A MICRO-INJECTION TECHNIQUE FOR BARNACLES

BY

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Although little is known of the endogenous mechanisms controlling cyclic activities in barnacles it is to be expected that they are similar to the general pattern of endocrine control in arthropods. Such a view is supported by the demonstration of neurosecretory cells in the supra-oesophageal and thoracic ganglia by Barnes & Gonor (1958 a, 1958 b), by the demonstration by Tighe-Ford (1967) of a possible mechanism for an endocrine control of breeding in Balanus balanoides (Linnaeus, 1761), and by the work of Sandeen & Costlow (1961) and Costlow (1963) which demonstrated that extracts of barnacle nervous tissue have a pigment-activating effect when injected into crabs. Although many of the arthropod endocrine systems have been elucidated by injections of extracts of glands and nervous tissue, such an approach has hitherto not been carried out on barnacles because of the calcareous shell surrounding the body. Barnacles have therefore not been injected with chemicals or tissue-extracts from other barnacles or other arthropods.

As part of an investigation into endogenous factors of barnacles an injection technique has been developed which permits barnacles to be injected, several times if required, with chemicals or tissue extracts, without the technique per se affecting their activity. The injections are made into the ovary mass for the following reasons, (i) to avoid damage to the prosoma and to cause minimal damage to the mantle and musculature and (ii) to ensure introduction of extracts into the haemo-coel for maximum dispersal throughout the body tissues.

DRILLING AND INJECTION TECHNIQUE

The experiments were carried out on first season Balanus balanoides (Linnaeus, 1761) which were settled on perspex panels and had an average carino-rostral length of 1.3 cm. This species was chosen because of its large size and membranous basis which made it possible to examine the ovary mass through the perspex panels. The hole was drilled with a mini-drill (Picard & Frère Ltd.,

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Crustaceana, 15
34-35 Furnival Street, London) fitted with a 0.655 mm hardened steel bit. The site of this hole was preferably at the rostral end of a lateral compartment to avoid the lateral scutal depressor muscles. Because of neighbouring barnacles this was not always possible, but drilling elsewhere through the shell appeared to have no adverse effects. The hole was drilled as near as possible to the base of the barnacle so that the needle penetrated along the depth of the ovarian tissue; at the time of these experiments the ovaries were fully developed so that it was relatively easy to inject into them without penetrating the inner cuticle lining the mantle cavity (this was confirmed by examining the underside of the barnacle through the perspex panel). A Hamilton microsyringe (25 microlitre) was then inserted into the hole to a depth of approximately two millimetres and two \( \mu l \) of fluid injected. As the hole was a possible source of bacterial infection a few grains of “Terramycin” (a wide-spectrum antibiotic, Pfizer Ltd.) were added. The hole was then dried and plugged with warmed gutta percha plasticised with cajeput oil. This hardened quickly forming a waterproof plug which could easily be removed with a hot needle for subsequent injections.

A non-injected control population was maintained in the laboratory during the experiments; during the twelve weeks of the experiments the mortality in this population was approximately 5%.

**SINGLE INJECTIONS OF DISTILLED WATER**

As a preliminary experiment ten specimens were injected once with two micro-litres of distilled water, “Terramycin” being added to four of them. The barnacles were then kept in a flowing tidal system maintained at 16 to 17°C. These were examined at regular intervals and were seen to be functioning normally — feeding and moulting. After nine weeks all of the barnacles were alive and active.

From this experiment it appeared that the injection technique had no serious effect on the activities of the barnacle and that small volumes of liquid could be injected into the ovarian tissue. To determine the feasibility of using the technique in endocrine experiments it was decided to investigate the preparation of small volumes of nervous system extracts and the effects of injecting such extracts into host barnacles. Although it would appear from the above experiment that addition of an anti-biotic was unnecessary, “Terramycin” was added in the experiments described below as a precaution against infection.

The 2 \( \mu l \) injection volume was chosen by empirical comparisons with prosoma and ovary volumes. As there is a slight loss of body fluid when the hole is drilled which may on occasions be greater than the injected volume, it is probable that the injected volume could be increased.

**SINGLE INJECTIONS OF NERVOUS SYSTEM EXTRACTS**

The extracts were prepared so that two \( \mu l \) contained the equivalent of three nervous systems, so that in endocrine experiments carried out later the injected