This was most apparent in the percentage of revival but there were also differences in the time required before activity appeared. A similar variation was found in the present work with regard to the ability of different gall populations to invade sweet corn. These variations were between the populations of galls which had been stored in the same container. Therefore the differences must have resulted from causes acting during the development of the galls or possibly from genetic differences.

The evidence presented in this paper supports the record that sweet corn has not been reported as a host of A. tritici, or at least the nematode is not one of its pests. However it seems possible that under unusual conditions sweet corn could be invaded in the field. The natural protection, which is the formation of the leaf tissues above the first node at the soil surface, could be lost if sweet corn, planted in infested soil, is cultivated when it is still small, in such a way that wet soil is thrown against the leaves. The same effect might be produced by infested, wet soil being washed over young plants by heavy rains.

Acknowledgments

I wish to express my appreciation to Dr. A. Morgan Golden of the U.S.D.A. Nematology Investigations for material, advice, and reading the manuscript, to F. A. Uecker of the Beltsville Mycology Laboratory for assistance with the photographs, and to Flora G. Pollack of the Mycology Laboratory, Plant Protection Institute, U.S.D.A. for library assistance.

Literature Cited


A Histochemical Study of Egg Shell Formation in the Monogenetic Trematode Octomacrum lanceatum

Mueller, 1934

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Abstract: The origin of shell precursors, their chemical nature, and the formation of egg shells were studied in the monogenetic trematode Octomacrum lanceatum by histochemistry. Shell precursors were identified as basic proteins, phenolic substances, and phenolases all found within vitelline cell globules. The presence of these compounds indicate the egg shell is a highly stable, quinone tanned protein. The egg shell is formed in the ootype and proximal uterus following coalescence of shell globules released from vitelline cells. Developing ova, the walls of the oviduct, ootype, and proximal uterus as well as the Mehlis' glands did not appear to add precursor components to the shell.

Most histochemical studies of egg shell formation in trematodes have dealt with digenetic trematodes (Stephenson, 1947; Johri and Smyth, 1956; Hanumantha-Rao, 1959; Smyth and Clegg, 1959; Burton, 1963; Coil, 1965, 1966, 1969; Coil and Reid, 1965; Madhavi, 1966, 1968; Wilson, 1967; and Nollen, 1971). Gerzeli (1968) studied the process in Aspidogaster conchicola, and, according to Smyth and Clegg (1959), Rennison investigated egg shell formation in the monogenetic trematode Diclidophora merlangi. Regarding other Monogenea, egg shell formation has been studied

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Results of these studies indicate that egg shells of many trematodes are sclerotin, a quinone tanned and inelastic protein formed through the action of phenolases on precursors having their origin from shell globules in vitelline cells. The chemistry of quinone tanning has been reviewed by Smyth and Clegg (1959) and Smyth (1966). However, it appears that not all trematodes possess a quinone tanning system (Smyth and Clegg, 1959; Madhavi, 1966, 1968; Nollen, 1971).

Since so few studies on egg shell formation have focused on monogenetic trematodes, such an investigation appeared desirable. The present study gives evidence for the existence of a quinone tanning system in the monogenetic trematode *Octomacrum lanceatum* Mueller, 1934.

**Materials and Methods**

Long nose suckers, *Catostomus catostomus* Girard, were collected by seine from Trout Creek immediately below Lake Manitou, Teller Co., Colorado. The trematodes were removed immediately from the gills and fixed in either 70% ethyl alcohol, phosphate buffered formalin (pH 7.3) or Carnoy’s fluid. Stains employed, following the methods of Johri and Smyth (1956), were: 0.1% aqueous catechol for detection of phenolases, 1.0% aqueous fast red B for detection of phenolic compounds (fixation in 70% ethyl alcohol) 1.0% aqueous bromphenol blue, and 0.5% aqueous malachite green as tests for basic proteins (fixation in buffered formalin). Treatment with aqueous catechol and fast red B were carried out prior to embedding in paraplast. Serial sections were cut at 5 to 10 µ. The PAS reaction was employed for the detection of polysaccharides, and control sections were treated with fresh saliva for glycogen determination. Flattened specimens were stained with Delafield’s hematoxylin and borax carmine for details of gross anatomical features.

**Observations**

The results of the various histochemical tests appear in Table 1. Vitelline cells contained numerous shell globules which stained intensely with bromphenol blue and malachite green indicating the presence of basic proteins. The same globules exhibited a strong positive reaction to fast red B and catechol, demonstrating the presence of phenolic compounds and phenolases, respectively. The vitelline cell cytoplasm was rich in glycogen, but shell globules were negative.

Fully formed eggs were oval with a long, anteriorly directed polar filament. Usually only one was present in the uterus at a given time. Shells of newly formed eggs showed positive reactions to catechol, fast red B, bromphenol blue, and malachite green. However, these reactions were weaker in shells of older eggs (those located more distal to the ootype).

Mehlis’ glands consisted of both mucous and serous cells. The latter were smaller and positioned very close to the ootype while mucous cells extended out into the adjacent parenchyma and were joined to the ootype by long, slender ducts. Mucous cells exhibited a strong PAS reaction before and after treatment with saliva, but both serous and mucous cells were negative to fast red B, catechol, bromphenol blue, and malachite green.

Small secretion granules were observed in serous cells along with some glycogen. Glycogen was also detected in the musculature of the ootype and proximal uterus. Maturing ova

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**Table 1. Results of histochemical tests on the female reproductive system of *Octomacrum lanceatum***

<table>
<thead>
<tr>
<th></th>
<th>Catechol</th>
<th>Fast red B</th>
<th>Bromphenol blue</th>
<th>Malachite green</th>
<th>PAS</th>
<th>PAS and saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitelline globules</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vitelline cell cytoplasm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vitelline cells in eggs</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Egg shell (newly formed)</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mehlis’ gland mucous cells</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mehlis’ gland serous cells</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Proximal uterine wall</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ootype wall</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Developing ova in ovary and oviduct</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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within the ovary and proximal oviduct were negative for all histochemical tests used.

**Discussion**

Egg shell formation in *O. lanceatum* parallels the process as demonstrated in digenetic trematodes having a quinone tanning system (Smyth and Clegg, 1959). The presence of basic proteins, phenolic compounds, and phenolases within the shell globules of vitelline cells coupled with the amber color of the shell strongly suggests a quinone tanning system. Further supporting evidence comes from the observation that older egg shells exhibited a decreased affinity for fast red B, malachite green, and bromphenol blue. Presumably this is because the phenols have been oxidized to quinones through the action of phenolases, and the free amino groups of the basic proteins have become bonded to quinones.

Though it is apparent that Mehlis’ gland cells do not contribute any of the shell precursors, we are not able to add any new knowledge as to their function. Several plausible ideas have been put forth by previous workers. Hanumantha-Rao (1959) suggested Mehlis’ gland secretions in *Fasciola hepatica* act on vitelline cells in such a way as to effect release of the shell precursors. More recently Ramalingam (1970, 1971) has shown in *Pricea multae* that the phenolic compounds existed in a masked state joined to acid mucopolysaccharides, and that the phenolases existed as proenzymes. Secretions from Mehlis’ glands are thought to free the phenols from acid mucopolysaccharides as well as to activate phenolases thereby allowing oxidation of phenols to quinones only at the proper time.

All of the necessary components of the egg shell (basic proteins, phenolic substances, and phenolases) are contained within the shell globules of vitelline cells of *O. lanceatum*. Developing ova, the walls of the oviduct, ootype, and proximal uterus as well as the Mehlis’ gland do not appear to contribute any of these basic components to the egg shell.

**Literature Cited**


Occurrence of Carbonic Anhydrase and its Relation to Ammonia Produced and Attraction of Both Sexes of Pelodera strongyloides

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United States Department of Agriculture

ABSTRACT: Histochemical methods for carbonic anhydrase, which were inhibited with 0.001 M Diamox, detected fine black granules in the intestine of both male and female Pelodera strongyloides cultured on nutrient agar only. When 0.001 M Diamox was incorporated into the nutrient agar, fewer fine black granules were detected so that the worms' intestines appeared unstained or lightly stained, and the ability of males and females to find one another was significantly reduced. In addition, NH₃ gas variably produced by both male and female worms was inhibited with 0.001 M Diamox.

Stringfellow (1974) reported that males of Pelodera strongyloides were attracted to the alkaline pH produced by aggregates of males and females. The present studies were undertaken to determine how these worms affect their microenvironment based on data of: (1) Scott and Whittaker (1970) who showed that NH₃ was highly concentrated in the medium used to culture P. strongyloides; (2) Dr. Eder Hanson (pers. comm.) who inquired whether NH₃ was involved in altering the worms' microenvironment; and (3) Carter (1972) who reviewed the little understood relationship between ammonium ion secretion and carbonic anhydrase.

Materials and Methods

The following experiment was run to determine the presence and distribution of the enzyme carbonic anhydrase in P. strongyloides as seen by the presence of fine black granules when suitable histochemical methods are used. Pellets of male and female P. strongyloides picked off nutrient agar with size No. 1 insect pins and calf pancreas fixed in cold acetone were stained with Haüsler's variant of Kurata's method for carbonic anhydrase as given in Lillie (1965). Pancreatic and worm tissues, incubated with Diamox* (2-acetamino-1,3,4-thiadiazole-5-sulfonamide), a specific inhibitor of carbonic anhydrase, or incubated without the substrate sodium bicarbonate, were used as controls.

An experiment was run to determine whether the enzyme carbonic anhydrase was qualitatively affected when male and female P. strongyloides were cultured for 24 hr on nutrient agar with and without 0.001 M Diamox incorporated into it. Male and female P. strongyloides from these cultures and from calf pancreas were stained as for carbonic anhydrase, described above.

Methods described by Stringfellow (1974) were used to determine whether 0.001 M Diamox affected the migration of male and female P. strongyloides under restrained conditions. Diamox at 0.001 M was considered

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