SOME HISTOPATHOLOGICAL EFFECTS OF ACANTHOCEPHALAN PARASITES ON CATOSTOMID FISHES

AIMORN CHAICHARN

Follow this and additional works at: https://scholars.unh.edu/dissertation

Recommended Citation
https://scholars.unh.edu/dissertation/819

This Dissertation is brought to you for free and open access by the Student Scholarship at University of New Hampshire Scholars' Repository. It has been accepted for inclusion in Doctoral Dissertations by an authorized administrator of University of New Hampshire Scholars' Repository. For more information, please contact nicole.hentz@unh.edu.
CHAICHARN, Aimorn, 1934—
SOME HISTOPATHOLOGICAL EFFECTS OF
ACANTHOCEPHALAN PARASITES ON
CATOSTOMID FISHES.

University of New Hampshire, Ph.D., 1966
Zoology

University Microfilms, Inc., Ann Arbor, Michigan
SOME HISTOPATHOLOGICAL EFFECTS OF ACANTHOCEPHALAN PARASITES ON CATOSTOMID FISHES

By
AIMORN CHAICHARN
B.Sc., Chulalongkorn University, 1957
M.S., University of New Hampshire, 1961

A DISSERTATION
Submitted to the University of New Hampshire
In Partial Fulfillment of
The Requirements for the Degree of
Doctor of Philosophy

Graduate School
Department of Zoology
September, 1965
This dissertation has been examined and approved.

Theodore T. Metcalf
George M. Moore
Philip J. Sawyer
Richard W. Strout
Arthur C. Benson
Paul A. Wright
William F. Bullock, Chairman

October 15, 1965

Date
ACKNOWLEDGEMENTS

I wish to express my sincere appreciation and gratitude to Prof. George M. Moore and Prof. Wilbur L. Bullock. Through the help of Dr. Moore I had an opportunity to have further study at the University of New Hampshire. I am grateful also for his wholehearted support and advice on both personal and scientific matters throughout my stay here. Prof. Bullock is my major advisor, who has devoted both his time and experience to the guidance of this dissertation. He suggested this problem, aided in collecting the specimens, loaned his books and reprints, corrected the early manuscript, and proof-read the final manuscript. Thanks are due for the first year that I was his research assistant, from which I gained experience in histological and histochemical techniques that I was able to use for my own research.

I also wish to express my thanks to Prof. Philip J. Sawyer of the Zoology Department and to Prof. Richard G. Strout of the Department of Animal Science for their careful reading of the manuscript during its preparation and for their many helpful suggestions.

Special thanks are due to Robert W. Hanks who spent many hours correcting the earliest manuscript and for his generosity in the use of his photographic equipment.

Acknowledgement is also made to all other members of the Department of Zoology for their cooperation and valuable assistance. Many thanks also for the continued interest and assistance of all friends and associates not mentioned by name.

This study was supported through a project assistantship from Research Grant AI-3166, "The Histopathology of Acanthocephalan Infections". This grant was made by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, U. S. Public Health Service.
# TABLE OF CONTENTS

**LIST OF TABLES** iii  
**LIST OF FIGURES** iv  
**SECTION I** INTRODUCTION 1  
**SECTION II** REVIEW OF LITERATURE 4  
**SECTION III** MATERIALS AND METHODS 20  
**SECTION IV** THE HISTOLOGY OF THE WHITE SUCKER INTESTINE 25  
**SECTION V** GENERAL PATHOLOGY AND HISTOPATHOLOGY DUE TO THE PRESENCE OF ACANTHOCEPHALA 42  
**SECTION V** GENERAL PATHOLOGY AND HISTOPATHOLOGY DUE TO THE PRESENCE OF ACANTHOCEPHALA 45  
*Pomphorhynchus bulbocolli* 45  
*Octospinifer macilentus* 50  
*Neoechinorhynchus cristatus* 53  
*Neoechinorhynchus prolixoides* 54  
Alkaline phosphatase 55  
**SECTION VI** DISCUSSION 56  
**SECTION VII** SUMMARY 61  
**SECTION VIII** FIGURES 63  
**SECTION IX** LITERATURE CITED 89  
**APPENDIX** 97  
*Formulary of Stains and Procedures* 97
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Details of each collection for the white sucker (<em>Catostomus commersoni</em>)</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Details of each collection for the creek chubsucker (<em>Erimyzon oblongus</em>)</td>
<td>21</td>
</tr>
<tr>
<td>III</td>
<td>The reaction of free border and cytoplasm of the absorptive cells to the various staining technics</td>
<td>22</td>
</tr>
<tr>
<td>IV</td>
<td>Comparison of PAS positive substance in the capsule and mucous of the goblet cell</td>
<td>28</td>
</tr>
</tbody>
</table>

Comparison of PAS positive substance in the capsule and mucous of the goblet cell 49
**LIST OF FIGURES**

<table>
<thead>
<tr>
<th>Number</th>
<th>Figure Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Pomphorhynchus bulbocolli</em> Linkins</td>
<td>64</td>
</tr>
<tr>
<td>2</td>
<td><em>Octospinifer macilentus</em> Van Cleave</td>
<td>65</td>
</tr>
<tr>
<td>3</td>
<td><em>Neoechinorhynchus prolixoides</em> Bullock</td>
<td>66</td>
</tr>
<tr>
<td>4</td>
<td><em>Neoechinorhynchus cristatus</em> Lynch</td>
<td>67</td>
</tr>
<tr>
<td>5</td>
<td>The white sucker <em>Catostomus commersoni</em> Lacepède</td>
<td>68</td>
</tr>
<tr>
<td>6</td>
<td>The creek chubsucker <em>Erimyzon oblongus</em> Mitchell</td>
<td>69</td>
</tr>
<tr>
<td>7</td>
<td>Cross section of the anterior part (intestine swollen) of the intestine of a white sucker</td>
<td>70</td>
</tr>
<tr>
<td>8</td>
<td>Cross section of the posterior part of the intestine of a white sucker</td>
<td>70</td>
</tr>
<tr>
<td>9</td>
<td>Columnar epithelium at the side of the mucosal folds of a white sucker intestine</td>
<td>71</td>
</tr>
<tr>
<td>10</td>
<td>Longitudinal section of the intestine of a white sucker showing alkaline phosphatase activity</td>
<td>71</td>
</tr>
<tr>
<td>11</td>
<td>Epithelium of the intestine of a white sucker showing the fat vacuoles in the absorptive cells</td>
<td>72</td>
</tr>
<tr>
<td>12</td>
<td>Epithelium near the tip of the fold of a white sucker</td>
<td>72</td>
</tr>
<tr>
<td>13</td>
<td>Cross section of the intestine of a white sucker showing the mitotic figure</td>
<td>73</td>
</tr>
</tbody>
</table>
14  The granular cell in the base of epithelium of a white sucker.......... 73
15  Rodlet cell in the epithelium of a white sucker.......................... 74
16  Early stage of development of rodlet cell................................. 74
17  The older stage of development of rodlet cell............................. 75
18  Polymorphonuclear wandering cell in the lamina propria of a white sucker...... 75
19  Macrophage in the lamina propria of a white sucker....................... 76
20  Longitudinal section of the intestine of the white sucker showing the stratum compactum................................. 76
21  Cross section of the intestine of a creek chubsucker....................... 77
22  Epithelium of the intestine of a creek chubsucker showing the goblet cell....... 77
23  Longitudinal section of the slender neck of Pomphorhynchus bulbocolli embedded in the intestinal wall of a white sucker.... 78
24  P. bulbocolli embedded in the intestine of a white sucker.................. 78
25  Intestinal portion of Figure 24 enlarged. 79
26  Characteristic of the capsular wall surround the proboscis and bulb of P. bulbocolli................................. 79
27, 28, 29  Stages in the repair of the capsule where the proboscis and bulb had been... 80-81
30,31 The repair sequence in the tunnel where the slender neck region was embedded................................. 82

32 Large number of Octospinifer macilentus present in the lumen of white sucker intestine................................. 83

33 Cross section of the white sucker intestine showing deep penetration by female O. macilentus................................. 83

34 Figure 33 enlarged................................. 84

35 Cross section of a white sucker intestine showing male O. macilentus attached to the mucosal layer................................. 84

36 Longitudinal section of the proboscis of Neoechinorhynchus cristatus partially embedded in the intestine of a white sucker................................. 85

37 Longitudinal section of the proboscis of N. cristatus attached to the mucosal layer of a white sucker intestine. This figure shows the marked increase of granular cells in the lamina propria around the proboscis................................. 85

38 The extremely elongated fibroblast nuclei near the proboscis of N. cristatus in lamina propria................................. 86

39 Longitudinal section of the proboscis of N. prolixoides partially embedded in the epithelium of a creek chubsucker intestine................................. 86

40-41 No additional alkaline phosphatase activity in the intestine when infected with N. cristatus and O. macilentus........ 87

42-43 Strong alkaline phosphatase activity in surrounding connective tissue of a white sucker intestine when P. bulbocolli and female O. macilentus were present........ 88
SECTION I

INTRODUCTION

The Acanthocephala are intestinal parasites of vertebrates. They often cause severe damage to the intestinal wall, and may even result in the death of the host.

The Acanthocephala were first distinguished from other intestinal worms in the late eighteenth century (Koelreuther, 1771). They have occupied an uncertain phylogenetic and systematic position for many years, having been included with the flat worms called "Intestinaux parenchymatoux" by Cuvier and in the Nematelmia by Vogt along with the gordiaceans, nematodes, and gordiaceans. Now it is generally accepted that the Acanthocephala constitute a separate phylum. Over 500 species of the Acanthocephala have been described. More species occur in fishes than in birds and mammals, and a few species are found in amphibians and reptiles.

The body of an acanthocephalan is divided into an anterior presoma, consisting of a proboscis and an unspined neck, and a posterior trunk. The proboscis as an organ of attachment, bears recurved hooks; it is capable of introduction into the proboscis receptacle. The body wall
consists of a cuticula beneath which is the syncytial subcuticula or hypodermis, and a subcuticular musculature. The trunk and presoma are demarcated by the two elongate structures called lemnisci. The lemniscus is an infolding of the cuticula and the derivative from the hypodermis which projects posteriorly into the body cavity. The nervous system consists of a brain-like proboscis ganglion and two lateral cords proceeding posteriorly from this, plus various minor nerves. A digestive system is absent. The excretory system, when present, consists of proto-nephridia opening into the posterior part of the reproductive system. The sexes are separate. The males possess a copulatory apparatus and cement glands. The eggs develop in the female body into a shelled larva which requires an arthropod intermediate host for its further development.

The geographical distribution of Acanthocephala is world wide. Host specificity is well established in some species. Acanthocephala have not been reported from elasmobranch fish, indicating that the Acanthocephala may have arisen after the appearance of the elasmobranchs (Moore, 1960).

The major emphasis of the present study has been on acanthocephalan histopathology in the catostomid fishes. The white sucker is host to a large number of parasites.
including a variety of acanthocephalan species.

Objectives of this study were to observe the details of normal histology of the hosts, and to investigate the host-parasite relationships, which are variable depending upon the species of parasites. This interpretation is based on:

1. The depth of penetration of proboscis.
2. Cellular reaction of the host.
3. The degree of scar tissue formation around a long established proboscis.
4. Some aspects of the dynamic physical relationship of parasite and host.
SECTION II

REVIEW OF LITERATURE

Our knowledge of the histopathology of the host intestine infected by the acanthocephala is quite inadequate. The following is a summary of previous studies of the pathogenicity of adult acanthocephalans in some vertebrate animals.

Wurmbach (1937) observed the pathogenic effects of Acanthocephalus anguillae and Pomphorhynchus laevis in the barbs. He demonstrated that P. laevis causes nodule formation continuous with the connective tissue of the fish intestine. A. anguillae does not cause nodule formation, but inflammation of the host intestine was observed.

Venard and Warfel (1953) observed the effects of Leptorhynchoides thecatus and Neoechinorhynchus cylindratus on the largemouth bass Huro salmoides (Micropterus). In the host tissue, at the site of attachment, the mucosa and submucosa are completely disrupted. Numerous groups of rod-shaped bacteria are also present as secondary infection. L. thecatus caused extensive damage to the pyloric ceca while less damage is evident in the intestine near the proboscis of N. cylindratus. The mucosa is destroyed only
in the immediate area of attachment for both species.

Connell and Corner (1957) reported heavy infection of a young male beaver, *Castor canadensis*, with *Polymorphus paradoxus*. The parasites were attached throughout the small intestine. No further details of pathology were presented.

Takos and Thomas (1958) reported that four of ten squirrel marmoset monkeys (*Saimiri o. orstedii*) died as a result of infection by *Prosthenorchis elegans*. Histological study revealed that the worms had destroyed the mucosa, muscularis mucosa, and submucosa, and had penetrated into the smooth muscle coat of the wall. The serosal nodules were located where the worm "tunnels" penetrated the mucosa of the bowel. Death followed peritonitis caused by perforation of the bowel. The process of perforation was a result of secondary bacterial infection—rather than the direct action of the acanthocephalan parasites.

In the same year Clark, O'Meara, and van Weelden (1958) reported heavy infections (up to 610 worms/bird) of *Polymorphus botulus* in dead and dying eider ducks (*Somateria mollisima dresseri*) along the New England coast. Many parasites penetrated the intestinal wall and protruded into the abdomen. The possibility of peritonitis was
indicated, if such penetration occurred before death.

Logachev and Bruskin (1959) described a histological study of intestinal changes in wild and domestic ducks as a result of invasion by the acanthocephalan *Polymorphus magnus*. They concluded that the effect of this parasite on the intestinal mucosa of the host is not only mechanical irritation but also "biological interaction" between parasite and host.

In 1960 Prakash and Adams studied the histopathology of the intestinal lesion induced by *Echinorhynchus laginiformis* in the starry flounder (*Platichthys stellatus*). They noticed that the female parasite penetrated deeper than the male, and induced formation of chronic nodules outside the intestinal wall. The wall was conspicuously deficient in eosinophils and, even in advanced necrosis, no bacteria were observed. They concluded that the severity of the infection was largely a matter of the degree of penetration by the parasite, and not necessarily due to the introduction of pathogenic bacteria.

Bullock (1963b) observed the histopathology of *Acanthocephalus jacksoni* in salmonid fishes. The most pronounced effects were the damage to the epithelium and the proliferation of connective tissue. There was no capsule formation around the parasite but a layer of
mucous was interposed between the parasite and the epithelium of the host.

The scientific and common names of fishes used in this investigation follow the American Fisheries Society, Special Publication No. 2, 1960.

**Acanthocephala from the Catostomid Fishes of North America**

Van Cleave (1919a) made the first intensive search for Acanthocephala in the fresh-water fishes of North America. However, in his study of the Illinois River, especially in the region of Havana, Illinois, he did not examine white suckers (*Catostomus commersoni*). He found no parasites in the one lake chubsucker (*Erimyzon sucetta oblongus*) collected. The same year Van Cleave (1919b), reported on more than 375 fishes which were collected during the summer of 1912. These specimens represented sixteen different species of fish from Douglas Lake, Michigan. Van Cleave found the following four species of Acanthocephala in the white sucker:

- *Pomphorhynchus bulbocolli* Linkins
- *Octospinifer macilentus* Van Cleave
- *Neoechinorhynchus crassus* Van Cleave
- *Echinorhynchus thecatus* Linton

Later, in 1924 Pearse made observations on parasitic worms from Wisconsin fishes. He found six species of
Acanthocephala in the white sucker. They are:

Echinorhynchus coregoni Linkins
E. thecatus Linton
Neoechinorhynchus crassus Van Cleave
N. cylindratus Van Cleave
Octospinifer macilentus Van Cleave
Pomphorhynchus bulbocolli Linkins

Van Cleave and Mueller (1934) made a survey of the worm parasites of Oneida Lake (New York) fishes. From the white sucker they reported two species of Acanthocephala:

Octospinifer macilentus Van Cleave
Pomphorhynchus bulbocolli Linkins

and from the lake chubsucker they reported

Neoechinorhynchus cylindratus Van Cleave
Pomphorhynchus bulbocolli Linkins

In 1936 Lynch examined 86 specimens of the western sucker (Catostomus macrocheilus) in western North America. Three species of Neoechinorhynchus were found and two were described as new species:

Neoechinorhynchus venustus n. sp. 62.5%
N. cristatus n. sp. 20%
N. crassus Van Cleave 17.5%
Hunter (1941) studied the parasites of fresh-water fishes of Connecticut. He found *Pomphorhynchus bulbocalli* and *Leptorhynchoides* sp. from the white sucker; *Neoechinorhynchus* sp. and *Octospinifer macilentus* from the creek chub-sucker (*E. o. oblonquus*).

In 1944 Fischthal (1947) surveyed the fish parasites of northwest Wisconsin. He examined 151 white suckers from various rivers, lakes, brooks, and creeks, and found 144 infected with at least one species of parasite. Only three species of Acanthocephala were found:

- *Neoechinorhynchus crassus*
- *Octospinifer macilentus*
- *Pomphorhynchus bulbocalli*

A very interesting ecological relationship was indicated since *O. macilentus* was found only in the white suckers from the river habitat, *N. crassus* occurred in a higher percentage of river specimens, and *P. bulbocalli* was found in the white suckers from various habitats.

Van Cleave (1949) studied the genus *Neoechinorhynchus* in the catostomid fishes of North America and his tabular compilation is reproduced below:
<table>
<thead>
<tr>
<th>SPECIES</th>
<th>HOSTS</th>
<th>LOCALITIES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Erimyzon oblongus</td>
<td>L. Pepin, Minn.</td>
</tr>
<tr>
<td>N. cristatus Lynch</td>
<td>Catostomus macrocheilus</td>
<td>L. Washington, Wash.</td>
</tr>
<tr>
<td>N. strigosus Van Cleave</td>
<td>Catostomus commersoni</td>
<td>L. Chetac, Wis.</td>
</tr>
<tr>
<td>N. venustus Lynch</td>
<td>Catostomus macrocheilus</td>
<td>L. Washington, Wash.</td>
</tr>
<tr>
<td></td>
<td>Erimyzon oblongus</td>
<td>Conn.</td>
</tr>
</tbody>
</table>

Fischthal (1950) made a second survey in 1949 on the parasites of northwest Wisconsin fishes. Seventy of the 72 white suckers were infected and two species of Acanthocephala were found: N. crassus and P. bulbocolli.

In 1950 Sinderman (1953) made a survey of the fish parasites in central Massachusetts:

...Two species, the white sucker (Catostomus commersoni) and chub sucker (Erimyzon succetta oblongus) were available in some number for examination. Both of these fish commonly support several species of spiny-headed worms (Acanthocephala). Such worms were found occasionally as juveniles
in the viscera and mesenteries of yellow perch and bullheads. This was especially true of one species (*Pomphorhynchus bulbocolli*); which has a survey index of 16.0 in the suckers.

De Roth (1953) worked on parasites from fresh water fishes of Maine. Fourteen white suckers were examined; twelve were infected with parasites. The only acanthocephalan found was *Pomphorhynchus bulbocolli*.

Hall (1953) studied the helminth parasites collected from fishes of the Squam Lakes, New Hampshire. *Pomphorhynchus bulbocolli* was the only species of Acanthocephala found in the intestine of white suckers.

In 1955 Bullock reported the occurrence of *Neoechinorhynchus cristatus* Lynch in the Bellamy River, Madbury, New Hampshire.

...nearly all the specimens of the white sucker *Catostomus commersoni* were infected with a species of *Neoechinorhynchus* which closely resembled *N. cristatus* in the presence of a prominent keel along the dorsal surface. Since that time numerous collections of these suckers have yielded many specimens of these acanthocephalan from several widely separated localities in southeastern New Hampshire.

Bullock (1963) described a new species *Neoechinorhynchus prolixoides* from Lake Masasecum, Bradford; Pawtuckaway Pond, Nottingham; and Winona Lake in West Center Harbor, New Hampshire.

The present study found three species of these parasites in the white suckers of southeastern New Hampshire.
1. *Pomphorhynchus bulbocolli* Linkins

2. *Octospinifer macilentus* Van Cleave

3. *Neoechinorhynchus cristatus* Lynch

Although *Neoechinorhynchus prolixoides* Bullock has been reported from the white sucker, I found it only in the creek chubsucker from Turtletown Pond, Concord.
Key to the species of Acanthocephala from catostomid fishes of New Hampshire

1. (a) Proboscis globular or pyriform, carrying but three circles of hooks.........................2

(b) Proboscis not globular, carrying more than three circles of hooks. Neck prominent with globular expansion just posterior to the proboscis....
   Pomphorhynchus bulbocolli (fig. 1)

2. (a) Six hooks in each of the three circles, Lemnisci markedly different in size................3

(b) Eight hooks in each circle. Lemnisci not markedly different in size..........................
   Octospinifer macilentus (fig. 2)

3. (a) Binucleate lemniscus about half the length of body........................................
   Neoechinorhynchus prolixoides (fig. 3)

(b) Binucleate lemniscus about one third the length of body, distinct subcuticular crest on dorsal body wall.............N. cristatus (fig. 4)
White Sucker

Other names: sucker, black sucker, common sucker, mullet, slender sucker, and white horse.

Description. The common white sucker _Catostomus commersoni_ (Lacépède) belongs to the family Catostomidae, order Cypriniformes, suborder Cyprinoidei. This fish has a slender cylindrical body tapering toward each end with a rather blunt snout. The body is covered with smooth-edged scales; the head is scaleless. The ventral surface of the body is wide and flat. The mouth is inferior in position, rather large and with protractile lips, thus enabling the fish to take food from the bottom of the stream or lake. The upper lip is thick with 2 or 3 rows of papillae, the lower lip is horse-shoe shaped with more rows of papillae than the upper. No teeth are present on the jaws; the pharyngeal bones are set with numerous teeth that are somewhat similar to those of a comb. The gill membranes are united to the isthmus, usually very broadly so that the gill openings are markedly restricted. The eyes are small. The lateral line is complete and nearly straight. The dorsal fin contains 10 or more soft rays but no spines; the caudal fin is forked; the pelvic or ventral fins are inserted far back on the abdomen; the pectoral fins are
inserted on the lower part of the body (fig. 5). The air bladder is large with two chambers. According to Eddy and Surber (1943), the white sucker is variable in coloration, particularly during the spawning season, when it is so dark as to receive locally the common name of the black sucker. During the spawning season the males have a well-marked, black lateral band. Below this band and parallel to it is a rosy salmon-colored lateral band. (Jordan and Evermann, 1896-1900; Eddy and Surber, 1943).

**Distribution.** The common white sucker ranges from the Mackenzie River to eastern Canada and southward to the Gulf states. It is abundant in all of the waters of eastern North America. It occurs in enormous numbers in the headwaters of tributaries to the Mississippi River. (Eddy and Surber, 1943).

**Breeding habitats and life history.** Adams and Hankinson (1928) stated that where the white sucker is abundant, the large ones live in the deeper waters and the small ones school on the marginal shallows and stream mouths. Early in the spring, soon after the ice has gone out of the lakes and streams and the water begins to warm, these suckers ascend streams to spawn. The spawning runs occur largely at night and are variable in the distance traveled. Many records are available which suggest the possibility
that suckers may spawn successfully in lakes. During the breeding season the male develops tubercles on the anal fin, the lower part of the tail and on the upper sides of the paired fins. Although the runs take place at night actual spawning goes on night and day. No nest is built and no parental care is exercised. Spawning occurs in riffle areas in shallow water. Usually the female is attended by more than one male with the males pressing in on either side of the female. The eggs and milt are released when the posterior ends of the bodies are vibrated rapidly. Females 14 to 16 inches in length produce 67,000 eggs (Vessel and Eddy, 1941).

After the completion of spawning the adults drop downstream into the lakes or, if a resident stream population, to the deep pools.

After an incubation period of about three weeks, the young are hatched. These young are probably the most important food item for the young of many of the game fish. The young attain a length of about 4 to 5 inches the first year. They continue to be a forage fish until they reach a size of about eight inches. They have been known to reach a length of 25 inches or more but these large specimens are quite rare except in a few of the larger natural lakes.

**Food Habits.** The common or white sucker feeds largely upon
aquatic plants, insect larvae, small mollusks, worms and other animals.

**Economic status of the white sucker.** The white sucker, an insectivorous fish, is a rival of the trout and bass, species of prime importance to man. The sucker eats many May-flies, so the competition may be keen.

Suckers are frequently berated by fishermen who claim they are spawn eaters. Actually the suckers which have migrated from lakes are usually not in trout streams until the eggs of the trout have hatched. On the other hand resident stream suckers can eat only the trout eggs which have not been properly covered with gravel and those eggs are doomed to death anyway.

As a food fish the white sucker is considered of little value. In the spring its flesh is firm and of agreeable flavor, but by June it becomes soft and undesirable. (Adam and Hankinson, 1928). The young suckers are very important food fishes for larger predacious species such as lake trout and a salmon.

**Creek Chubsucker**

**Description.** The creek chubsucker, *Erimyzon oblongus* (Mitchill), belongs to the family Catostomidae, order Cypriniformes, suborder Cyprinoidei. This fish has an
elongate body, more compressed and more hump-backed than the white sucker. The small short head is not covered with scales. The mouth is moderate in size and subinferior. The upper lip is well developed, freely protractile, while the lower lip is infolded. The gill rakers are rather long; the pharyngeal bones are weak; the teeth are quite small, slender and weak. The eyes are moderate. The body is covered with rather large scales, more or less crowded forward. The dorsal fin, rather short and high, usually has 11 or 12 rays; the pectoral fins are moderate; the anal fin high and short, more or less bilobed in adult males; and the caudal fin is moderately forked; its 2 lobes are about equal (fig. 6). The air bladder has two chambers. There is no lateral line at any ages. The color is dark olive above. The young have a light strip on top of the head and a dark strip along side of the body, the latter is replaced by faint vertical bars in the adult. The fish may reach a length of 15 inches (Jordan, 1896; Carpenter and Siegler, 1947).

**Distribution.** The creek chubsuckers are found in the Atlantic coast drainage from Nova Scotia and New Brunswick (and possibly from the St. Lawrence River) to Virginia (Hubbs and Lagler, 1949).
Life History. There have been no extensive studies of the life history of the species so there is little actually known about it. Maine sportsmen frequently observe spawning concentrations at outlets of lakes in May and early part of June. During this period the males develop three prominent tubercles on each side of the head (Everhart, 1961).

Food habits. The creek chubsucker feeds on immature aquatic insects, aquatic plants, and minute crustaceans (Everhart, 1961).
SECTION III -

MATERIALS AND METHODS

Sixty-four white suckers (*Catostomus commersoni*) and four creek chubsuckers (*Erimyzon oblongus*) were collected during the period from 1960 to 1964 by seining, trapping and the use of rotenone. The fish came from Rangeley River, Oquossuc, Maine; Round Pond, Wakefield, New Hampshire; Winnicutt River, Stratham, New Hampshire; Oyster River, Durham, New Hampshire; and Turtletown Pond, Concord, New Hampshire. Details of each collection are shown in Table I for the white sucker and Table II for the creek chubsucker. The fish were transported alive to the laboratory at the University of New Hampshire, except those obtained by the use of rotenone which were examined at the site of capture.

Some suckers were killed and examined soon after reaching the laboratory, in order to obtain fresh acanthocephalan specimens for whole mounts (see Appendix). Most fish were held in recirculating aquaria, maintained at 55°F for several days in order to remove undigested food and debris from the intestinal tract. The fish were killed by severing the spinal column at the base of the skull. Then
Table I. Details of each collection for the white sucker (*Catostomus commersoni*).

<table>
<thead>
<tr>
<th>Identification Location</th>
<th>Date of capture</th>
<th>Date of examination</th>
<th>Gear</th>
<th>Standard size of fish (cm.)</th>
<th>Condition of intestine while examined and fixed</th>
<th>Acanthocephala</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP19-21 Rangeley River Oquossuc, Maine</td>
<td>8/5/60</td>
<td>8/5/60</td>
<td>Trap</td>
<td>21.7-25</td>
<td>Food throughout the digestive tract</td>
<td>Octospinifer macilentus and Neoechinorhynchus cristatus</td>
</tr>
<tr>
<td>HP27-29 Round Pond Wakefield, N. H.</td>
<td>10/18/60</td>
<td>10/18/60</td>
<td>Rotenone</td>
<td>33.5-40.2</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>Pomphorhynchus bulbocolli</td>
</tr>
<tr>
<td>HP 26 Winnicut River Stratham, N. H.</td>
<td>4/11/64</td>
<td>4/11/64</td>
<td>Common sense minnow seine, 10' long.</td>
<td>23.4</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>O. macilentus</td>
</tr>
<tr>
<td>Sk1-24</td>
<td>6/10/64</td>
<td>6/10/64</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>3.9-7.8</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>O. macilentus and N. cristatus</td>
</tr>
<tr>
<td>Sk 45</td>
<td>9/25/64</td>
<td>10/1/64</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>8 (9 gm.)</td>
<td>Injected two times with trypan blue, and killed when almost dead one day after second injection.</td>
<td>O. macilentus</td>
</tr>
<tr>
<td>Sk 46-51</td>
<td>9/25/64</td>
<td>10/2 — 10/17/64</td>
<td>&quot; &quot;</td>
<td>7.4-14</td>
<td>No food left in the digestive tract</td>
<td>O. macilentus</td>
</tr>
<tr>
<td>Sk 52</td>
<td>9/25/64</td>
<td>10/7/64</td>
<td>&quot; &quot;</td>
<td>15.2 (62 gm.)</td>
<td>Injected three times with trypan blue, No food left in the digestive tract</td>
<td>N. cristatus</td>
</tr>
<tr>
<td>Sk55-55</td>
<td>9/25/64</td>
<td>10/7/64</td>
<td>&quot; &quot;</td>
<td>12-16</td>
<td>No food left in the digestive tract</td>
<td>O. macilentus</td>
</tr>
<tr>
<td>Sk57,60,61 Turtletown Pond Concord, N. H.</td>
<td>10/13/64</td>
<td>10/13/64</td>
<td>Rotenone</td>
<td>22-23</td>
<td>Food throughout the digestive tract</td>
<td>N. cristatus</td>
</tr>
<tr>
<td>Identification Number</td>
<td>Location</td>
<td>Date of capture</td>
<td>Date of examination</td>
<td>Gear</td>
<td>Standard size of fish (cm.)</td>
<td>Condition of intestine while examined and fixed</td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------------------</td>
<td>-----------------</td>
<td>---------------------</td>
<td>-----------------------------------</td>
<td>----------------------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>Sk 2</td>
<td>Winnicutt River</td>
<td>6/10/64</td>
<td>6/10/64</td>
<td>Common sense seine, 10' long, 1/4&quot; stretch mess</td>
<td>6.4</td>
<td>Food throughout the digestive tract</td>
</tr>
<tr>
<td>Sk 31</td>
<td>&quot; &quot; &quot;</td>
<td>7/3/64</td>
<td>7/9/64</td>
<td>&quot; &quot;</td>
<td>8.2</td>
<td>No food left in the digestive tract</td>
</tr>
<tr>
<td>Sk 58-59</td>
<td>Turtletown Pond</td>
<td>10/13/64</td>
<td>10/13/64</td>
<td>Rotenone</td>
<td>24-26</td>
<td>Food throughout the digestive tract</td>
</tr>
</tbody>
</table>
a ventro-lateral incision was made from the operculum to
the anus exposing the internal organs. The viscera were
taken out, placed in Ringer's solution, and examined under
the binocular microscope. In small fish, acanthocephala
can be seen through the semitransparent intestinal wall.
The parasitized posterior section of the fish intestine was
excised. If required, these portions were opened and
washed free of food material. These tissues were fixed
immediately in a variety of cold fixatives: Bouin's,
nearl buffered formalin, Helly's, Zenker's, 85% ethyl
alcohol, and Gendre's. Appropriate washing was used for
each fixative. Then each tissue was dehydrated and
embedded in "paraplast" (see Appendix for procedure) and
serial sections (7-8 microns) were made on a rotary
microtome.

For the histological study these following staining
techniques were employed:

- Ehrlich's hematoxylin and eosin
- Safranin and fast green
- Thionin
- Gomori's trichrome
- Verhoeff's elastic tissue stain
- Mallory's phosphotungstic acid
- Krutsay's iron alum hematoxylin
- Basic fuchsin and picro-indigo carmine
- Fast green Van Gieson
- Azure A and C
- Carmalum
- Goldman's iron alum-picric acid hematoxylin
Histochemical procedures included:

The periodic acid—Schiff (PAS) for polysaccharide
Bauer-feulgen for glycogen
Feulgen nuclear reaction for DNA
Alcian blue for acid nucopolysaccharide
Cameron and Steele simplified aldehyde-fuchsin
Buffered azure eosinate (pH 4 and pH 5)

Alkaline phosphatase distribution was determined by
the Gomori technique as modified by Bullock (1953, 1958).

To demonstrate macrophages in the lamina propria, two
white suckers (9 and 62 gm.) were injected subcutaneously
with a freshly prepared 0.5% (Ringer's) solution of trypan
blue. Injections of 0.5 ml. per 20 gm. of body weight, as
suggested by Lee (1946), were given at intervals of three
to five days. After the second injection, the 9 gm. fish
was moribund and was killed a day after that. The 62 gm.
fish was fairly active. It was killed after the third
injection. The intestinal tissues were fixed in Bouin's
solution, found by Lee to be the best fixative for the
preservation of trypan blue dye contained in the vacuoles
of the macrophages. The carmalum counter stain was used,
giving an excellent pink background to distinguish
macrophages containing trypan blue particles.

Every procedure and formulary of stains used will
be found in the Appendix.
SECTION IV

THE HISTOLOGY OF THE WHITE SUCKER INTESTINE

The general histology of the sucker intestine has been described by Weisel (1962) for *Catostomus catostomus*. Stewart (1926) studied the development of the intestinal coils in the post larva of *Catostomus commersoni*. The results of the present observations of the basic histology of the white sucker intestine are similar to those reported by these earlier workers.

The elongation of the alimentary tract is achieved by complex coiling. The first limb of the tract, which is larger in circumference than the rest of the digestive tract, proceeds as a straight tube to the region of the vent. It then doubles back to the anterior portion of the body cavity, thereby forming the third coil before straightening into the limb that leads to the vent. The intestinal coils are located chiefly on the right side of the body.

Not all fishes have a stomach, a portion of the digestive tube with a typically acid secretion and a distinctive epithelial lining different from that of the intestine. In the families Catostomidae, Cyprinidae and Cyprinodontidae for instance, the epithelial tissue of the esophagus grades
directly into that of the intestine.

The sucker also lacks a distinct rectum. The intestine is provided with unbranched longitudinal zigzag folds arranged in an antero-posterior direction. These folds are tall at the beginning of the tract and reduced in the portion near the anus (Fig. 7-8). There are no crypts or multicellular glands in the wall of the tract. The intestine is made up of the following coats:

I. Mucosa
   A. Epithelium
   B. Lamina propria

II. Stratum compactum

III. Muscularis
   A. Inner circular
   B. Outer longitudinal

IV. Serosa

I. Mucosa

A. Epithelium

There are two basic kinds of cells; the principal absorptive cells (columnar or cylindrical cells) interrupted by mucous-secreting or goblet cells. Other cells found scattered among the true epithelial elements are: wandering leucocytes, rodlet cells, and granular cells.

a. Absorptive cells. The absorptive or columnar cells are elongated and slender, tapering towards their
base. They vary slightly according to the position in the mucosal folds, measuring 30 to 45 microns in length and about 3 to 6 microns in breadth for young fish and average from 50 to 57 microns in length by 3 to 6 microns across for the old fish.

There is a distinct free border (top plate or striated border) over the surface of the absorptive cells, which is continuous along the whole epithelium, interrupted only where the goblet cells open into the lumen. The free border in the white sucker varies from 1.5 to 5.32 microns in thickness. Al-Hussaini (1949b) described the free border as divided into three layers: the superficial layer; the canal layer; and the granular layer. Only the last two: the canal layer and the granular layer can be clearly distinguished in the white sucker (Fig. 9) when two adjacent folds come close together. Here the free border appears much thicker than usual and the canals appear stretched. Al-Hussaini (1949b) suggested that the free border is elastic and capable of expansion and that this has been effected by the temporary adhesion of the free surfaces so that when the muscularis layer contracts during the normal peristaltic movement of the intestine, the free border is slightly stretched. On the other hand the reverse may occur, and the free border may appear in places to be very
# TABLE III

The reaction of free border and cytoplasm of the absorptive cells to the various techniques

<table>
<thead>
<tr>
<th>No.</th>
<th>Fixative</th>
<th>Staining</th>
<th>Free border</th>
<th>Cytoplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>sk11s</td>
<td>Bouin's</td>
<td>Trichrome</td>
<td>dark green</td>
<td>green</td>
</tr>
<tr>
<td>sk57Bf</td>
<td>Zenker's</td>
<td>Haematoxylin and eosin</td>
<td>purple-pink</td>
<td>purple-pink</td>
</tr>
<tr>
<td>sk11Azs</td>
<td>Helly's</td>
<td>Azure-esoinate (pH 4)</td>
<td>pale pink or unstained</td>
<td>purple</td>
</tr>
<tr>
<td>sk16A1f&quot;</td>
<td>Helly's</td>
<td>Azure-esoinate (pH 5)</td>
<td>light green</td>
<td>purple</td>
</tr>
<tr>
<td>HP19A6</td>
<td>Helly's</td>
<td>Goldman's</td>
<td>brown</td>
<td>dark brown</td>
</tr>
<tr>
<td>HP19A22</td>
<td>Helly's</td>
<td>Fast green Van Gieson</td>
<td>unstained</td>
<td>light purple</td>
</tr>
<tr>
<td>HP19A24</td>
<td>Helly's</td>
<td>Safranin and fast green</td>
<td>green</td>
<td>green</td>
</tr>
<tr>
<td>HP19A25</td>
<td>Helly's</td>
<td>Thionin</td>
<td>green</td>
<td>light blue</td>
</tr>
<tr>
<td>HP26B6</td>
<td>Bouin's</td>
<td>Fuchsin and picro indigo carmine</td>
<td>dark green</td>
<td>green</td>
</tr>
<tr>
<td>HP20,1</td>
<td>Helly's</td>
<td>Azure C 10%</td>
<td>light green</td>
<td>bluish purple</td>
</tr>
<tr>
<td>HP27C25</td>
<td>Helly's</td>
<td>Verhoeff's</td>
<td>brown purple</td>
<td>brown purple</td>
</tr>
<tr>
<td>sk57Blg'</td>
<td>Gendre's</td>
<td>Aldehyde-fuchsin-Halmi's</td>
<td>green</td>
<td>green</td>
</tr>
<tr>
<td>sk57Blm'</td>
<td>Gendre's</td>
<td>Mallory's phosphotungstic acid hematoxylin</td>
<td>light brown</td>
<td>light brown</td>
</tr>
</tbody>
</table>
much contracted so it is hard to see the details. These changes in the free border may aid the passage of digested food into the epithelial cells. The reaction of these layers to the various staining techniques is shown in Table III.

The presence of non acid mucopolysaccharide in the free border was indicated by marked staining with PAS but unaffected salivary digestion; it fails to stain with alcian blue. Alkaline phosphatase appeared to be localized in the absorptive cells. The reaction is much more intense in the cells at the top of the fold which are directly in contact with the intestinal contents, and becomes progressively weaker as the cells approach the crypts (Fig. 10, 40, 41). The concentrated staining reaction occurred in the free border, which was often completely blackened.

The cytoplasm of the absorptive cell was slightly granular. In most cases in the sub-border region the granulation appeared to be heavier than in the other parts of the cell. The reaction of the cytoplasm to the various staining techniques is also shown in Table III. The supranuclear cytoplasmic region showed intense alkaline phosphatase staining.

In the fed fish, each absorptive cell contained one or more vacuoles in the sub-border region (Fig. 11). There
was granular cytoplasm present between the vacuole and the nucleus and also between the granular layer of the free border and the vacuole. These are probably fat globules but in paraffin preparations the fat is dissolved out. These vacuoles differ from the mucous of the goblet cell in three ways.

1. The mucous occupied the distal end of the goblet cell so that no cytoplasm could be seen between the mucous and the free border.

2. The mucous caused a distinct bulge in the wall, while the vacuoles remained small and occupied a definitely limited area within each cell.

3. The vacuoles did not stain with any of the stains used but remained perfectly clear. In fact, sections stained with PAS showed goblet cells filled with bright red mucous at the same time that the vacuole in the absorptive cells remained unstained.

The supranuclear region does not stain with azure-eosinate, azure A or C so it appeared as a clear band over the nucleus. This area has been interpreted as the Golgi zone (e.g. Bullock, 1963b).

The nuclei are vesicular and comparatively large, club-shaped or oval, and average from 10 to 17 microns in length and 3 to 5 microns in width for both big and small
fishes. Their outlines are easily distinguished by the thickness of the nuclear membrane. The nuclei of absorptive cells are usually located in the basal half of the cells. No nucleolus is distinguishable. Almost all of the nuclei contain a few clumps of irregular chromatin masses, among which are smaller chromatin granules, which stain deeply with basic dyes.

During cellular division the chromatin granules are condensed into visible discrete chromosomes. The mitotic figures were usually found above the nuclear level toward the luminal edge of the base or side of the fold. They were never found at the tip (Fig. 13). The chromosomes of active cells and the chromatin granules of resting cells contain desoxyribose nucleic acid (DNA) which was seen as Feulgen positive material.

The cytoplasm of the infranuclear region exhibits the same reaction as the supranuclear region but is less granular. Usually this area was narrow or obscure because the nuclei of the absorptive cells occupied the second half of the cells and also there are many non-absorptive cells located in this basal region.

b. **Goblet cells.** The goblet cell, a unicellular gland, secretes mucin which forms mucus with water. It has a typical goblet outline, i.e. it is constricted at the
middle and tapers basally to where the oval nucleus is embedded. The dilated portion of the cell consists of a thin protoplasmic wall which is filled with reticular-like mucin. It is usually located at the luminal end. The height of the goblet cell is as long as the absorptive cells but the swollen part varies in size, depending upon the amount of mucus in them. Some goblet cells measured about 14 microns long by 10 microns in the broadest portion. The goblet communicates with the lumen of the intestine by a conspicuous neck and a small pore. These cells are scattered here and there among the absorptive cells. They are not very numerous in any part of the intestine except toward the anus. They may be found in the bottom of a crypt or on the sides or top of a fold. Their contents stained bright red with PAS; deep red with safranin; dark purple with Ehrlich's hematoxylin and eosin, light purple with azure eosinate, and dark purple with aldehyde fuchsin. The dilated part of the goblet cell, containing mucous, showed no alkaline phosphatase reaction (Figure 10). The mucus in the goblet cells contains acid mucopolysaccharide as indicated by intense staining with alcian blue.

c. Wandering leucocytes. The wandering leucocytes are quite common among the epithelial cells (Fig. 8). They appear chiefly within the fluid-filled intracellular space
around the base of the absorptive cells. The majority are scattered, but they are sometimes found in groups of two or three cells. They may be located between the cells, but rarely near the surface. They never appear to be escaping from the epithelial coat into the lumen of the intestine. Their nuclei are quite distinct from the epithelial nuclei in being smaller, rounded, and darkly staining. The diameter of these nuclei averages from 3 to 6 microns. Occasional large, irregularly oval nuclei may be found. Their cytoplasm can hardly be seen when they are crowded in the basal area but it can be recognized when they are located separately between the absorptive cells near the luminal edge. The diameter of the cell is about 8 microns.

The wandering leucocytes are numerous at the level of the infranuclear region of absorptive cells. The same type of cell is also found in the lamina propria in considerable numbers.

No polymorphonuclear wandering cells were seen at the base of the epithelium but such cells were found in the lamina propria. Polymorphonuclear cells were found in this region in trout by Bullock (1963b).

d. **Granular cells.** Many investigators have described the presence of large granular cells between the epithelial cells and elsewhere in the wall of the digestive tract of
the fishes. Their function in the tract is not known (Bolton, 1933, Al-Hussaini 1949b). These cells are numerous in the epithelium of the white sucker. They are more abundant in old fish than in the young ones.

The granular cells of the sucker are relatively large, either circular or irregularly oval in outline varying in size between 6 and 13 microns. The nucleus is always pushed to one side of the cell or eccentric in position (Fig. 14). Its structure and size appears the same as the nucleus of the wandering leucocyte which ranges between 2 and 6 microns. The cytoplasm contains minute rounded granules which stain red with PAS; pink with hematoxylin and eosin; brown with Goldman's; green with trichrome; green with fast green; light pink with azure-eosinate pH 4, light blue with pH 5, and violet with aldehyde fuchsin. They are abundant in the lamina propria and are also found at the basal part of the epithelial layer. The granular cells are scarce above the level of the nuclei of the absorptive cells. They are also found in various parts of the intestine, e.g. underneath the stratum compactum, in or between the layers of the muscular coat and between the latter and serosa.

E. Rodlet cells. These cells have been observed by many investigators in various fishes. They have been
generally reported from the intestinal mucosa but some have been found in blood vessels, the heart, collecting ducts of kidney, the bile ducts, etc. Their precise significance is obscure.

Kato (1935) described "A-cells" from the intestinal epithelium of a stromateoid fish (*Nomeus gronovii*). His illustration and description are similar to the rodlet cells which are found in the sucker. Al-Hussaini (1949b) illustrated "pear-shaped" cells in some cyprinid fishes but gave no detail. Weisel (1962) compared the digestive tract of a sucker and a minnow. One of the granulocytes in his illustration showing the microscopic anatomy of the epithelial lining of the sucker's intestine looks like a rodlet cell. Bullock (1963b) found these cells in the salmonids, American eel and the white sucker. He also referred to them as "rodlet cells" and, on the basis of their morphology and histochemistry identified them with the "stäbchendrüsenzellen" of Plehn (1906).

The rodlet cell found in the sucker is oval in shape, narrower at the distal end and with a prominent cell membrane (Fig. 15). These cells are located between the absorptive cells in the intestinal epithelial. The number of rodlet cells is much less than the goblet cells and the distribution is highly irregular. The vast majority of
the cells are situated near the lumen into which they appeared to open by a small pore. The size of the cells is larger in young fish. They are about $\frac{1}{2}$ to $\frac{1}{3}$ of the height of the absorptive cells in the young ones but $\frac{1}{3}$ to $\frac{1}{5}$ for the older ones. Usually average dimensions are 13 to 16 microns in length and 6 to 9 microns in width.

The spherical nucleus of the rodlet cell typically occupies the basal (proximal) position of the cell. Irrregularly shaped nuclei are not common. Opposite one pole of the nucleus the cytoplasm contains long slender rodlets that are parallel to each other and to the long axis of the cell. The rodlets stain neither with thionin nor with azure-eosinate but stain brown in Goldman's, red in hematoxylin and eosin, green in safranin and fast green, purple in fast green Van Gieson, pink to purple in Bauer-Feulgen; bright red in the periodic acid-Schiff reagent, even after preliminary treatment with saliva; dark purple with aldehyde fuchsin; and green with trichrome. They do not stain with alcian blue.

In the intestine of the white sucker the rodlet cells appear to originate from wandering leucocytes situated at the base of the mucosal fold, where a few intermediate stages may be found. The earliest recognizable stage of this transformation are typical wandering leucocytes with
inconspicuous cell membranes. The cytoplasm contains small acidophilic masses. The nuclei are irregularly spherical. This stage is usually found in the infranuclear area of epithelial cells (Fig. 16). In the next stage the cell becomes elongated and the nucleus is rounder. The acidophilic mass becomes larger (Fig. 17). This stage is generally found in the absorptive nuclei level. And it finally develops into the mature stage in which the cell membrane is very distinct. The acidophilic mass transforms into many small rod-like structures. The nucleus is rounded. The mature cells are usually found near the free border of the absorptive epithelial cell but sometimes are encountered in the same level as the younger ones (Fig. 15).

B. Lamina propria

The lamina propria is a layer of vascular connective tissue, located between the epithelium and the stratum compactum. This layer is usually as thick as the epithelial layer (85 to 95 microns) but appears thicker in the region in which it is projected into the epithelial fold for which they form a supporting core (130 to 140 microns). The fibers are apparently all collagenous. They are acidophilic and stain red with hematoxylin and eosin, pale red with Mallory's phosphotungstic acid hematoxylin; brown with Goldman's; purple-red with PAS; green with fast green; blue
with indigo carmine. The fibers are coarse. The cells present are wandering leucocytes, granular cells, polymorphonuclear wandering cells, and fibroblasts which are scattered among the fibers in abundance. There are also a few macrophages. The structure of the wandering leucocytes and granular cells is the same as found in the epithelial layer. The polymorphonuclear wandering cells are 5 - 7 microns in diameter. The nucleus typically has several lobes forming a horse-shoe or broken circle shape near the periphery of the cell membrane (Fig. 18). The cytoplasm was slightly granular and acidophilic staining pink with PAS. The fibroblasts have elongated nuclei, and an average size about 8 to 9 microns long and 2 to 3 microns wide. The macrophages are scattered among the other kinds of cells. The macrophages can be best identified in the trypan blue injected fish. After repeated injection, the dye particles are engulfed in the macrophages vacuole and become numerous which develops a distinct macroscopic color. The shape of the cells varies from rounded or oval to elongated spindle-shaped with an average size about 6 to 9 microns long and 4 to 6 microns wide. The nuclei are usually irregular, oval or kidney shaped, and contain coarse chromatin particles (Fig. 19).
In the lamina propria, the capillary endothelium showed a strong positive alkaline phosphatase reaction. Connective tissue elements are usually negative with the exception of those immediately underneath the epithelium which are occasionally positive (Fig. 10).

In the young fish the lamina propria layer is much thinner than the epithelial layer except at the region of the epithelial folds, where it is usually thickened.

II Stratum compactum

The stratum compactum is a layer between the lamina propria and the muscularis layer. It is a fairly uniform band, its thickness is relatively constant, varying between 15 to 26 microns. This layer is a dense non-cellular layer of large, wavy collagenous fibers which forms a thick heavy sheath, and appears acidophilic, stains red with PAS and hematoxylin and eosin; dark orange with Mallory's phosphotungstic acid hematoxylin; blue with indigo carmine; brown with Goldman's; pink with Bauer-Feulgen; green with fast green; and green with trichrome.

The surface of the stratum compactum that is toward the lumen of the intestine is marked by a continuous single layer of fibroblast cells. On the basal surface of the stratum compactum the collagenous fibers regularly arrange
themselves as perpendicular bands which penetrate into the circular muscle layer (Fig. 20). The stratum compactum always shows a negative alkaline phosphatase reaction (Fig. 10).

The stratum compactum was only about 1.5 to 5 microns thick in the 4 to 5 cm. fish.

III Muscularis

There are two well-developed muscular layers of the intestine, an inner circular and an outer longitudinal layer, both composed of smooth muscle. The circular muscle layer is about 53 to 158 microns in thickness while the longitudinal is 9 to 39 microns thick. In between the two layers there is a loose connective tissue in which are embedded blood vessels and the nerve plexus of Auerbach. The circular muscularis, just beneath the stratum compactum usually shows alkaline phosphatase (Fig. 10).

In the young fish the muscular layers are very thin. The longitudinal layer possesses only a few layers of smooth muscle fibers. The circular muscle layer is thicker. The nuclei of the smooth muscle are elongate and relatively large.

Glycogen was not found in the intestinal wall except a small amount was observed in the muscularis layer.
IV Serosa

The serosa is composed of a single layer of flat mesothelium which is separated from the longitudinal muscular layer by a thin layer of connective tissue and blood vessels. It is continuous with the serosa of other adjacent visceral organs and the mesenteries.
GENERAL HISTOLOGY OF CREEK CHUBSUCKER INTESTINE

The general histology of the intestine of the creek chubsucker is similar to that of the white sucker. The intestinal wall is composed of the typical four layers: (1) mucosa (epithelium and lamina propria); (2) stratum compactum; (3) muscularis; and (4) serosa (Figure 21). This study is based on one 26 cm. fish.

Mucosa

The mucosal folds are larger and relatively lower than those found in the white sucker.

A. Epithelium. There are two basic kinds of cells, the absorptive cells and the goblet cells. Other cell types found in the epithelium are similar to those of the white sucker except that no rodlet cells were seen.

(a) Absorptive cells. The absorptive cells are elongated, measuring 38 to 60 microns in length and 3 to 6 microns in breadth. The nuclei are larger irregular or regular ovoids, averaging from 9 to 15 microns long and 2 to 5 microns wide. The nuclei are usually located at the middle of the cell in the top of the mucosal fold and near the base of the cell in the bottom of the fold. The other structural details are similar to those of the white sucker.
(b) **Goblet cells.** They are numerous in the epithelium and structurally similar to the goblet cells of the white sucker. However, the dilated portion containing mucin, is more round and located very close to the lumen of the intestine (Fig. 22). These cells are about 9 to 12 microns long by 6 to 8 microns wide in the broadest portion.

(c) **Wandering leucocytes.** They appear similar to those found in the white sucker but are less abundant at the base of the absorptive cells.

(d) **Polymorphonuclear wandering cells.** These cells are very rare in the basal region of the epithelium but there are considerable numbers inside the blood vessels of the lamina propria. Morphologically these cells appear similar to those found in the white sucker.

(e) **Granular cells.** They are larger (averaging between 7 to 10 microns) and more abundant than in the white sucker. These cells are numerous near the basal region of the absorptive cells and the number decreases progressively on approaching the free border region of the cells.

B. **Lamina propria.** The lamina propria of the creek chubsucker is 5 to 7 times (250 to 350 microns) thicker than the epithelium (42 to 60 microns) and therefore structurely different from the white sucker. It also has
more granular cells scattered among the collagenous fibers and is very rich in blood vessels of relatively large size (diameter between 9 and 16 microns) which contain polymorphonuclear wandering cells.

The stratum compactum, muscularis and serosa are similar to those of the white sucker.

The description of the general histology of the creek chubsucker intestine has been based on only one 26 cm. fish. From the abundance of the granular cells as well as the thickness of the lamina propria it would appear that the histology of the intestine of this fish is quite different from that of the white sucker. However, it is possible that this may represent an abnormal individual. The general histology of the creek chubsucker should be further studied.
SECTION V

GENERAL PATHOLOGY AND HISTOPATHOLOGY

DUE TO THE PRESENCE OF ACANTHOCEPHALA

Pomphorhynchus bulbocolli

**General pathology**

The white suckers captured from Round Pond were heavily parasitized by *P. bulbocolli*. Up to 18 worms were counted in a square centimeter of the infected intestinal area. The Acanthocephala usually inhabited the area between the middle and posterior intestine. Females were more abundant than males. The parasite has its trunk in the lumen; its proboscis, with a long slender neck, penetrates the mucosa and stratum compactum and extends through the muscularis layers. The anterior end of the parasite becomes encapsulated beneath the serosa or in the mesentery. Macroscopically, the fibrous capsules surrounding the proboscis and neck bulb were clearly visible from the outside.

**Histopathology**

The most pronounced reaction of the intestine to the presence of *P. bulbocolli* was the formation of a collagenous
fibrous capsule. In the host intestine, at the site of penetration, the mucosa, the stratum compactum and muscularis layers are completely disrupted to form a "tunnel" enclosing the long slender neck of *P. bulbocollis*. The proboscis and spherical enlargement of the neck are embedded in the terminal part of the capsule, which may protrude several mm. above the intestinal wall. The capsule is covered by the mesentery and is often embedded in the adjoining liver and pancreas.

Typically, the host intestine shows a marked connective tissue reaction to the penetration of the Acanthocephala by developing collagenous fibers around the slender neck and destroying the normal architecture of the intestinal wall. The epithelial layer is totally destroyed at the site of attachment, but adjacent to the trunk of the worm there is only cellular compression. These compressed cells exhibited no histochemical changes from the adjacent normal cells.

The lamina propria, the stratum compactum, and the muscularis are likewise destroyed at the site of penetration; the long slender neck is usually embedded in these layers (Fig. 23). The region of disorganized tissue extends some distance from the tunnel. In the tunnel, no mucous nor any debris is present between the cuticula of the parasite and the host tissue. The cuticula of the neck is in direct
contact with the connective tissue of the host and a dense accumulation of fibroblast cells from the lamina propria is arranged in epithelioid fashion. Several layers of these fibroblast cells surround the tunnel and extend into the capsule in the serosa of the intestine (Fig. 24). Next to the epithelioid layer the connective tissue forms dense interlacing strands of thick collagenous fibers. Numerous granular cells are commonly scattered among the collagenous fibers. These cells are most abundant between the fibrous tissue of the capsule and the normal tissue of the intestine (Fig. 25). Proliferation of other cell types was not recognized.

The capsule around the proboscis and bulb is composed of several layers of fibroblasts which appear to grow out from the intestinal surface, the pancreas, or the liver. The capsular wall can be separated into two layers. The innermost layer of the capsule is composed of few to several layers of epithelioid fibroblasts. These fibroblast cells are characterized by having large and pale staining nuclei. The nucleus has an oval or cylindrical form with rounded ends. It contains small pale chromatin granules. The cytoplasm appears homogeneous and acidophilic as indicated by staining with eosin and fast green but does not stain with thionin, azure C, and azure-eosinate.
Externally the capsule is covered with a loose circumferential arrangement of fibroblastic elements. There is a mucous-like substance present between the proboscis and bulbous neck region, and the epithelioid layers of the terminal capsule (Fig. 26). This substance is different from the mucous of the goblet cells as shown by the reaction to various staining technics in Table IV.
TABLE IV

Comparison of PAS positive substance in the capsule and mucus of the goblet cell

<table>
<thead>
<tr>
<th>stain</th>
<th>substance around proboscis and bulb</th>
<th>mucus in the goblet cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS</td>
<td>red</td>
<td>red</td>
</tr>
<tr>
<td>Thionin</td>
<td>unstained</td>
<td>dark purple</td>
</tr>
<tr>
<td>Azure-eosinate</td>
<td>unstained</td>
<td>violet</td>
</tr>
<tr>
<td>Alcian blue</td>
<td>unstained</td>
<td>blue</td>
</tr>
<tr>
<td>H - E</td>
<td>pink</td>
<td>dark purple</td>
</tr>
</tbody>
</table>

This substance is PAS positive but unaffected by salivary digestion; it fails to stain with alcian blue for acid mucopolysaccharide. That PAS positive substance is secreted by the inner layer of epitheloid fibroblast cells is indicated by the presence of similar staining material in the cytoplasm of some of these cells. There is no evidence of inflammation.

In many sections there are more capsules in the intestinal wall than living worms in the lumen. Apparently the presoma of *P. bulbocolli* remains after the death of the worm and the elimination of the trunk region from the
intestine. The presoma is embedded in the capsule and destroyed there. In heavy infections there are areas that appear to be undergoing a process of repair. After the proboscis and bulb are destroyed the region becomes filled with the mucoid secretion from the epithelioid fibroblast cells lining the capsule (Fig. 27-28). This was followed by the entrance of the connective tissue cells appearing as a loose reticulum (Fig. 29). These stages of repair are similar to that found in salmonid fishes infected with *Acanthocephalus jacksoni* (Bullock, 1963). The repair sequence differs in the tunnel where the slender neck region was embedded. After the destruction of the neck, this area is invaded by fibroblasts first and then collagenous fibers. The tunnel is later filled with heavy fibrous connective tissue (Fig. 30-31).

**Octospinifer macilentus**

**General pathology**

White suckers (4.6 - 12 cm.) captured from the Oyster River and the Winnicutt River had light to moderate infections with *Octospinifer macilentus*. In the heaviest infections as many as 11 Acanthocephala (Fig. 32) were found in one intestinal cross section. However the fish appeared normal and showed no sign of sickness or malnutrition. All
of the *O. macilentus* were found in the posterior part of the intestine. Nodule formation could be observed on the exterior of the infected intestine. Dissection of the intestine showed that the nodules on the outer surface were produced only by the female worm which penetrates deeper than the male. This difference in the degree of penetration between males and females had been observed for *Echinorhynchus lageniformis* by Prakash and Adams (1960) who interpret this as an adaptation for greater mobility of the male parasite allowing copulation with the female.

**Histopathology**

The female of *O. macilentus* penetrates the mucosa and stratum compactum, but never through the muscularis. However the muscular layer is disrupted at the site of penetration, and infiltrated with connective tissue forming a nodule on the intestinal surface (Fig. 33).

The intestinal wall at the site of penetration by the Acanthocephala shows a complete denudation of the epithelium, lamina propria and underlying stratum compactum. These cells and tissues are mechanically cut off by gradual penetration, resulting in scattered necrotic epithelial and connective tissue in the lumen. The proboscis and sometimes the anterior trunk of the acanthocephalan is in
direct contact with the host tissue (Fig. 34). There is no evidence of mucus surrounding the parasite as was observed by Bullock (1963b) for *Acanthocephala jacksoni* in four species of salmonids he examined. There is no cellular reaction in the mucosal layer, but a thickening of the lamina propria occurs near the site of penetration.

Nodule structure is similar to the capsule produced by the presence of *P. bulbocelli*. Both nodule and capsule are formed by the reaction of the host tissue due to the penetration of the acanthocephalan. They are the result of fibrosis, and the major components are collagen fibers, fibroblasts and numerous granular cells interspersed between the collagen fibers, which become thicker toward the periphery of nodule or capsule. Both nodule and capsule were covered by the mesentery and were not sharply separated from the surrounding connective of the pancreas and liver. Neither inflammation nor bacteria were observed in either case. There are two major differences between the nodule and the capsule; first, there are no epithelioid fibroblasts lining the nodule, and second, no PAS positive substance present between the proboscis and the host tissue.

The most pronounced reaction of the fish intestine is due to the penetration of the female, resulting in nodule
formation. In contrast, the male does not penetrate so deeply. Often the proboscis reaches only the stratum compactum and nodule formation seldom occurs (Fig. 35). The mucosa is destroyed as in female attachment and the stratum compactum is either normal in thickness or somewhat thinner. The muscularis is infiltrated with some connective tissue.

**Neoechinorhynchus cristatus**

**General pathology**

*Neoechinorhynchus cristatus* was found in white suckers obtained from Winnicutt River, Oyster River, Rangeley River, and Turtletown Pond. The infected fish showed no sign of sickness or malnutrition. Infected fish range from 4 to 23 cms. long. The fish, examined immediately after collection, had moderate to heavy infections with *N. cristatus*, but the number of parasites decreased and disappeared after the fish were maintained several days in aquaria. *N. cristatus* inhabited the posterior part of the intestine. The infected intestine appeared normal with no nodule or capsule formation. Dissection of the intestine showed that these acanthocephalans were loosely embedded in the mucosa which may account for the loss of parasites in captivity.
Histopathology

*Neoechinorhynchus prolixoides* never penetrates the stratum compactum. This acanthocephalan appears to destroy only the epithelium and a small portion of the underlining lamina propria. These effects are limited to the site of attachment (Fig. 36). Usually there was no evidence of tissue reaction due to the presence of *N. cristatus*. The only cellular reaction is shown by the granular cells which always increase in number in the lamina propria around the proboscis (Fig. 37). In one series of sections, extremely elongated fibroblast nuclei (21 microns) were observed near the site of attachment (Fig. 38). Again, there is no evidence of bacterial infection.

*Neoechinorhynchus prolixoides*

**General pathology**

The creek chubsuckers captured from Turtletown Pond had light to moderate infections of *Neoechinorhynchus prolixoides*. The infected fish appeared normal. The acanthocephalans were scattered in the posterior part of the intestine, which outwardly showed no nodule or capsule formation. When the intestine was opened the acanthocephalan often sloughed off. After fixation only a few acanthocephalans were left embedded in the intestine.
Histopathology

*N. prolixoides* were observed only in the epithelium and seldom penetrated into the lamina propria. The epithelium is disrupted or destroyed only in the immediate area of attachment (Fig. 39). There is no evidence of any tissue or cellular reaction due to the presence of *N. prolixoides*.

Alkaline phosphatase

The general distribution of alkaline phosphatase in the infected intestine appeared similar to that in uninfected sections. No additional alkaline phosphatase activity was found when tissue was infected with *N. cristatus* (Fig. 40), or male *O. macilentus* (Fig. 41). In contrast, there was strong alkaline phosphatase activity in the surrounding connective tissue when either *P. bulbocollis* (Fig. 42) or female *O. macilentus* (Fig. 43) were present.
SECTION VI

DISCUSSION

Histological changes in fish intestine, due to the presence of Acanthocephala, are variable depending upon the species of parasite. The reactions of the host tissue are specific and of value in determining the parasite species from serial sections. The most significant character is the depth of penetration, which initiates capsule or nodule formation and some other tissue or cellular reactions.

The capsule and the nodule produced by host tissue is in direct response to the depth of penetration of P. bulbo-colli and female O. macilentus which penetrate the muscular layers and stratum compactum respectively. There is no sign of capsule or nodule formation in the other acanthocephalans studied where penetration never reached the stratum compactum.

The depth of penetration also initiated host cellular reaction. For instance, the depth of penetration of N. cristatus is as far as the lamina propria and there are clumps of granular cells around the proboscis, while N. prolixoides just penetrated the epithelial layer and there is no sign of any cellular reaction of the host tissue.
Granular cells are present in great numbers around the proboscis of *N. cristatus* in the lamina propria and scattered among the collagenous fibers of capsules and nodules which are formed by the presence of *P. bulbocollis* and female *O. macilentus*. The granular cells are always found infiltrated between the fibrous layer of the capsule and nodule, and adjacent normal tissue of the intestine. The presence in great numbers of these granular cells indicates an important function concerned with the physiological defense mechanism. There is no difference in the number of wandering leucocytes, polymorphonuclear leucocytes, and macrophages in response to the presence of acanthocephala.

Previous workers have described the pathological effects of acanthocephalan parasites. Some of these investigators indicated that even a low number in a bird or fish may be serious or even fatal. Thus, Webster (1943) implied that one or two *Plagiorhynchus formosus* could kill a robin. Most of the suckers examined had moderate to heavy infections of Acanthocephala. None of these fish seemed to be suffering from the effects of the parasites, however. The histopathological effects observed indicate that *P. bulbocollis* and female *O. macilentus* are the pathogenic acanthocephala of the white suckers, and *N. cristatus* and *N. prolixoides* are less harmful, destroying only the epithelium
at the site of attachment. However, this factor may combine with heavy infections to interfere with the metabolism of the white sucker.

In many of the Acanthocephala the proboscis is the only specialized hold-fast organ, anchoring individuals against the movement of food masses, which tend to carry them out of the host intestine. Among these four species of Acanthocephala found in white suckers, *P. bulbocolli* has the greatest morphological development of the presoma. *P. bulbocolli* has a secondary modification in the inflated bulb and the filiform structure connecting the bulb with the trunk both developing relatively late (Van Cleave, 1952). These accessory anchors, embedded permanently in the intestinal wall, permit long-established infections as previously described. *O. macilentus, N. cristatus* and *N. prolixoides* had a simple globular proboscis bearing on their surfaces three rows of sharply pointed recurved hooks; *O. macilentus* is able to penetrate deeper into the host tissue than *N. cristatus* and *N. prolixoides* which often are eliminated from the host under conditions of captivity.

The relationship between these acanthocephalans and the white suckers observed are similar to those described by previous investigators. For *P. bulbocolli*, the histopathological reaction of host tissue is most like that
described by Wurmbach (1937) for P. laevis in the barbs, and for Prosthenorchis elegans of squirrel marmoset monkeys by Takos and Thomas (1958). In the hosts mentioned above, the worms had destroyed the mucosa, the stratum compactum and muscular layer, and formed serosal capsules. O. macilentus is similar to the Echinorhynchus lageniformis in the starry flounder (Platichthys stellatus (Prakash and Adams, 1960). The female parasite appears to penetrate more deeply than the male and induces formation of a chronic nodular outgrowth outside the intestinal wall. N. cristatus as well as N. prolixoides are comparable to N. cylindratus in the largemouth bass (Venard and Warfel, 1953). The mucosa of the host is destroyed only in the immediate area of attachment and there is neither nodule formation nor deep penetration beyond the lamina propria of the infected gut. In no case were secondary bacterial infections observed as reported for N. cylindratus by Venard and Warfel (1953), and for Prosthenorchis elegans by Takos and Thomas (1958). The other important aspect of the relationship is the ability of the acanthocephalans to move and hence to produce multiple wounds in the intestinal wall of the host. N. cristatus, N. prolixoides and male O. macilentus temporarily attach to the intestinal wall and are able to move and re-attach again. Therefore each worm
may be responsible for more than one wound in the mucosa. This phenomenon is supported by some wounds which are seen where the Acanthocephala are no longer present. From the characteristics as well as the depth of penetration of the proboscis inside the capsule or nodule produced by P. bulbocolli and female O. macelentus one can conclude that these worms stay in place for a very long time once they have become attached. The serious effects on fish by the movement of some Acanthocephala have been reported by previous workers (Wurmbach, 1937; Bullock, 1963). However, the effect on the fish of the movement of N. cristatus, N. prolixoides and male O. macilentus are not as serious as the effect of Acanthocephalus anquillae in the barb or Acanthocephalus jacksoni in the trout. For A. anquillae and A. jacksoni not only move but also penetrate deeply into the host tissue, while N. cristatus, N. prolixoides and O. macilentus have shallow penetration.
SECTION VII

SUMMARY

1. The catostomid fish examined had neither stomach nor rectum. The alimentary tract is a series of coils about two times the standard body length.

2. The intestinal wall is composed of four typical layers: (a) mucosa (epithelium and lamina propria); (b) stratum compactum; (c) muscularis; and (d) serosa. Histological study revealed a similarity of structure between the white sucker and the creek chubsucker, except for minor details.

3. In the mucosa, the epithelium contains two principal types of cells: the absorptive cells and goblet cells. Other cell types associated with the epithelium are wandering leucocytes, and granular cells. Rodlet cells are also found in considerable numbers among the columnar epithelium of the white sucker, but were absent in the creek chubsucker.

4. The four species of Acanthocephala commonly found in the catostomid fishes in New Hampshire are:
Pomphorhynchus bulbocolli (Linkins); Octospinifer macilentus (Van Cleave); Neoechinorhynchus cristatus (Lynch); and N. prolixoides (Bullock).

5. The most pronounced effects of *P. bulbocolli* infection are (a) the damage of the mucosa, the stratum compactum and muscularis at the site of penetration, and (b) the proliferation of connective tissue to form a capsule around the presoma.

6. Female *O. macilentus* penetrate the mucosa and the stratum compactum, but never the muscularis layer. However the muscular layer is disrupted at the site of penetration and infiltrated with connective tissue forming a nodule. Male *O. macilentus* does not penetrate the stratum compactum and nodule formation seldom occurs.

7. *N. cristatus* and *N. prolixoides* destroy only the epithelium and underlying lamina propria. These effects are limited to the site of attachment. There may be a concentration of granular cells in the lamina propria around the proboscis of *N. cristatus*.

8. No evidence of bacterial infection or inflammation was observed in any cases.
SECTION VIII

FIGURES
Figure 1  *Pomphorhynchus bulbocolli* Linkins
a. Proboscis with inflated neck bulb
b. Entire male
   (after Van Cleave and Mueller, 1934)
Figure 2  *Octospinifer macilentus* Van Cleave

a. Proboscis

b. Entire male

(after Van Cleave and Mueller, 1934)
Figure 3  *Neoechinorhynchus prolizoides* Bullock

a. Proboscis

b. Entire male

(after Bullock, 1963a)
Figure 3
Figure 4 *Neoechinorhynchus cristatus* Lynch
a. Proboscis
b. Entire male
   (after Lynch, 1936)
Figure 5 The white sucker *Catostomus commersoni* Lacepede (after Forbes and Richardson, 1920)
Figure 6  The creek chubsucker *Erimyzon oblongus* Mitchell
(after Forbes and Richardson, 1920)
Figure 7  Cross section of the anterior part (intestine swollen) of the intestine of a white sucker, showing the high simple mucosal folds. Helly's, PAS. X 100

Figure 8  Cross section of the posterior part of the intestine of a white sucker showing the low, simple mucosal folds. Note the presence of a cross section of an acanthocephalan in the lumen. Bouin's, Trichrome. X 100
Figure 9  Columnar epithelium at the side of the mucosal folds of a white sucker intestine, showing the canal layer (c.l.) and the granular layer (g.l.) of the free border. Bouin's, H-E. X 1,000

Figure 10  Longitudinal section of the intestine of a white sucker showing alkaline phosphatase activity. Note the intense activity in the free border of the absorptive cell and the capillary endothelium in the lamina propria, but the dilated part of the goblet cells and the stratum compactum show negative reaction. X 100
Figure 11 Epithelium of the intestine of a white sucker showing the fat vacuoles in the absorptive cells. Helly's, PAS. X 450

Figure 12 Epithelium near the tip of the fold of a white sucker. Note the abundance of dark staining nuclei of the wandering leucocytes at the base of absorptive cells. Helly's Azure-eosinate. X 450
Figure 13  Cross section of the intestine of a white sucker, showing the mitotic figure above the nuclear level toward the luminal edge of the base of the fold. Bouin's, Trichrome. X 450

Figure 14  The granular cell in the base of epithelium of a white sucker. Bouin's, PAS. X 1,000
Figure 15  Rodlet cell in the epithelium of a white sucker. Bouin's, PAS. X 1,000

Figure 16  Early stage of development of rodlet cell in the epithelium of a white sucker. Bouin's, PAS. X 1,000
Figure 15

Figure 16
Figure 17  The same section as figure 12 showing the older stage of development of rodlet cell in the epithelium of the white sucker. Bouin's, PAS. X 1,000

Figure 18  Polymorphonuclear wandering cell in the lamina propria of a white sucker. Note the horse-shoe shape nucleus near the periphery of the cell membrane. Bouin's, Goldman's. X 1,000
Figure 17

Figure 18
Figure 19  Macrophage in the lamina propria of a white sucker. Note the dark staining vacuoles containing trypan blue particles in the cytoplasm. Bouin's, Carmalum. X 1,000

Figure 20  Longitudinal section of the intestine of the white sucker showing the stratum compactum. Note the non-cellular layer of thick heavy collagenous fibers. Helly's, Mallory's phosphotungstic acid hematoxylin. X 200
Figure 21  Cross section of the intestine of a creek chubsucker showing the epithelium, lamina propria, stratum compactum, muscularis and serosa. Note the very thick layer of lamina propria with the abundance of granular cells scattered among the collagenous fibers. Gendre's, PAS. X 200

Figure 22  Epithelium of the intestine of a creek chubsucker showing the goblet cells. Gendre's, PAS. X 1,000
Figure 23  Longitudinal section of the slender neck of Pomphorhynchus bulbocolli embedded in the intestinal wall of a white sucker, showing complete destruction of the mucosa, the lamina propria and the muscularis at the site of penetration. Bouin's, Trichrome. X 100

Figure 24  P. bulbocolli embedded in the intestine of a white sucker. Note the marked increase in the connective tissue forming capsule around the presoma. Helly's, PAS. X 20
Figure 25  Intestinal portion of the same section as shown in Figure 24 enlarged. Note that the granular cells are abundant between the fibrous tissue of the capsule and the normal tissue of the intestine. X 200

Figure 26  Longitudinal section of the proboscis and bulb of *P. bulbocolli* in the capsule showing the characteristic of the capsular wall which can be separated into two layers: the innermost layer is composed of few to several layers of epithelioid fibroblasts; the external layer is a loose circumferential arrangement of fibroblastic elements. Note there is a PAS positive substance present between the proboscis and bulbous neck region, and the capsule. Helly's, PAS. X 100
Figures 27, 28, and 29

Stages in the repair of the capsule where the proboscis and the bulb had been.

Figure 27-28 As the proboscis and bulb are destroyed the space is filled with the PAS positive secretion from the epithelioid fibroblast cell lining the capsule. Helly's, PAS. X 100
Figure 29 Beginning of connective tissue cells infiltrated into the PAS positive substance of the capsule. Helly's, H-E. X 100
Figures 30 and 31

Showing the repair sequence in the tunnel where the slender neck region was embedded.

Figure 30  The cross section of the slender neck of *P. bulbocollis* embedded in the tunnel of the capsule. Bouin's, Trichrome. X 1,000

Figure 31  Cross section of the capsule where the slender neck had been. Note the tunnel is filled with heavy fibrous connective tissue as a stage of repair. Bouin's, PAS. X 1,000
Figure 32 A cross section of the posterior portion of a white sucker, showing large number of *Octospinifer macilentus* present in the lumen. 4% formalin in 1% acetic acid, Carmalum. X 50

Figure 33 Cross section of the white sucker intestine showing deep penetration by female *O. macilentus*. Note the formation of nodule at the site of penetration. Bouin's, H-E. X 20
Figure 34 Same section as shown in Figure 33 enlarged showing the complete denudation of the epithelium, the lamina propria and the stratum compactum. The nodule formation is the result of fibrosis. Note that the proboscis and the anterior trunk of the *O. macilentus* is in direct contact with the host tissue, there is no PAS positive substance surrounding the presoma. X 50

Figure 35 Cross section of a white sucker intestine showing male *O. macilentus* attached to the mucosal layer. Note the depth of penetration is as far as the stratum compactum and no nodule formation. Bouin's, Aldehyde-fuchsin. X 100
Figure 36  Longitudinal section of the proboscis of *Neoechinorhynchus cristatus* partially embedded in the intestine of a white sucker. Note the worm destroys only the epithelium and a small portion of the underlining lamina propria. Gendre's, H-E. X 100

Figure 37  Longitudinal section of the proboscis of *N. cristatus* attached to the mucosal layer of a white sucker intestine. Note the marked increase of granular cells in the lamina propria around the proboscis. Zenker's, PAS. X 100
Figure 38  *N. cristatus* attached to the mucosal layer of a white sucker intestine. Note the presence of extremely elongated fibroblast nuclei near the proboscis. Helly's, H-E. X 100

Figure 39  Longitudinal section of the proboscis of *N. prolilixoides* partially embedded in the epithelium of a creek chubsucker intestine. Note the limited destruction of epithelium at the site of attachment and the lack of tissue or cellular reaction to the parasite. NBF, Trichrome. X 100
Figures 40 and 41

Intense alkaline phosphatase activity in the free border of mucosal fold. Note no additional alkaline phosphatase activity was found when tissue was infected with *N. cris tatus* in Figure 40 and male *O. macilentus* in Figure 41. Note also the absence of phosphatase in the worm.
Figures 42 and 43

Strong alkaline phosphatase activity in surrounding connective tissue of a white sucker intestine when *P. bulbocolly* (Figure 42) or female *O. macilentus* (Figure 43) were present. Note also the presence of phosphatase in the outer subcuticula of *P. bulbocolly* at the right of Figure 42.
SECTION IX

LITERATURE CITED


Chan, V. M. 1941. The histology of the alimentary tract of the deep-water gurnade Peristedion longispatha (Goode & Bean). Univ. of Nebraska Studies, 41:(1) 1-29.


Curry, E. 1939. The histology of the digestive tube of the carp (Cyprinus carpio communis), J. Morph. 65: 53-78.


FORMULARY OF STAINS AND PROCEDURES

**Bouin's Fixative**
- Saturated, aqueous picric acid : 75.0 ml.
- 40% formaldehyde : 25.0 ml.
- Gracial acetic acid : 5.0 ml.

**Demke's Fixative**
- 40% formaldehyde : 5.0 ml.
- Glacial acetic acid : 5.0 ml.
- Glycerine : 10.0 ml.
- Ethyl alcohol : 24.0 ml.
- Distilled water : 46.0 ml.

**Helly's Fixative**
- Distilled water : 100.0 ml.
- Mercuric chloride : 5.0 g.
- Potassium dichromate : 2.5 g.
- 40% formaldehyde : 5.0 ml.
- Anhydrous sodium sulfate : 1.0 g.

**Neutral Buffered Formalin (NBF)**
- 40% formaldehyde : 100.0 ml.
- Distilled water : 900.0 ml.
- Acid sodium phosphate, monohydrate : 4.0 g.
- Anhydrous disodium phosphate : 6.5 g.

**Ringer's Solution**
- Sodium chloride : 8.5 g.
- Potassium chloride : 250.0 mg.
- Calcium chloride : 300.0 mg.
- Distilled water : 1000.0 ml.
Zenker's Fixative

Distilled water 100.0 ml.
Glacial acetic acid 5.0 ml.
Mercuric chloride 5.0 g.
Potassium dichromate 2.5 g.
Anhydrous sodium sulfate 1.0 g.

Tissue Processing

85% alcohol overnight
95% alcohol 1 hour
Absolute alcohol 1 hour
Absolute alcohol/benzene 1/2 hour
Benzene I 1/2 hour
Benzene II 1/2 hour
Paraffin I 1 hour
Paraffin II 1 hour
Paraffin III 1 hour
Embed 1 hour

Ehrlich's Acid Hematoxylin (Cavanaugh, 1956, p. 18)

Hematoxylin 2.0 g.
Ethyl alcohol, 100% 100.0 ml.
Glacial acetic acid 10.0 ml.
Glycerine 100.0 ml.
Distilled water 100.0 ml.
Aluminum ammonium sulfate 100.0 ml.
Let mixture ripen in the light until dark red.

Hematoxylin and Eosin (H-E)

Procedure: Various fixatives.

1. Bring sections from xylol to water, using iodine in 85% alc. if necessary
2. Ehrlich' hematoxylin 1:9 in distilled water 30 min.
3. Wash in running water 30 min.
4. Dehydrate
5. Counterstain in 0.05% alcoholic eosin 15-30 sec.
6. Complete dehydration, clear and mount.
**Gomori's trichrome**

Chromotrope 2R 0.6 g.  
Fast Green 0.3 g.  
Phosphotungstic acid 0.7 g.  
Glacial acetic acid 1.0 ml.  
Distilled water 100.0 ml.  

Procedure:  
Good after Bouin's, Gendre's and N.B.F.  
Fair (uneven) after Helly's  
Poor after Zenker's

1. Bring sections from xylol to water, using iodine in 85% alc. if necessary  
2. Stain in trichrome stain 8-15 min.  
3. Rinse in 1% acetic acid in 90% alc. until excess stain no longer flows from slide  
4. Dip twice in 100% alc.  
5. Dehydrate in second change of 100% alc. 30 sec.  
6. Xylol 1 min.  
7. Mount in Permount.

**Safranin and Fast Green**

1. Bring sections from xylol to water, using iodine if necessary  
2. Fast Green, 1:5,000 in distilled water 3 min.  
3. 1% acetic acid 1 min.  
4. Safranin 0 1:1000 45 sec.  
5. Acetone I 3-5 min.  
6. Acetone II 3-5 min.  
7. Acetone/xylol 3-5 min.  
8. Xylol and mount.

**Fast Green-Van Gieson**

1. Bring sections from xylol to water, using iodine if necessary  
2. Stain in Ehrlich's hematoxylin 1:9 15 min.  
3. Wash in tap water 15 min.  
4. Stain in 0.1% aqueous fast green 4 min.  
5. Wash in 1% acetic acid  
6. Stain in 0.2% acid fuchsin in saturated aqueous picric acid 10-15 min.  
7. Wash in 1% acetic acid 2 min.  
8. Rinse in distilled water  
9. Dehydrate, clear and mount.
Azure A or C (Casselman, 1959)

1. Usually after N.B.F. or Helly's, not after Bouin's
2. Bring sections down to water using iodine if necessary
3. Stain in 0.1% Azure A or C 10 min.
4. Rinse in water 1 min., 70% alc. 1 min., and dehydrate in two changes of 100% alc. 2 min. each and 2 changes of xylol. Mount.

A nuclear stain which exhibits in varying measure the property of metachromasia or of staining cartilage matrix, mucin, and the granules of mast cells in a more violet or redder tone than they do nuclei. Called a basic aniline dye.

Trypan blue preparation (Lee, 1946)

Dissolve 0.5 g. of trypan blue in 100 ml. of distilled water, or saline. Filter. Heat at temperature of boiling water for 10 min. to sterilize old solution should not be used as alterations in colloidal state occur after a time, and the dye becomes more toxic. It is undesirable to use solutions more than a month old. The dye solution is best kept in an ice safe.

Rawitz's Carmalum

Ammonium alum 20.0 g.
Distilled water 150.0 ml.
Glycerine 150.0 ml.
Carminic acid 2.0 g.

The ammonium alum should first be dissolved in the distilled water, then the carminic acid added, and the mixture heated to assist in dissolving. After cooling the glycerine is added, and the mixture filtered. Recommended by Ludford for counterstaining trypan blue material.

Procedure:

1. Bring sections from xylol to water
2. Stain in carmalum 15 min.
3. Distilled water 5 min.
4. Dehydration, clear and mount.
Krutsay's iron alum hematoxylin (Krutsay, 1962)

A. Hematoxylin 1.0 g.
   Pot. alum 50.0 g.
   Pot. iodate 0.2 g.
   HCl (25% aqueous) 5.0 ml.
   Distilled water 1000.0 ml.

B. Iron alum 2.0 g.
   HCl (25% aqueous) 0.5 ml.
   Distilled water 100.0 ml.
Add 8 volumes of B to 100 volumes of A just before use. Fresh mixture brown - no good if orange.

Procedure:
1. Bring sections from xylol to water, using iodine if necessary
2. Krutsay's hematoxylin 5 min.
3. Running water 5 min.
4. Dehydration, clear and mount.

Thionin

Procedure:
1. Bring sections from xylol to water, using iodine if necessary
2. Stain 1-2 min. in thionin (1 : 1000)
3. Rinse in distilled water
4. Dehydrate in acetone, clear in xylol, and mount.

Goldman's iron alum - picric acid hematoxylin (Goldman 1950)

Procedure:
1. Remove paraffin from sections and hydrate in the usual manner
2. Mordant in 4% aqueous iron alum containing 1% glacial acetic and 0.12% conc. sulfuric acids for 30 min.
3. Wash in running water for 10 min.
4. Transfer to saturated aqueous picric acid containing 0.1% conc. sulfuric acid for 3 hrs.
5. Rinse in distilled water for 1-2 min.
6. Stain in 0.5% aqueous hematoxylin for about 1 hr.
7. Wash in running water for 15 min.
8. Transfer to 70% alcohol containing five drops of saturated aqueous lithium carbonate solution for 10 min.
9. Dehydrate and mount in usual manner.

**Verhoeff's elastic tissue stain** (Galigher, 1934)

**Procedure:**

1. Fix material in Zenker's, formalin, or Bouin's. Tissues should not be treated with iodine solution before staining, as excess mercury is removed the stain.
2. Embed in paraffin or cellodin and section in the usual way.
3. Stain for 15 min. in the following mixture.
   - Hematoxylin crystals 1.0 g.
   - Absolute alcohol 20.0 g.
   - Dissolve by heating, filter, and add the following:
     - Ferric chloride, 10% aqueous solution 8.0 ml.
     - Lugol's sol. (Iodine 2 g., potassium iodine 2.0 g., water 100 ml.) 8.0 ml.
4. Wash sections a few minutes in running water. Then place them in 2% aqueous solution of ferric chloride until the stain is extracted from all structures but the elastic fibers and the nuclei. The former should be black, the latter gray. Keep the slides moving during the process. Differentiation occurs very rapidly, and must be carefully controlled. Slower differentiation may be obtained by using a weaker solution.
5. Wash slides for at least 30 min. in running water then run them up to 90% alcohol. They should remain there for several min. in order to remove all traces of iodine.
6. Counterstain lightly with eosin, clear, mount in balsam. Elastic fibers are stained jet black, nuclei gray, other structures various shades of pink.
**Mallory's phosphotungstic acid hematoxylin** (Lillie, 1954)

Hematoxylin 1.0 g.
Phosphotungstic acid 20.0 g.
Distilled water 1000.0 ml.

This ripens in several weeks and the naturally ripened product is thought to be best.

**Procedure:** Zenker's fixed paraffin sections

1. Iodine in 95% alcohol (0.5%) 5 min.
2. 0.5% sodium thiosulfate 5 min.
3. Wash in tap water
4. 0.25% potassium permanganate 5 min.
5. Wash in water
6. 5% oxalic acid 5 min.
7. Wash in running water 1-2 min.
8. Stain in phosphotungstic acid hematoxylin overnight (12-24 hrs.)
9. Dehydrate rapidly in 95% and 100% alcohol or acetone, clear with a 50% mixture of the dehydrating agent and xylene, then two changes of xylene. Mount in balsam or clarite.

**Aldehyde - fuchsin** (Gabe's method) (Cameron and Steele)

Boiling 200 ml. water, add 1 g. of basic fuchsin boil 1 min., cool, filter. To the filtrate add 2 ml. each of conc. HCl and paraldehyde. Leave stoppered at room temperature. Each day after preparation of this solution withdraw a drop and place it on a filter paper. The fuchsin will be found to decrease and the precipitate to increase in quantity. When the solution has lost its reddish fuchsin color (3-4 days), filter it, and discard the filtrate. Dry the precipitate on the filter paper (the paraffin oven at its usual temperature is suitable for this). The dry crystals can then be removed from the filter paper and stored in a reagent bottle. The yield is about 1.9 g.

To make the staining solution, dissolve 0.25 g. of dry stain in 50 ml. of 70% alcohol. This solution will keep for at least 6 months with no apparent change in behavior.
Halmi's mixture (Cameron and Steele)

- Light green S.F. yellowish: 0.2 g.
- Orange G: 1.0 g.
- Chromotrope 2R: 0.5 g.
- Phosphotungstic acid: 0.5 g.
- Glacial acetic acid: 1.0 ml.
- Distilled water: 100.0 ml.

Simplified Aldehyde - Fuchsin (Cameron & Steele, 1959)

Procedure:

1. Deparaffinize and hydrate section in the usual way.
2. Oxidize in Gomori's fluid (1941) for 1 min. (0.15 g. of KMnO₄ in 50 ml. of water containing 0.1 ml. of conc. H₂SO₄)
3. Rinse in 2.5% Sodium bisulfite few seconds.
4. Rinse in distilled water.
5. Transfer through 30% to 70% alc.
6. Stain in the aldehyde - fuchsin 2-10 min.
7. Wipe back of slide (should be quick) and rinse in 95% alc.
8. 95% alc. 2-5 min. (until no more stain comes out)
9. Bring to water through 70% and 30% alc.
10. Counterstain in Halmi's mixture 20-30 min.
11. Wipe back of slide and differentiated in 95% alc. containing 0.2% acetic acid until no more stain comes away (2-3 min.)
12. Rinse in 95% alc.
13. Bring through 2 washes of absolute alc. to xylene and mount in balsam.

Alcian blue (Barka & Anderson, 1963)

Procedure: N.B.F. Carnoy's fixative

1. Bring sections from xylol to water.
2. Stain in 0.1-0.2% Alcian blue in 0.01 N. HCl for 10 min.
3. Rinse briefly in 3 changes of distilled water.
4. Rinse in 0.01 N. HCl or 1-2% acetic acid.
5. Rinse in distilled water.
6. Counterstain with safranin.
7. Dehydrate and mount.
**Schiff Reagent** (Lillie, 1954)

Dissolve 1.0 g. of basic fuchsin in 1000.0 ml. boiling water.
Filter at 50-60°C
Add 2.0 g. sodium bisulfite and 20.0 ml. n HCl.
Stopper tightly and store in the dark overnight at room temperature.
Add 300.0 mg. activated charcoal
Shake one minute and then filter.
Store in the refrigerator.

**Periodic Acid - Schiff** (PAS) (Lillie, 1948)

Procedure: Various fixatives

**For Experimental:**

1. Bring sections from xylol to water, using iodine if necessary
2. Hydrolyze 7 or 8 min. in 0.5% periodic acid
3. Rinse 5 min. in running water
4. Schiff reagent 15 min.
5. 3 changes of dilute sulfuric acid (10 ml. sodium bisulfite, 10 ml. 1 N HCl, and 180 ml. distilled water)
   At least 2 min. each change
6. Running water 1 min.
7. Ehrlich's hematoxylin (1:9 in distilled water)
   30 min.
8. Running water 20-30 min.
9. Dehydrate, clear, and mount.

**For Controls:**

1. Bring sections from xylol to water, using iodine if necessary
2. Saliva:- strained through cheesecloth 1 hr. at 37°C
3. Wash in running water 5 min.
4. Proceed as for experimentals beginning with step #2.
Bauer - Feulgen stain for glycogen (Lillie, 1948)

Procedure:

1. Bring sections from xylol to water, using iodine if necessary
2. Hydrolyze 1 hr. in 5% chromic acid. Mix every 10 min.
3. Running water 5 min.
4. Proceed as for PAS, step #4-9

Note: Saliva digested controls should be run as in the PAS technic.

Feulgen Nucleal Reaction (DiStefano, 1952)

Procedure: Various fixatives but not after Bouin's

1. Bring sections from xylol to water, using iodine if necessary
2. 10% perchloric acid, room temperature 12-24 hrs. (18 hrs.)
3. Drain slides and put in Schiff reagent for 20-30 min.
4. Wash in 3 two min. baths of dilute sulfurous acid (see PAS)
5. Wash 10 min. in running water
6. Dehydrate, counterstaining in 0.01% Fast green in 95% alc. (1 min.)
7. Xylol and mount.

Buffered azure - eosinate (Lillie, 1948)

Procedure: Various fixatives but not after Bouin's

1. Bring sections from xylol to water, using iodine if necessary
2. Stain in buffered azure - eosinate 1 hr.
3. Rinse in distilled water
4. Dehydrate in 2 changes of acetone 1 min. each
5. Acetone/xylol 1 min.
6. Xylol 2 changes 2-5 min. each
Azure - eosinate stock

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azure eosinate stain</td>
<td>1.0 g.</td>
</tr>
<tr>
<td>Glycerol</td>
<td>50.0 ml.</td>
</tr>
<tr>
<td>Methanol</td>
<td>50.0 ml.</td>
</tr>
</tbody>
</table>

Buffered azure - eosinate

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azure - eosinate stock</td>
<td>0.5 ml.</td>
</tr>
<tr>
<td>Acetone</td>
<td>5.0 ml.</td>
</tr>
<tr>
<td>Buffer</td>
<td>2.0 ml.</td>
</tr>
<tr>
<td>(1.3-1.1 M/10 Citric acid: 0.7-0.9 M/5 disodium phosphate - for formalin fixative)</td>
<td></td>
</tr>
<tr>
<td>(1.2-1.0 M/10 Citric acid: 0.8-1.0 M/5 disodium phosphate - for Helly's)</td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>32.0 ml.</td>
</tr>
</tbody>
</table>

Basic fuchsin and Picro - Indigo - Carmine (Shumway, 1926)

Procedure: Containing formalin fixative as Bouin's

1. Basic fuchsin saturated aqueous solution 20 min. Without rinsing, place in the following mixture.
2. Indigo-carmine, saturated aqueous solution 1 part, picric acid, saturated aqueous solution 1 part 5 min.
3. To decolorize, transfer to 70% alc. for a few seconds only until sections appear pink to the eye.
4. Dehydrate rapidly in 95% alc. then absolute alc. for a few seconds until sections appear blue or green to the eye, then
5. Clear in xylol

Alkaline Phosphatase

Procedure: Fix in cold 85% alc. 24 hrs. in refrigerator

1. Dehydrated through a graded series of acetones
   (a) 50% acetone 1 hr.
   (b) 70% acetone 1 hr.
   (c) acetone I 1 hr.
   (d) acetone II 1 hr.
   (e) acetone III 1 hr.
   (f) acetone/benzene 1 hr.
   (g) benzene I 1 hr.
   (h) benzene II 1 hr.
(i) paraffin I 1 hr.
(j) paraffin II 1 hr.
(k) paraffin III 1 hr.
(l) embedding 1 hr.

2. Sectioning within 24 hrs. of embedding 7 microns
3. Bring sections from xylol to water
4. Place the tissues in the substrate mixture for 30 min. at 37°C
5. 2% Ca(NO₃)₂ 3 min.
6. 2% Cobalt²⁺ chloride 10 min.
7. Wash briefly in distilled water
8. 2% yellow ammonium sulfide solution 15 min.
9. Two changes 35% alc. 5 min. each
10. Distilled water 5 min.
11. Carmalum 15 min.
12. Distilled water 5 min.
13. Dehydration, clear and mount

**Substrate mixture for Alkaline Phosphatase**

**For Controls:**
- 2% Ca(NO₃)₂ 20.0 ml.
- 1% sodium barbital 10.0 ml.
- Distilled water 50.0 ml.

**For Experimental:**
- 2% Ca(NO₃)₂ 20.0 ml.
- 1% sodium barbital 10.0 ml.
- Distilled water 50.0 ml.
- 2% sodium glucerophosphate 20.0 ml.
Procedure for Processing Acanthocephala

1. After careful removal from host intestine place worms in distilled water until the proboscis is fully extended. This may take from a few minutes to many hours. Difficult specimens can be left overnight in the refrigerator.

2. Fix in an alcohol-formalin acid mixture (such as Demke's) for a few hours or overnight.

3. Store in 70% ethyl alcohol with glycerine (5%).

4. Using entomological "minutennadeln", carefully prick the body wall of each specimen. Use 2 or 3 pricks for small specimens, 4 or 5 for medium size, etc. Be sure to include the regions near the extremities but do this carefully so as not to destroy important internal structures.
   Note: The small specimens require this pricking even more than the larger ones.

5. In the morning (i.e. before 9 AM) replace alcohol with undiluted Grenacher's borax-carmine.

6. In late afternoon (between 4:30 and 5:30 PM) carefully add one drop of concentrated HCl for each 5 ml. of stain. Quickly mix by inverting vial several times but be careful that all worms are returned to the fluid and do not remain on the cork or the upper portion of the vial. Leave overnight.

7. The next day (preferably in the morning) replace the stain with 1% HCl in 70% alcohol and destain until a light pink color has been obtained.
   Note: In order to prevent the loss of small worms it is best to pour the contents of the vial (containing precipitated stain) into a Syracuse watch glass or Petri dish and carefully transfer worms to acid alcohol in vial. During the destaining process replace acid alcohol frequently. The destaining process usually requires several hours but may take up to 2 days. It is best not to allow the destaining to proceed unattended overnight. (Replace acid alcohol with 70% and then return to acid alcohol the next day).
8. After destaining is completed transfer to 85% alcohol for 18-24 hours. Be sure to change the 85% alcohol several times during this period to remove excess acid.

9. Transfer to 95% alcohol for 6-18 hours.

10. Transfer to 100% alcohol for 6-18 hours.

11. Transfer to 100% alcohol - terpineol:

   - 25% terpineol for 6-18 hours.
   - 50% terpineol for 6-18 hours.
   - 75% terpineol for 6-18 hours.

12. Transfer to pure terpineol for 18-24 hours. **Note:** These terpineol mixtures can be re-used. Decant from the vial into a small beaker, allow all debris to settle, and then decant back into stock bottle.