inhibited by SRIF, but not by other similar synthetic peptides, we can consider that the substance revealed by the immunological reaction corresponds to SRIF, or at least to a very closely related peptide. In the lizard, the system which reacts specifically with SRIF antiserum originates in a group of PVN cells whose axons partially join the hypothalamo-hypophysial tractus, but terminates almost exclusively in the external zone of the median eminence. It would therefore seems that somatostatin, which intervenes in regulating the hypophysial somatotropic function, posses through the capillaires of the hypophysial portal system to the reactive sites (STH cells) of the anterio lobe.

In the lizard, the immunocytological reactions with SRIF and neurophysin antisera reveal that the PVN comprises at least 2 immunologically distinct cells. These observations tally with those carried out, under the same conditions, on an amphibian, Xenopus laevis[12], revealing a cross-reaction with SRIF antiserum in a small group of neurophysin positive cells of the preoptic nucleus. They also agree with those of Dubois and Kolodziejczyk[13], who observed cells reacting with both SRIF and neurophysin antibodies in the PVN of the rat. The results for the lizard, as previously for the amphibian, demonstrate the existence, in the Poïkilotherms, of an SRIF-like regulatory system, and give a very clear idea of its localization.

Binding of ferritin-lectin conjugates to C-type virus in intact cells[1]

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Summary. Ferritin-Ricin II and Ferritin-Concanavalin A bound to budding as well as mature C-type viral particles. No differencies in binding between the viral coat and adjacent plasma membrane were detected with either lectin conjugate. Aggregation of viral particles by lectin conjugates was observed, and linking of virus to the plasma membrane resulted in phagocytosis of viral particles.

C-type particles budding from the surface of a cell characteristically display an outer electron dense coat arranged in spikes whereas the adjacent plasma membrane of the cell is uncoated or smooth. The viral coat presumably contains unique glycoprotein(s) that distinguishes it from the rest of the cell membrane. After infection by C-type viruses new glycoproteins have been found on the cell membranes[2]. The question of whether there are restricted sites on the cell membrane that will permit viral budding, and if such sites are associated with specific glycoproteins is still largely unsolved. Plant lectins interact with specific saccharide residues and thus might be useful for earmarking glycoproteins containing such residues. In the present study we have used the plant lectins Ricinus communis agglutin, specific for β-galactopyranosyl-like residues[3] and Concanavalin A, which is specific for α-D mannopyranosyl-like residues[4,5], in order to find a) if there are restricted or clustered binding sites on the cell membrane and b) if the membrane of the viral bud or of the mature virus can be distinguished from the rest of the cell membrane. Ferritin-lectin conjugates purified by affinity chromatography[6] were used for high resolution studies of binding sites in intact cells producing C-type particles[7]. Very recently, affinity chromatography of membrane fragments of C-type virus infected cells on Ricinus communis columns has shown that 2 viral glycoproteins were bound and largely purified from many other membrane proteins[8]. Concanavalin A was studied because it has been used as the basis for a high yield isolation of infectious C-type particles from culture media[9].

Materials and methods. Ricin II was prepared from beans as described[10] and was stored at a concentration of 25 mg/ml in 50 mM $NaPO_4$ pH 7.0. Concanavalin A (Con A) was purified by affinity chromatography on Sephadex G-75 or G-100[4] and stored under similar conditions. Conjugates of ferritin-Ricin II and ferritin-Con A were cross-linked with glutaraldehyde essentially as described[6]. 1 ml of stock Ricin II or Con A was mixed for 2 min with 1 ml (25 mg) of 6 × recrystallized ferritin using a magnetic stirrer. 0.1 ml of 0.5% (v/v) glutaraldehyde in 50 mM $NaPO_4$ pH 7.4 was added dropwise over a period of 5 min and stirred 45 min at 22 °C. The conjugate was dialyzed against 1 l of 50 mM NH_4Cl at 22 °C for 3–4 h followed by 1 l of 50 mM $NaPO_4$ pH 7.2 at 4 °C overnight. Prior to affinity chromatography the solution was clarified at 3,000 rpm for 30 min and the precipitate discarded. For ferritin-Ricin II affinity chromatography, supernatant was added to a 2.2 × 20 cm column of Sepharose 6B in 25 mM $NaPO_4$ pH 7.2. The column was washed with 50 ml of 25 mM $NaPO_4$ pH 7.2 and the conjugate of ferritin-Ricin II then eluted from the column with 0.1 M galactose in 25 mM $NaPO_4$ pH 7.2. 5.5 ml fractions were collected (figure 1, top) and the second peak eluted by galactose (Fx 25–28) was concentrated using a XM 50 Amicon filter to about 2 ml final. The concentrate was diluted, refiltered 1–2 × and centrifuged for 15 min at 10,000 rpm. The supernatant contained almost pure ferritin-Ricin II conjugate and the small amount of aggregated ferritin-Ricin II it contained was removed by gel

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filtration on Sepharose 6B equilibrated in 50 mM $NaPO_4$ pH 7.5 containing 50 mM galactose (figure 1, bottom). Ferritin-Con A was similarly purified except that affinity chromatography was done using Sephadex G-100 equilibrated in 50 mM $NaPO_4$ pH 7.2 and the elution buffer contained 0.1 M α-methylmannoside.

TC 509 derived from a methylcholanthrene induced mouse glioblastoma producing C-type particles[7] and other lines were maintained as reported[11]; all cells were in log phase at the time of exposure to conjugates. 3 different lots each of ferritin-Con A and ferritin-Ricin II were prepared and tested. Monolayers of cells were washed 3×5 min at 22 °C with isotonic phosphate buffered saline (PBS) to completely remove protein and sugars of the feeding medium; incomplete washing resulted in very limited binding of conjugate to cells and visible aggregation of conjugate in the supernatant. Lectin-ferritin conjugates were diluted in PBS and tested at concentrations of 0.06–0.17 mg ferritin/ml. Conjugates were allowed to bind to cells at 22 °C for 15 min, the monolayer was washed for 5–10 min with 3 rinses of excess PBS at 22 °C to remove unbound material and cells fixed as a monolayer with glutaraldehyde as described[12] for electron microscopy.

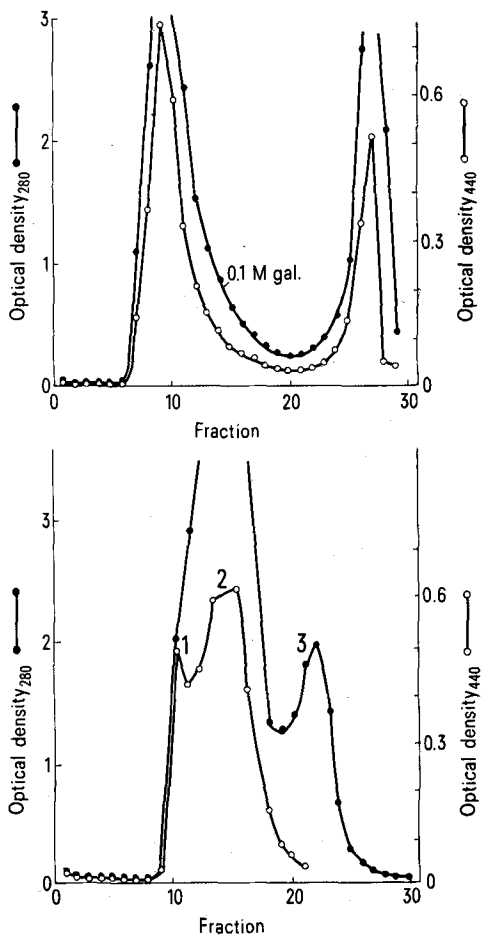

Fig. 1. Top is from affinity purification of conjugated ferritin-Ricin II from free ferritin and inactive ferritin-Ricin conjugate on Sepharose 6B. Second peak (Fx 25–28) eluted after addition of 0.1 M galactose was separated from aggregates and free Ricin on Sepharose 6B equilibrated with galactose, shown at bottom. Peak 1 represents aggregates of ferritin-Ricin II, 2 is ferritin-Ricin II used for EM studies and 3 is free Ricin II. Optical density 440 (-○-○-○-) and 280 (-●-●-●-).

In control experiments to check if the binding of conjugates to cells could be completely inhibited by the appropriate saccharide, 0.1 M galactose or 0.1 M α-methylmannoside were added to the PBS containing the lectin conjugate. Only galactose inhibited binding of ferritin-Ricin II and only α-methylmannoside abolished ferritin-Con A binding. In other experiments to rule out non-specific binding of ferritin, excess unconjugated ferritin (0.22 mg/ml PBS) was applied to cells and in this case there was no labelling of the cell surface. A second method was also employed to check results with Con A since there was some variability between different lots of ferritin-Con A conjugate. Cells were exposed to Con A, washed, fixed and then reacted with peroxidase. This method is based on the high affinity of peroxidase for a site on the Con A molecule[13]; conditions for the study were essentially as described[13]. In addition to TC 509 other normal and glioblastoma lines were labelled at the same time under the same conditions[14].

Results. In all experiments, at all concentrations, and with all batches of conjugates of ferritin-Ricin II, avid binding to the surface of TC 509 cells was observed. Ferritin particles studded all exposed surfaces of the cell. Microvilli, portions of the cell surface and regions with pseudopodial or 'ruffled' surfaces of the plasma membranes showed equivalent density of label. The ferritin was evenly distributed and there was no obvious clustering of particles in most sections (figure 2, A). In regions where cells were closely apposed, penetration and binding of conjugate to the membrane was also noted. Pinocytotic vesicles were often crammed with ferritin particles (figure 2, B).

C-type particles in TC 509 in all stages of maturation were studied and compared to adjacent membrane. Early stages of viral budding at the surface of the cell revealed equally dense and even labelling with ferritin-Ricin II as adjacent sections of membrane with no virus. Immature virus on a slender stalk (figure 2, A) as well as mature viral particles containing a dense core (figure 2, B) also revealed equivalent label both to each other and to adjacent portions of the cell. Mature viral particles in the supernatant were also heavily labelled.

Ferritin-Ricin II labelled pinocytotic vesicles occasionally contained clusters of labelled virus. Such clusters often appeared aggregated as if cross-linked by ferritin-Ricin II molecules (figure 2, B) and these clusters appeared to be ingested by the cell. Other cell vacuoles containing budding virus were generally not labelled. Control experiments with excess ferritin (unattached to a lectin) showed no surface label (figure 2, E); in such preparations in contrast to lectin conjugates, ferritin particles were also only rarely observed in pinocytotic vesicles or cell vacuoles. Studies of other glioblastoma cells producing no C-type particles including glioblastomas derived from a different species (human) showed similar intense binding of ferritin-Ricin II as TC 509 (figure 2, C). In all control studies there was inhibition of binding when excess galactose was added to the medium (figure 2, D).

Experiments with ferritin-Con A were more variable. Some batches of ferritin-Con A revealed patchy labelling of the cell surface of TC 509, and this could not be related to the concentration of conjugate employed. In such preparations aggregates of ferritin particles were occasionally associated with C-type particles or clusters of particles (figure 2, F, G), however, many unlabelled particles as well as cell surfaces were observed. Dense fluffy material on the surface of the cell was also labelled (figure 2, G). In contrast to TC 509 other human glioblastoma cells prepared and labelled identically always revealed more

avid binding of ferritin-Con A. In all cases α-methyl-mannoside completely abolished ferritin-Con A binding. In order to rule out the possibility that such poor binding was due to alteration of Con A during preparation of the ferritin conjugate, labelling of cells with untreated Con A and subsequent binding of peroxidase to Con A sites were studied. In such preparations, as in the ferritin-Con A experiments, cells of TC 509 revealed only weak and patchy binding of this lectin and viral particles were only occasionally labelled. In contrast other glioblastoma cells without C-type virus as in experiments with ferritin-Con A were more intensely and reliably labelled than TC 509 cells.

Discussion. The foregoing experiments demonstrate that conjugates both of Ricin II or Con A can be localized to spiked regions of the plasma membrane coating C-type virus as well as to smooth regions devoid of morphologically distinct specific viral structures. These results cannot be ascribed to artefacts of manipulation since these intact cells were studied under physiological conditions without any harsh disruptive measures. One possibility

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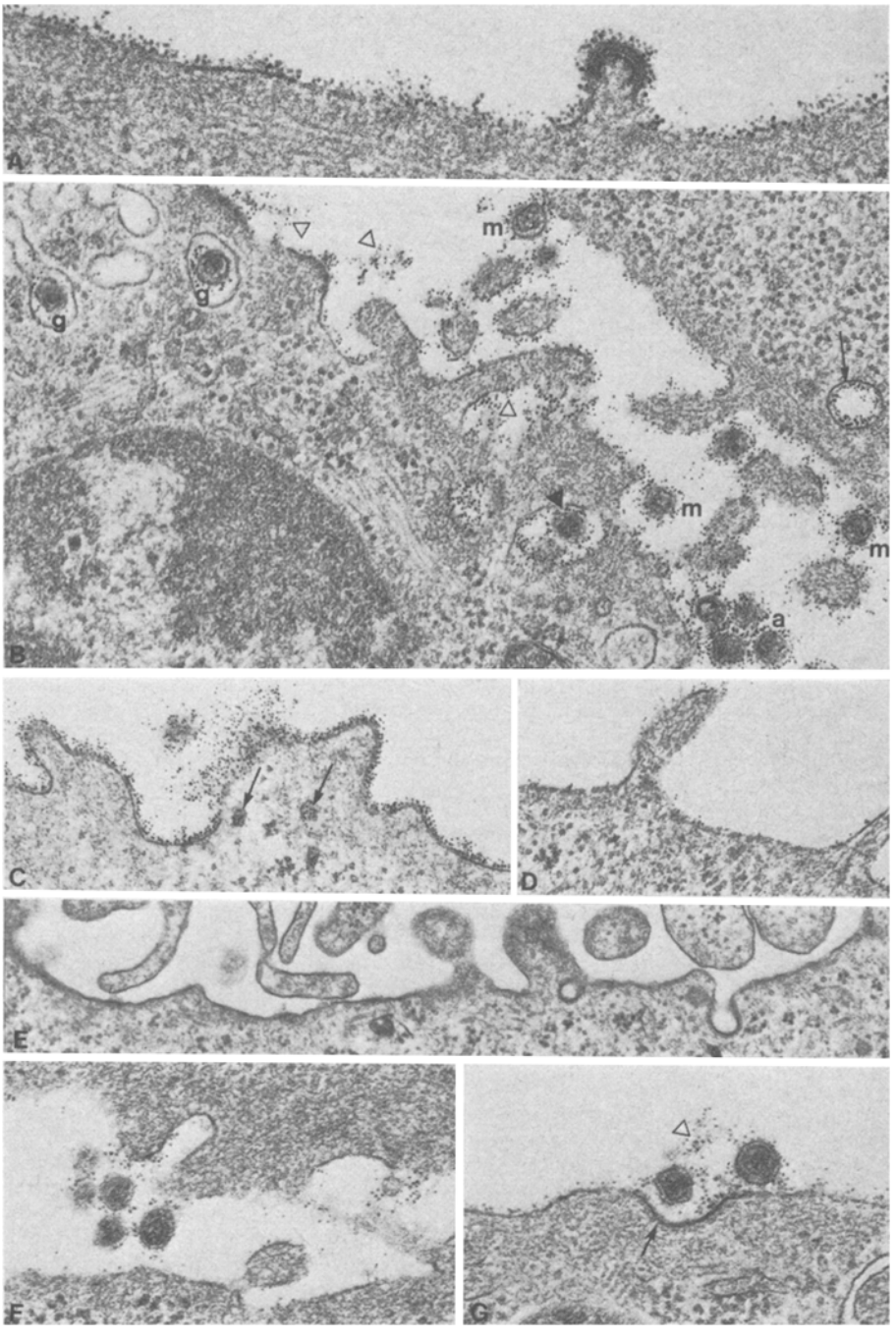

Fig. 2. *A* Immature particle budding from surface of TC 509 is labelled by ferritin-Ricin II as is stalk and adjacent region of plasma membrane. ×92,615. *B* Mature particles with dense cores (m) appear to be joined by ferritin-Ricin II to adjacent plasma membrane; aggregates of mature particles (a) appear similarly linked to each other by ferritin-Ricin II. 1 labelled mature virus appears in a phagocytotic vacuole (▲) and others are seen in the Golgi apparatus (g). Pinocytotic vacuole is labelled (arrow) as is fuzzy material at the surface of the cell (△). ×56,350. *C* Human glioblastoma with no virus shows similar intense label of plasma membranes by ferritin-Ricin II and conjugate is also seen in pinocytotic vesicles (arrows). ×45,475. *D* Inhibition of ferritin-Ricin II binding in the presence of galactose. ×44,806. *E* Addition of excess unconjutaged ferritin resulted in no label of plasma membrane. ×29,759. *F* TC 509 treated with ferritin-Con A; only patchy label of virus and cell membrane is seen and binding was always less intense than with Ricin II conjugate. ×66,093. *G* Mature virus particles labelled with ferritin-Con A; 1 particle appears to be in early stage of ingestion by underlying membrane with pinocytotic specialization (arrow). Fuzzy material at surface of cell is labelled (△). ×66,093.

is that the entire membrane of these cells contains viral glycoprotein(s) capable of binding these 2 lectins; a specific murine glycoprotein has been found in the membrane both of virus shedding cells[15] as well as in transformed but non-virus producing cells[16] suggesting some diffuse viral glycoprotein insertion in the plasma membrane in the absence of viral budding. However, in the present study human glioblastoma cells that were not infected also similarly bound these lectins thus indicating that these lectins are not sufficiently specific to detect or distinguish specific viral glycoprotein(s) in an intact preparation.

Affinity chromatography using Ricin II has been successfully employed for purification of specific membrane glycoproteins in C-type infected cells[8]. The apparent difference in specificity in the biochemical purification with lectin affinity columns as compared to the lack of specificity observed in the present study in intact cells should be considered in light of 2 factors: 1. Glycolipids with specific sugar residues will probably add to the background of lectin binding in the intact cells; such glycolipids are discarded in the biochemical isolation of glycoproteins, and 2. purification of glycoproteins from other proteins during lectin affinity chromatography can enrich viral glycoprotein components without the lectin having to possess absolute specificity for viral glycoprotein.

Use has been made of lectins in purifying C-type particles[9], however, the present studies would not indicate specific binding to viral components which could serve as the basis for their purification. Rather our results indicate that these lectins induce aggregates of viral particles. Lectins conjugated with ferritin in the present study appear to retain their polyvalent character allowing them to form a bridge between 2 or more virus particles. Production of viral aggregates would facilitate the purification of virus through an alteration in their sedimentation characteristics. The present studies also indicate Ricin II and Con A conjugates induce phagocytosis of virus by the cell, probably by linking them to the plasma membrane. Such conjugates, or lectins alone, thus might be useful as other surface active agents that induce phagocytosis[17,18] in stimulating an increased uptake of virus.

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A reticulum of nerve-like cells from trochophores of Phyllodoce mucosa (Polychaeta)[1]

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Summary. The positions of and connections between the 12 principal cells of the larval reticulum are described from Golgi-Cox preparations and sections.

A network of large, multipolar cells or 'larval reticulum' has been reported in trochophores of Lopadorhynchus that is supposed to be neural in nature[3]. We describe here initial attempts to trace the axon-like processes of cells of a similar reticulum in trochophores of Phyllodoce mucosa and to determine the relation between the reticulum and the remainder of the larval nervous system using light and electron microscopy and methods specific for nerves.

Methods. Our studies are on larvae 150 µm in height and larger collected from plankton tows in Passamaquoddy Bay, New Brunswick, during June. Cholinesterase was demonstrated using the acetylthiocholine method described by Thompson[4]. Golgi-Cox preparations were made using the rapid method described by Ramón-Moliner[5]. Preparation for EM followed standard procedures except that, for good tissue preservation, it was necessary to fix larvae first in 1% glutaraldehyde for less than 1 min and then add the postfixative (2% OsO_4) directly to the specimens in glutaraldehyde.

Results. The acetylthiocholine method for cholinesterases is a convenient means of demonstrating the larval nerve trunks in whole mounts (figure 1). Enzyme activity appears to be associated more with the surfaces of cells surrounding nerves, in the case of the prototroch nerve with the enveloping folds of the prototroch cells, than with the nerves themselves. In electron micrographs, each nerve trunk appears as a bundle of from 6 to several dozen axons depending on developmental stage. Axons in the arched connective can be traced to cell bodies lying in the paired rudiments of the cerebral ganglion located immediately behind the 2 eyes and to a cluster of 4 large apical cells presumably derived from apical tuft.

In late stage trochophores, the Golgi-Cox method demonstrates a number of large, multipolar structures scattered through the larval ectoderm with branched and interconnecting surface processes. Among these are structures having surface processes only. In sections these are seen to be single cells with large, uniformly stained nuclei and a cytoplasm filled with large (0.1–0.2 µm diameter), empty vesicles. In advanced larvae, 300–500 µm in height, there may be as many as 2 dozen such cells of which only a few impregnate in any larva. Progressively younger larvae have fewer of these cells.

As many as 4 impregnated structures may be found in the larvae having, in addition to surface processes, a single inwardly-directed process that branches once (figures 2 and 4). These are located in the ganglionic rudiments (1 in each) and on either side of the mouth. In sections we find that each of the structures comprises in fact a pair of closely apposed cells. The more distal of each pair is of the vesicle-filled sort described above and bears most of the surface processes. The more proximal (figure 3) is smaller, has fewer large vesicles and invariably gives rise to the single inwardly-directed process. Branches from this process in each case travel along the arched connective. This means that branches connecting the 2 proximal cells in the ganglionic rudiment travel through the developing commissure. Besides a single pair of reticular cells, each rudiment in the 150 µm larva con-