The results of regression analysis carried out on the data obtained are shown in the table and graphically in the figure.

The curves (fig.) corresponding to the flower and leaf-generations (FL and L, respectively) allow us, knowing the number of adults captured, to anticipate the level of infestation on the respective vegetative stages of the tree, which are normally low in both cases in the area. However, during the fruit-generation, the resulting percentage of infestation is usually very high in the biotope, due to several oeco-biological factors, mainly the low incidence of population reduction factors intervening during the precedent anthophagous generation[5].

1 The authors want to acknowledge Dr Owen T. Jones, Southampton University, for his assistance in drafting this paper.
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Experimentally induced encapsulation of *Diplostomum phoxini* (Faust) in the fish host

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Summary. Experimentally induced encapsulation of *Diplostomum* by the host *Phoxinus,* is recorded for the first time and requires the metacercariae to develop in fish at a temperature (23–26 °C) which is above that naturally encountered.

The metacercariae of *Diplostomum phoxini* (Trematoda) live in the brain of the second intermediate host, a fish, *Phoxinus phoxinus.* These parasites do not encyst and may occur anywhere in the brain although most of them lie beneath the ependyma of the IVth ventricle[2,3]. Naturally infected fish are common even though they may support numerous metacercariae; 1296 being the maximum number recorded[3,4].

The occurrence in the brain of so many active, feeding metazoan parasites which have little if any recorded effect upon the host[3] is a paradox made the more extraordinary by the absence of an effective host response against the parasites. An accumulation of rounded, vacuolated cells around the parasites is, however, a feature of this infection[2,3].

Parts of the brain may be immunologically privileged[5] and this, together with a lack of connective tissue cells in the brain[6], could account for the host's failure to encapsulate the parasite and for the need of a parasite derived cyst. However, the logic of this argument is weakened because *Diplostomum baeri eucaliae* and *Ornithodiplostomum ptychocheilus* which live in the brain of other fish are, respectively, encapsulated in a 'tumor' by their host[7] or encyst there[8]. Therefore, the assumption that *P. phoxinus* is incapable of encapsulating the metacercariae of *D. phoxini* was tested. The simplest method to perturb the relationship between the host and parasite without at the same time damaging the morphologic basis for the privileged nature of the brain was to raise the temperature of experimentally infected fish above that existing in natural conditions.

Materials and methods. Naturally infected snails *(Lymnaea peregra)* emitting cercariae of *D. phoxini* were collected from Fron Goch Pool, near Aberystwyth, Wales, the source used by Rees[9]. Uninfected fish *(P. phoxinus)* were collected from a lake (Pen dam) from which no infected fish have been recorded[9]. To experimentally infect each fish with 30–60 parasites, a single infected snail was exposed to light in 200 ml of lake water. After 1 h the snail was removed and

Induction of capsule around developing metacercariae (23–25 °C)

Days after infection	Experiment 1 (13/11/1979)				Experiment 2 (23/2/1980)				Experiment 3 (16/6/1980)			
	1	2	3	4	1	2	3	4	1	2	3	4
10	35	–	–	35	34	–	–	34	16	–	–	16
20	46	–	–	46	30	–	–	30	40	–	–	40
22	40	–	–	40	41	–	–	41	48	–	–	48
24	38	–	–	38	39	–	–	39	50	–	–	50
26	50	–	–	50	51	–	–	51	34	–	–	34
28	54	–	25	29	36	–	20	16	55	–	17	38
30	52	–	30	22	42	–	28	14	31	–	18	13
32	36	–	25	11	45	20	12	13	27	15	10	2
34	48	28	15	5	41	25	12	4	50	27	12	11
36	35	15	10	10	18	10	6	2	45	29	4	12
38	42	23	12	7	29	12	9	8	19	19	–	–
40					35	17	8	10	32	20	8	4
50					52	28	14	10	46	23	10	13
60					30	21*	2	7	28	15**	8	5

1 Total number of parasites recovered; *2* number of encapsulated specimens; *3* number of parasites with abnormal accumulation of cells; *4* number of normal parasites. * 18 dead; 3 living parasites, ** 15 dead parasites.

the water containing the cercariae was added to 5 l lake water with 10 *P. phoxinus*. After 1 h exposure to infection, the fish were transferred to aquaria at 17–18 °C for 48 h. The fish were then divided into 2 groups. A control group was kept at 17–18 °C and the experimental group at 23–26 °C.

At known intervals after infection, fish were killed with MS 222 (Sandoz). Parasites were recovered from excized brains in Hanks saline. Living specimens were examined by light microscopy, whole brains were fixed in formal calcium, embedded in wax and sectioned. Specimens for electron microscopy were prepared by the methods detailed in 9 and examined in an AEI 6 B electronmicroscope.

Results. When experimentally infected fish were kept at 23–26 °C about half of the developing metacercariae were encapsulated in the brain 32–35 days after infection (table). Before this time they were not encapsulated. However, from 28 days post-infection there was an increase in the number of the rounded cells which normally lie between the metacercariae and the neurons (table). The judgement to score these as abnormal was subjective and not clear cut as when a capsule was present.

With increased time, up to 60 days post-infection, the proportion of encapsulated parasites grew but all parasites were never encapsulated.

In living specimens, the capsule consisted of a thin, refractile layer (fig. 2). The parasite within the capsule could be seen to move in a fluid which contained lipid-like droplets and cells. The existence of a capsule was confirmed in sectioned brains (Fig. 3). By day 60 post-infection, most of the encapsulated parasites were dead.

At 32–35 days post-infection, the capsule wall was seen by electron microscopy to consist of a layer of overlapping, thin cells linked by desmosomes (fig. 4 and 5). The cells are electron translucent with few, widely scattered organelles and bundles of fibrils which extend through the cell as an interconnecting network. This appearance is typical of astrocytes of mammals[11,12]. Between the capsule and the parasite lie quite different electron dense cells believed to be the rounded cells normally lying against the parasite.

Although no visible change was observed in the structure of the capsule around dead metacercariae by light microscopy, the intercellular space between overlapping astrocytes was

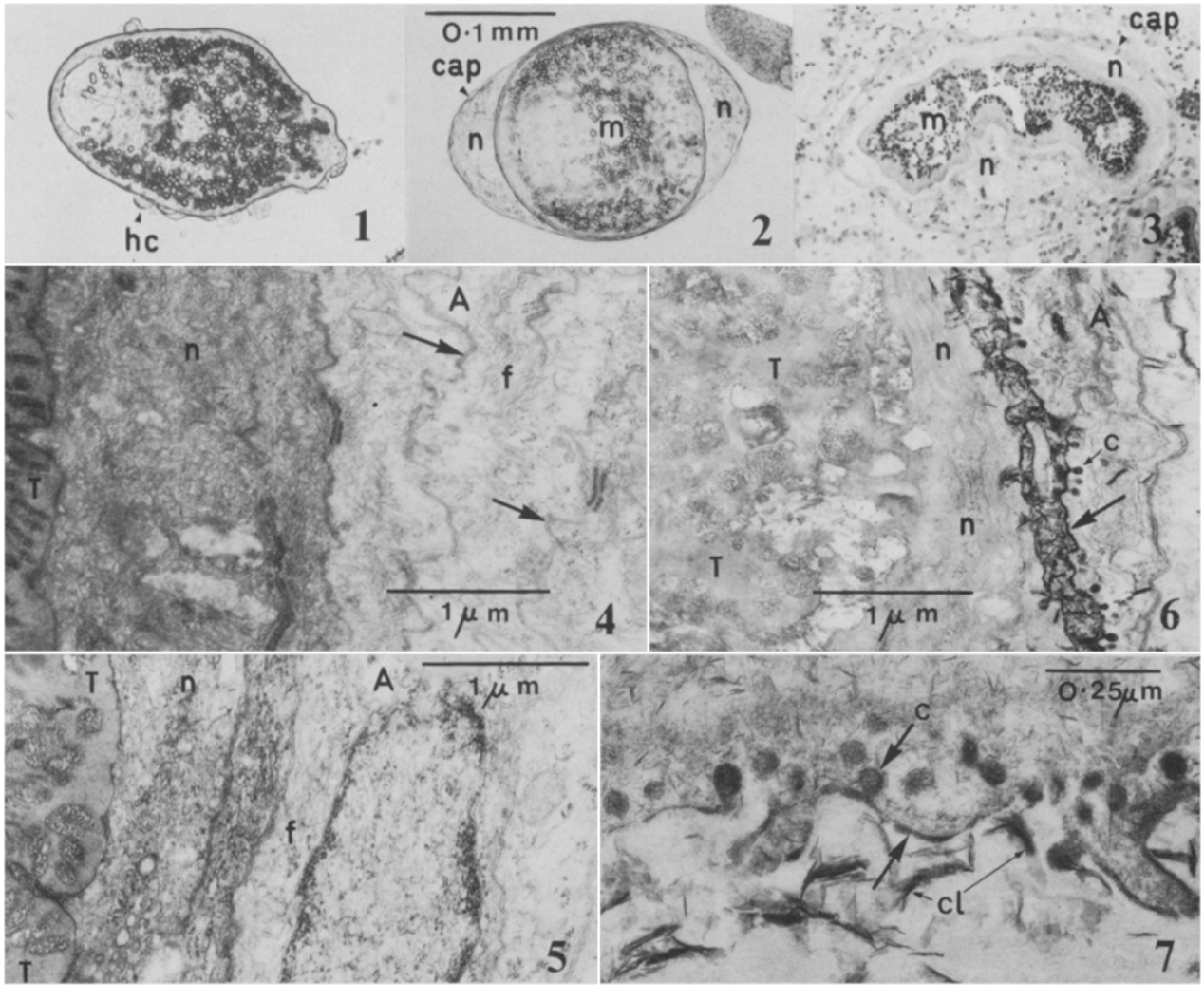

Figure 1. Normal metacercaria with host cells (hc) adherent to surface, natural infection. Figure 2. 35 days post-infection, metacercaria (m) and host tissue (n) encapsulated (cap). Figure 3. 35 days post-infection, section of infected brain stained by haematoxylin and eosin: metacercaria (m) within capsule (cap) which also encloses host tissue (n). Figures 4–7. Electronmicrographs: *4* and *5* 35 days post-infection, outer surface of metacercaria (T); encapsulated host cells (n); astrocytes (A) with fibrils (f) and electron translucent intercellular matrix (arrows). *6* and *7* 60 days post-infection, degenerating parasite (T) and encapsulated cells (n) within astrocytes (A) containing crystalline-like inclusions (cl). Electron dense intercellular matrix (arrows) and evidence of cytosis (c).

filled with a strongly electron dense material (fig. 6 and 7). The presence of dense, crystalline-like material within the cells and the endocytotic vesicles was indicative of cytosis. Within the capsule the parasite and encapsulated cells were disrupted. No collagen was seen in any section. No encapsulated metacercariae were found in the brain of experimentally infected fish that had been kept at 17–18 °C for up to 6 months. Around excized metacercariae from these fish was the normal number of the rounded cells always accompanying experimental and natural infections (fig. 1).

Discussion. Explanations drawing upon the supposed immunologically privileged nature of the brain for the survival, unencapsulated, of *D. phoxini* are clearly inadequate. Similarly, derived explanations for the survival of other unencapsulated parasites, as in the pericardium of *Xenopus*[13], must also be questioned.

The capsule is formed from a small number of overlapping cells identified from their ultrastructure as astrocytes. Whether the deposition of the electron dense intercellular matrix is the cause of the death of the parasites, or a consequence of its death, remains to be determined. Furthermore, the reasons for the lack of synchrony in the encapsulation of all parasites in one fish must be sought.

A mechanism does exist in *P. phoxinus* to wall off and kill *D. phoxini*. It is proposed that the vacuolated cells around the parasites, which have so often been noted, are part of the natural processes of repair in a brain damaged by the activity of the trematode parasites. In natural conditions, the repair process is not fully effective and as a direct consequence, the parasite survives. The parasites and fish from Wales used in these experiments would not naturally experience temperatures much, if at all, in excess of 20 °C. Therefore, the factor leading to encapsulation is high temperature during the complex development[9,14] of the metacercaria from the cercaria.

1 Acknowledgment. It is a pleasure to thank M.R.L. Johnston for his advice.
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A pesticide-resistant mutant of the N_2-fixing blue-green alga *Nostoc muscorum*

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Summary. The fungicide dithane Z-78 (zinc ethylene bisdithiocarbamate) has been found to be an inhibitor of growth and heterocyst differentiation in *Nostoc muscorum*. Its inhibitory effect has been reversed by exogenous glucose. A spontaneous mutant resistant to a toxic concentration of the fungicide grows in the presence of dithane at a dose normally applied in fields and does not require an exogenous carbon source for its growth.

The success of modern agriculture depends on the extensive use of pesticides[3] which usually kill their target organisms by being either an inhibitor of photosynthesis, or respiration, or growth[4]. The blue-green algae, besides being able to fix nitrogen, possess photosynthetic machinery identical to the chloroplasts of higher plants[5,6]. Pesticides are therefore expected to interfere with the photosynthetic machinery of naturally occurring blue-green algae. A majority of blue-green algae, which are heterocystous and filamentous, are of immense value in rice technology[7]. Heterocysts and their adjacent vegetative cells depend upon each other. The heterocysts supply fixed nitrogen to vegetative cells, and in return utilize their carbohydrates and energy for N_2 fixation[5]. Pesticides which inhibit photosynthesis thus indirectly affect the N_2-fixing machinery of blue-green algae. This prompted the authors to investigate which pesticides are capable of inhibiting the photosynthetic process in blue-green algae, and also to raise a mutant population of this group of nitrogen-fixers resistant to such pesticides.

The biological effects of different concentrations of dithane Z-78 (obtained from the Plant Protection Department, Govt. of India, Muzaffarpur), which was prepared by the method described previously[8], on growth and the frequency of heterocysts of *Nostoc muscorum* in N_2 and NO_3^- media were examined. Culture conditions, and growth and heterocyst measurement methods were the same as those described previously[8].

Experiments in general were done with 5 replicates and the results obtained were analyzed statistically using the calculation of the standard error[9] in order to assess the biological significance and reproducibility of the findings.

In contrast to the control, the dithane-supplemented cultures of *Nostoc muscorum* did not show significant growth in N_2 and NO_3^- media (fig. 1). In addition, dithane significantly inhibited the heterocyst frequency of the alga in N_2

Maximum heterocyst frequency* of parent *Nostoc muscorum* and its dithane-resistant mutant strain in N_2 medium containing or lacking glucose (500 ppm)

Dithane dose (ppm)	Heterocyst frequency (%) Parent		Mutant	
	Without glucose	With glucose	Without glucose	With glucose
0 (control)	5.35 ± 0.18	5.72 ± 0.17	5.28 ± 0.15	5.55 ± 0.05
10	2.25 ± 0.16	5.38 ± 0.13	5.28 ± 0.16	5.55 ± 0.08
25	1.76 ± 0.09	5.31 ± 0.16	5.28 ± 0.09	5.55 ± 0.12
50	0.0	5.15 ± 0.12	5.28 ± 0.12	5.55 ± 0.09
75	0.0	5.08 ± 0.08	5.28 ± 0.08	5.55 ± 0.11
100	0.0	5.02 ± 0.09	5.28 ± 0.10	5.55 ± 0.07

* The number of heterocysts per 100 vegetative cells each based on a random sampling of 12 filaments. The values are the means ± SE of 5 independent readings.