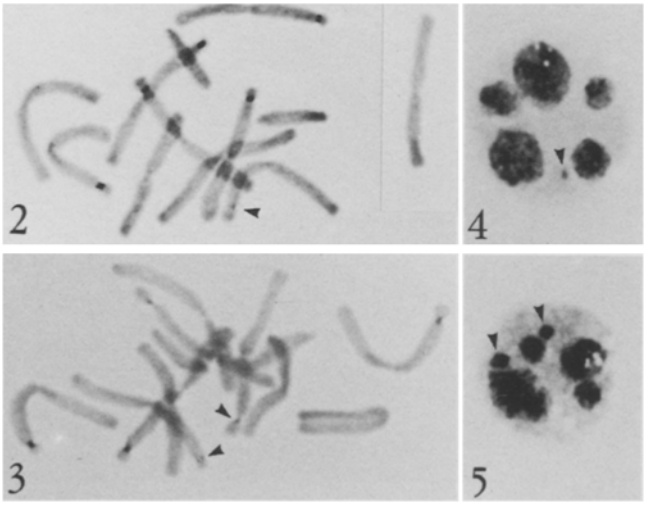
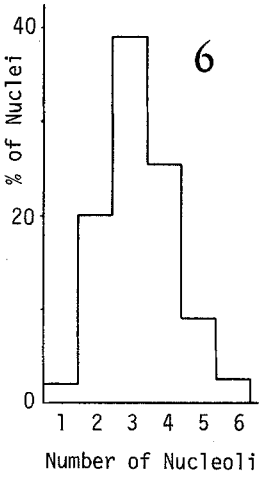
Figure 1. Metaphase chromosomes of *Nigella damacena* stained with Giemsa. Arrow heads indicate the secondary constrictions. × 1200.





Figures 2 and 3. Metaphase chromosomes of *N. damacena* stained with silver nitrate. The smallest stained regions, indicated by arrow heads, varied in number among seedlings. × 1450.
Figures 4 and 5. Interphase nuclei of *N. damacena* stained with silver nitrate. Arrow heads indicate the smallest nucleoli. × 1100.
Figure 6. Histogram of frequency of nuclei with each nucleolar number. Nucleoli were examined from 1032 of 10 seedlings.

Petri dishes at 20 °C in the dark. After a week, actively growing primary root tips were harvested and pretreated in 0.05% colchicine solution at 20 °C for 2.5 h, then fixed in an ethanol-glacial acetic acid (3:1) mixture at 5 °C for 1 h. The fixed materials were treated with both cellulase and pectinase prior to flame-drying. The slides were stained with a 50% $AgNO_3$ solution at 60 °C for 1–3 h (see Hizume et al.[3] for details). Some slides were stained with 2% Giemsa solution for 10 min for standard karyotype analysis.

Results and discussion. The chromosome complement (2n = 12) was composed of 5 pairs of metacentric chromosomes and a pair of telocentric chromosomes. The secondary constrictions appeared at or near terminal regions in 3 metacentric pairs of chromosomes (No. 3–8, fig. 1). After Giemsa staining in some cells of some clones the secondary constrictions were not apparent, but after silver staining all the 6 secondary constrictions were always heavily stained (figs 2 and 3). While the size of the stained regions in the complement was typically variable, 1 or 2 regions were larger than the others. The smallest stained region seemed to be located in the 4th pair of chromosomes. 1–6 nucleoli, usually 2–4, are visualized as darkly-stained spherules in the nucleus after staining with silver nitrate (fig. 6). In the nucleus with 6 nucleoli their size was graded from large to small, and 2 nucleoli were typically larger while 1 or 2 were typically smaller (figs 4 and 5). This appearance of nucleoli was strongly correlated with the size of the silver stained region on the metaphase chromosomes. The maximum number of nucleoli per nucleus is coincident with the number of the silver stained regions and the secondary constrictions; therefore, we concluded that all the secondary constrictions of *Nigella damacena* chromosomes were NORs.

1 We would like to thank Dr C.R. Parks (Department of Botany, The University of North Carolina at Chapel Hill, USA) for some helpful comments during manuscript preparation.
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Cytochemically rich neuroglia invest metacercariae in the brain of *Phoxinus phoxinus* L.

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Summary. Histochemical determination of neuroglia investing parasites in naturally infected fish depends upon recognition of the highly soluble or diffusible nature of the cytoplasm.

Remarkably, minnows survive although the brain is parasitized by the trematode, *Diplostomum phoxini* (Faust, 1917). Cercariae invade fish, migrate to the brain[2] and grow from 0.22 × 0.03 mm^3 to metacercariae, 0.35 × 0.13 mm in size[3] or larger[4]. They live anywhere in the brain but most lie beneath the ependyma of the IVth ventricle[2]. Parasitized fish live for more than 2 years in the laboratory[5]; all mature minnows in a population can be infected[2–6], while reports of parasite-induced aberrant behaviour[7,8] or death of the host[3] are rare.

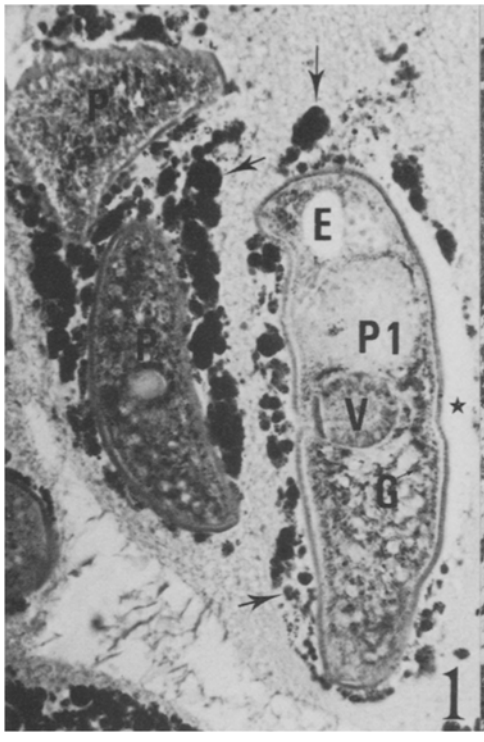

Figure 1. Light micrograph (× 174), *D.phoxini* in brain fixed by 'experimental' method stained by PAS. Arrows indicate cytochemically active neuroglia around parasites (P). In L.S. 1 parasite (Pl) shows excretory bladder (E) ventral sucker (V) and glycogen (G). Tissue shrinkage causes an artifact at ★.

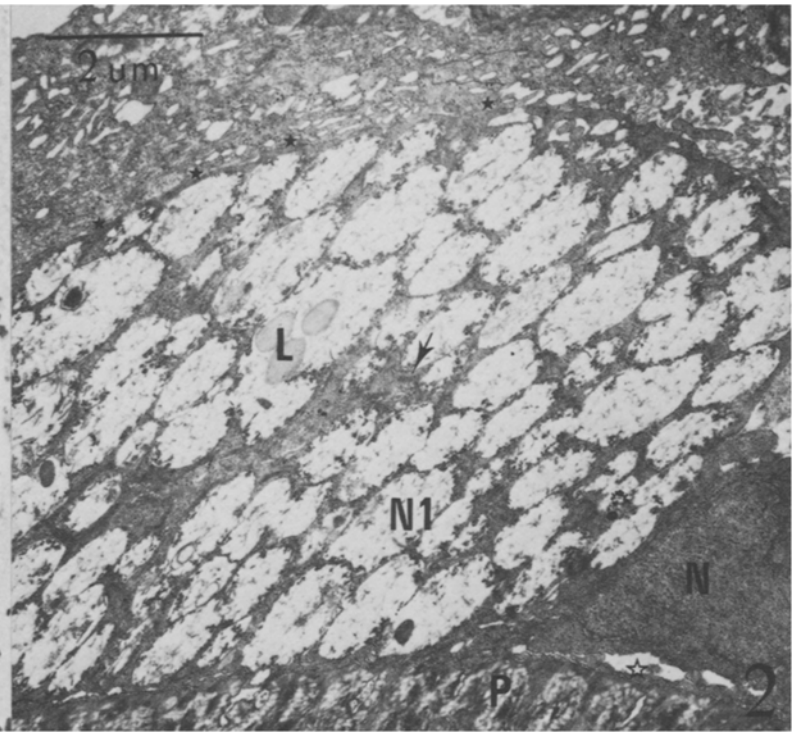

Figure 2. Electron micrograph shows *D.phoxini* (P) invested by small (N) and large (Nl) neuroglial cells (L-lipid; arrow-golgi) with thin extensions (☆). Parasites are isolated from nervous tissue (above ★★).

The fish does not control the number of parasites; minnows freed from re-infection remain parasitized for more than 2 years[5]; from a few to 1296 metacercariae[3] occur naturally in a fish. The parasite does not encyst but remains in contact with host tissue, and feeds[8].

Encapsulation by the host under natural conditions has not been recorded. To account for this by invoking the concept of an unresponsive brain[9] is wrong because experimentally-induced encapsulation[10] can lead to the death of *D.phoxini* when astrocyte-like cells invest both the parasite and some host cells in a thin-walled cyst.

Minnows, therefore, have the capacity to respond to *D.phoxini* but usually do not fully express this response. However, the proliferation of vacuolated cells around metacercariae has been recorded for 50 years[3,9–12]. An hypothesis to account for the incomplete response proposed the vacuolated cells to be the 1st part of the repair process[10]. Hence cells accumulate around the parasite and possibly protect the neurons from damage. The vacuolated cells may, therefore, have a crucial role to play in the maintenance of host and parasite and the present knowledge of them as 'rounded' and 'vacuolated' is inadequate. Some fish cells, such as mast cells, have unusually soluble contents[13,14]. To test whether the vacuolated cells investing *D.phoxini* in a natural infection contain water soluble or diffusible substances required an experiment on the effect of aqueous fixatives and histological methods compared with methods using water sparingly, if at all.

Materials and methods. All *Phoxinus phoxinus* caught in Frongoch Pool near Aberystwyth were infected with *D.phoxini.* Minnow brains and whole heads (total 17) were fixed and embedded in wax by methods used before[3,5,8,10,11], these methods are designated 'standard' in the table. 9 brains were fixed while infected fish were under deep anaesthesia induced by 'Epontal' (Bayer, Leverkusen)[15]. The brain was exposed and fixed in Rossmans's fixative[16] while the anaesthetized fish lay in a dish of shallow water, gill ventilation continued until the applica-

Test	Reference to method	Results Standard preparation	Experimental preparation	Comments
PAS	18	∓	+	Aqueous periodic acid
PAS	19	∓	+ + + +	Alcoholic periodic acid
PAS/dimedone	18	Not done	+	Aqueous dimedone
PAS/dimedone	16	Not done	+ + +	Alcoholic dimedone
Best's carmine	18	∓	+ +	Parasites stain strongly
Alcian blue	18	∓	+ +	Aqueous A-blue
Alcian blue	–	∓	+ + + +	Alcoholic A-blue
Alkaline phosphatase	16; 18	+ + + +	Not done	Gomori and Azodye methods
Glycogen phosphorylase	16	+ + +	Not done	Mauve-purple colour

Cytochemical response of neuroglia associated with *D.phoxini:* related to method of fixation and use of aqueous and alcoholic tests.

tion of fixative; perfusion of these small fish was not attempted. The brain was then quickly excised, placed in Rossmans' fixative precooled to −35 °C and kept at that temperature for 24 h. Brains were then passed through 3 changes of ethanol at 4 °C each of 1 h duration. Following this, specimens were cleared in xylene and embedded in wax. This method is designated 'experimental' in the table.
Sections from all blocks were cut at 7 µm, floated on water (standard method) or alcohol (experimental method) and stained by methods listed in the table. Hydration was avoided wherever possible in 'experimental' methods. A brain was fixed for electron microscopy[17] while the fish was anaesthetized.
Results. In the brain of fish fixed and stained by methods used by others the parasites were surrounded by vacuolated cells which were cytochemically inactive except for alkaline phosphatase (table). These results confirm the earlier work[3,5,8,10,11].
In contrast, cells around parasites stained strongly (fig. 1) in sections prepared and stained by methods which minimize post-mortem change and the loss of soluble or diffusible substances (table). These cells were seen also at some distance from the parasites, especially in the meninges and under the ependyma. The PAS reaction was markedly reduced when aqueous solutions of periodic acid were used.
Electron microscopy showed parasites to be in contact with neuroglia[17] and extensions of these cells could be very thin (fig. 2). Some neuroglial cells were small (fig. 2), had little cytoplasm, contained glycogen, long profiles of endoplasmic reticulum and mitochondria, but little else, whereas large cells were filled with vesicles (fig. 2), subdivided into groups by the scanty cytoplasm; also present were glycogen, Golgi bodies, mitochondria and lipid inclusions. Cells existed that were intermediate in size and cytology between these extremes.
No equivalent PAS-rich cells were detected in the uninfected fish although serial sections of the whole brain were studied.
Discussion. Anaesthesia was used in the experimental method because post-mortem changes in brain are well known to be rapid. The avoidance of incubation of tissue in aqueous solutions permits the demonstration of cytochemically rich cells where otherwise unreactive, vacuolated cells were seen. By their staining reaction, such cells could be detected elsewhere in the infected brain, in addition to those in contact with the parasites. The less-differentiated cells resemble microglia[20] but certain identification of neuroglia in fish cannot be made; the need for a systematic study of the pathology of fish nervous tissue has been recognised[14]. 2 sites of origin account for cells which respond to brain lesions[20]: astrocytes and oligodendrocytes arise from ectodermal precursors beneath the ependyma, whereas microglia have their origin in the mesoderm. Phagocytes from the blood following vascular injury may further contribute to the cellular response.
To be determined next is the origin of the cells that invest the parasites and appear to buffer it from neurons.

1 Acknowledgment. M.R.L. Johnston advised me during the course of this work.
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Studies on the modification of *Escherichia coli* ribosomal protein L7/L12 by succinic anhydride[1]

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Summary. Lysine modification by increasing quantities of succinic anhydride in the *Escherichia coli* ribosomal protein L7/L12 produces loss of its ability in reconstitution of elongation-factor-G-dependent GTP hydrolysis and polyphenylalanine synthesis activities, showing lower antigenicity and loss of antigenic determinants.

The *Escherichia coli* L7/L12 ribosomal protein may be removed from ribosomes quite specifically by ethanol-ammonium chloride[2], giving particles (cores) unable to support most of elongation-factor-dependent functions[3,4]. These particles efficiently rebind L7/L12, achieving the normal stoichiometry of 4 L7/L12 monomers in dimeric structure per ribosome[2,5].
Chemical modification of amino acid side chains within proteins has yielded extensive information about the role of particular amino acid residues and maintenance of native structure and antigenicity[6,7]. Succinic anhydride is able to react with lysine and tyrosine, but only lysines remain succinylated over pH 5.0, replacing a positive by a negative charge[8]. In the present study the L7/L12 modification by succinic anhydride has been examinated in relation to its functional activity and antigenicity.
Materials and methods. Escherichia coli strain MRE 600 was collected in logarithmic phase and ribosomes prepared as previously described[9]. Ribosomal protein L7/L12 was obtained from ribosomes by treatment with 1 M ammonium chloride and 50% v/v ethanol[2]. The isolated protein dissolved in 0.05 M HEPES[10], 0.02 M $MgCl_2$, 0.0005 M DTT