XCI. A NOTE ON THE PHYSIOLOGY OF THE SHIP-WORM (*TEREDO NORVEGICA*).

By CHARLES ROBERT HARINGTON.

From the Laboratory of the Marine Biological Association, Plymouth.

(Received November 25th, 1921.)

CHEMOTAXIS.

THE duration of the free-swimming larval stage of the wood-boring mollusc, *Teredo*, is a point of some importance in connection with the problem of the protection of timber against its attacks, since it is only during this free-swimming period that infection can be conveyed from one piece of timber to another.

In the course of some experiments with a view to the determination of the maximum length of the larval life of the animal, the larvae were kept in large dishes of sea water in which were placed pieces of wood; it was hoped that some of the larvae might settle on the wood and commence to bore into it.

Although the object was not attained it was noticed constantly during these experiments that there was an aggregation of the larvae upon the surface of the wood. This aggregation took place even when the wood was in the less strongly illuminated part of the dish, although the larvae are normally positively phototropic.

This observation of the apparent tendency of the larvae to aggregate upon the surface of wood, suggested the possibility that a definite chemical attraction for the larvae might be exercised by some normal constituent of wood. The following experiments were undertaken in the attempt to elucidate this point. In the first place some ordinary deal sawdust was extracted in a Soxhlet apparatus with ether for four hours and then with alcohol for a further four hours. Both the solutions on evaporation left a gummy residue, which was thoroughly freed from all traces of solvent. Small patches of these residues were then introduced into dishes of sea water containing some of the larvae, the latter being evenly distributed throughout the dish. After 24 hours the vast majority of the larvae in both cases were closely aggregated around the wood-extracts (see photograph).

In order to determine whether this were only a mechanical effect, the experiments were repeated, substituting in one case small stones, and in another glass beads, for the wood-extracts. In neither of these cases was any attraction to be observed. The further possibility of the extracts exerting a paralytic effect upon the larvae and hence acting as a trap was discounted

by the fact that movement could still be observed even among those larvae which were closely packed around the substance in question.

The results of the experiments were so striking that it was decided to extend them to a number of other substances—both those which might be expected to be toxic and those which might conceivably be present in the wood. Where possible the more dependable method used by Pfeffer for fern spermatozoids was used, which consists in placing a capillary tube filled with a solution of the substance under investigation in a dish with some of the larvæ and observing the effect upon the distribution of the latter. The results of experiments with various solvents are shown in the accompanying table and Fig. 1.

Experiments on Attraction of Larvæ—Direct Method.

	Substance employed	Effect upon distribution of larvæ	Condition of larvæ
A	Small pieces of stone	None	Living
B	Glass beads	None	Living
C	Alcoholic sawdust extract	Very marked aggregation of larvæ in the course of 24 hours	Living
D	Ethereal extract of sawdust	Same as C	Living
E	Crystal of dinitro-naphthalene	Pronounced aggregation around crystal in 24 hours	Living except immediately around crystal
F	Crystal of chloro-dinitrobenzene	No aggregation after 24 hours	Dead
G	Crystal of T.N.T.	Very slight aggregation	Dead
H	Drop of creosote	Some aggregation	Dead
K	Crystal of calcium malate	Apparent aggregation in both cases, but effect difficult to observe on account of rapidity of diffusion	(Living
L	Crystal of calcium succinate		) Living

Fig. 1. Photograph showing distribution of larvæ in dish about patch of alcoholic extract of wood ($\times 80$). After 24 hours.

Experiments on Attraction of Larvae—Capillary Tube Method.

Contents of tube	Resulting distribution of larvae	Condition of larvae
I. Sea water	No larvae in tube after 48 hours	Living
II. Distilled water	A few larvae in tube after 48 hours	Living, except those in tube
III. Sea water saturated with alcoholic sawdust extract	After 48 hours numerous larvae have entered the tube	Living
IV. Sea water saturated with ethereal sawdust extract	After 48 hours the mouth of the tube is blocked with a mass of the larvae	Living
V. Sea water which has been boiled with sawdust	Similar result to IV	Living
VI. Sea water containing 0.1 % sodium acetate	Very few larvae in tube after 48 hours; after 72 hours some have entered	Living
VII. Sea water containing 0.1 % sodium citrate	Similar result to VI	Living
VIII. Sea water containing 0.1 % acetic acid	Large number of larvae enter tube	Living
IX. Sea water containing 0.1 % hydrochloric acid	Similar result to VIII	Living
X. Sea water containing 1 % malic acid	There is a rapid re-arrangement of the larvae on introducing the tube into the dish; in a few hours many larvae have entered the tube and many more are gathered about its mouth	Living except for those which have actually entered the tube in XI
XI. 1 % malic acid in distilled water		
XII. 0.1 % malic acid in sea water	Similar to XI and X, but to a less marked degree	Living
XIII. 1 % tartaric acid in distilled water	No perceptible aggregation of larvae	Living
XIV. 1 % citric acid in distilled water	Very slight attraction	Living
XV. 1 % succinic acid in distilled water	No attraction	Living
XVI. 0.1 % vanillin in sea water	No attraction	Living
XVII. 1 % glucose in distilled water	No attraction	Living
XVIII. Saturated picric acid in distilled water	No attraction	Dead

From these it will be seen that a number of the substances experimented with show a certain power of attraction for the larvae, but that by far the most rapid and intense effect was obtained with malic acid; this acid is one which is very widely distributed in the plant kingdom, and may conceivably be present in the wood; but, without more evidence, it is impossible to express an opinion as to whether this is the substance which is responsible for the apparent attraction exercised by the wood. It is a noteworthy fact that both the alcoholic and ethereal extracts of wood are distinctly acid in reaction, the latter being the more markedly so. The toxic substances used behaved as would be expected, with the exception of dinitronaphthalene; the aggregation which took place around this substance is difficult to explain; probably the substance, which is very slightly soluble in water, acts as a trap for the larvae, those which happen to come within the small area of water in which the concentration of the dinitronaphthalene is toxic, being at once paralysed, and, by making a close ring round the crystal, causing the diffusion of the poison to be still slower, so that the larvae in the other parts of the dish remain unaffected. The aggregation around the drop of creosote may be partly accounted for

in the same way, but in this case vigorous diffusion currents are set up on introducing the oil into the dish, which may also help to account for the phenomenon.

The general conclusions to be drawn from these experiments seem to be as follows:

There is some substance present in wood, and extracted therefrom by water, alcohol and ether, to which *Teredo* larvae are positively chemo-tropic; it is just possible that this substance may be malic acid, but further work is required in order to establish its identity.

Since this positive chemo-tropism exists, the question arises as to what part it plays in the life of the animal.

In view of the natural conditions which prevail in the sea it is not possible to imagine that the larvae can be attracted towards wood by a chemical mechanism, since they must be at the mercy of the wash of the water. It would, however, obviously be of advantage to them if they possessed some means of recognising wood when they came into contact with it.

It would appear from the experiments described above that such recognition is rendered possible by chemical means, and that the larvae are dependent upon this chemical stimulus to enable them to distinguish between wood and stone or other surfaces into contact with which they may chance to be brought. In connection with this phenomenon it is interesting to compare the work of Dakin [1910] on the larvae of *Pecten*.

THE ENZYMES OF *TEREDO*.

The question of the source from which *Teredo* derives its nourishment is an interesting one. The fact that all the wood borings pass through the alimentary canal would naturally lead one to expect that some nutriment would be derived therefrom. On the other hand it has been held that the sole source of food is the plankton which is drawn in through the inhalant siphon and that the wood is passed out of the body chemically unchanged. In order to try and elucidate this point it was decided to make some experiments with a view to the detection, in the digestive gland of *Teredo*, of any ferments which might be capable of attacking wood; more particularly of one which could hydrolyse the celluloses or hemi-celluloses of wood with the production of glucose.

The difficulty in these experiments was to obtain sufficient material. The "liver" of *Teredo* is a thin skin of tissue extending over the surface of the gonad and is difficult to free completely from the latter; moreover, a very large number of individuals are necessary in order to provide sufficient liver substance for a satisfactory experiment.

The first experiment tried in this direction was as follows. The livers of six *Teredo* were excised, and freed as completely as possible from intermixed gonad; the tissue was then quickly and thoroughly minced up and mixed with distilled water to which toluene was added to inhibit bacterial action. The

mixture was allowed to stand for 24 hours and then filtered through muslin; the extract of the liver thus obtained was used for the following experiments:

- (1) Tube containing 0.16 g. filter paper (pure cellulose) + 8 cc. water + 2.5 cc. extract.
- (2) The same boiled in order to destroy enzymes.
- (3) 2.5 cc. starch solution + 2.5 cc. water + 2.5 cc. extract.
- (4) Same, boiled.
- (5) 0.2 g. fibrin + 5 cc. water + 2.5 cc. extract.
- (6) Same, boiled.

In all cases toluene was added as an antiseptic.

The contents of tubes 1-4 were examined at intervals of 24 hours for reducing substances. At the end of 72 hours the contents of tube 3 gave a marked reduction with Benedict's reagent, and the iodine reaction had completely disappeared; on treatment with phenylhydrazine hydrochloride in presence of acetic acid, a plentiful crop of glucosazone crystals was obtained. The contents of tube 4 at the end of the same period gave no reduction with Benedict's solution and still gave an intense iodine reaction.

The contents of tubes 1 and 2 gave no reduction with Benedict's solution even after a fortnight. No evidence of the digestion of the fibrin could be obtained from the contents of tubes 5 and 6. A second experiment was carried out in which the livers of 12 *Teredo* were excised and extracted as before, and the resulting extract (10 cc.) was divided into two equal portions, to each of which was added 5 cc. of water and 0.5 g. of finely sifted sawdust. One of these tubes was then boiled in order to destroy any enzymes which might be present. After keeping for a week, with toluene as an antiseptic, the contents of the unboiled tube gave a distinct reduction with Benedict's reagent, while those of the boiled tube gave a very slight reduction. Both solutions were then treated under the same conditions with phenylhydrazine hydrochloride and acetic acid, with the result that the contents of the unboiled tube gave a fairly large amount of glucosazone crystals, whereas those of the boiled tube only gave a few crystals of this substance.

Owing to the shortage of material and the consequent impossibility of running rigorous controls, it can hardly be claimed that the results are conclusive, except in the case of the starch digestion; here at least we have a clear demonstration of the fact that the liver contains an enzyme or enzymes which can hydrolyse starch completely to glucose.

In the case of the experiment with sawdust it might be argued that the possibility of the glucose having originated from the liver itself was not excluded. Any formation of glucose from the liver would probably, however, have taken place during the process of extraction, and this may account for the small quantity of glucose which was found in the control experiment. It seems difficult to account for the very much larger amount of glucose which was present in the unboiled tube, unless we assume it to have been formed from the wood by the action of an enzyme.

The failure, in the first experiment, to obtain any evidence of hydrolysis of cellulose is not such a contradiction of this hypothesis as at first appears. In the first place the liver extract used was very much less concentrated than that used on the wood and, secondly, there are present in wood hemi-celluloses which one would expect to be more readily hydrolysed than pure cellulose. Moreover, there is the further possibility that there may be other substances present in wood which are necessary as co-enzymes to the ferment in question.

Without more data it is impossible to draw definite conclusions, but the balance of evidence so far obtained is certainly in favour of the view that *Teredo* has in its liver an enzyme capable of producing glucose from some constituent of wood, and hence one may suppose that the wood, at least to this extent, is made use of as a source of nourishment.

It is hoped, by further experiments, to obtain more definite evidence upon this point.

The experiments described above form part of a research under the auspices of the Scientific and Industrial Research Department, undertaken on behalf of the Sea-Action Committee of the Institution of Civil Engineers, to whom I am indebted for permission to publish the results. I also wish to express my gratitude to Dr E. J. Allen, F.R.S., for assistance and advice, and to Dr J. S. Dunkerley for taking the photograph.

REFERENCE.

Dakin (1910). *Mitt. Zool. Stat. Neapel*, 20