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HISTOPATHOLOGY OF THE INTESTINE OF THE CROW-PHEASANT INFECTED WITH *PORRORCHIS INDICUS* (SCHMIDT AND KUNTZ[1]). (SYN. *PSEUDOPORRORCHIS INDICUS* DAS[2])

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ABSTRACT

Histological and histochemical alterations in the intestine of crow-pheasants (*Centropus sinensis*) infected with *Porrorchis indicus* (Acanthocephala) are described in this paper. Desquamation of mucosa and damage to the lamina propria and muscularis occur at the region where lesions are formed due to the penetration of proboscis. The intestinal wall gets perforated in heavily infected birds. A thick layer of mucus and cell debris of the host tissue envelops the proboscis of the parasite. There is a pronounced hypertrophy of the cellular elements (goblet cells and connective tissue cells) of the host tissue surrounding the embedded proboscis. Whereas the goblet cells of the normal intestine elaborate predominantly sulfomucins, those in the infected regions elaborate large amounts of sialomucins and sulfomucins.

INTRODUCTION

THE investigations by Von Brand[3], Nicholas[4], Pflugfelder[5], Clark *et al.*[6], Prakash and Adams[7], Bullock[8], Chaicharn and Bullock[9] show that the degree of injury to host tissue due to acanthocephalan infection depends upon several factors such as the number of worms infecting the host, age and diet of the host, concurrent presence of other parasites, host specificity and toxic effect due to infection. The activity of some enzymes in acanthocephalan parasites are known through the works of Bullock[10–12], Crompton[13,14], Crompton and Lee[15], Bryant and Nicholas[16]. Since very little is known about the histochemical changes consequent on acanthocephalan infection, it was felt that a detailed histochemical analysis of the infected sites in the intestines of crow-pheasants would be of considerable interest. This paper deals with the mucin histochemistry of the infected region.

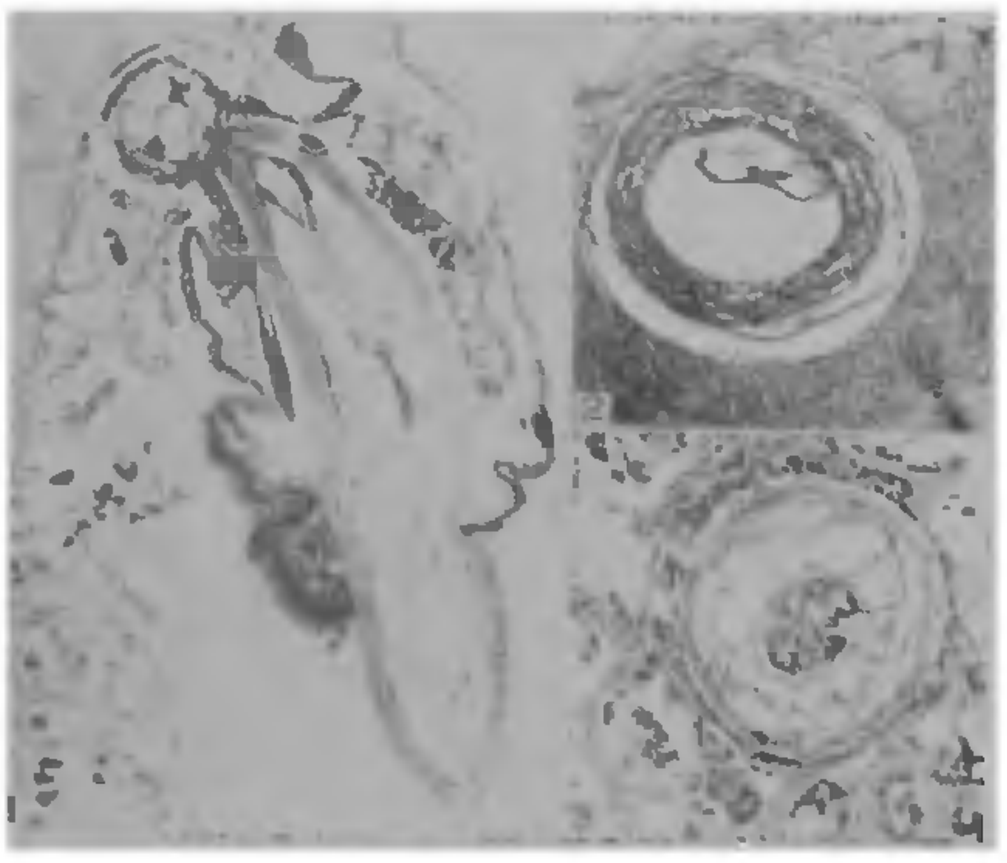
MATERIAL AND METHODS

Crow-pheasants infected with the acanthocephalan worm, *Porrorchis indicus*, were examined for the present study. In all the cases the proboscis was embedded in the wall of the posterior half of the intestine of the host. The infected regions of the crow-pheasants were fixed in chilled calcium acetate formalin for 24 hrs. After a thorough washing with distilled water, the tissues were dehydrated by passing through graded ethanol, embedded in paraffin and sectioned at 5-6 μ. The sections were stained with Harris haemotoxylin-eosin. Standard histochemical procedures were employed for the demonstration of various types of mucosubstances[17–32] (Table I).

RESULTS

Histological Changes :

The parasite normally infects the posterior part of the intestine where digested food of the host is available for the parasite for absorption. In the infected region the proboscis of the parasite penetrates the mucosa and submucosa and, in some cases, it reaches even the muscularis layer (Figs. 1and 2) of the intestine. The hooks are embedded in the wall of the intestine (Fig. 3). The goblet cells of the host tissue are reduced in number and their nuclei get pycnotic and there is an hyperplasia of the connective tissue. The cells of the intestinal epithelium become lifted up from the basement membrane and undergo progressively degeneration. A thick layer of mucus and cell debris consisting of fragments of epithelial cells, deformed goblet cells, degenerating erythrocytes and leucocytes and mucus envelops the proboscis of the parasite (Fig. 3).



FIGS. 1–3. Fig. 1. L.S. of proboscis of *Porrorchis indicus* inserted in the mucosa of the host tissue. Fig. 2. C.S. of proboscis embedded in the muscle. Fig. 3. C.S. of proboscis embedded in the mucosa.

Histochemical Observations :

The data obtained from histochemical reactions employed in the present investigation are recorded in Table I. The intensity of staining is expressed in terms of plus signs as usual.

TABLE I

Comparative histochemical reactions of the mucosubstances in the intestine of the host containing acanthocephalan worm

Sl. No.	Histochemical Reaction	Region away from parasite; un-infected region. Goblet cells.	Mucous layer surrounding parasite	Parasite attachment region. Infected region. Goblet cells.	Muscularis of host intestine	Parasite body
1.	PAS	++++ P	++++ P	++++ P	+± P	++ P
2.	Phenylhydrazine + PAS	++++ P	++++ P	++++ P	–	–
3.	Diastase + PAS	++++ P	++++ P	++++ P	–	+ P
4.	AB pH = 1·0	++++ B	+++± B	++± B	–	–
5.	AB pH = 1·0 + PAS	++++ B	++± B	++± B	+± P	++ P
6.	AB pH = 2·5 + PAS	++++ B	++++ B	++++ B	–	–
7.	AB pH = 2·5 + PAS	++++ B	++++ B	++++ B	+± P	++ P
8.	AF	++++ P	++++ P	++++ P	–	–
9.	AF + AB pH 2·5	++++ P	++++ PB	++++ PB	–	–
10.	Azure A pH 1·5	++++ P Metachromatic	++ P Metachromatic	++ P Metachromatic	– Orthochromatic Blue	– Orthochromatic Blue
11.	Azure A pH 3·0	++++ P Metachromatic	+++ P Metachromatic	+++ P Metachromatic	,,	,,
12.	Azure A pH 4·5	++++ P Metachromatic	++++ P Metachromatic	++++ P Metachromatic	,,	,,
13.	Mild methylation AB pH 2·5	++++ B	++++ B	++++ B	–	–
14.	Mild methylation saponification AB pH 2·5	++++ B	++++ B	++++ B	–	–
15.	Active methylation AB pH 2·5	–	–	–	–	–
16.	Active methylation saponification AB pH 2·5	–	++ B	++ B	–	–
17.	AB pH 5·6	++++ B	++++ B	++++ B	–	–
18.	AB pH 5·6 + 0·1 M Mg^{++}	++++ B	++++ B	++++ B	–	–
19.	AB pH 5·6 + 0·2 M Mg^{++}	++++ B	++++ B	++++ B	–	–
20.	AB pH 5·6 + 0·4 M Mg^{++}	++++ B	+±	+±	–	–
21.	AB pH 5·6 + 0·5 M Mg^{++}	++ B	–	–	–	–
22.	Acid hydrolysis AB pH 2·5	++++ B	++ B	++ B	–	–
23.	Hyaluronidase AB pH 2·5	++++ B	++++ B	++++ B	–	–

Abbreviations : P = Pink; B = Blue; PB = Purple-blue. PAS = Periodic acid Schiff; AB = Alcian Blue; AF = Aldehyde fuchsin.

The mucous film between the body wall of the parasite and the host tissue is intensely PAS-positive and this is not altered by either diastase digestion or prior phenylhydrazine treatment. These initial results reveal the presence of mucosubstances bearing acidic groups. With alcian blue (AB) (pH 1·0) the mucous film exhibited moderate alcianophilia and with AB (pH 2·5) it exhibited more intense alcianophilia thus indicating the presence of sulfate esters and carboxyl groups in the mucus. In the sequential AB (pH 1·0) —PAS and AB (pH 2·5)—PAS staining procedures the mucous layer reacted only with AB, exhibiting

moderate to intense blue staining, there being no trace of pink colouration. With aldehyde fuchsin (AF)–AB (pH 2·5) sequential staining procedure the mucus reacted with both the stains exhibiting purple-blue staining thereby indicating the predominance of carboxyl groups. This confirmed the presence of sulfated esters and carboxyl groups in the mucus. The mucous film exhibited intense alcianophilia in the presence of 0·1 M Mg^{++} and this remained practically unaltered at 0·2 M Mg^{++} concentration. At 0·3 and 0·4 M concentration of Mg^{++} there was some decrease in the alcianophilia which was completely abolished at 0·5 M. These reactions indicated the presence of both sialomucins and sulfomucins.

With mild methylation no significant decrease in the intensity of alcianophilia could be detected, but with active methylation the alcianophilia was completely abolished, and this could be partly restored by saponification. The partial loss of alcianophilia indicated the presence of sulfate groups in it since sulfate groups are hydrolytically removed. The partial restoration of alcianophilia indicated the presence of carboxyl group which were esterified during methylation and recovered after saponification. The carboxymucins were identified as sialomucins as could be judged from the partial loss of alcianophilia following acid hydrolysis. The metachromatic staining technique using Azure A also confirmed the presence of sulfomucins and sialomucins in the mucus. The mucous layer exhibited alcohol-stable metachromasia at lower pH (1·5 to 2·5) and partly alcohol-labile metachromasia at higher pH (3·0 to 4·5) as revealed by a greater intensity of staining at higher pH than at the lower pH. Hyaluronidase digestion had no effect on the basophilia of the mucus. This indicates the absence of hyaluronic acid in the mucus. These reactions also revealed the absence of neutral mucins from the mucus.

The goblet cells in the uninfected regions showed the presence of only sulfomucins and absence of sialomucins. The characterization of sulfate groups in them was based on their alcianophilia at pH 1·0 without its increase at pH 2·5, only purple staining with AF – AB (pH 2·5) sequence and the complete abolition of alcianophilia after active methylation and its non-restoration after saponification.

Discussion

The mode of attachment of parasite to the wall of intestine has been described earlier in *Acanthocephalus anguillae*[33], *Acanthocephalus ranae*[34], *Echinorhynchus* and *Lageniformis*[7]. Perforations of the wall of the intestine by the acanthocephalan worm *Polymorphus botulis* has also been observed by Clark *et al.*[6]. The formation of a capsule was noticed around the presoma of *Polymorphus boschalis*[5] and *Polymorphus bulbocoli* which infect the intestine of ducks[7] and infect fishes[9]. Bullock[6] did not notice any capsule formation in *Acanthocephalus jacksoni* which infect fishes. The present studies reveal that capsule formation does not take place around the presoma of *Porrorchis indicus*.

The present studies also show that the acanthocephalan infection induces alterations in the synthesis and secretions of the mucosubstances in the intestine of the host. Bullock[8] had noticed the formation of mucoid material between the host mucosa and the worms in salmonid fishes infected with *Acanthocephalus jacksoni*. The mucoid material was tentatively described as an acidic polysaccharide but no confirmatory evidence except its PAS positive nature was given. The present studies show that there is a copious secretion of mucus forming a film between the parasite and the host mucosa. The histochemical reactions have revealed the presence of sulfomucins and sialomucins in the mucus. The goblet cells seem to elaborate predominantly sulfomucins. These substances are known to have a protective function in the alimentary tract[35].

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INDIAN NATIONAL SCIENCE ACADEMY, NEW DELHI

AWARDS OF 'SCIENCE ACADEMY MEDALS FOR YOUNG SCIENTISTS' FOR THE YEAR 1979

The Indian National Science Academy instituted in 1974 Science Academy Medals for Young Scientists to give recognition to the scientific achievements of scientists below the age of 30, in any branch of science and technology within the purview of the Academy.

The Council of the Indian National Science Academy has selected 8 scientists for the award for the year 1979. The presentation will be made at the time of the Annual General Meeting of the Academy, during the 67th Session of the Indian Science Congress to be held in January 1980 at Gauhati.

The following Scientists have been selected for the award of Science Academy Medals for Young Scientists for the year 1979 :

(1) Dr. (Miss) Manju Bansal, Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012; (2) Sri. Samir K. Brahmachari, Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012; (3) Dr. Kamanio Chattopadhyay, Department of Metallurgical Engineering, Banaras Hindu University, Varanasi 221 005; (4) Dr. Yogesh Jaluria, Department of Mechanical Engineering, Indian Institute of Technology, Kanpur 208 016; (5) Dr. Opender Krishen Koul, Department of Entomology, Regional Research Laboratory, Jammu (Tawi) 180 001; (6) Dr. Jagdish Kumar Ladha, Department of Plant Sciences, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021; (7) Dr. (Miss) Amita Pal, Chromosome Research Centre, Department of Botany, University of Calcutta, Calcutta 700 019 and (8) Sri. Sundaram Ramakrishnan, Department of Biochemistry, All India Institute of Medical Sciences, New Delhi 110 016.

BORLAUG AWARD

Dr. M. S. Swaminathan, former Director-General of the Indian Council of Agricultural Research and now Secretary to the Union Agriculture Ministry, has been selected for the Borlaug Award for 1978. The award was instituted by Coromandel Fertilisers in honour of Dr. Norman E. Borlaug's great work in the advance of wheat revolution and increased production in various parts of the world. The award consists of a gold medal and Rs. 10,000 in cash.

Dr. Swaminathan's research interests have been wide and extensive and he has been hailed as leader of a productive school of research workers. He has received international recognition for his many contributions to genetics and breeding of crop plants and application of agricultural sciences which have resulted in improving crop production.
