Mast cells enter a teleost's brain by Xth cranial nerve in response to *Diplostomum phoxini* (Trematoda)

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Summary. Experimental infection by *Diplostomum phoxini* of the brain of laboratory hatched and reared *Phoxinus phoxinus* induces the migration along the Xth cranial nerve of periodic acid Schiff positive granular leucocytes (PAS-GLs). These differentiate and grow into cells that lie between the parasite and the neurones of the host. The transformed cells are associated with elevated levels of heparin and serotonin in the brain; little histamine was detected. The reactive cell is identified as a mast cell.

More than 1000 metacercariae of the trematode *Diplostomum phoxini* can live in the brain of the teleost *Phoxinus phoxinus* (the European minnow) and although the parasite is associated with a host response[2] it survives for at least 2 years[3]. The host can be induced to encapsulate and kill *D. phoxini* in the brain[4] but it has not been recorded to do so in natural conditions. The cells of the capsule, but not those of the normal response, were identified as astrocytes[4] but in view of the reported abundance of ependymal cells in the teleost brain[5] the participation of ependymal cells in capsule formation cannot be ruled out. The origin and nature of the cells that contribute to the normal response have not been determined although following an appreciation of the extreme solubility of the cell's contents, some histochemical data was recorded[2]. The course of the normal host cell reponse has now been studied through the experimental infection with *D. phoxini* of young, laboratory hatched and raised minnows.

Materials and methods. Spermatozoa stripped from 1 male *P. phoxinus* were used to fertilize ova from a single female minnow collected from Lake Frongoch. This lake has been the source of material used in a number of studies of *D. phoxini*[6-9]. Artificially fertilized eggs were maintained in the laboratory at 18 °C in running lake water and they hatched in 7 days. The young fish were kept at 18 °C and they measured 1.4-2.3 cm and weighed 60-123 mg at 89 days of age. The gonads in the young fish at this time were developing and primary oocytes were observed in females; the fish were judged to be physiologically normal. These fish were then infected with about 50 cercariae of *D. phoxini* from 1 specimen of *Lymnaea peregrer* collected in Lake Frongoch. The infected fish were kept at 18 °C and individuals were killed 4, 6, 8, h; 3, 4, 6, 8, 10, 12, 15, 20, 25, 29, 30, 34 days post-infection. The fish were fixed in Rossman's fixative[2], embedded without decalcification in Paraplast (Lancer, Oxford), most serial sections were stained with PAS, the use of aqueous solutions was avoided[2]. A few sections were stained in PAS using the standard technique[10] to determine the water solubility of intracellular granules. Because the fish were small it was possible to transversely section whole animals. Furthermore, because they had been kept isolated from other fish in the aquarium they were free of pathogens except *D. phoxini* and so any cell response could be associated with that trematode. There was no evidence for concomitant infections with bacteria or fungi carried in with the cercariae as they invaded the fish. Uninfected fish were studied as controls.

Quantitative analyses for heparin[11], histamine[12] and serotonin[13] were carried out on brains of naturally infected minnows from Lake Frongoch, on brains of uninfected fish from Lake Pen Dam and, in the case of the serotonin analysis, on isolated metacercariae (~ 200).

Results. All sections were examined for the presence of the water soluble, histochemically rich cells associated with mature metacercariae of *D. phoxini* (2). They were detected 8 days after infection, but not earlier, when they were near to the parasite in the brain. On their detection and for the next 20 days the parasites probably ingested the cells as, during this time only, the gut contained cells with PAS positive contents which were water soluble. When the parasite started to feed on these cells, growth in the metacercariae was detected for the first time. The initiation of growth and metamorphosis was recorded at 7 days in an earlier study[14] but the significance of the delay between invasion and growth was not discussed. Although the cells were to remain a constant and common feature of the infection at no time was cell division seen nor was there evidence for migration of the cell into the brain. From about 30 days post infection the parasites and the cells were identical to those seen in natural infections of mature metacercariae[2]. In contrast to the heavily granular nature of the cell in contact with the parasite's tegument in the mature infections, some cells in contact with the tegument of parasites appeared damaged during the time *D. phoxini* fed on host cells. That is, the damaged cells stained less strongly with PAS.

Determination of serotonin, heparin and histamine in mature, naturally infected *P. phoxinus* is listed in the table. Parasitism is associated with an increase in serotonin and heparin, histamine is at a low level and serotonin cannot be accounted to the metacercariae.

From 4 h post infection a change occurred in *P. phoxinus* in the number and distribution of a cell previously identified in several species of teleost[15-17] and later confirmed as a cell type in the gut and also around and in the thymus of *Cyprinus carpio* and the gut of *Barbus conchonus*[18]. This cell was termed periodic acid Schiff positive granular leucocyte (PAS-GL)[15]. In uninfected young *P. phoxinus* the cell was seen in connective tissue throughout the body but it was especially common near to lymphatics and lymphatic tissue such as the pronephros (head kidney) and thymus. The cell was also seen in the pineal region of the brain in uninfected fish.

At 4 h post infection the PAS-GL cell was seen in large numbers in the Xth cranial nerves; they apparently issued into the brain and, although the exact relationship of the cells to the tissue was not determined, it appeared that most were lying in the meninges. The invasion of the parasite from the skin to the brain is rapid as parasites were seen in sections of the brain at 4 h post infection.

Determination of serotonin, heparin and histamine in naturally infected and in uninfected minnows. For comparison, values for laboratory mouse tissue are tabled

Tissue	Quantity in $\mu g\ g^{-1}$ tissue[a]		
	Uninfected brain	Infected brain	Comparative material
Serotonin	4.2; 7.2	22.5; 18	None detected[b]
Heparin	2.0; 2.0	13.5	
Histamine	None detected	0.18; 0.2; 0.16; 0.25	60[c] 0.8[d]

[a] Each value represents the analysis of 1 animal.
[b] 200 metacercariae *D. phoxini*.
[c] Laboratory mouse ear; a value of 63.3 given in Anton and Sayre[28].
[d] Laboratory mouse lung; a value of 0.29 given in Anton and Sayre[28].

Until 8 days post infection there was no sign of a cell response to the parasite itself, but on the 8th day PAS-GL host cells were seen for the first time close to the parasite. Among these cells were others which were larger and these cells had more granular contents that were water soluble. By 10 days postinfection, large numbers of the typical reactive cell were around *D. phoxini* whereas the PAS-GL cells were some distance away from the developing metacercariae. Throughout the infection, undifferentiated PAS-GL cells occurred in the tissues of the fish such as the brain, the Xth cranial nerve, head kidney and thymus. The differentiated, granular cell was to be seen in the vicinity of *D. phoxini* and not elsewhere.

Discussion. The separate elements of this study are now interpreted as a continuous sequence. In response to infection with *D. phoxini* one cell type (termed PAS-GL), previously identified in several species of teleost, migrates within hours into the brain. After a period of 6–8 days it moves towards the parasite and transforms into the cytochemically rich cell typical of the mature infection. Natural infection of mature metacercariae is associated with elevated levels in the brain of serotonin and heparin, but not histamine.

The PAS-GL cell in other fish was termed a mast cell[16–18]. Unlike the mammalian mast cell, however, it did not contain detectable quantities of histamine or heparin, although a substance related to heparin was suspected. In its transformed state in *P. phoxinus* the PAS-GL cell acquires the extreme solubility in water which was a characteristic of the fish mast cell identified on morphological grounds by Michels[19]. The cytological differentiation seen in *P. phoxinus* could be associated with a build up of heparin and serotonin which are both constituents of some mast cells; histamine on the other hand does not occur in the mast cells of fish and amphibia[20]. It is concluded that the host cell responding to *D. phoxini* is a mast cell, but strict homology with the mammalian mast cell is not implied.

The mast cell of *P. phoxinus* shows a marked response to stimuli when it migrates and differentiates. A study of these responses in comparison with events in mammalian mast cell clones when they mature[21] appears justified.

Whether this transformation is a feature of other teleost mast cells, whether the undifferentiated PAS-GL cell has a functional role and what effects the differentiated cell may have on *D. phoxini* are to be investigated. Some insight may be gained from studies on the mammalian mast cell which is known to attach to *Schistosoma mansoni* in vitro[22]; furthermore, the parasite has an anti-mast cell mechanism that prevents degranulation[23]. The mast cell may be important as an item of food and serotonin itself may act to initiate development in *D. phoxini* because *S. mansoni* is known to make use of its mammal host's serotonin to activate an adenylate cyclase system[24]. The absence of growth until mast cells assemble about *D. phoxini* is indicative of the brain's inadequacy as a source of nutrients in early development.

No evidence was seen of any route into the brain other than the Xth cranial nerve, but a population of the cells was observed in the pineal before infection. The speed and apparent directional nature of the migration was notable. This could indicate rapid communication between the brain, damaged by invading *D. phoxini*, and the PAS-GL cells. The message could be bloodborne but that, by way of the heart, would not be as direct a route to the connective tissue sites of the PAS-GL cell in head, head kidney and thymus as one by the lymphatic system. A blood route would not provide a chemical gradient for the migrating cells. The head of teleosts is richly supplied with lymphatic drainage[25] and although the brain does not have lymphatic vessels, intercellular fluid in the brain (this could include substances released from brain tissues damaged by *D. phoxini*) may pass out of the brain and gain access to true lymphatics as has been argued by Földi[26].

Mast cells were not recorded in the central nervous system[27] but that book is a review of studies on mammals; however, mast cells do exist in the endoneurium but not in nerve roots central to the sensory ganglia of mammals. Regeneration of the central nervous system in a mammal is very limited; in teleosts and amphibia, on the other hand, extensive repair and replacement is possible. If a mast cell response similar to that seen in *P. phoxinus* to *D. phoxini* is general this may be why teleosts and amphibians are parasitised by *D. phoxini* and its relatives and why the host is not disabled by neurological damage.

Next to be determined is the route taken by the PAS-GL cell to the Xth cranial nerve and during its migration along the nerve into the brain.

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