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-

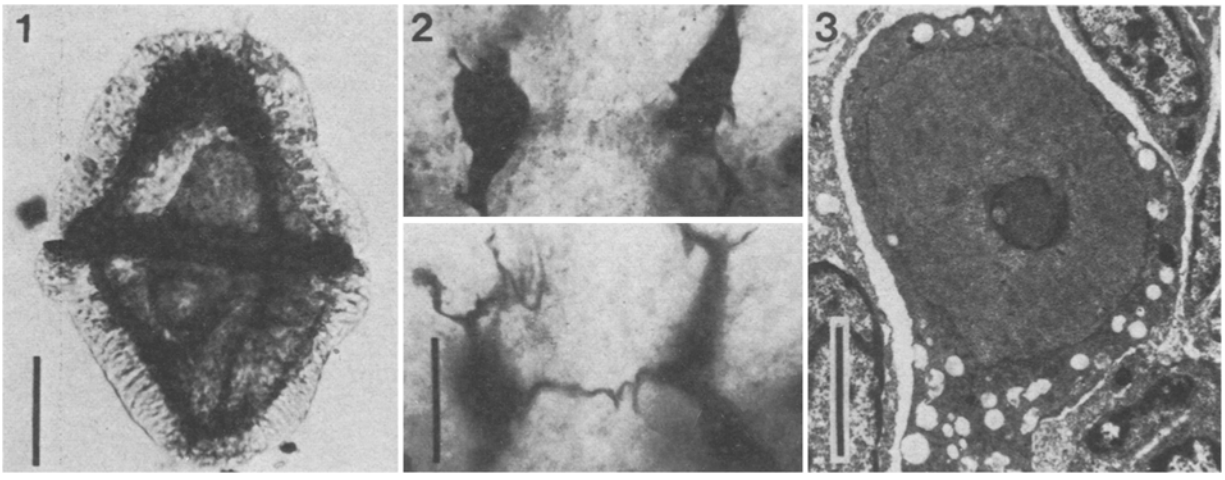

Fig. 1. Whole mount of a 300 µm P. mucosa trochophore showing localization of the enzyme cholinesterase. The circular prototroch nerve (running left to right in the figure) and arched connective are both sharply delineated, and some enzyme activity is apparent around cells of the mouth. Bar: 75 µm. Fig. 2. Whole mount of a Golgi-Cox preparation, dorsal view in 2 planes of focus of a 540 µm P. mucosa larva showing the 2 multipolar structures located in the 2 rudiments of the cerebral ganglia and the internal connection between them. Each of these structures is in fact a pair of cells, and the more proximal cell of 1 such pair is shown in figure 3. Bar: 50 µm. Fig. 3. Electron micrograph of the proximal cell of the reticular pair from the cerebral ganglion rudiment of a 200 µm P. mucosa trochophore. The limited cytoplasm and uniformly stained nucleoplasm are characteristic. Bar: 5 µm.

tains a number of neuroblasts, but not more than 4 or 5 well-differentiated, unipolar neurones of the kind typically found in well-developed ganglia. The reticular cells are therefore present very early in development when the nervous system comprises comparatively few cells.

The arrangement of the 4 pairs of cells and their processes is shown in figure 4. Cell bodies occupy the same positions in all larvae. Surface processes go to roughly the same regions in all larvae, but differ somewhat in branching pattern. The pattern formed by the internal processes is invariant except for details of the distribution of small spines and swellings along their length. The internal branches are shown schematically running parallel to one another in the figure. In fact they are in intimate contact over the greater part of their length in most preparations, and gaps are sometimes bridged by small spines with bouton-like terminals. The shrinkage problem inherent in the Golgi-Cox method makes it impossible to draw any conclusions about the exact morphology of these contacts however. 2 additional pairs of reticular cells lie in the dorsal region of the larva just posterior to the prototroch. One of these is shown in the side view in figure 4. Both pairs lack internal processes, but have extensive surface processes. These, together with the surface processes of the reticular cells flanking the mouth, form a girdle around the entire larva. We do not know whether the 2 Y-shaped structures immediately adjacent to the mouth are separate cells or specialized processes of the larger reticular cells. The arms of the Y's border a row of ciliated cells that surrounds the mouth.

Discussion. The system of 2 interconnecting, orthogonal nerve trunks is known from other trochophores[6], but a nerve net or reticulum-like structure has been previously described only for Lopadorhynchus. Our evidence shows that 12 of the reticular cells in P. mucosa, arranged in pairs, deserve special attention. These 12 are the first elements of the reticulum to appear during development, and connection between 4 of them (between the 4 proximal cells) via central pathways is an invariant feature from very early stages onward. More reticular cells are added peripherally as development proceeds, but the central connections remain essentially unchanged. To our knowledge, the P. mucosa larva is the smallest and

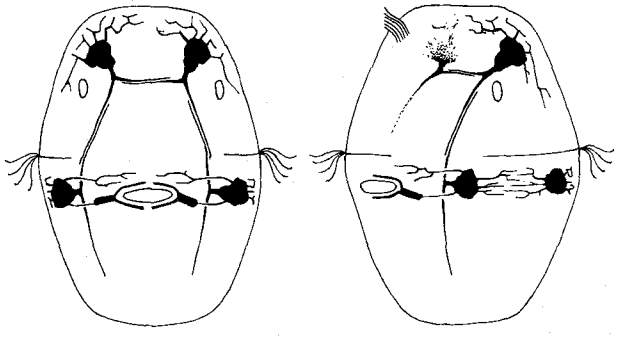

Fig. 4. A reconstruction of the reticular system in a P. mucosa larva 250 µm in height seen from the front (ventral surface) and in oblique side view, compiled from 40 Golgi-Cox preparations showing varying degrees of partial impregnation. The internal processes are in apparent contact with one another over varying proportions of their length, but are shown here schematically running parallel to one another. The form and number of branched surface processes vary from preparation to preparation and so are also shown schematically. Eyes, mouth, prototroch and the ventral sensory organ are also shown.

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simplest organism in which it has yet been possible to demonstrate such constancy of pattern and position at the level of individual nerve cells. If, as in development of the nematode ventral cord, a nerve cell's lineage determines what cell contacts it will make[7,8], the pattern of central connections seen in the P. mucosa reticulum could be understood simply as a consequence of like-to-like contact between cells having equivalent positions in the cell lineage, as proximal cells presumably do. The close association and morphological similarity between unipolar neurones of the cerebral ganglion and 2 of the proximal cells raises the possibility that similar like-to-like patterns of contact involving nerve cell types other than reticular cells might be expected to occur in the CNS. Without functional information, it is not possible to say whether the morphological contacts between reticular cells are of any functional significance or even whether reticular cells are nerve cells at all. Reticular cells in Lopadorhynchus larvae are supposed to be neurones because of their morphology, staining properties and the fact that they send processes to roughly those areas of the larva that are contractile[3]. The last is also true for P. mucosa; contractions are initiated at the top of the larva and circumferentially at a point just posterior to the prototroch. The supposition that reticular cells are neurones is further supported by their location, in P. mucosa, in ganglionic rudiments.

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Pigment in the spleen of C57BL/10ScSn and related mice[1]

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Summary. Pigment present in the spleen of C57BL and related mice, after the stress of experimental procedures, was shown to be melanin.

During experiments designed to examine the response of mice to inoculations of different species of mycoplasma (Hill, unpublished results), it was noticed that in an experiment with C57BL/10ScSn mice, at necropsy dark areas were visible in the spleens of some mice. This experiment was repeated with similar results. It was thought unlikely that the specific mycoplasma species used (strain 58B) should have caused this appearance. Other strains of mice, BALB/c, +nu, nu nu and A2Hr were unaffected when inoculated with the same species of mycoplasma. Therefore, it was decided to examine this anomaly.

Materials and methods. The animals used in these experiments were 4–6 weeks old mice from a specified pathogen free unit, category 4[2]. After inoculation the mice were housed in filter boxes[3] and kept in a conventional animal room. The medium used was as follows: 70 ml Difco PPLO broth, 1 ml penicillin G 100,000 units, 2 ml thallium acetate 2.5%, 10 ml yeast extract 25%, 20 ml horse serum, 1 ml glucose 10%, 1 ml phenol red 0.2%. Mycoplasma pulmonis, M. neurolyticum and mycoplasma strain 58B were freshly isolated from the lung of a rat, the conjunctiva of a mouse and the conjunctiva of a rat respectively. These isolates were subcultured in liquid media twice and then frozen in ampoules at –70 °C. 0.02 ml of each inoculum was given intranasally to each mouse as shown in table 1 and 2. Components of the media were diluted in PBS to give the same concentration as used in the medium. 6 uninoculated mice, housed under the same conditions, were kept as controls. The mice were kept for 3 weeks and then killed. A cut surface of spleen and nasopharyngeal swabs were rubbed over the surface of mycoplasma agar plates which were incubated at 37 °C in a humid atmosphere for 3 weeks. Portions of spleen were fixed in 10% formalin, and sections of the tissue stained with haematoxylin and eosin. Portions of liver, kidney and adrenals, from mice showing dark areas in the spleen, were also fixed and sections examined. Sections of spleen showing dark areas were bleached by the methods shown in table 3 and then stained with haematoxylin and eosin. Sections bleached by potassium chlorate and potassium permanganate methods were also stained with Schmorl and Masson-Fontana's silver stain. Further sections were stained (table 4).

Results and discussion. 3 weeks after inoculation all animals appeared healthy. At necropsy 20 out of 93 spleens showed dark areas partially banding the spleens (one on each) transversely but not exceeding $^1/_2$ of the whole spleen (figure 1). The size of these spleens were within normal limits. The other organs appeared normal, and no histological abnormality was seen on any of the livers, kidneys or adrenals examined. No deposits were seen in

Table 1. Strains of mice and inocula

Inoculate	Strain	Site	No. of mice	No. affected
M. pulmonis	C57BL/10ScSn	Nasal	6	0
M. neurolyticum	C57BL/10ScSn	Nasal	6	2
M. 58B	C57BL/10ScSn	Nasal	6	2
Whole media	C57BL/10ScSn	Nasal	6	1
$^1/_{100}$ phenol red 0.2%	C57BL/10ScSn	Nasal	5	2
$^1/_{50}$ thallium acetate 2.5%	C57BL/10ScSn	Nasal	5	0
$^1/_{100}$ penicillin G 100,000 units	C57BL/10ScSn	Nasal	5	1
$^1/_{10}$ yeast extract 25%	C57BL/10ScSn	Nasal	5	1
$^1/_5$ horse serum	C57BL/10ScSn	Nasal	5	3
M. 58B	C57BL/10ScSn	Nasal	6	3
M. 58B	C57BR	Nasal	6	0
M. 58B	C57BL	Nasal	6	1
M. 58B	C57BL	Conjuctiva	6	2
Distilled water	C57BL	Nasal	20	2
Total			93	20