to constitute the functional determinants of a variety of cell surface antigens (Winzler, 1972; Hughes, 1973) and otherwise influence cell surface antigenicity (Apffel and Peters, 1970); ionized acidic sugar moieties contribute substantially to the negative surface charge present on cell types thus far examined (Cook, 1968; Cook and Stoddart, 1973; Weiss, 1973); and surface-associated carbohydrate is believed to exert important control over the social behavior of cells (Roseman, 1970; Cook and Stoddart, 1973; Emmelot, 1973; Hakomori, 1973).

Examination of the body surfaces of a variety of parasitic platyhelminths using cytochemical, autoradiographic, and biochemical techniques has provided results consistent with the presence of a glycocalyx in association with the tegumental surface membrane (reviewed by Lumsden, 1975). Physiological and immunological considerations would suggest that detailed information concerning the nature of the body surface membrane and associated carbohydrate coat would provide a better understanding of the nature of the host-parasite relationship.

A considerable amount of information is available detailing the pathological and serological status of the host in paragonimiasis (Sadun et al., 1959; Capron et al., 1965; Yogore et al., 1965; Yokogawa, 1965; Seed et al., 1966 and 1968; Tada, 1967; Lumsden and Sogandares-Bernal, 1970; Chung, 1971; Sogandares-Bernal and Seed, 1973), but little is known concerning the physiology of the trematode itself. In the case of pulmonary disease, adult worms may survive for several or many years. The infection assumes a chronic course and inflammatory changes transform airways harboring

adult worms into fibrotic pulmonary lesions or cyst-like structures. Moreover, sera of infested hosts are known to contain antibodies reactive against worm antigens. Adult worms, however, at least those from primary infections, are not rejected by host defense mechanisms. We report here observations on the body surface fine structure and topochemistry of *Paragonimus kellicotti* that may relate to the failure to reject and to other features of host-parasite interaction in pulmonary paragonimiasis.

MATERIALS AND METHODS

Adult *Paragonimus kellicotti* were removed from the lungs of domestic cats three or four years following the administration of 15 to 30 metacercariae. The worms were fixed in 5.0% glutaraldehyde in Millonig's phosphate buffer containing 3.0% sucrose and washed in buffer containing 5.0% sucrose. For ultrastructural study, material was post-fixed in Millonig's phosphate buffered 1.0% osmium tetroxide containing 2.0% sucrose, dehydrated with ethanol, and embedded in epoxy resin (Lumsden, 1970). Sections were stained with aqueous 2.0% uranyl acetate and lead citrate (Reynolds, 1963) and examined in a Siemens 1A electron microscope operated at an accelerating voltage of 80kv.

Cytochemical procedures included incubation of glutaraldehyde-fixed material in ruthenium red (Luft, 1971) and polycationic ferritin (Miles-Yeda Ltd., Rehovoth, Israel) according to the method of Danon et al. (1972). Staining with cationic and anionic iron colloids was performed according to methods described by Gasic et al. (1968). Procedures intended to reduce surface negative charge prior to cytochemical staining included incubation of tissue in neuraminidase (Sigma Chemical Co., type V; 4 hour incubation at 37°C in 0.05M acetate buffer, pH5.5, containing 0.85% NaCl and 10.0 units/ml of neuraminidase), poly-L-lysine-HBr (New England Nuclear, molecular weight 160,000; 1 hour incubation in phosphate buffered saline, pH7.2, containing 10.0mg/ml of poly-lysine), and methanolic-HCl (Lillie, 1954). Additional material was incubated in native (i.e., anionic) ferritin (K&K Laboratories; 1 hour incubation in phosphate buffered saline, pH7.2, containing 10.0mg/ml of ferritin).

Methods employed for the detection of concanavalin A binding sites were those previously described by Stein and Lumsden (1973) and McCracken and Lumsden (1975). In brief, specimens previously exposed to this lectin were incubated in a solution of horse radish peroxidase, transferred to a mixture of diaminobenzidene and hydrogen peroxide, and then osmicated. Control procedures included incubation of concanavalin A-labeled tissue in 0.1M alpha-methyl-D-mannoside prior to transfer to peroxidase and completion of the reaction sequence. Other material was incubated in ferritin-conjugated concanavalin A in phosphate buffered saline (Nicolson and Singer, 1971).

Thin sections collected on nickel or steel grids were treated with periodic acid (0.8gm dissolved in 100ml of 70% ethanol containing 0.02M sodium acetate) followed by alkaline bismuth subnitrate (Ainsworth et al., 1972) in order to detect 1,2-glycols of polysaccharide containing macro-molecules. Control procedures included blocking of periodate-engendered aldehydes with 1.0M m-aminophenol prior to treatment with alkaline bismuth subnitrate, removal of osmium from tissue sections with 1.0% hydrogen peroxide, and omission of the oxidation step from the reaction sequence.

RESULTS

Electron microscopic examination revealed that the body surface of *Paragonimus kellicotti* adults is a cytoplasmic syncytium containing numerous mitochondria and discoidal, membrane-bound inclusions (figure 1). Cytoplasmic processes connect this protoplasmic surface with nucleated tegumentary cytons located within the deeper parenchymal tissues of the worm (figure 2). The perinuclear cytoplasm is typically filled with inclusions similar to those observed in the surface syncytium (figures 2 and 3). Also present are mitochondria, a well developed granular endoplasmic reticulum, Golgi membranes, and free ribosomes (figure 3). The folded body surface is bounded by a trilaminar plasmalemma, the outer dense aspect of which is invested with a thin hirsute coat (figure 4).

Incubation of tissue in ruthenium red resulted in the formation of a uniform and continuous layer of reaction product along the outer surface of the tegument. The ruthenium-osmium complex was localized at the outer aspect of the surface membrane and was confined to the region occupied by the hirsute coat seen on conventionally stained material (figure 5). Examination of unstained thin sections through the body surface revealed that certain of what appear to be vesicular cytoplasmic inclusion bodies reacted with ruthenium red as well (figure 6). Since ruthenium red does not typically cross intact membranes, however (Luft, 1971), these may represent cross sectioned canalicular extensions of the free surface plasmalemma.

Polycationic ferritin employed at pH 7.2 uniformly covered the body surface and was bound by the hirsute coat associated with the surface plasmalemma (figure 7). Methylation or treatment of tissue with poly-lysine prior to incubation in the positively charged ferritin resulted in a considerable reduction in the amount of polycationic ferritin subsequently bound (figure 8).

Uniform and dense staining of the body surface membrane was obtained when cationic colloidal iron was employed at pH 2.0 (figures 9 and 10). Prior incubation of material in poly-lysine largely prevented the subsequent binding of positively charged iron micelles (figures 11 and 12). It is to be noted as well that the integrity of the surface membrane was preserved following treatment with poly-lysine (figure 12). Essentially no binding of anionic colloidal iron was observed when this stain was employed at pH 5.5. Treat-

FIGURES 1-12 ON FOLLOWING PAGES.

FIG. 1. SURVEY MICROGRAPH OF ADULT *Paragonimus kellicotti* body wall illustrating fine structural features of the tegument. Note the numerous mitochondria and discoidal inclusion bodies. The free tegumental surface is folded, and cell boundaries are absent. Section stained with uranyl acetate and lead citrate. ×28,000.

FIG. 2. SURVEY MICROGRAPH OF BODY WALL illustrating several tegumentary cytons (TC). The cytoplasm of tegumental cell bodies is filled with discoidal granules (gr). Section stained with uranyl acetate and lead citrate. ×10,500.

FIG. 3. CYTOPLASMIC ORGANELLES OF TEGUMENTAL PERIKARYA including mitochondria (m), free ribosomes (r), granular endoplasmic reticulum (er), and Golgi membranes (go). Discoidal granules (gr) are also present. (Nucleus, N). Section stained with uranyl acetate and lead citrate. ×36,000.

FIG. 4. TRILAMINAR BODY SURFACE PLASMA MEMBRANE. Note the hirsute coat associated with the outer dense leaflet of the membrane. Section stained with uranyl acetate and lead citrate. ×210,000.

FIG. 5. TEGUMENTAL SURFACE STAINED WITH RUTHENIUM RED. Note that opaque reaction product is localized at the outer surface of the plasmalemma and occupies the region of the hirsute coat. Section stained with uranyl acetate and lead citrate. ×129,000.

FIG. 6. TISSUE INCUBATED *EN BLOC* IN RUTHENIUM RED. Cytoplasmic inclusion bodies (*) different from the discoidal granules (gr) are stained by this cytochemical reagent. Section otherwise unstained. ×57,000.

FIG. 7. BINDING OF POLYCATIONIC FERRITIN TO THE OUTER FACE of the body surface membrane is uniform and dense. Incubation at pH 7.2. Section stained with uranyl acetate and lead citrate. ×76,500.

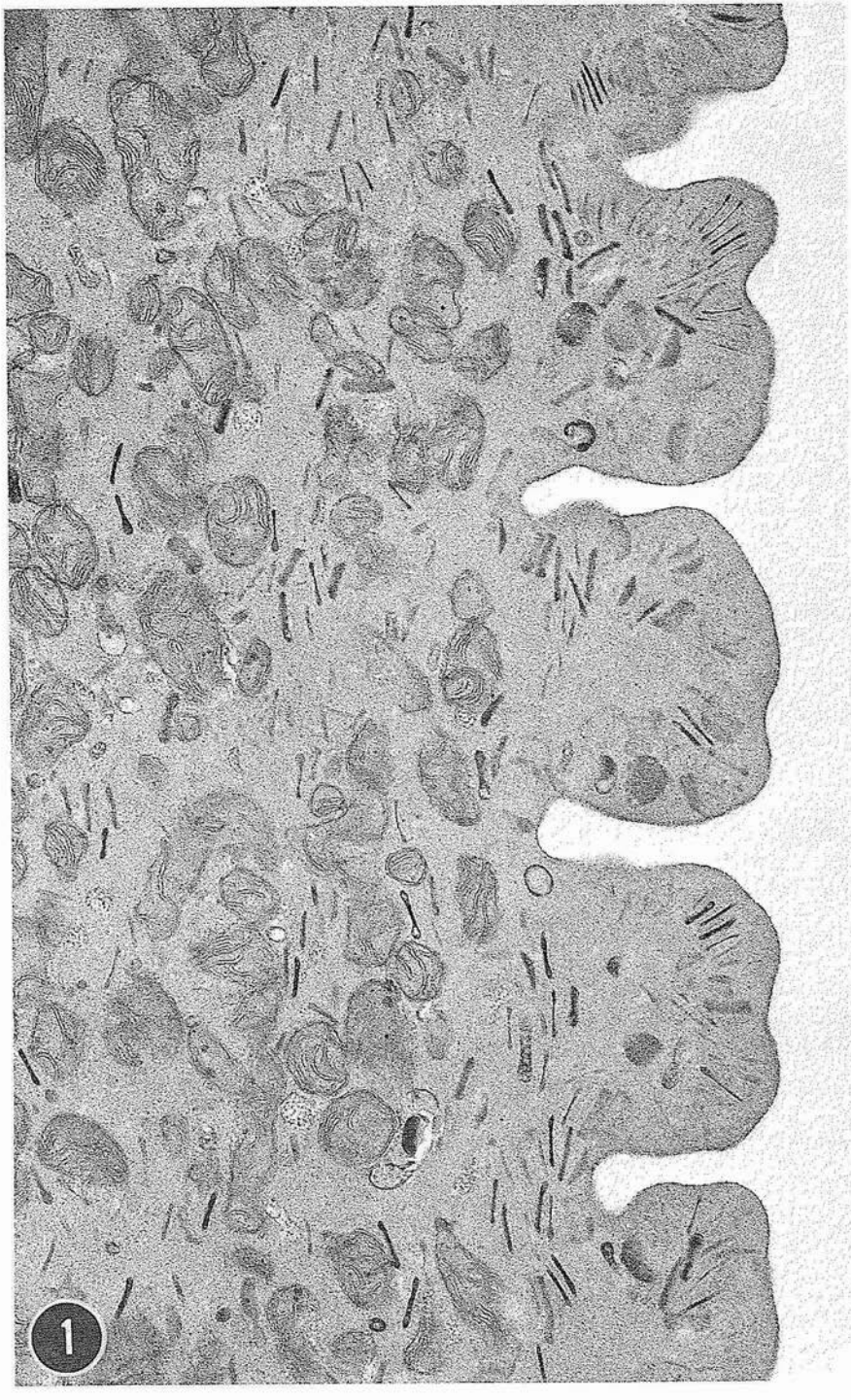
FIG. 8. SPECIMEN TREATED WITH METHANOLIC HCl prior to incubation in polycationic ferritin. Note the decrease in binding of cationic ferritin when compared with results illustrated in figure 7. Section stained with uranyl acetate and lead citrate. ×115,000.

FIG. 9. SURVEY MICROGRAPH ILLUSTRATING UNIFORM AND DENSE STAINING following treatment with cationic colloidal iron at pH 2.0. Section stained with uranyl acetate and lead citrate. ×33,800.

FIG. 10. TISSUE STAINED WITH CATIONIC COLLOIDAL IRON AT pH 2.0. Note that the iron micelles are bound to the outer dense leaflet of the plasmalemma. Section stained with uranyl acetate and lead citrate. ×185,000.

FIG. 11. SPECIMEN TREATED WITH POLY-L-LYSINE prior to staining with positively charged colloidal iron. Note the considerable reduction in binding of iron micelles when compared with results illustrated in figures 9 and 10. Section stained with uranyl acetate and lead citrate. ×57,500.

FIG. 12. MATERIAL TREATED WITH POLY-L-LYSINE prior to staining with cationic colloidal iron. The surface coat appears thickened, presumably due to the presence of electrostatically bound poly-lysine. Morphological integrity of the surface plasmalemma is preserved. Section stained with uranyl acetate and lead citrate. ×167,000.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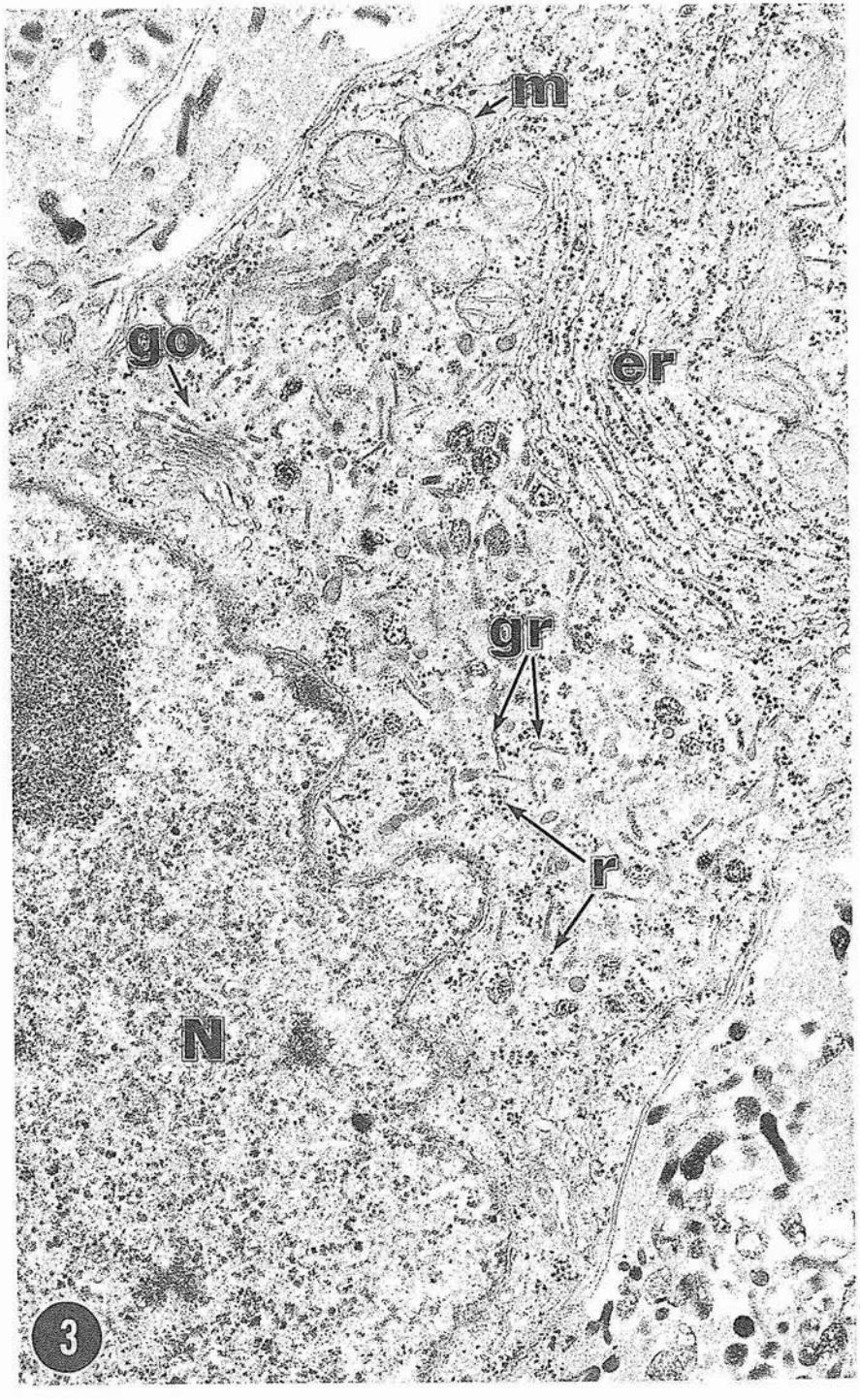
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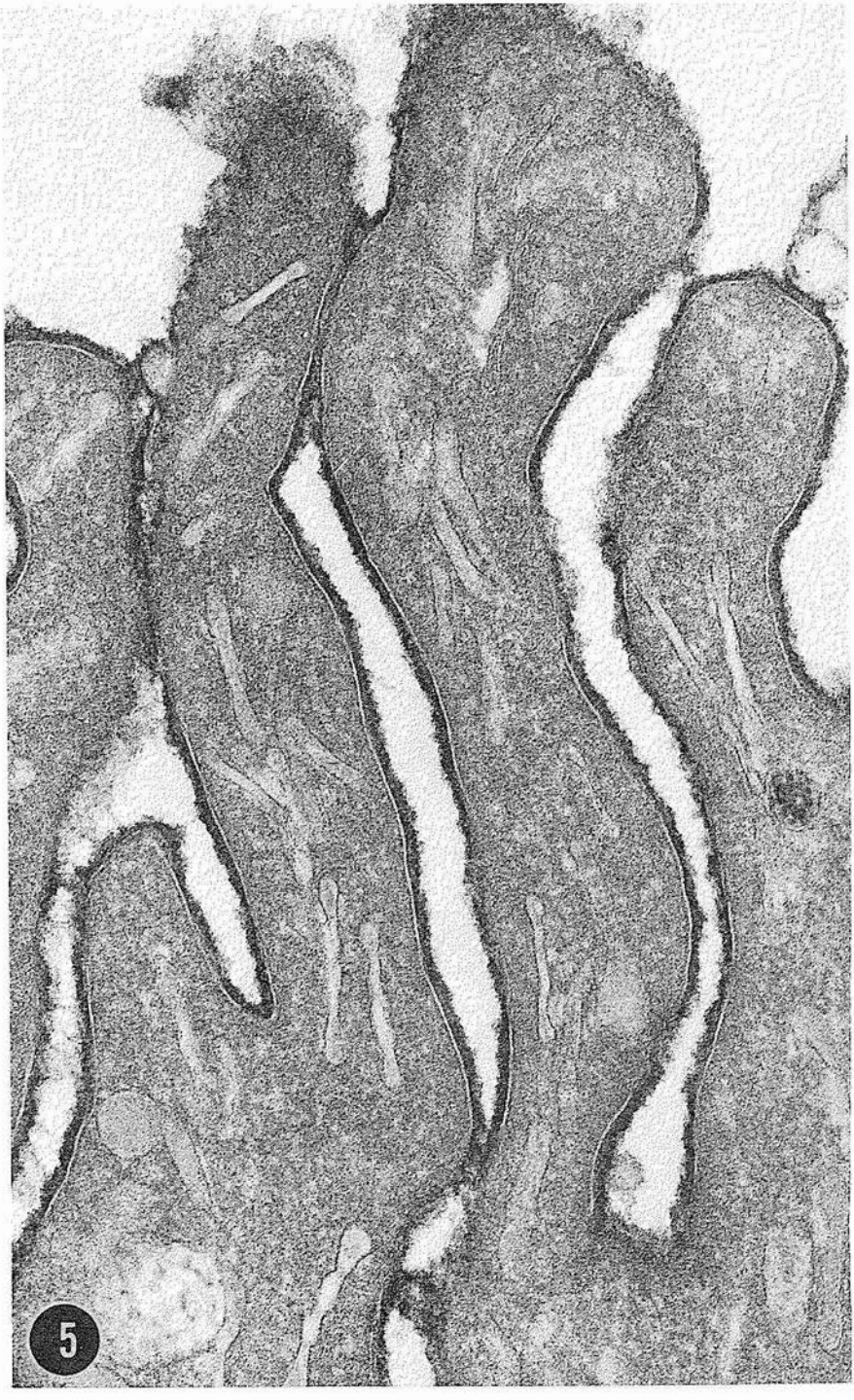
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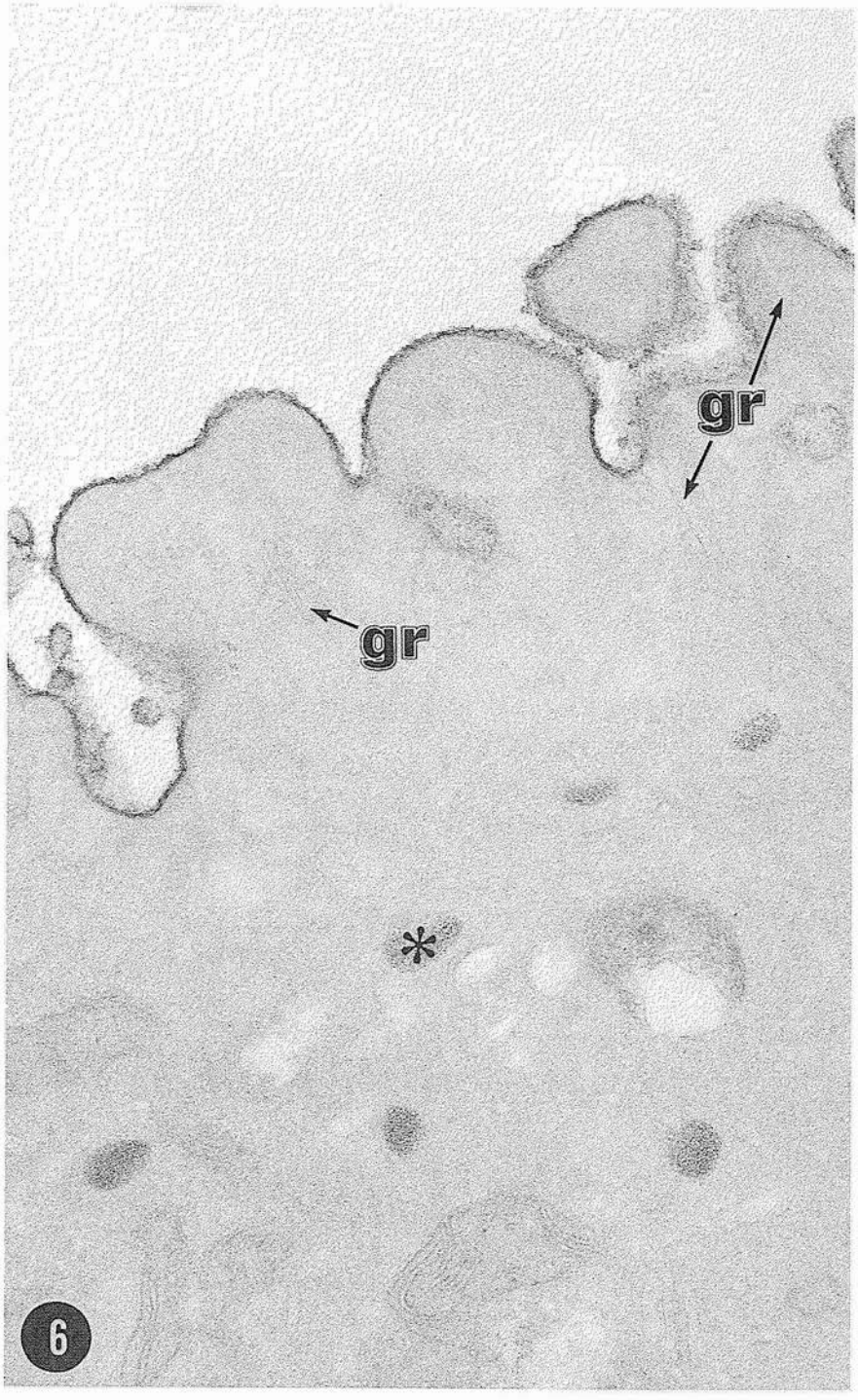
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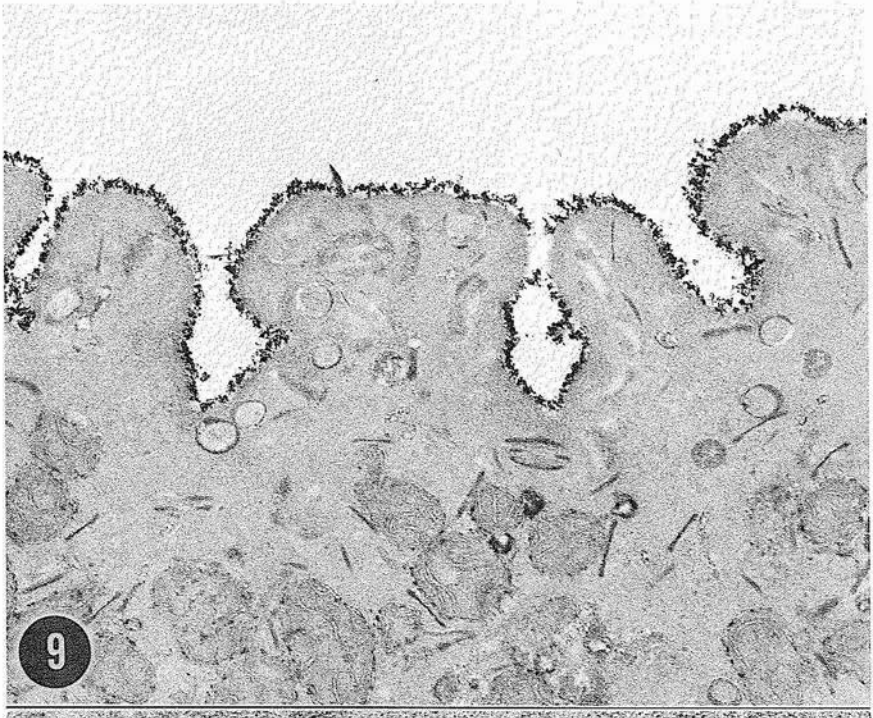
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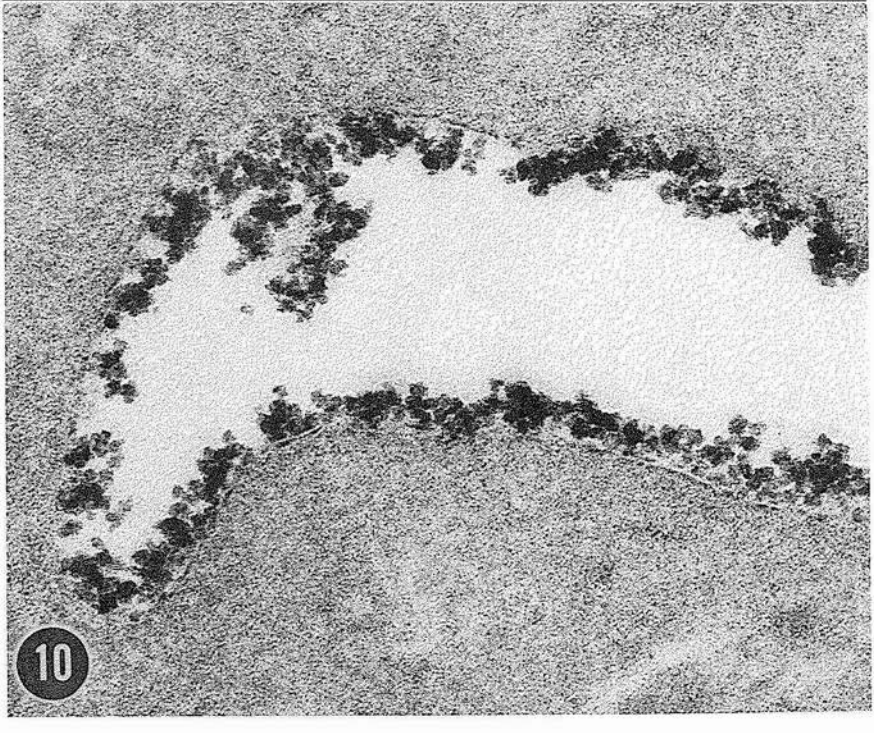
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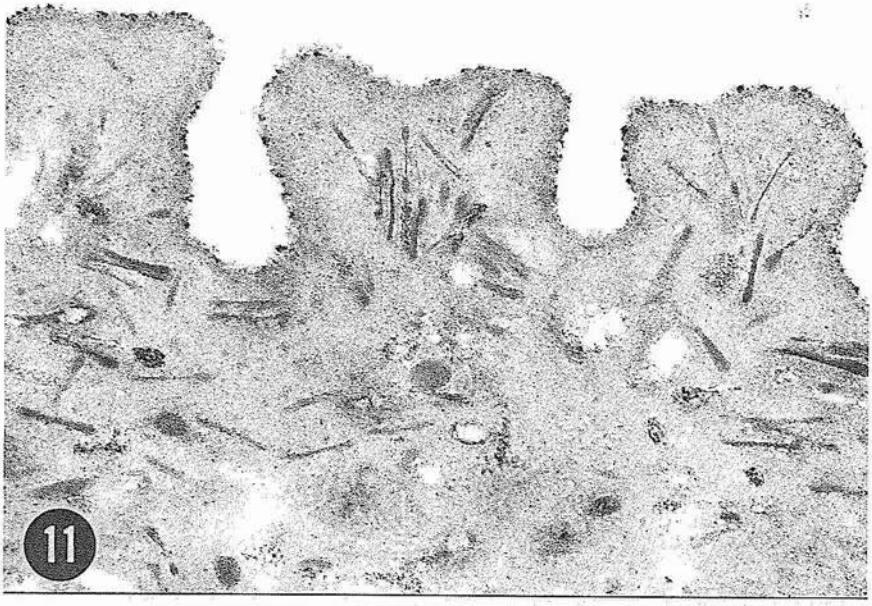
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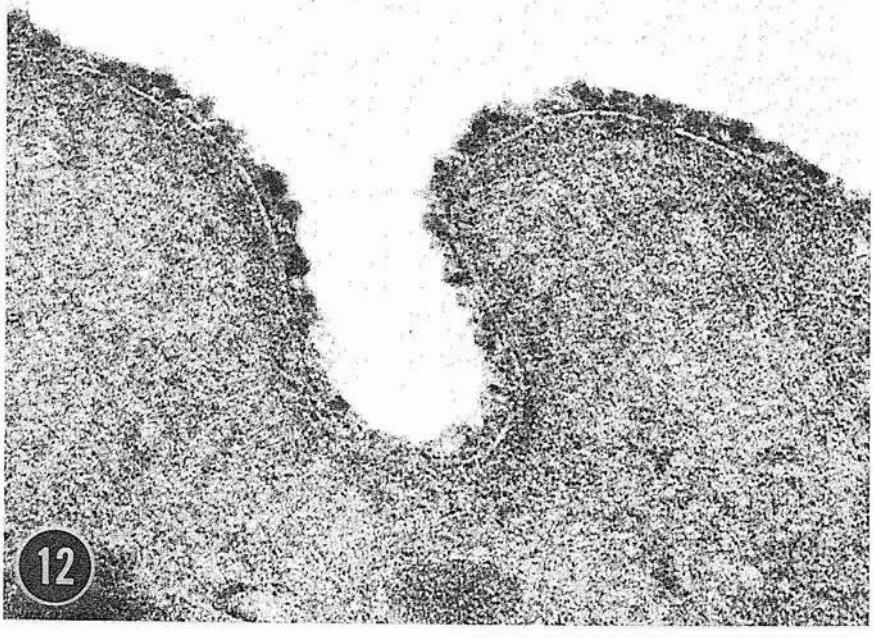
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ment of tissue with neuraminidase did not unequivocally reduce the staining by cationic iron nor did it substantially increase the affinity of the surface membrane for the anionic colloid.

Binding of native (i.e., anionic) ferritin to the body surface was negligible and was not increased following methylation.

The osmium black reaction product of the concanavalin A-horse radish peroxidase-diaminobenzidene procedure was visualized as a continuous and dense precipitate associated with the outer leaflet of the plasmalemma (figures 13 and 14). The specificity of the procedure was confirmed by the observation that no precipitate was visible when concanavalin A-treated tissue was incubated in methyl-mannoside prior to completion of the reaction sequence with peroxidase, diaminobenzidene, and osmium tetroxide. Lectin binding sites were directly made visible by treating samples with ferritin-conjugated concanavalin A (figure 15).

The tegumental surface membrane and cytoplasmic inclusions were uniformly stained by reduced bismuth subnitrate following periodate oxidation (figure 16). Golgi membranes and inclusions present within perikarya reacted similarly (figures 17, 18, 19, and 20). Images such as those illustrated in figures 17, 19, and 20 suggest a secretory mechanism whereby budding vesicles limited by membranes derived from Golgi saccules undergo a condensation process resulting in the formation of the discoidal granules present in the perikarya and tegumental syncytium.

Survey micrographs illustrate palisade-like arrays of these membrane bound inclusions adjacent to the tegumental surface plasmalemma (figure 1), and examination of tissue sections that had been oxidized and treated with bismuth reagent revealed a continuity of cytochemically reactive substance(s) between some of these inclusions and the tegumental surface (figure 21).

Blockage of periodate-engendered aldehydes with m-aminophenol prevented the reduction of bismuth subnitrate. In addition, specific bismuth staining did not occur when the periodate oxidation step was omitted. Non-specific bismuth staining was prevented when sections were treated with 1.0% hydrogen peroxide in order to remove osmium from the tissue.

DISCUSSION

The body surface of adult *Paragonimus kellicotti* is a syncytial epithelium consisting of an outer protoplasmic layer continuous with nucleated cytons located in the underlying parenchyma. This cyto-architecture conforms to that previously described for the tegument of other digenetic trematodes (reviewed by Lee, 1966 and 1972; Inatomi et al., 1970; Lumsden, 1975).

The free tegumental surface of *P. kellicotti* is bounded by a trilaminar plasmalemma. The outer layer of this membrane is invested with a thin hirsute coat, which appears by its cytochemical staining properties to be rich in acidic glycans. The presence of carbohydrate in this coat is specifically

FIGURES 13-21 ON FOLLOWING PAGES.

FIG. 13. ELECTRON-DENSE REACTION PRODUCT uniformly coats the body surface following treatment of concanavalin A-incubated tissue with peroxidase and diaminobenzidene. Section stained with uranyl acetate and lead citrate. ×72,600.

FIG. 14. OSMIUM BLACK REACTION PRODUCT localizes lectin binding sites to the outer surface of the tegumental surface membrane following treatment of concanavalin A-incubated tissue with peroxidase and diaminobenzidene. Section stained with uranyl acetate and lead citrate. ×336,000.

FIG. 15. DIRECT LOCALIZATION OF CONCANAVALIN A BINDING SITES is achieved by incubating tissue in ferritin-conjugated lectin. Note that lectin binding sites are associated with the body surface hirsute coat. Section stained with uranyl acetate and lead citrate. ×177,000.

FIG. 16. SURVEY MICROGRAPH ILLUSTRATING UNIFORM STAINING of the tegumental surface and cytoplasmic inclusion bodies by bismuth subnitrate following periodate oxidation. Section otherwise unstained. ×65,000.

FIG. 17. FOLLOWING PERIODATE OXIDATION, Golgi saccules (go), budding vesicles (v), and cytoplasmic granular inclusions (gr) of the tegumental perikarya are stained by reduced bismuth subnitrate. Section otherwise unstained. ×92,400.

FIG. 18. CYTOPLASMIC GRANULES OF TEGUMENTAL PERIKARYA stain specifically following treatment with periodic acid and bismuth subnitrate. Section otherwise unstained. ×98,500.

FIG. 19. IMAGES SUCH AS THE ONE ILLUSTRATED HERE suggest a secretory mechanism whereby vesicles (v) derived from Golgi saccules (go) condense to form recognizable discoidal granules (gr). Note the variations in opacity of the condensing bodies and the close association of granular inclusions with the Golgi apparatus. Bismuth reagent stains the matrices of granular inclusions as well as membranes of Golgi saccules and vesicles. Section treated with periodate and alkaline bismuth subnitrate. ×71,300.

FIG. 20. GOLGI APPARATUS OF TEGUMENTAL PERIKARYON illustrating specific bismuth staining of Golgi membranes, budding and condensing vesicles, and granular inclusions. Continuity of a vesicular body (v) with a granular inclusion suggests that vesicles derived from Golgi saccules condense to form the carbohydrate-containing granular inclusions. Section treated with periodate followed by bismuth subnitrate. ×61,800.

FIG. 21. REDUCED BISMUTH SUBNITRATE is deposited over the tegumental surface (*) following periodate oxidation. Cytoplasmic discoidal granules (gr) stain specifically with bismuth reagent as well. The continuity of cytochemically demonstrable material between the tegumental surface and granular inclusion illustrated here suggests that these carbohydrate-containing granules may contribute their contents to the glycocalyx which invests the body surface of *P. kellicotti*. Section otherwise unstained. ×184,800.

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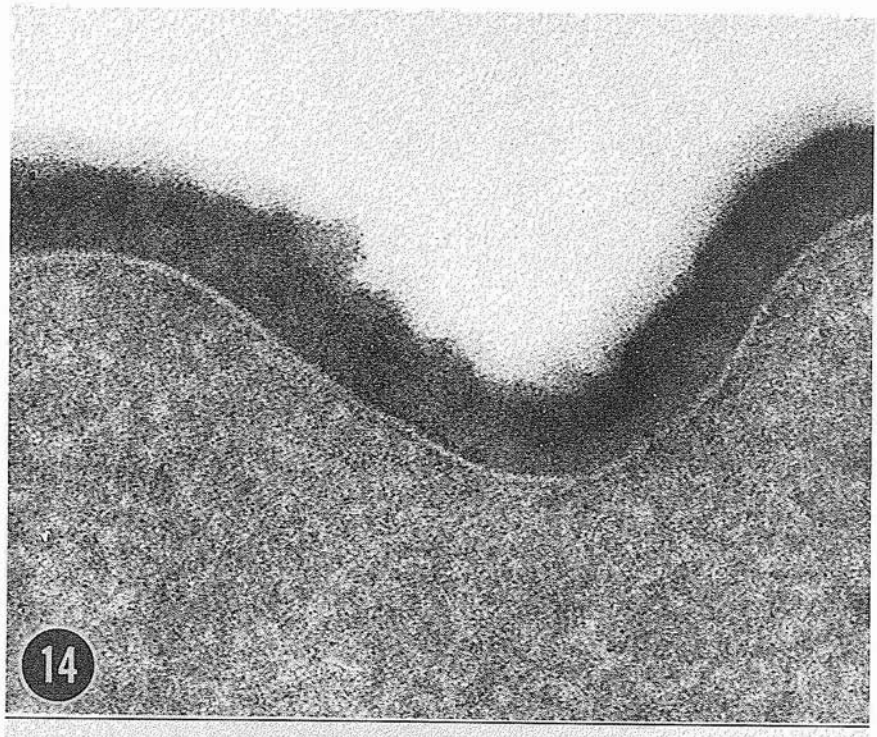
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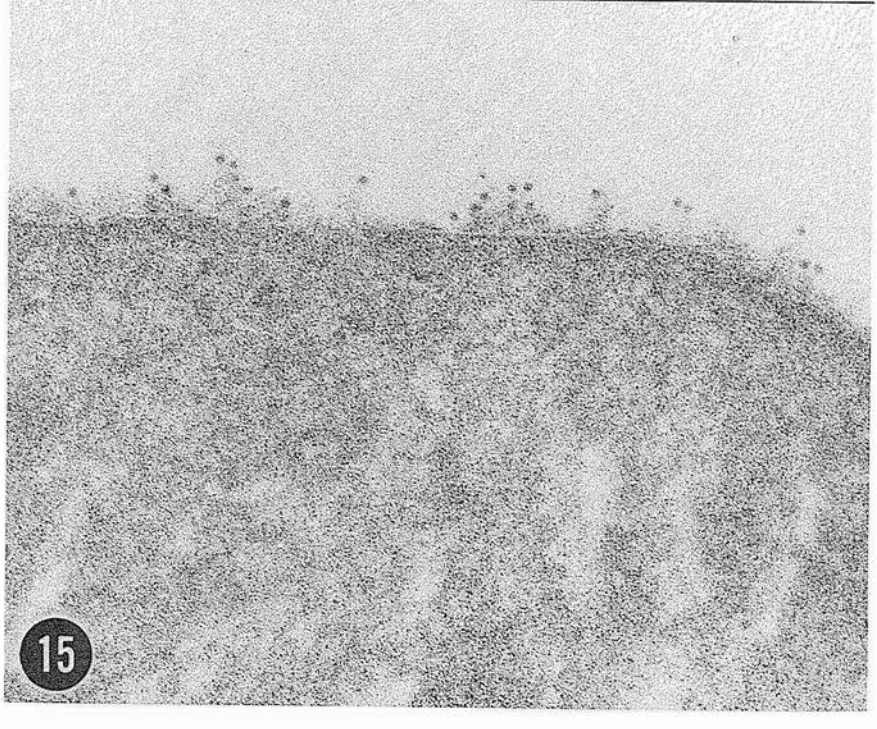
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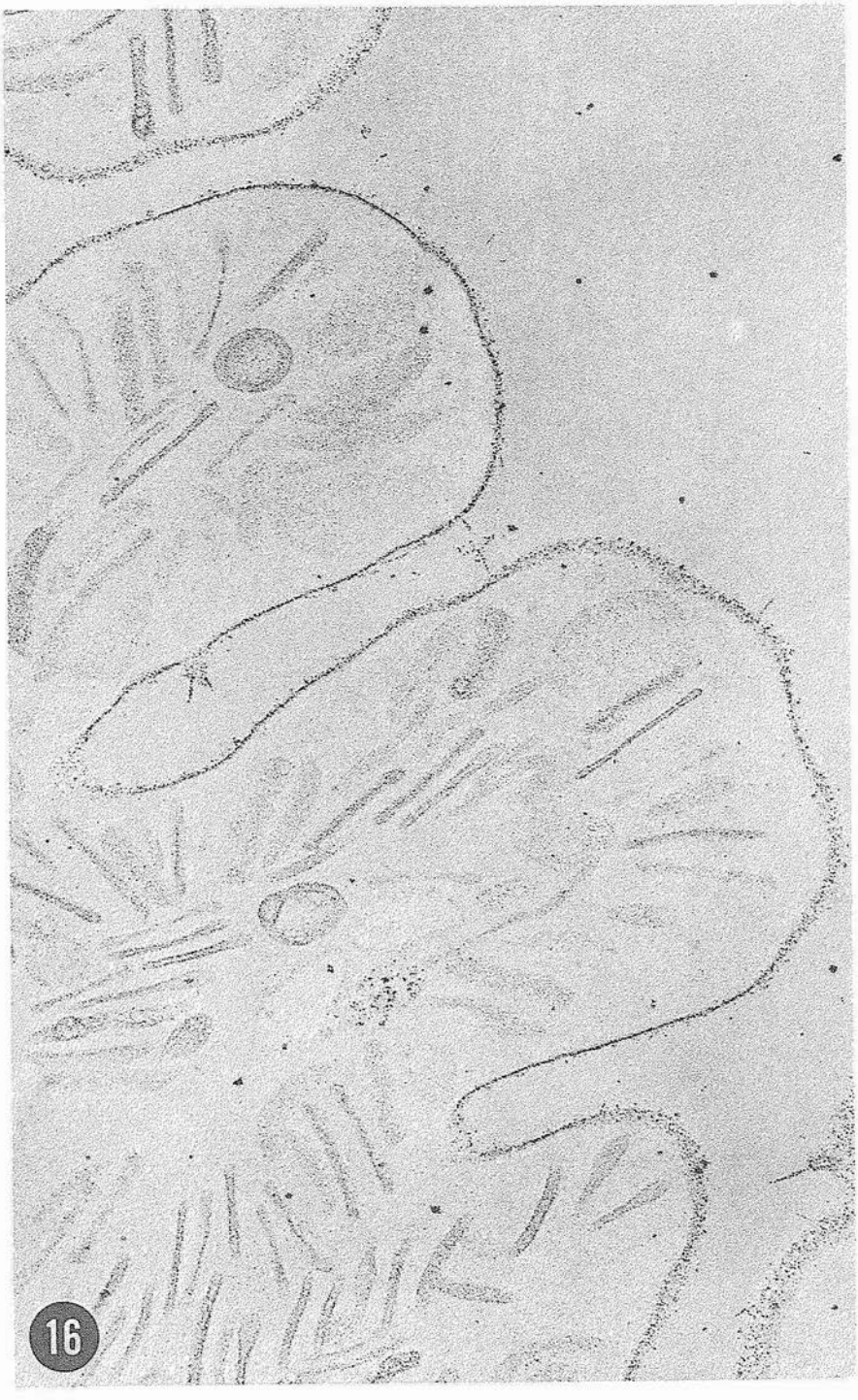

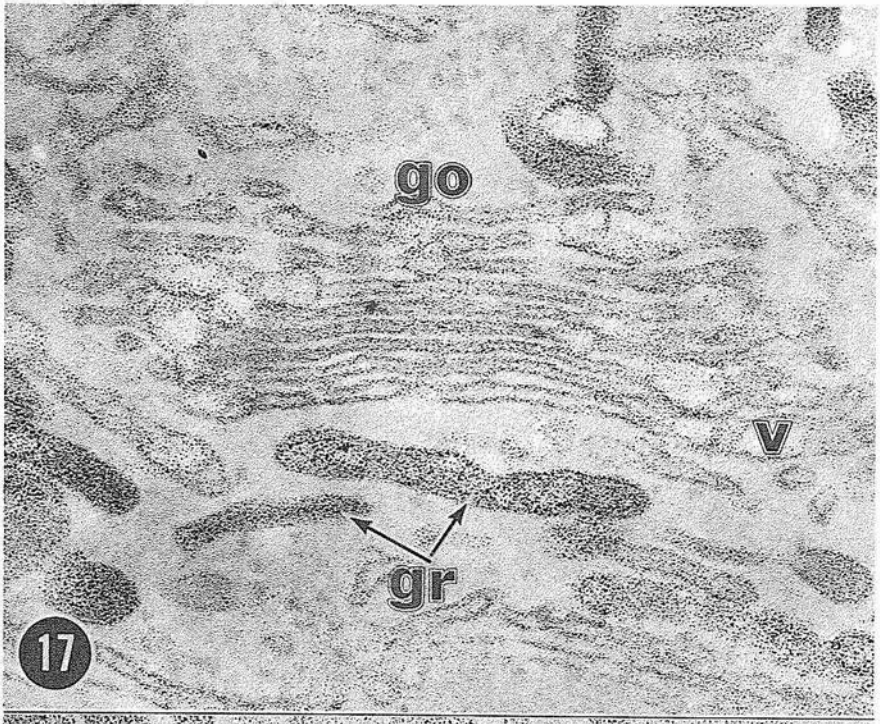
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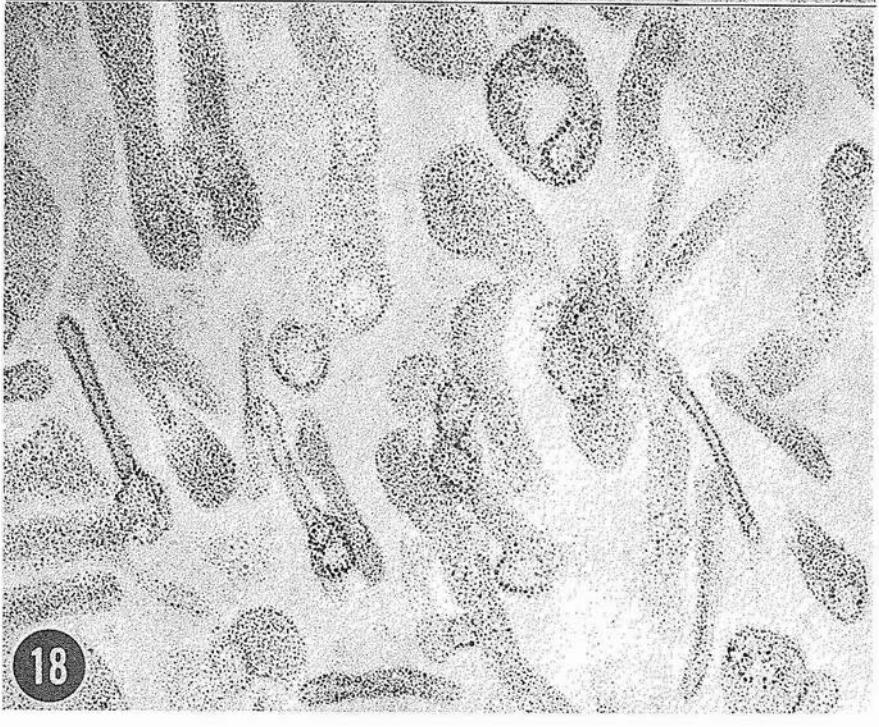
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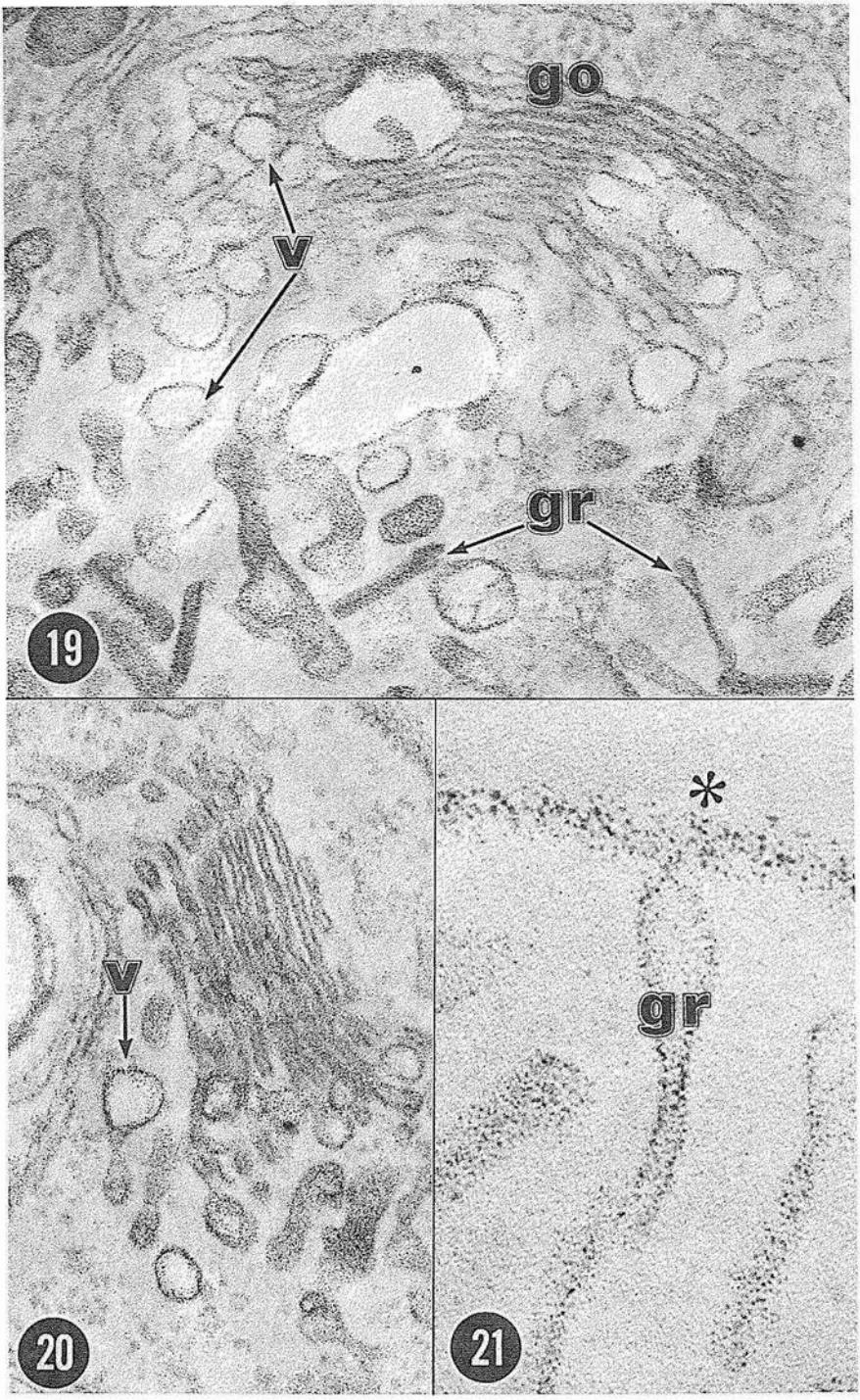
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indicated by its affinity for the lectin concanavalin A, which binds to sugars having the D-arabinopyranoside configuration at C_3, C_4, and C_6 (Lis and Sharon, 1973). As indicated by the use of ferritin or peroxidase-conjugated concanavalin A, lectin binding sites at the tegumental surface of *P. kellicotti* are numerous, uniformly distributed, and associated with the hirsute coat. Further confirmation of the carbohydrate content of this coat was obtained with the periodic acid-bismuth subnitrate technique of Ainsworth et al. (1972), which is considered to yield results for electron microscopy comparable to those obtained with the periodate-Schiff procedure employed for light microscopic demonstration of carbohydrate-containing macromolecules.

The polyanionic nature of the surface coat is indicated by its staining with cationic ferritin, colloidal iron, and ruthenium red. Pretreatment of tissue with poly-lysine greatly reduced staining by cationic iron and ferritin, presumably because this strongly basic polypeptide electrostatically neutralized many of the acidic substances associated with the body surface (Mamelak et al., 1969; Lumsden et al., 1970; Lumsden, 1972). Furthermore, treatment of tissue with methanolic HCl, which destroys sulfate groups and esterifies carboxylic acid functions (Lillie, 1954), caused a marked reduction in the binding of colloidal metal cations, presumably due to a net reduction in surface electronegativity. Attempts to stain the surface with anionic colloidal iron and native ferritin (negatively charged at neutral pH) proved unsuccessful. The failure to stain might be explained by the occurrence of a charge repulsion and/or masking effect due to the presence of coat-associated anions (Lumsden et al., 1970) or to a paucity of cationic constituents accessible to the reagents employed. Indicative of the latter possibility is the observation that methylation procedures did not convincingly increase the binding of negative colloidal iron or anionic ferritin.

Considered collectively, the results of cytochemical tests employing ruthenium red, polycationic ferritin, and cationic colloidal iron indicate that acidic carbohydrate is a constituent of the body surface membrane hirsute coat. At the low pH used for staining with cationic colloidal iron, the ionization of free carboxyl groups of proteins is suppressed, whereas more strongly acidic sulfate, carboxyl, and phosphate groups bound to carbohydrate or lipid may remain ionized (see Lumsden et al., 1970; Lumsden, 1973, for discussion) and may thus account for the body surface electronegativity. Also, we regard as untenable the view that the cell surface negative charge may be attributed in its entirety to membrane phospholipids (Seaman and Heard, 1960; Wallach and Eylar, 1961; Eylar et al., 1962; Cook, 1968; McLaughlin et al., 1971).

Numerous studies (see, for example, Cook and Stoddart, 1973; Weiss, 1973) have demonstrated that neuraminic (sialic) acids are important ionogenic species at the surface of animal cells. Sialic acid is a constituent of membrane glycoprotein and glycolipid (Cook, 1968), and the carboxyl group

of this acidic sugar (pK_a 2.6) is responsible for a significant portion of the negative surface charge of animal cells (Wallach and Eylar, 1961; Eylar et al., 1962; Cook, 1968). Our attempts to detect the presence of sialic acid at the tegumental surface of *P. kellicotti* (by demonstration of a reduction in staining with cationic iron and polycationic ferritin following treatment of material with neuraminidase) were inconclusive. Furthermore, no substantial increase in the binding of anionic iron was observed following enzymatic digestion. Such qualitative studies do not, however, rule out the possibility that sialic acid may be present, since the incubation medium was not assayed for enzymatically liberated sugar. Lumsden et al. (1970) noted that chemical fixation interfered with subsequent enzymatic removal of carbohydrate from the body surface of the cestode *Hymenolepis diminuta*, and it is conceivable that cross-linking of membrane constituents by the glutaraldehyde used in the present study might have induced conformational changes, thereby rendering the alpha ketoside linkage of sialic acid non-labile to the action of neuraminidase. It has been noted as well that certain sialic acid residues bound to glycoprotein resist cleavage by neuraminidase (Labat and Schmid, 1969). Furthermore, sialic acid bound to glycolipid is not susceptible to hydrolysis by neuraminidase (Weinstein et al., 1970). The possibility that a substantial portion of the surface negative charge may be due to the presence of membrane-bound ionogenic species other than the carboxyl groups of neuraminic acid, such as sulfate and phosphate, must also be considered (Hauser et al., 1969; Monis et al., 1969; Rothman and Elder, 1970; Allen, Winzler et al., 1971; Allen, Ault, et al., 1974).

Membranous granular inclusions of the type seen in the tegument of *P. kellicotti* are ubiquitous among trematodes and cestodes, and have been implicated in several diverse functions (see Lumsden, 1975, for review). The morphological associations between the Golgi apparatus and these inclusions and their common staining with bismuth subnitrate following periodate oxidation suggest that carbohydrate, probably as glycoprotein, is incorporated into the granule matrix via the Golgi apparatus of the tegumentary cytons. The presence of palisaded accumulations of these granules immediately below the tegument surface membrane and the apparent fusion of such granules with it would suggest a mechanism whereby the contents of such granules may contribute to the maintenance or renewal of the glycocalyx. Molecular constituents of a wide variety of cell surface membranes, including those of other parasitic helminths, undergo continuous turnover (Warren and Glick, 1968; Oaks and Lumsden, 1971; Hughes et al., 1972), the carbohydrate components in many cases being elaborated in the form of membrane-limited vesicles originating from the Golgi apparatus (see, for example, Bennett and Leblond, 1970; Bennett et al., 1974).

The failure of the tegument granules of *P. kellicotti* to stain for the acidic moieties so demonstrated in the glycocalyx is not inconsistent with this view.

It is to be noted that staining with ruthenium red, colloidal iron, and ferritin was carried out *en bloc* rather than applied to sections, and these substances typically do not cross intact membranes. Nor were the granules stained in the tissues incubated *en bloc* with concanavalin A. This lack of staining is also most likely due to the inability of the concanavalin A and/or the ferritin/peroxidase markers to pass through the tegument plasmalemma and vesicle membranes, rather than to an absence of lectin binding material in the vesicles.

It would appear that the glycocalyx of the *P. kellicotti* tegument is not simply a layer of adsorbed host mucins. Repeated washing failed to reduce subsequent staining with the cytochemical reagents employed, and there is evidence (noted above) for the elaboration of surface glycans by the worms. However, binding of extrinsic (i.e., host) glycans is not excluded by these observations. A glycan sharing antigenic specificity with the AB antigens of host erythrocytes has been identified in the surface coat of schistosomula and adults of *Schistosoma mansoni* (Clegg, 1972). These as well as Forssman antigens (which are glycolipids) have been shown by Dean and Sell (1972) and by Dean (1974) to be passively adsorbed by these worms *in vitro*.

The similarities in topochemistry between adult *P. kellicotti* and *S. mansoni* are noteworthy in considering the functional implications of the *Paragonimus* glycocalyx. As noted by Stein and Lumsden (1973) and Lumsden (1975), the highly acidic glycocalyx of the blood-dwelling schistosomes would be expected to militate against their entrapment by clot formation and inflammatory elements. The pulmonary capsules in which the adults of *P. kellicotti* reside are highly vascularized and the central space contains an exudate of plasma and extravasated formed elements. The flukes' survival in this environment may be due, at least in part, to the electronegative potential of the body surface, which could serve to repel similarly charged inflammatory or immunocompetent cells (Currie and Bagshawe, 1967; Hause et al., 1970), platelets, and fibrinogen (Sawyer and Pate, 1953; Sawyer, Pate, and Weldon, 1953; Mattson and Smith, 1973). The possibility that acidic carbohydrates may reduce the immunogenicity of body surface constituents or otherwise impair the functioning of humoral factors produced by the immune system of the host (Apffel and Peters, 1970) must also be considered. Whether or not potentially protective host antigenic determinants (Clegg, 1972) or blocking antibodies (Feldman, 1972) may coat the body surface as extrinsic components of the glycocalyx remains to be established.

Little information is presently available regarding the biochemistry of *Paragonimus* (see Hamajima, 1971, 1972, 1973a, and 1973b), the nutritional requirements of these flukes, and the possibility that the tegumental membrane may function as an absorptive surface. Evidence that the activities of hydrolytic enzymes and transport systems associated with the body surfaces of enteric parasitic platyhelminths are dependent upon cationic cofactors (see, for example, Dike and Read, 1971; Lumsden, 1973; Lumsden and

Berger, 1974) would suggest that hydrophilic and acidic carbohydrate moieties present at the body surface might function to bind essential ions or water molecules and so facilitate surface enzyme or transport activity. Similar functional roles have been attributed to carbohydrates present at the surfaces of other cell types (Seaman et al., 1969; Langer and Frank, 1972).

ACKNOWLEDGMENT

Research was supported by grants (AI 08673 and AI 0002) from the National Institutes of Health.

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