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Stabilization and Chemical Nature of Clamp Sclerites of *Heteromicrocotyle indicus* Ramalingam, 1960 (Monogenea : Microcotylidae)

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ABSTRACT. The chemical nature of clamp sclerites of the monogenetic trematode *Heteromicrocotyle indicus* parasitizing the gills of the marine fish *Carangoides bajad* was investigated using histochemical techniques. Both types of clamp sclerites are stabilized by the formation of bimers of tyrosine and basic proteins are also present. In addtion, the clamp material gives positive reaction to elastin stains. Other structural proteins such as collagen, proteins having-S-containing amino acids and sclerotin are absent.

The term monogenean sclerites refers to the hard parts of monogenea, *e.g.* marginal hooks, hamuli, accessory bars, accessory sclerites and clamp sclerites. The clamp sclerites occur only in the more specialized polyopisthocotylina (Llewellyn 1963) and are acquired at a late post larval stage (Lyons 1966). These supporting sclerites are not all of the same composition and biochemical differences between them have been reported (Lyons 1966). Although the morphology of these sclerites has been studied in detail and used in taxonomy (Sproston 1946, Bychowsky 1957, Yamaguti 1963), few studies have been carried out to elucidate their chemical nature (Lyons 1966, and Ramalingam 1973). Consequently, the chemical nature of the clamp sclerites of *Heteromicrocotyle indicus*, which has two types of clamp sclerites, was investigated.

Material and Methods

Heteromicrocotyle indicus were collected from the gills of the marine fish *Carangoides bajad* (Carangidae) obtained from the inshore waters of the sea coast of Dammam. Paraffin sections as well as unfixed entire clamps were used for

histochemical tests. The procedures for the various tests carried out were adopted from Pearse (1968) and McManus and Mowry (1960), unless otherwise stated, and are summarized in Table 1.

Results and Discussion

The results of the histochemical tests performed on the sections as well as whole clamp sclerites of *H. indicus* are presented in Table 1.

The clamp sclerites gave negative results for phenoloxidase, phenol and quinone, indicating the absence of quinone-tanning. Tests for other structural proteins were then performed. The clamp protein was negative to the tests for collagen as it gave red color with Mallory's triple stain and was refractory to the Van Geison's stain as well as to Gomori's trichome stain. The red staining following the Mallory's triple stain confirmed the absence of quinone-tanning because tanned structures are refractory to this stain (Dennell and Malek 1956). Similar results have been reported for the clamp sclerites of some other monogeneans (Lyons 1966 and Ramalingam 1973). The clamp sclerites gave negative results with the tests for proteins having-S-containing amino acids following DDD, Ferric ferricyanide and performic acid alcian blue methods. However, they gave positive results for basic proteins following aqueous bromophenol blue and malachite green stains and also for aromatic amino acids with Millon's test.

The clamps stained sapphire blue with toluidine blue-light green in phosphate buffer and deep blue with methylene blue in glycerol and water for dityrosine. These results are also in accordance with the results obtained for the clamp sclerites of *Tripathia chorinemi, Protomicrocotyle mannarensis, Pseudaxine indicana* and *Pricea multae* (Ramalingam 1973). Lyons (1966) concluded that the clamp substance differs from other scleroproteins like sclerotin, vertebrate elastin, reticulin, collagen and keratin, but is possibly similar to an elastin-like protein, while Ramalingam (1973) reported this to be dityrosine, similar to that found in the ligament cuticle of insects and the elastic nature of the clamps is due to the presence of dityrosine.

The clamp sclerites of *H. indicus* were PAS negative unlike those of *Diclidophora luscae* (Lyons 1966). The clamp sclerites gave positive results for elastin following Aldehyde fuchsin and Verhoeff's stain and these results are similar to those reported in *D. luscae* (Lyons 1966). On the basis of tryptophane content and more intense staining of clamp material with Schiff's reagent than with Aldehyde fuchsin, she (*Loc. cit.*) suggested that this protein differs from vertebrate elastin but the clamp sclerites in *H. indicus* are PAS negative and Aldehyde fuchsin positive.

Table 1. Results of histochemical tests performed on clamp sclerites of *Heteromicrocotyle indicus*.

Staining reaction for	Tests	Clamp sclerites of both sides
Phenol-oxidase	Catechol method (Smyth, 1954)	–
	Catechol after heat treatment at 80 °C (Hackman and Goldberg, 1967)	–
	Catechol after diethyldi-thiocarbamate treatment	–
Phenols	Diazo test (Johri and Smyth, 1956)	–
	Ferric chloride (Lison, 1936 as referred by Smyth, 1954)	–
	Toluidine blue method (Ramalingam and Ravindranath, 1970)	–*
Collagen	Van Geison's stain	Yellow refractory
	Gomori's trichome stain	refractory
	Mallory's triple stain	red
Protein having S-containing amino acids	DDD method	–
	DDD after sodium thioglycollate treatment	–
	Ferric ferricyanide method	–
	Performic acid alcian blue	–
Basic proteins	Aqueous bromophenol blue	+
	Malachite green (Johri and Smyth, 1956)	+
Tyrosine	Millon's test	+

Table 1. (Continued).

Staining reaction for	Tests	Clamp sclerites of both sides
Dityrosine	Methylene blue in glycerol and water (1:1) (Andersen and Weis-Fogh, 1964)	++
	Toluidine blue-light green in phosphate buffer of pH 7.2 (Andersen and Weis-Fogh, 1964)	++
Glycogen	PAS reaction	–
	PAS after diastase treatment	–
Elastin	Aldehyde fuchsin stain	+
	Verhoeff's stain	++

\+ = Positive
++ = Intensely positive
– = Negative
–* = Produced no metachromasia.

Conclusion

The negative results for the precursors of quinone-tanning, S-S linkage and collagen together with the positive results for dityrosine and basic proteins suggest that the clamp sclerites of both sides in *H. indicus* are stabilized by dityrosine and that basic proteins are also present. Bearing in mind these results, together with other histochemical reports on the clamp sclerites of other monogenea, it can be surmised that the clamp sclerites in all the higher monogenea may be stabilized by bimers of tyrosine.

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طبيعة المكونات الكيميائية وتصلب الدعائم السكليريتينية فى الطفيل المونوجينى

***Heteromicrocotyle indicus*, Ramalingam, 1960**

(Monogenea: Microcotylidae).

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باستخدام التقنيات الهستوكيميائية، تمت دراسة طبيعة المكونات الكيميائية للدعائم السكليريتينية الموجودة فى الممص الخلفى للطفيل المونوجينى *Heteromicrocotyle indicus* الذى يتطفل على خياشيم سمك البياض المسمى علميا بالـ *Carangoides bajad* .

أوضحت هذه الدراسة أن عملية تصلب كل من النوعين المختلفين من الدعائم السكليريتينية تتم بواسطة تكوين بيمرات التيروسين، وأن هذه الدعائم السكليريتينية تحوى بروتينات قاعدية وتعطى نتائج إيجابية لصبغات الإلاستين كما أوضحت أيضا بأن هذه الدعائم السكليريتينية لاتحتوى على بروتينات شبيهة بالكولاجين ولا على بروتينات تحوى أحماضاً أمينية بها كبريت، ولاتحتوى أيضا على سكليروتين.