

Tissue Damage and Leukocytic Infiltration Following Attachment of the Mite *Unionicola intermedia* to the Gills of the Bivalve Mollusc *Anodonta anatina*

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Unionicola intermedia lives as a parasite in the gill region of *Anodonta anatina*. It attaches to the gills of its host by means of the pedipalps which cause displacement, rupture, and erosion of the gill epithelium. The pedipalps are sunk deeply into the underlying connective tissue of the gills and produce a leukocytic infiltration into the damaged area and a consequent edema of the gill filaments.

INTRODUCTION

Unionicola intermedia is a water mite belonging to the superfamily Pionae. Mitchell and Pitchford (1953) studied the mites parasitizing *Anodonta* spp. in England and indicated that *U. intermedia* normally parasitized the duck mussel, *A. anatina*, whereas *U. ypsilophora* was limited to the bay mussel, *A. cygnea*. Jones (1967) has listed the known hosts of parasitic water mites and also includes *U. intermedia* with *A. anatina*. Soar and Williamson (1927) and Mitchell and Pitchford (1953) have given descriptions of these mites. The anatomy and life cycle of members of the Unionicolidae has been fully described by Mitchell (1955).

Marshall and Staley (1929), Feng and Hoeppli (1933), Jones (1950), Hoeppli and Schumacher (1962), Schumacher and Hoeppli (1963), Arthur (1965), and Baker (1973) have all described histological changes in and damage to host tissue caused by mites, mainly with regard to the effects of salivary secretions and the formation of a tissue canal in the host.

The blood cells of lamellibranchs, in particular oysters, have been studied by a large number of workers including Takatsuki (1954), Bang (1961), Feng et al. (1971), Rudolph (1971a, b, c), and Foley and Cheng (1972). Hill and Welsh (1966) and Cheng and Baker (1970) have produced good recent reports on molluscan blood cells.

There have been a number of reviews dealing with cellular immunity in invertebrates and in particular the internal defense reactions produced in the tissues of molluscan hosts by their metazoan parasites (Cheng, 1967; Brooks, 1969; Cheng and Rifkin, 1970).

MATERIALS AND METHODS

Freshwater mussels were collected from Wintersett, a large man-made lake in Yorkshire, England. They were quickly opened alive and the mites removed attached to a small piece of gill tissue. This was fixed in Susa's fixative to which was added a small crystal of iodine to remove any precipitate formed by the mercury. The material was subsequently dehydrated using alcohol, cleared in xylol, and embedded in paraffin wax of 56°C melting point. Sections were cut at 8 μ m and stained in either Masson's trichrome stain or Delafield's hematoxylin and eosin.

RESULTS

Structure of the Pedipalps and Attachment to the Host

As with other mites, the basal segments or coxae of the pedipalps fuse to form the gnathosomal base. The remaining five segments are free, articulated to the gnathosoma, and referred to as the trochanter, femur, genu, tibia, and tarsus. The femur is



Unionicola intermedia showing the distal end of a filament.

masses of detached tissue forming groups of epithelial cells, and nuclei are seen on the outside of individual filaments as the cells at the gill surface are injured and destroyed (Fig. 2).

Responses of the Mussel

Inflammatory infiltration and epithelialization have been observed in different mussel gill tissue and are believed to be different cellular reactions due to the presence of the mite.

An increase in blood cell number occurs in the epithelium in the wounded areas of parasitized mussels (Fig. 4). The blood cells include square, rectangular, triangular, and spindle-shaped forms. The nuclei are also variable in shape and may be centrally placed or at one side of the cell. There are at least two kinds of blood cells. The most frequently observed form is of an irregular shape, a centrally placed nucleus, and a granular cytoplasm which does not stain with eosin. A second type has a spindle or only finely granular cytoplasm and a spindle-shaped nucleus. The cytoplasm of these cells stains green with Masson's trichrome and the nuclei stain red with Masson's trichrome. A third type has a spindle-shaped nucleus, a granular cytoplasm stained red with Masson's

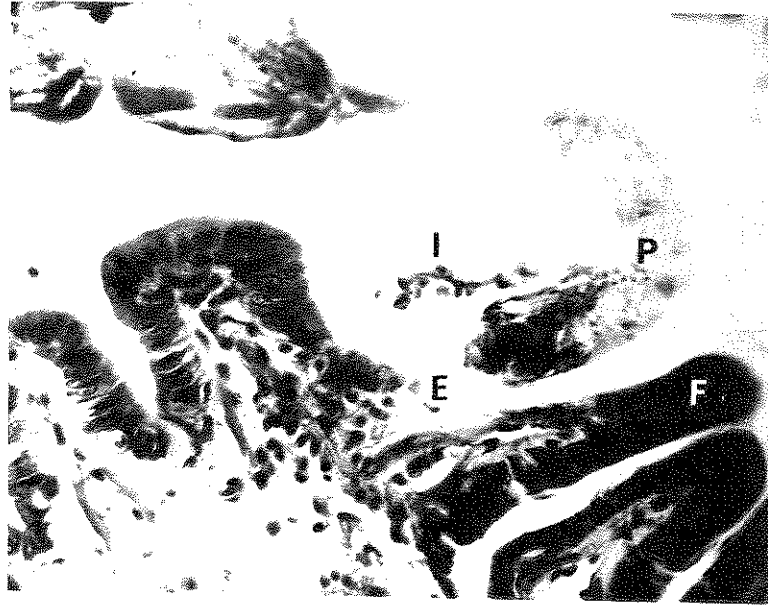


Fig. 2. Section through the gill filaments of *Anodonta anatina* showing displacement of individual filaments (F), injured epithelium (E), and an isolated patch of host tissue (I). Part of a pedipalp (P) from the mite can also be seen.



Fig. 3. Section through the gill filaments of *Anodonta anatina* showing the proliferation of a mass of epidermal cells (arrowed) at the base of one of the filaments (F). This is believed to be a reaction in response to the presence of the mite (P); $\times 328$.



FIG. 4. Leukocytic infiltration (arrowed) into the connective tissue of the gill (T) of *Anodonta anatina*, below the point of attachment of the pedipalps (P) of the mite (M); $\times 155$.

stain, collect below the area of attachment and are arranged parallel to the long axis of the gill filaments. The two main types of blood cell do not appear to be markedly different in size.

The gill filaments in the infected area become edematous, swelling appreciably, and

projecting distally beyond the normal surface (Fig. 5). Internally they are packed with numerous blood cells which account for the edema observed.

In parts of the infected area, production of new epithelial tissue was observed towards the base of and between individual gill filaments.



FIG. 5. Four gill filaments of *Anodonta anatina* showing noticeable swelling of one filament (F) in the region where the mite (M) is present. Note the amoebocytes (= leukocytes) within the filaments; $\times 330$.

ments (Fig. 3). This mass is composed of elongated cells having nuclei at the ends and forms a compact and discrete aggregation of cells. Cilia are present on the outer cells of this mass and in this case are located further down the gill filament than is normally the case. Some cell proliferation and overgrowth of epithelial tissue may have occurred.

DISCUSSION

Bequaert (1925) referred to the mite *Unionicola* as found fixed on to the gill. The evidence from the present study indicates that *Unionicola* uses the pedipalps for its purpose. In the Acarina, the pedipalps are essentially sensory appendages but are modified for grasping food and other purposes in certain species. In *Listrioides*, the pedipalps are expanded to form a clasp (Hughes, 1954), and the hypostome originates from the fusion and development of the pedipalpal coxae.

The accumulation of a plug of cells on the surface of the damaged epithelium is thought to represent a response due to the presence of the mite. Leukocytic infiltration towards the source of an irritation is described by Pauley and Sparks (1967), Des Voigne and Sparks (1967), and other workers and is one of a number of cellular responses demonstrated in molluscs. Pauley and Heator (1967), however, found a lack of a pronounced cellular response in *A. oregonensis* to surgical incisions, but this was thought to be unusual.

The damaged gill area of *A. anatina* is invaded by an influx of blood cells and is believed to have the potential to regenerate other kinds of tissue. Agranular eosinophilic leukocytes have been observed in the connective tissue below the point of attachment of *Unionicola*, and Ruddell (1971) has shown that this type of cell contributes to the formation of an amoebocyte plug in wounds. It appears that more than one type of cell is involved in leukocytic infiltration in the case of *A. anatina*, but it is not yet clear if any of these cells are involved in wound repair.



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ble swelling of one filament (F) in the region of attachment of the mite (M) to the gill filaments; $\times 330$.

ments (Fig. 3). This mass is composed of elongated cells having nuclei at different levels and forms a compact and distinct aggregation of cells. Cilia are present on the outer cells of this mass and in this position are located further down the gill filament than is normally the case. Some cellular multiplication and overgrowth of existing epithelial tissue may have occurred.

DISCUSSION

Bequaert (1925) referred to the fact that *Unionicola* is found fixed on to the gills of its host. The evidence from the present work is that *Unionicola* uses the pedipalps for this purpose. In the Acarina, the pedipalps are essentially sensory appendages but may be modified for grasping food and other functions in certain species. In *Listrophorus*, broad expansions of the pedipalpal coxae are directed inwards to form a clasping organ (Hughes, 1954), and the hypostome of ticks originates from the fusion and development of the pedipalpal coxae.

The accumulation of a plug of cells below the surface of the damaged epithelium is thought to represent a response due to the presence of the mite. Leukocytic infiltration towards the source of an irritation or injury is described by Pauley and Sparks (1965), Feng (1967), Des Voigne and Sparks (1968), and other workers and is one of a number of cellular responses demonstrated by molluscs. Pauley and Heaton (1969), however, found a lack of a pronounced cellular response in *A. oregonensis* following surgical incisions, but this was thought to be unusual.

The damaged gill area of *A. anatina* is flooded by an influx of blood cells which are believed to have the potential to produce other kinds of tissue. Agranular eosinophilic amoebocytes have been observed in the inflammatory tissue below the point of attachment of *Unionicola*, and Ruddell (1971b) believes that this type of cell contributes to the amoebocyte plug in wounds. It appears that more than one type of cell is involved in leukocytic infiltration in the case of *A. anatina*, but it is not yet clear if any or all are involved in wound repair.

It was not possible to study the sequence of events such as that described for the inflammatory reaction in *Crassostrea gigas* by Pauley and Sparks (1965) and for wound repair in *A. oregonensis* by Pauley and Heaton (1969). This was because of the difficulty in estimating the length of time of attachment of the mites prior to fixation. The observed cellular reactions therefore were not of standard form, different mussels showing varying responses resulting, it is believed, from the length of time of attachment.

The increase in the number of epithelial cells observed in *A. anatina* may be the result of cellular multiplication in this tissue or the conversion of leukocytes to epithelial cells. Des Voigne and Sparks (1968) describe wound healing in *C. gigas* and maintain that leukocytes transform into a pseudoepithelium and replace damaged host digestive or mantle epithelial tissue. Ruddell (1971a), reviewing previous work, maintains that wound repair in *C. gigas* involves both epithelial cells and amoebocytes, the latter filling up the wound with a plug of tissue and the epithelial cells migrating over this plug. It seems clear that these additional epithelial cells of *A. anatina* play an important role in wound repair but their origins are not yet clear.

A tissue reaction described by Marshall and Staley (1929) in mosquitos was caused by aquatic larval hydrachnids and was believed to be a protective reaction against the bite of the parasite. Histopathological changes in host tissue caused by various species of mite were likewise considered by Feng and Hoeppli (1933) to be due partly to the secretion of the parasites and partly a defence reaction on the part of their hosts. The host reactions reported for *A. anatina* may be partly the result of the feeding activities of the mite but the damage caused and the inflammatory response induced appear to be related more to the pedipalps than to the chelicerae.

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Oral Infection of *Hylobius*

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Microfeeding of *Hylobius pales* with ascarid larvae that excluded any possibility of infection. When larger doses were employed, the insects were able to penetrate the host from within the digestive tract. *In vitro*, conidia mixed with the liquid contents of the gut occurred even though both yeasts and bacteria were present. Hyphae contained inside their digestive

INTRODUCTION

Infection of insects by bacteria, protozoans and viruses is generally initiated by ingestion of the pathogen. This route of infection is, however, unusual for most entomophilic nematodes and fungi. Only one fungus, the yeast *Monosporella unicuspidata*, is believed to invade its host, a dipterous insect, exclusively from within the intestinal tract (Kellin, 1920). The needle-shaped ascospores of this fungus supposedly assist in penetrating through the gut wall.

With other fungi, there is little or no available evidence as to what role, if any, oral and/or intestinal infections play. Histochemical proof is often lacking or unconvincing because the experimental procedures usually employed by investigators did not exclude the possibility of integumental infections.

Until the present time, no research has shown that *Metarrhizium anisopliae* has invaded the host from within the digestive system (Mogler, 1965). Evidence in favor of cuticular penetration, if not intestinal, infection does exist. Notini and Mathlein (1944) as well as Green (1966) noticed hyphae of *Metarrhizium* penetrating proximal portions of mouthparts.

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