

STUDIES ON THE MOUTHPARTS OF
SOME SPECIES OF ENTOMOBRYA
RONDANI, 1861 (INSECTA: COLLEMBOLA)
WITH SPECIAL REFERENCE
TO ULTRASTRUCTURE.

by

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To My Parents

ABSTRACT

A.M. Zein El Abdin: Studies on the mouthparts of some species of Entomobrya Rondani, 1861 (Insecta: Collembola) with special reference to ultrastructure.

The detailed morphology and fine structure of the mouthparts in the genus Entomobrya Rondani, 1861 (Entomobryidae: Collembola) is described mainly by the aid of the scanning and transmission electron microscopes. Few variations have been observed in the morphology of the mouthparts of seven British species of the genus and the validity of these variations in taxonomy are discussed. The mouthparts are of the chewing and scraping type with well developed mandibular molar plates and less complex maxilla head. The structure of the hypopharynx and the hitherto unknown ventral lingual structures has been fully described and a role of these structures in conveying salivary juices is suggested. The complex epipharyngeal structures, together with the oral valve, formed by a tongue-like protrusion of the lingua and an epipharyngeal fold and the relationship of these structures with the feeding mechanisms has been discussed for the first time.

Some of the histological and histochemical characteristics of the mouthparts are described. The posterior part of the hypopharynx shows some enzymic activity. A fine structural analysis revealed a fairly uniform cellular organisation in both hypopharynx and epipharynx. Intrinsic longitudinal and

transverse muscles are located in the hypopharynx. Two functionally different types of sensilla on the labium concerned in mechanoreception and gustation are distinguished and their articulation and innervation clarified.

Behavioural observations utilizing multi-choice experiments for different chemicals are made and a particular affinity of insects to salt solutions has been observed.

Electrophysiological recordings from a single chemoreceptor, carried out for the first time in Collembola, confirm the presence of more than one chemosensory neural units and one mechanosensory neurone in the sensilla. Variability of response to a constant stimulus is appreciable both between and within sensilla, and individual neurones are capable of responses to compounds of different chemical classes. The specificity to various modalities appears to be more at the level of sensilla than at the level of neurons.

The feeding habits of living animals are examined and detailed descriptions are given of their method of feeding. Both solid and fluid foods are taken and this is made possible by the action of the epipharyngeal muscles forming a negative pressure. This method of feeding is compared with that of other Collembola.

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LIST OF GENERA AND SPECIES WITH SYNONYMS

COLLEMBOLA

ARTHROPLEONA

A. Poduroidea

Poduromorpha

1. Poduridae

Podura Linnaeus, 1758

P. aquatica Linnaeus, 1758

2. Hypogastruridae

Anurida Laboulbène, 1865

A. maritima (Guérin - Ménéville, 1836)

Brachystomella Årgen, 1903

B. parvula (Schäffer, 1896)

Friesea Dalla Torre, 1895

F. mirabilis (Tullberg, 1871)

Neanura MacGillivray, 1893

Protanura Börner, 1906

P. carpenteri Mukerji, 1932

Xenylla Tullberg, 1869

3. Onychiuridae

Onychiurus Gervais, 1841

Tetrodontophora Reuter, 1882

B. Entomobryoidea

Entomobryomorpha

1. Isotomidae

Archisotoma Linnaniemi, 1912

Folsomia Willem, 1902

F. candida Willem, 1902

Isotoma Bourlet, 1839

I. cinerea (Nicolet, 1841)

Isotomurus Börner, 1903

2. Entomobryidae

Entomobrya Rondani, 1861

E. albocincta (Templeton, 1835)

E. corticalis (Nicolet, 1841)

E. intermedia (Nicolet, 1841)

E. maritima Reuter, 1891

E. multifasciata (Tullberg, 1871)

E. nicoleti (Lubbock, 1867)

E. nivalis (Linnaeus, 1758)

E. purpurascens Packard, 1873)

Lepidocyrtus Bourlet, 1839

Orchesella Templeton, 1835

O. cincta (Linnaeus, 1758)

3. Tomoceridae

Tomocerus Nicolet, 1841

Pogonognathellus Paclt, 1944

T. longicornis (Miller, 1776)

T. plumbeus Nicolet, 1841

SYMPHYPLEONA

1. Neelidae

Neelus Folsom, 1896

1. INTRODUCTION

No other anatomical character tells so much about an animal's habits as its mouthparts (Macnamara, 1924): They vary in form to a greater extent than almost any other organs, the variation being correlated with the method of feeding and the nature of food taken. The functional interpretation of the structure of the mouthparts of insects is throwing much light on their probable evolutionary history and taxonomy. However, the mouthparts of Collembola have received little attention due to their minute size, considerable complexity and their entognathous or enclosed condition. For this reason, our knowledge of the functional morphology of the collembolan mouthparts, the feeding habits and the mechanism of food intake is meagre and in certain aspects erroneous..

1.1 Review of the collembolan mouthparts literature

The first papers on the structure of the mouthparts of Collembola include, among others, the works of Nicolet (1841), Lubbock (1873) and Stummer-Traunfels (1891). Despite the fact that the descriptions and drawings they gave were somewhat inaccurate, their works are of some importance. Folsom (1899, 1900) was the first to describe, in some detail, the mouthparts of Orchesella cincta (Linnaeus, 1758) and their musculature, and to draw the attention to the rotary movements of the mandible. He was followed by Willem (1900) who was the first to give some description of the nerve supply to the muscles in the head. Hoffmann (1905, 1908, 1911) gave an extensive and accurate account of the structure and musculature of the mouthparts of Tomocerus (Pogonognathellus) longicornis (Müller,

1776) under the name Tomocerus plumbeus L. and correctly stressed the rotatory movements of the mandibles. Imms (1906) gave a brief account of the mouthparts of Anurida maritima (Guérin-Ménéville, 1836) and the structural similarities in the different collembolan species. Börner (1908, 1909) gave for the first time an interpretation of the complex structure of the maxilla head of Tomocerus longicornis.

Other works on collembolan mouthparts include those of Philiptschenko (1906, 1908) on the anatomy of the mouthparts of Isotoma cinerea (Nicolet, 1841), Denis (1928, 1949) on the comparative anatomy of the head and the mandibular and maxillary musculature in different genera of Collembola and Mukerji (1932) on the mouthparts of Protonura carpenteri Mukerji, 1932. The more recent works on the subject comprise those of Manton (1964) who has given a comprehensive account of the mandibular mechanism of Tomocerus longicornis while discussing the jaw mechanisms of representatives of the major classes of arthropods, Singh (1969) on the anatomy of the mouthparts of Tomocerus longicornis, Adams and Salmon (1972) on the methods of feeding and the structure of the mouthparts of Brachystomella parvula (Schäffer, 1896), one of the rare species with no mandibles and Goto (1972) who provided an extensive description of the fine structure of the mouthparts of Folsomia candida Willem, 1902, in which the structure of the maxilla and of the mandibular molar plate was clarified and hence the grinding function attributed to the latter was disputed.

1.2 Development of entognathy

The embryonic bases of entognathy have been studied by Claypole (1898), Folsom (1900) and Garaudy (1967) for Anurida maritima, by Philiptschenko (1912) for Isotoma cinerea and Jura (1972) in Collembola in general. The last four authors are in agreement that

entognathy comes about through the formation of two independent folds (plicae orales) appearing on each side of the head in the region of the mandibles from a pair of lateral evaginations of the germ band and proceeding forward and backward to the bases of the labrum and labium. In this way, they form a kind of enclosing box which, by further growth, comes to surround the remaining mouthparts. According to Claypole, however, plicae orales develop from intercalary appendages, but this view has not been generally accepted. Paclt (1956) and Denis (1949) support the opinion of Folsom and the others in opposing the statement by Snodgrass (1938) that hexapod entognathy develops from a sinking of the mouthparts into the head.

Manton (1964, 1972, 1973) has discussed the evolution of Arthropoda with reference to the mandibular and locomotory mechanisms of some representatives of the phylum and has suggested that the entognathous condition in the three groups concerned (i.e. Collembola, Diplura and Protura) has probably evolved independently from a more primitive Myriapod-like stock. On the other hand, other entomologists like Crampton (1922) believe that the Crustacea rather than the Myriapoda are the nearest representatives of the ancestors of insects. However, Hennig (1969), Tuxen (1959, 1970), Lauterbach (1972) and Kristensen (1975) unite the three entognathous orders into one supposedly monophyletic group, the Entognatha, which they contrast with the Ectognatha (= Thysanura + Pterygota).

1.3 The cephalic endoskeleton of Collembola

Despite the works of Folsom (1899), Hoffmann (1905), Denis (1928), Hansen (1930), Tuxen (1959), Manton (1964, 1972), François (1971), Hutasse-Jeannenot (1974) and Chaudoneret (1973 a, b), the cephalic

endoskeleton of Collembola, which is closely related to the mouthparts, is not fully understood. Manton (1972) describes anterior and posterior tentorial arms and transverse segmental "tendons" between the mandibles and maxillae; but others have denied that the cephalic apodemes constitute a true tentorium (Denis, 1928; François, 1971; Hutasse-Jeanont, 1974; Chaudoneret, 1973, a, b).

1.4 The mouthparts and the taxonomy of Collembola

The various modifications which the mouthparts of insects undergo are of considerable taxonomic importance. Stummer-Traunfels (1891) was among the first to realise the systematic value of the collembolan mouthparts. Since then, many of the mouthparts and surrounding features were used as taxonomic criteria, both in descriptions and keys. Szeptycki (1967) found that the armature of the labrum could be used to separate several species of the genus Lepidocyrtus Bourlet, 1839, while Yosii (1976) used the same character in a taxonomic study of the different families of Collembola. Other characters derived from the mouthparts vary from the chaetotaxy of the base of the labium, used by Gisin (1964, 1965) to identify species of the genus Lepidocyrtus, to the complex structures of the maxilla head, used by Cassagnau (1955) and Massoud (1967) in the taxonomy of Neanuridae, Poinso (1965) in the revision of the genus Archisotoma Linmaniem, 1912, Gisin (1960) on species of the genus Anurida Laboulbène, 1865 and Massoud and Ellis (1974) on the genera Tomocerus Nicolet, 1841 and Pogonognathellus Paclt, 1944.

Considerable confusion exists in the literature on the taxonomy of the genus Entomobrya Rondani, 1861. The identification of taxa within the genus is based on very superficial characters such as the purple or

purple-black pigment patterns on the dorsal side of the body, as well as measurements and ratios of various parts of the body (Gisin, 1948; Christiansen, 1954). The morphology of the mouthparts has been very little used to provide taxonomic characters. Christiansen (1958) used the form and the relative length of the "external differentiated seta" of the outermost labial papilla for the separation of species of Entomobrya. He also used the shape of the labral papillae for the same purpose. Other workers followed his example and used these structures in the identification of members of the genus (Gisin, 1960; Stach, 1963; Paclt, 1974). However, South (1959, 1961) in his work on the taxonomy of the British species of Entomobrya, disputed the reliability of using these mouthpart structures in the taxonomy of the genus as he did not observe a considerable variation in the shape of the labral papillae in different species and that the form of the external seta and its relative length varied with age within the same species.

1.5 Feeding habits of Collembola

Studies on the feeding habits of Collembola started by the differentiation of the group into two forms according to the structure of the mouthparts. This dates back as early as 1872, when Tullberg recognised that certain Collembola possess no mandibular molar plate and suggested therefore that in these forms, the mouthparts are adapted for sucking. Macnamara (1924) recorded two groups of Collembola based on the form of the mouthparts. He considered that the species possessing a well developed molar plate chewed their food and were probably vegetarian, while those forms which have no molar plate, were suctorial and probably carnivorous. In this respect,

Maddison (1969) has reported observing Friesea mirabilis (Tullberg, 1871) feeding on the eggs of other Collembola. Singh (1964) showed that suctorial Collembola can be vegetarian, while Wolter (1963) distinguished two sucking types of mouthparts: (1) piercing and sucking where the labrum and labium together form a conical tube enclosing the rest of the mouthparts, the latter being modified into stylets e.g. Neanura MacGillivray, 1893 and its allies and (2) rasping and sucking (or what Wolter called ritzend-saugend) where the maxilla head is toothed e.g. Anurida maritima. Sharma and Kevan (1964) proposed a division of Collembola into four groups, based on the different types of feeding. These are: (1) biting and chewing (2) rasping and sucking (3) piercing and sucking and (4) anomalous. Their first three groups correspond fairly closely to those proposed by Wolter and their proposal for a fourth anomalous group is not quite clear.

1.6 The food of Collembola

The soil and soil-surface living Collembola, having chewing mouthparts, consume a range of items, of which fungal hyphae and spores, bacteria, algae, yeasts, cells of living and dead mosses, angiosperms and probably the plants especially foliage and root hairs, faeces of other soil animals and more or less, amorphous material like humus are the most important (Poole, 1959; Singh, 1969; Bødvarsson, 1970). The diet of the same species varied considerably between individual localities and at different times (Agrell, 1940; Singh, 1964; Tillbrook, 1970). On the other hand, gut content analysis generally have not shown species-specific differences in food selection and all lead to the conclusion that Collembola are, more or less, indiscriminate feeders (Knight and Angel, 1967; Gilmore and Raffensperger, 1970; McMillan, 1976). Different authors do not agree upon the importance

of the Collembola in the process of decomposition of the litter material and the formation and maintenance of the soil (Peterson, 1971; Goto, 1972).

As for the food of Entomobrya, South (1959) examined the gut contents of the British members of the genus and found that the most common foods were fungal spores, mycelia, Pleurococcus sp. and pollen grains.

1.7 Histology and histochemistry of the collembolan mouthparts

Details of the structure of the collembolan mouthparts and their associated musculature, together with the interrelationships of the different parts can be elucidated from a study of serial sections. On the other hand, a full understanding of the functioning of the mouthparts can only come through an accurate knowledge of their chemical constitution and organization gained from histological and histochemical studies. New techniques have been developed recently by which sections can be obtained for light microscopy from tissues embedded in epoxy resins and processed primarily for electron microscopy (Dodge, 1964). They have proven to be of advantage over the conventional paraffin wax sections in reducing tissue shrinkage and enabling much thinner sections to be cut, thus providing a better resolution under the light microscope. The hardness of the epoxy resin was much more suitable for cutting sections of tough structures like the mouthparts. Differential staining of cytoplasmic components in such sections has been accomplished by the use of histochemical techniques (Jurand and Goel, 1976).

Although some work has been done on the histochemistry of the cephalic endoskeleton of Collembola (François, 1971), no attempt

has been made to reveal the chemical nature of the mouthparts.

1.8 Behavioural response of Collembola to chemicals

A logical procedure for studying an animal's feeding behaviour would be to observe it in the field, ascertain by laboratory experiments and finally search for underlying physiological mechanisms. Many field studies (Milne, 1962; Usher, 1969; Joose, 1970) have shown that there is a correlation between various environmental factors and the density of Collembola. In the few experimental studies performed to analyse causes of aggregation, the distribution of the environmental factors: relative humidity, moisture, food and soil type appeared to be important (Boyd, 1967; Christiansen, 1970; Joose, 1971; Joose and Verhoef, 1974; Barra and Christiansen, 1975; Usher and Hider, 1975). On the other hand, Verhoef et al. (1977) demonstrated the presence of aggregation pheromones in Collembola which cause an orthokinetic reaction in perceiving animals.

Little work has been carried out on the effect of chemicals on the feeding responses of Collembola. Mochizuki (1952) tested the chemotaxis of Onychiurus yagii Kinoshita, 1917 to some carbohydrates and the olfactory response of Entomobrya purpurascens Packard, 1873 to potato extracts was tested by Boyd (1967)*.

1.9 Receptors on the collembolan mouthparts

In order to obtain a thorough understanding of food selection in any animal, a complete study of the mechanisms and sense organs involved in feeding is necessary. The sense organs or receptors (Dethier, 1963) are those structures whereby the energy of a stimulus

* B.A. (Sci.) thesis. Trinity College, Dublin.

is transformed into a nervous impulse which, after transmission to one of the central ganglia, usually results in a change in the behaviour of the insect or in the maintenance of some existing activity.

The physiology of the chemical senses in insects and the receptors involved have been reviewed by Hodgson (1958, 1965, 1968, 1974), Dethier (1963, 1977), Schneider (1969, 1971), Lewis (1970) and others. Receptors sensitive to stimulation by chemical solutions applied directly have been identified positively in Diptera, Lepidoptera, Hymenoptera and Coleoptera (Lewis, 1954, 1972; Dethier, 1963). They are trichoid sensilla located on the legs and mouthparts and are the sense organs which make the final chemical assays of potential food materials before these materials are either ingested or rejected. In this capacity, they have enormous economic, as well as theoretical, importance. A typical sensillum consists of a minimum of four cells: (1) the trichogen or hair-forming cell (2) the tormogen or socket-forming cell (3) the bipolar neurone (4) the reniform neurilemma cell (Wigglesworth, 1933). Mechanical deformation of some part of the receptor can stimulate a mechanoreceptor cell attached to the base of the hair (Dethier, 1963; Schwartzkopff, 1974; McIver, 1975), but only the extreme tip of the hair is sensitive to chemical stimulation (Dethier, 1955). Adams et al. (1965) have found that, in the stable fly, Stomoxys calcitrans (Linnaeus, 1758), the dendrites extend through a pore in the cuticle.

The Arthropoda occupy a unique position in the study of the taste receptors. Theirs were the first chemoreceptors which proved amenable to electrophysiological analysis and they continue to yield important data concerning the relationships between sensory input and

animal behaviour (Blaney, 1974; Mitchell, 1974). The best known taste receptors are the labellar chemoreceptors of flies. Prominent hairs on the labellum contain dendritic endings of two, three or four bipolar neurones (Dethier, 1955).

Electrophysiological techniques were first developed by Hodgson et al. (1955) and Hodgson and Roeder (1956) to make electrical measurements of the nerve impulses involved in chemoreception. The first two labellar taste receptors to be studied electrophysiologically were a receptor for sugars and one for salts. There is now electrophysiological evidence for the function of four sensory neurones of the labellar contact chemoreceptors of flies: a sugar receptor, a salt receptor, a mechanoreceptor and a water receptor (Dethier, 1977). Electrophysiological recordings from individual sensilla (or even individual sense-cells) are now being made increasingly often and provide the best evidence of their function, but conclusions from the ultrastructure of the sensilla (Thum, 1968; Gnatzy and Tautz, 1977) and from the results of behavioural experiments (Blaney and Chapman, 1968; Dethier, 1977) can also now be drawn with increasing confidence.

Few studies have been made on the fine structure of the sensory organs of Collembola. These include the works of Altner et al. (1970), Dallai (1971) and Karuhize (1971) on the postantennal organ; Altner and Thies (1972) on the sense organs of the antenna of Onychiurus Gervais, 1841 and Eisenbeis (1976) on the sense organs of the ventral tube of Tomocerus and Orchesella Templeton, 1835. To the best of the author's knowledge, there has been no published work on the fine structure, functional aspects or the electrophysiology of the sense

organs on the collembolan mouthparts.

1.10 Objectives of the present study

From the above review of the literature on the mouthparts of Collembola, it is apparent that information is lacking in many areas such as the functional morphology, feeding habits and mechanism of food intake. Inadequate understanding of the structure of the different components of the collembolan mouthparts led to some confusion in the systematics of the Collembola (Goto, 1972). Lawrence (1968) has given a warning of the possibility of misinterpretation of such complex structures as those found on the collembolan maxilla.

In view of this, and because of the atypical features of the collembolan mouthparts, together with the nature of the food consumed, it has been thought worthwhile to provide a more detailed account on the ultrastructure and physiology of the mouthparts in one of the common British genera of Collembola, Entomobrya. The studies on ultrastructure were largely carried out with both the scanning and the transmission electron microscopes which provide useful higher magnifications and better resolution and depth of focus than the light microscope. In these studies, the stress has been made on the little-known components of the mouthparts such as the epipharynx, the hypopharynx and the sense organs. On the other hand, a histochemical investigation was undertaken in an attempt to provide information about the chemical nature of the individual components of the mouthparts. In order to determine the response of the Collembola to various chemicals and try to correlate

their behaviour to the response of the sensilla on the mouthparts, behavioural as well as electrophysiological investigations were carried out. Finally, a study of the feeding mechanisms was made based on serial sections of the mouthparts and associated structures together with observations made on living animals confined in various ways.

2. MATERIALS AND METHODS

2.1 Collection and maintenance of cultures

The species described and illustrated in this study include seven British members of the genus Entomobrya Rondani, 1861:

E. albocincta (Templeton, 1835).

E. corticalis (Nicolet, 1841).

E. intermedia (Nicolet, 1841).

E. maritima Reuter, 1891.

E. multifasciata (Tullberg, 1871).

E. nicoleti (Lubbock, 1867).

E. nivalis (Linnaeus, 1758).

The majority were taken from wild populations from Imperial College Field Station, Sunninghill, Berkshire, by beating of branches, searching under and on the bark of trees with a pooter, sweeping of grass and treating cores of soil and litter by means of a modified Tullgren funnel.

Cultures of the insects were kept alive until required for treatment to ensure fair cleanliness of the parts examined. The containers used were essentially similar to those described by South (1959). They consisted of heat resisting glass dishes 3 X 5 cms, fitted with corks, lined from the inside by a layer of thin polythene sheeting to prevent the growth of fungi on the corks and to render the vivaria airtight. A filter paper moistened with distilled water was put on the bottom of each dish to provide the maximum relative humidity. Food was provided in the form of the fungus Cladosporium herbarum Fr., cultured on potato agar and cut into 2cm squares which were placed on the filter papers.

2.2 Light microscopy studies

To examine the mouthparts within the head using conventional light microscopy, the specimens were first killed by exposure to ethyl acetate vapour for 1 - 2 minutes. The whole insect was then fixed in Gisin's fixative for 2-3 days and the head was transferred to a mixture of 50% lactic acid and a few drops of Chlorazol black E which was employed as a stain (Goto, 1964). The heads were macerated in this mixture overnight at 60°C. After the treatment, all the entognathous mouthparts and the posterior arms of the tentorium became grey-black and could be seen easily through the cranial cuticle. The specimens were then washed thoroughly in 70% alcohol and permanent mounts were made in a modified version of Salmon's (1949) polyvinyl lactophenol (Goto, 1964) using thin slides (0.8/1.0mm) and No.0 cover slips. All observations with light microscopy were carried out by means of a Wild phase-contrast photomicroscope.

2.3 Scanning electron microscopy studies

Specimens, including adults of both sexes and first instar nymphs, were taken from the seven species under study to be treated for the examination of the external features of the mouthparts. They were killed in ethyl acetate vapour and then processed for dehydration in a series of acetone:water mixtures of 35, 50, 70, 90 and 100% acetone. The heads were then separated from the rest of the body and were either left to dry in air or transferred for further thorough dehydration. The latter consisted of using a Polaron critical point drying apparatus where the absolute acetone

was replaced by liquid carbon dioxide (Hayat and Zirkin, 1973). The temperature was then raised and the carbon dioxide released in the form of gas, leaving the specimens absolutely dry. It was observed that air-dried specimens showed very little distortion, especially if they were left to dry less rapidly in the acetone vapour just above the surface of the absolute acetone. Heads dehydrated by both methods, were then fixed to a standard Cambridge Stereoscan stub using double-sided Sellotape or colloidal silver in iso-butyl methyl ketone (Acheson's 'dag' 915). Fixation on double-sided Sellotape was found to be more convenient, was fairly stable under the electron beam and caused almost no "charging" when a Polaron Sputter Coater was used to coat the specimens in vacuo with pure gold. In order to preserve some of the delicate structures, some specimens were fixed before dehydration in 2% glutaraldehyde in 0.1M phosphate buffer at 4°C for 24 hours. Other specimens were treated with silver nitrate to reveal the permeable areas at the tip of the labial sensilla (Slifer et al., 1957).

The enclosed components of the mouthparts such as the mandibles, maxillae, hypopharynx and associated fulcral arms were first dissected out of the head capsule before examination with the scanning electron microscope. The heads were first macerated in the mixture of 50% lactic acid and Chlorazol black E and the whole complex of the enclosed mouthparts could be removed with comparative ease using fine pointed needles. Little distortion was observed in the hard parts like the mandibles and maxillae after the maceration procedure. The more delicate hypopharynx was very much affected by the same process, and so it was found more appropriate to dissect

it intact from already dry heads fixed to stubs with silver 'dag' or examined in situ after the removal of the labium. The epipharyngeal structures on the ventral side of the labrum could be examined in the same way after the removal of both the labium and the hypopharynx.

A Cambridge Instrument Company Mark II Stereoscan scanning electron microscope was used at an accelerating voltage of 10 KV. The micrographs were taken on 35mm Ilford Pan F film.

2.4 Transmission electron microscopy studies

Specimens consisting of whole heads of the species E. nivalis were used for selected sectioning and viewing under the transmission electron microscope. Two fixation methods were used. The first involved the use of the method recommended by Graham and Karnovsky (1966) in which the material was primarily fixed for one hour at 4°C in 5% glutaraldehyde in 0.1M sodium cacodylate buffer at pH 7.4, to which had been added paraformaldehyde (2gm) and calcium chloride (25 mgm). The material was then washed twice (20 minutes each) in buffer and post-fixed for one hour in 2.5% osmium tetroxide. After washing again for 10 minutes in buffer, the material was dehydrated through a graded acetone series (5 minutes in each solution except for a 12 hour stain in 1% phosphotungstic acid and 1% uranyl acetate in 70% acetone). After three 15 minute washes in absolute acetone, the material was transferred for embedding.

In the second method, the material was first fixed in buffered 2.5% glutaraldehyde, post-fixed in Palade's (1952) veronal/acetate buffered osmium tetroxide for 2-4 hours and dehydrated through the graded acetone series.

For embedding, the fixed material was transferred to a mixture of 75% acetone and 25% Araldite for 6 hours (Glauret and Glauret, 1958). The percentage of Araldite was then increased by 25% every 6 hours, until the material was in 100% Araldite, where it was left for 12 hours. It was then transferred to fresh Araldite and oriented in plastic vials. The material was polymerized in a 60°C oven for 8-10 hours.

Ultrathin sections were taken with an LKB Ultratome III using glass knives. They were floated on a 10% acetone solution and picked up on 0.3% formvar-covered 200 mesh copper grids. Sections were then double stained with 2% aqueous uranyl acetate and lead citrate (Reynolds, 1963) for 10 minutes in each, washed in two changes of 0.02M sodium hydroxide, rinsed in distilled water and dried on filter paper. Sections were examined in a Philips EM 300 electron microscope at 60-80 KV. The micrographs were taken on sheet film.

2.5 Preparation of serial sections

Serial sections of the head of Entomobrya nivalis were cut in two planes, from tissue blocks originally prepared for transmission electron microscopy. The sections, 0.5 μ m - 1 μ m thick, were taken by an LKB Ultratome III and a group of 4-5 sections were transferred in a drop of water with a copper loop to a clean glass slide. The sections were heated over a spirit lamp flame for a few seconds to stretch and dry. They were stained by a drop of 1 per cent aqueous solution of toluidine blue in 1 per cent borax and the slide was heated again over the flame till the toluidine blue had completely dried up. The slide was then washed thoroughly with

distilled water, left to dry and then the sections were mounted in a drop of "Depex" (Gurr) and covered with a glass slip.

2.6 Histological and histochemical studies

Epoxy resin sections prepared as in Section 2.5 for serial sectioning were used for general staining as well as the staining of cellular secretions and products. The gentle heating of slides over a spirit lamp flame, make the sections adhere firmly to the slides and the various histochemical staining procedures could be carried out on them.

2.6.1. General stains

A variety of general staining procedures previously developed for epoxy sections were tested. Good results were accomplished by the use of the following stains:

- (a) Toluidine blue (Trump et al., 1961; Chandra & Skelton, 1964; Germain, 1974).
- (b) Crystal violet (Grimley et al., 1965)
- (c) Methylene blue (Huber et al., 1968)
- (d) Heidenhain's iron haematoxylin (Schantz & Schecter, 1965)
- (e) Chlorazol black E. (Hoefert, 1968).

Staining was carried out either at room temperature or on a hot plate at 90°C after flooding the slides with the appropriate stain. The slides were then left until the stain had evaporated completely, washed thoroughly with distilled water, dried on a hot plate and mounted in a suitable mounting medium. A considerable degree of

transparency was obtained with "Depex" (Gurr) specially at high magnifications as a result of the closeness of its refractive index to that of epoxy resins (Aoki, 1966). Nevertheless, "Depex" tended to destain the sections more rapidly than immersion oil, in which sections had retained their stain for over six months.

The general stains could be used advantageously in sequence with other stains, if they were buffered to a slightly acid reaction. Thus, toluidine blue, crystal violet and methylene blue could be combined with basic fuchsin (Aoki and Gutierrez, 1967; Lee and Hopper, 1965), while haematoxylin gave better results with safranin O (Schantz and Schecter, 1965).

2.6.2. Staining of cellular products

The following histochemical tests were performed to stain the cellular secretions and products.

(a) Polysaccharides and mucopolysaccharides

Polysaccharides and mucopolysaccharides were localized in the mouthparts by means of two procedures the periodic acid-Schiff (PAS) technique and Grimley's (1964) tribasic stain method, specially developed for epoxy sections.

The PAS test was performed in the same way as in conventional paraffin wax sections (Mahoney, 1973) on both oxidized and diastase-digested controls. The diastase was used as a 1% aqueous solution and the sections were incubated at 37°C for one hour.

In the tribasic stain method, the sections were immersed in a saturated ethanolic solution (8%) toluidine blue and malachite green for 2-5 minutes in Coplin jars until cell nuclei were well stained. The excess dye was washed from the slides by gentle flooding with tap water. A saturated (4%) aqueous solution of basic fuchsin served as a counterstain and differentiator. After rinsing with water, the sections were dried and mounted in "Depex".

(b) Lipids

Epon sections were found to be suitable for this test. Slides were placed into either a saturated solution of Sudan black B in 70% alcohol or a 1% aqueous solution of Nile blue A in Coplin jars and allowed to stain for 2 hours at room temperature (McGee-Russell and Smale 1963). Slides were then removed, rinsed with distilled water, drained and mounted in Farrant's medium as "Depex" was found to destain the sections.

(c) Proteins

1% alcoholic bromphenol blue was used as a general protein stain (Pearse, 1968). The sections were placed in this dye for 2 hours at room temperature.

(d) Nucleic acids

The presence of both RNA and DNA can be detected by the use of 0.2% aqueous solution of methyl green-pyronin (Jurand and Goel, 1976). Araldite sections stain distinctly after immersion for one hour.

(e) Enzymes

Two representative enzymes were selected for testing: succinic dehydrogenase - an oxido-reductive enzyme and non-specific esterase - a hydrolytic enzyme.

Although studies on histochemical localization of enzymes are usually carried out on tissue sections, yet because of their relatively small size and thinness, the whole-mount preparation of the collembolan mouthparts has been found superior to conventional sectioning for the direct determination of enzyme distribution in its various parts. The use of whole-mount preparations for enzyme histochemistry has been reported by Bartman and Dixon (1966) in vertebrate material and by Raychaudhuri (1968) in the reproductive system of an insect.

Large and physically active insects of Entomobrya nivalis were first immobilized by carbon dioxide and their heads were severed. Dissection was performed under a binocular microscope at a magnification of 50 and in a few drops of ice-cold saline solution containing 9gm sodium chloride; 0.42gm potassium chloride; 0.25gm calcium chloride per litre. By holding the insect's head under the salt solution on a cavity slide, the whole complex of the enclosed mouthparts could be quickly dissected out using fine needles. This was transferred to a 24 x 24mm No.1 coverslip and after it had been left to dry at room temperature for 2 minutes it was stored over ice in a refrigerator. Each cover slip containing 4 or 5 preparations was kept at this low temperature until ready for enzyme incubation.

The incubating medium for succinic dehydrogenase consisted of 0.2M phosphate buffer, pH 7.6; 0.2M sodium succinate and Nitro-BT, proportioned 1:1:2 by volume. Incubation was carried out for both the control and the enzymatic activity for 8-10 minutes at 37°C. After the blue colour became visible in active material (which demonstrated presence of diformazan deposits), the incubation was stopped by rinsing the cover slips in 0.85% NaCl. The reaction products were fixed in 10% formal-saline for 10 minutes, and the cover slips were then mounted on slides using glycerol jelly.

The incubating medium for non-specific esterase consisted of 0.1 ml of 1% naphthol AS (anilide of 2-hydroxy-3-naphthoic acid) acetate in acetone; 10 ml of 0.05 M phosphate buffer, pH7 and 10 mgm of fast blue BB salt. This medium was stirred and filtered, then applied by dropping onto the previously dried specimens. The reaction was allowed to continue for 20 minutes at room temperature. After incubation, the specimens were washed for 1 minute and then mounted in glycerol jelly.

Succinic dehydrogenase was also run after pretreatment with 0.01 M iodo-acetate and esterase reaction was run after pretreatment with 0.01 M eserine sulphate.

Inactivated specimens were prepared by immersion in boiling water for 5-10 minutes and these were used as control.

2.7 Behavioural studies

Individuals of Entomobrya nivalis used for this study were collected, as required, from the field and were confined without food in sterilized glass tubes, containing a plaster of Paris and charcoal mixture substrate (Goto, 1961) for up to 12 hours prior to experiments. A relative humidity of almost 100% was maintained within the tubes by moistening the substrate with a few drops of distilled water. Only active and apparently healthy individuals, representing a range of ages, were used as experimental animals.

The experiments were conducted in covered, sterilized 9 cm petri dishes, with a thin layer of paraffin wax at the bottom (Figure 1). The chemical solutions were presented to the animals in the form of soaked filter paper discs 15mm in diameter, and were introduced into the petri dishes one hour before the animals. The discs were laid out on the paraffin wax surface at random, but with equal distances between each two neighbouring discs. Fifty animals were released into a clear area in the centre of each petri dish (Figure 1).

Experiments were run for 16 hours and counts of animals on each specific disc were carried out at hourly intervals. Ten replicates of dishes were used for each experiment at one time and each experiment was repeated twice. The positions of the petri dishes were chosen, as far as possible, to get uniform light during the period of the experiments as a precaution against any effects of light.

The test chemicals and compounds were grouped in four experiments,

- (a) 0.2 M solutions of different sugars (i.e. sucrose, glucose and fructose), starch, oxalic acid and sodium chloride.

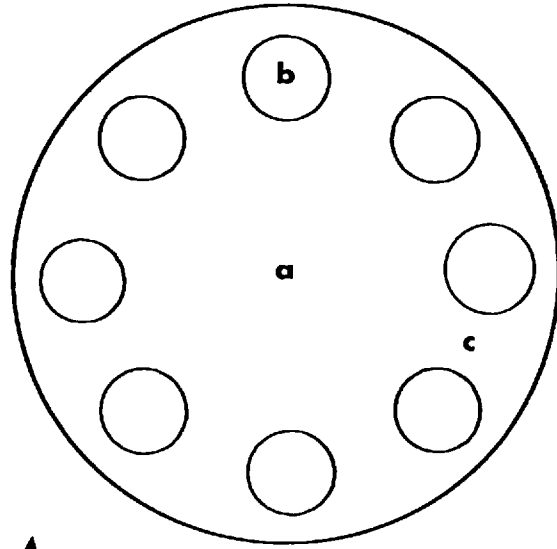
Figure 1 Test dish used in the multi-choice behaviour experiments.

A. Dorsal view.

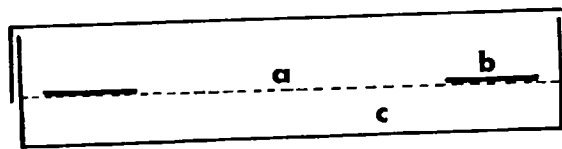
B. Lateral view.

a, site of release of insects; b, filter paper discs;

c, paraffin wax layer.



A



B

- (b) Different concentrations of sodium chloride i.e. 0.001 M, 0.01 M, 0.1 M, 1 M and 4 M solutions.
- (c) 0.2 M solutions of different salts i.e. sodium chloride, potassium chloride, lithium chloride, calcium chloride and magnesium chloride.
- (d) 0.2 M solutions of different chemicals and compounds i.e. glycerol, hydrochloric acid, casein, quinine, acetone, ethyl alcohol and neem, (Melia azadirachta L.) seed extract, (1%).

The chemical solutions of stable chemicals such as sugars, salts and acids were made up as stock solutions and diluted as necessary; extract of the neem seed was made fresh for each set of experiments by grinding the seeds in a mortar with a little distilled water. In each of the above experiments, a disc soaked in distilled water acted as a control.

After each hourly record of animals on test discs, the petri dishes were tapped gently to encourage dispersal. In this way, each new record represented a fresh distribution rather than a cumulative one. Aggregation data were analysed by a standard Chi-square goodness-of-fit test to determine whether the numbers of insects on test discs were a measure of a true aggregation or of normal variation.

2.8 Electrophysiological Studies

2.8.1 The Insects

All the experiments were carried out on adults of both sexes of Entomobrya nivalis, taken from the field and kept without food for 2 hours before preparation for electrophysiology. This was carried out to ensure that the sensilla on the labium had regained the readily functional state after feeding (Bernays et al., 1972).

2.8.2 Recording technique

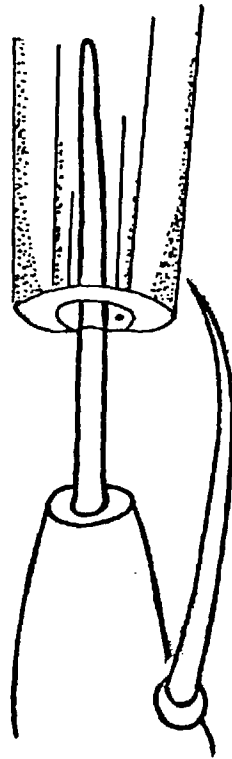
Electrophysiological recordings were obtained using a modified version of the techniques of Hodgson et al. (1955) and Hodgson and Roeder (1956). Silver/silver chloride electrodes were used to detect electrical changes within the sensilla (Figure 2A). The indifferent electrode (ie) was connected to earth, while electrical signals from the sensilla were fed from the recording electrode (re) through a CFD 18121 Pre-amplifier. Amplified signals were displayed on a two-channel storage Telequipment DM64 Oscilloscope and recorded on Kodak oscillograph paper using a u.v. recorder in a Bell and Howell 5-137 Oscillograph. A schematic diagram of the recording situation is given in Figure 2B.

2.8.3 Solutions and pipettes

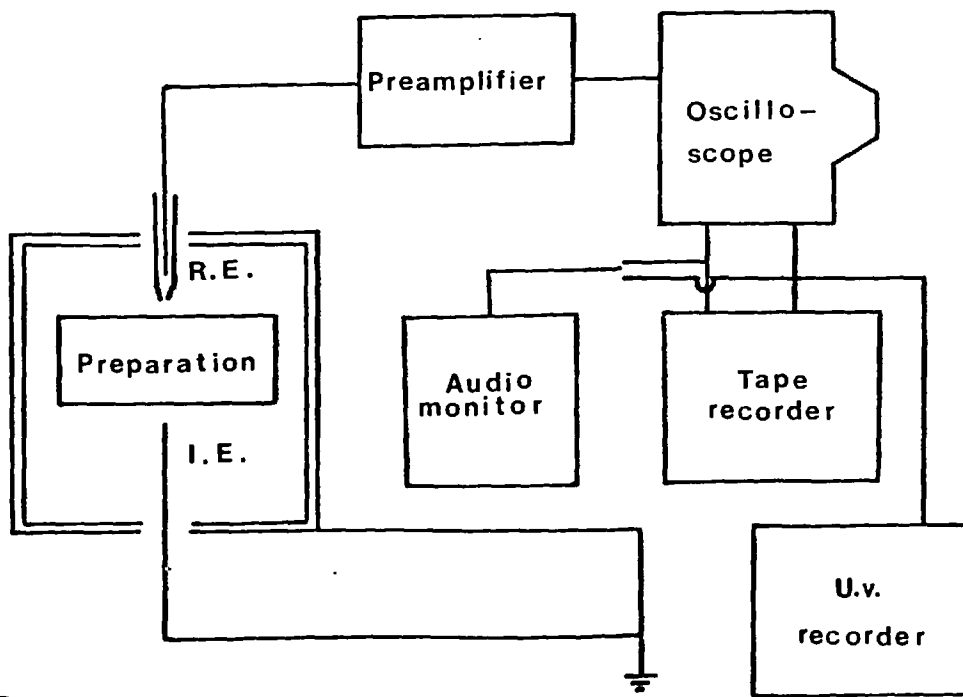
The method of hair tip recording (Figure 2A) prevented the testing of compounds which were not electrolytes except by the addition of an electrolytic substance to the stimulating solution. In practice,

- Figure 2 Electrophysiology experimental preparation.
- A. Position of recording electrode with reference
 to sensillum during tip recording.
 - B. Schematic representation of the recording
 arrangements.
- R.E., recording electrode; I.E., indifferent electrode

A



B



all substances other than salts were presented in a solution of 0.1M sodium chloride. The stimulating solutions of stable chemicals were made using Analar compounds in glass-distilled water. They were stored when not in use at 4°C. Extracts of the fungus Cladosporium herbarum and neem (Melia azadirachta) seed were made fresh for each set of experiments by grinding the fungus or plant tissue in a mortar with a little distilled water and adding a measured amount to the electrode fluid.

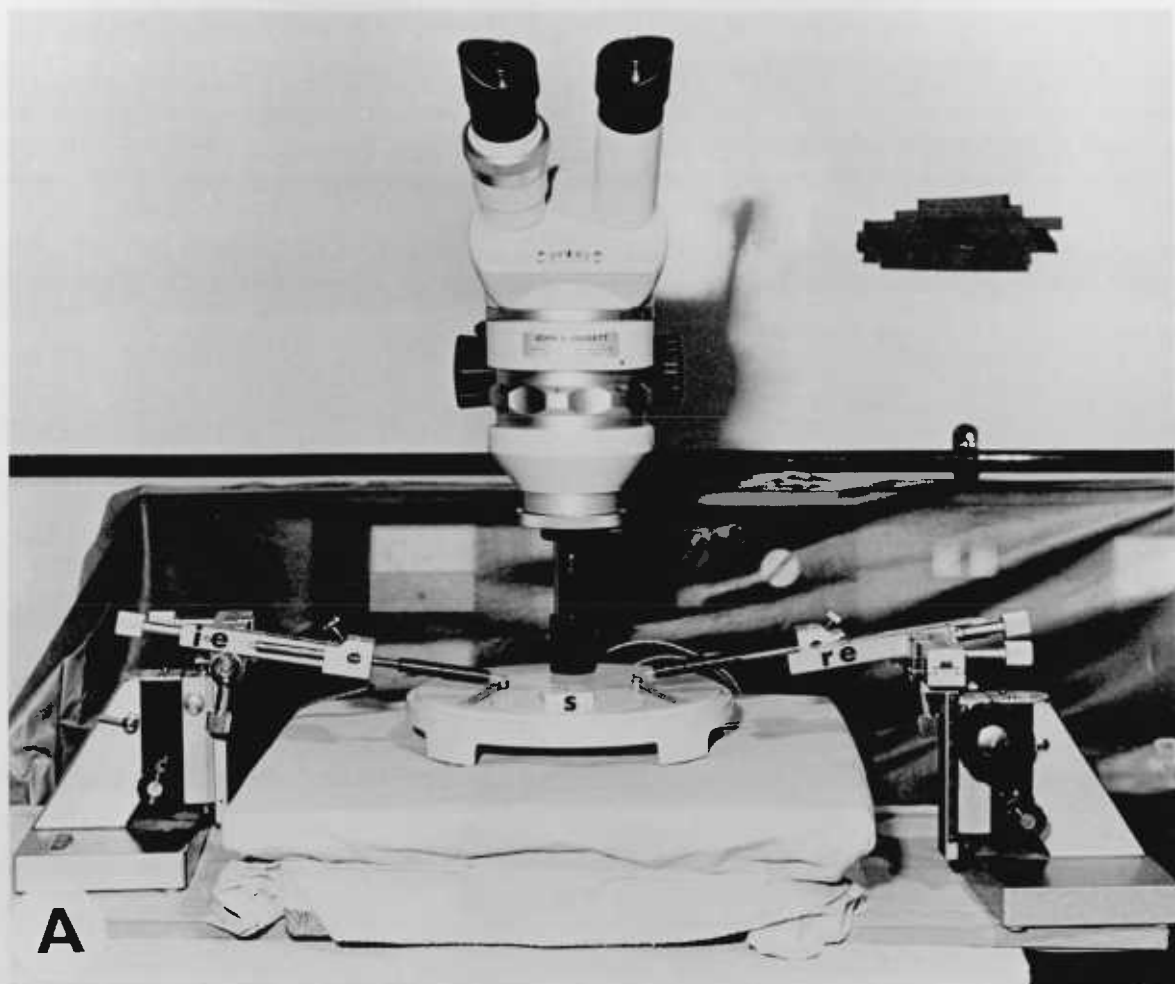
The test solution, with the recording electrode, was applied to the tip of the sensillum in a glass capillary drawn and cut to give a tip diameter of 10µm. The indifferent electrode was made in the same way, but had a relatively larger tip diameter of 30µm.

2.8.4 Method of preparation

Insects were lightly anaesthetized with carbon dioxide and then fixed dorsal side down on a horizontal Perspex block with the legs, antennae and furca outstretched and the head and thorax immobilized by means of double-sided Sellotape (Plate 1B). In this position, the labial sensilla were exposed for recording. The indifferent electrode, containing 0.1M sodium chloride, was inserted into the haemolymph of the head, near the neck, through a small hole in the cuticle made with a fine metal probe (tip diameter 20µm). Chemoreceptor responses were obtained by placing the recording electrode, containing the stimulating solution, gently over the tip of one of the labial sensilla by the aid of a Prior micromanipulator (Plate 1A). In order to ensure that the concentration applied to the sensillum was typical of the solution in the recording electrode,

Plate 1: Set up for electrophysiological investigation
of labial chemoreceptors.

- A. Microscope stage to show Perspex cradle(s),
indifferent electrode (ie) and recording
electrode (re).
- B. Collembola in position for recording,
showing method of restraint.



fluid was drawn from the tip of the capillary by means of lens tissue, immediately before each stimulation. Mechanoreceptor responses were obtained by manipulation of the sensillum tip, involving the lateral movement of the whole sensillum from its resting position to about 45° .

Preliminary experiments showed that the use of intact animals for recordings was more convenient and gave more repeatable results than when the head was removed and mounted separately. The latter preparations tended to deteriorate more rapidly through drying and seemed to produce an injury effect, arising from interference with conduction of receptor potential along the dendrites (Rees, 1967).

No special arrangements were made to maintain the temperature of the preparations at a constant level, but the range of air temperatures during the experiments was low, of the order of $18 - 20^{\circ}\text{C}$. All investigations were performed under a Wild binocular microscope at a magnification of 100 (Plate 1A).

2.9 Feeding mechanism studies

The general feeding behaviour of the genus Entomobrya was studied by observing individuals in glass vivaria using a dissecting microscope. More close observations were made by confining specimens in the cell of a cavity slide sealed with a cover slip. Food was applied in the form of fungal spores suspended in a droplet of distilled water. A drop of distilled water applied to the edge of the cover slip would flow by capillarity under the cover slip and would ensure a high relative humidity within the cell. A thin coat of vaseline was applied around the edges of the cover slip to reduce the loss of water through evaporation. Specimens were

frequently found to live in these cells for several hours, during which time, observations were made either directly through the microscope or recorded on a Sony Video tape. The activity of the specimens within the cells was restrained by keeping the slide on ice for a few minutes. Young and newly moulted individuals of the genus Onychiurus were found useful in observing the movement of the enclosed mouthparts due to the relative transparency of their heads.

3. OUTLINE TOPOGRAPHY OF THE MOUTHPARTS IN THE GENUS ENTOMOBRYA

The head in the genus Entomobrya shows no definite signs of segmentation, well suiting a life within woodland litter and under the bark of trees (Manton, 1964). The head capsule is extended in the form of a buccal cone (Plate 2A, B) formed by the labrum and labium and is situated antero-ventrally on the head. The secondary mouth-opening (Goto, 1972) forms a transverse opening on the distal border of the buccal cone. The head is prognathous and the mouthparts entognathous. The mandibles and the majority of the maxillae are enclosed in the cavity formed by the fusion of the lateral folds (the plicae orales or pleural folds) with the labrum and the labium (Folsom, 1900; Denis, 1949; Paclt, 1956). The buccal cavity (Mundh le of Hoffmann and atrial cavity of Denis) can be divided into two parts: the gnathal pouch, which contains the posterior parts of the mandibles and maxillae and the pre-oral cavity, comprising the space enclosed between the epipharynx dorsally and the labium ventrally and contains the hypopharynx and the anterior parts of the mandibles and maxillae. These parts can be protracted through the opening of the pre-oral cavity, which is a characteristic of entognathous insects. The relative positions of the main components are shown in Figure 3.

The anterior free parts of the mandibles lie above and parallel to the hypopharynx and their posterior parts or shafts, to which mandibular muscles are attached, are loosely suspended from their posterior ends by the mandibular suspension (ms) from the lateral head wall. The mandibular shaft bears the mandibular cavity (mc), which opens towards the median line of the head in a triangular fashion for allowing the mandibular muscles to enter and insert. No

Plate 2: General view of the oral region and the labrum.

- A. Dorsal view of the oral region, showing relative positions of the labrum, labium, outer lobe of maxilla and the secondary mouth opening. Note the prominent frontal sclerite at the back of the labrum. (E. maritima X 1,350)
- B. Lateral view of the secondary mouth region. Note labrum, labium and plica oralis behind the outer lobe of maxilla. (E. intermedia X2,000)
- C. Dorsal view of labrum, showing the labral setae, the anterior smooth area and the labral papillae. (E. maritima X2,400)
- D. Dorsal view of labrum with six setae in the median row. Note the free posterior margin of the smooth area. (E. albocincta X3,200)
- E. Anterior part of labrum showing the smooth area, papillae and the two mechanosensitive hair sensilla arising from elongate deep sockets within the smooth area. (E. intermedia X3,800)

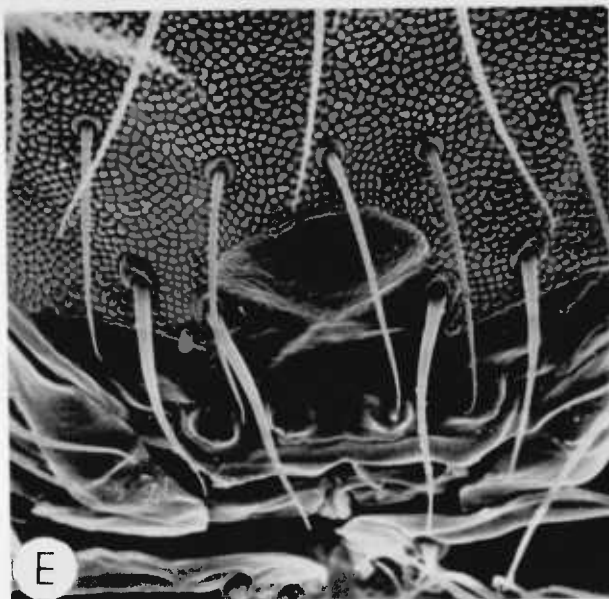
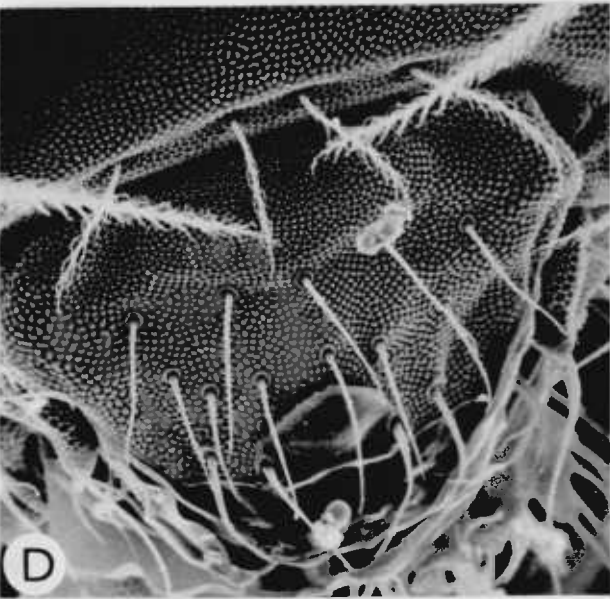
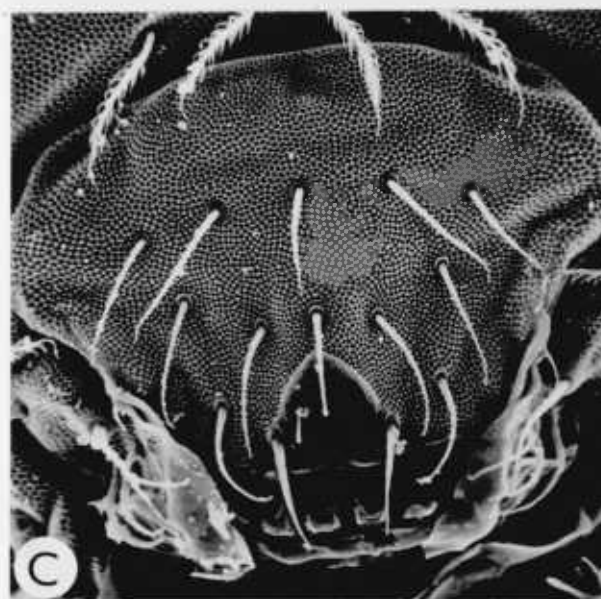
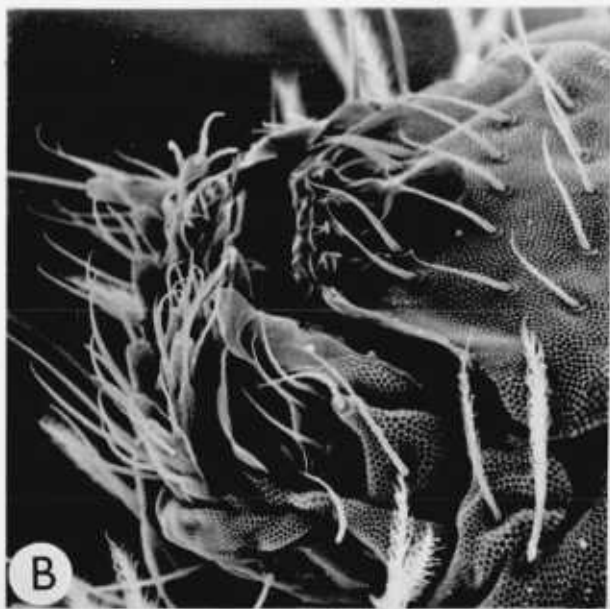
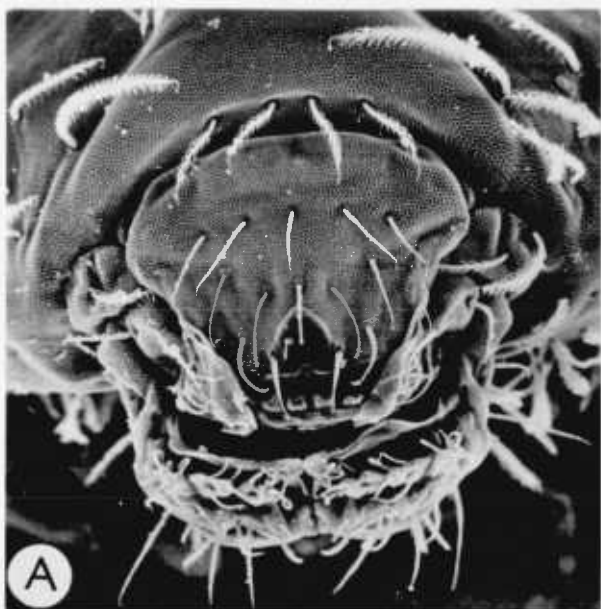
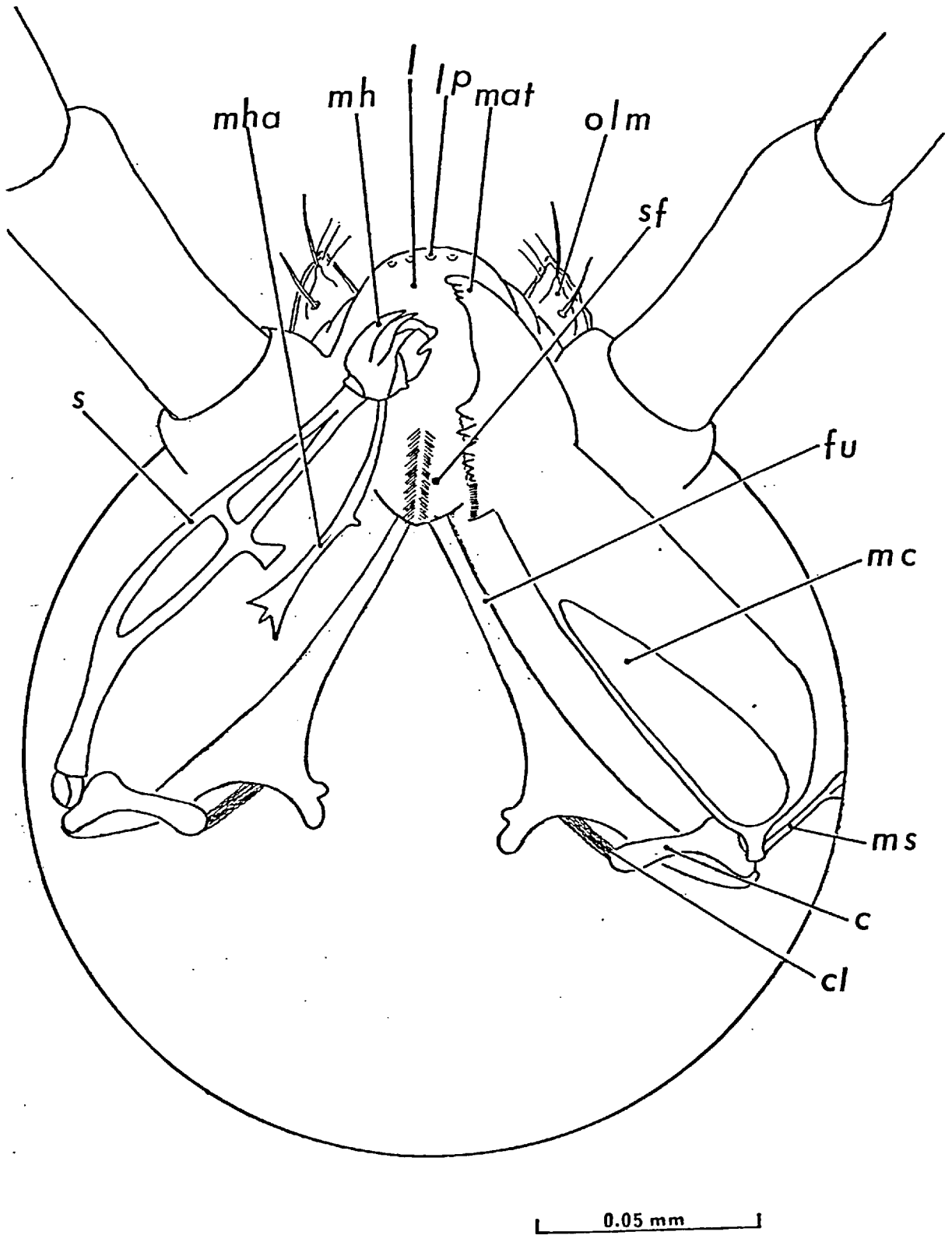


Figure 3 Diagrammatic representation of the mouthparts and associated structures in dorsal view of the genus Entomobrya. The mandible has been removed on the left side.

c, cardo; cl, cardo ligament; fu, fulcrum;
l, labrum; lp, labral papilla; mat, mandibular
apical teeth; mc, mandibular cavity; mh, maxilla
head; mha, maxilla head apodeme; ms, mandibular
suspension; olm, outer lobe of maxilla; s, stipes;
sf, superlingual filaments.



ridges for dorso-ventral suspension of the mandibles, as was claimed by Singh (1969), were observed. Although it is difficult to assess the exact positions of the different components as they move through protraction, yet it seems that the mandibles do not extend so far back as has been illustrated by Goto (1972) in Folsomia candida.

The framework of the maxilla, formed by the stipes, lies parallel to the mandible. The anterior part of the maxilla, known as the maxilla head or capitulum (mh) lies on both sides of the hypopharynx and moves on the dorsal surface of the superlinguae. Anteriorly the stipes also bears a structure known as the outer lobe of maxilla (olm). The capitulum articulates with the maxilla head apodeme (mha), which serves as the point of insertion of some maxillary muscles which are capable of moving the maxilla head independently.

The hypopharynx occupies a median position in the pre-oral cavity and its dorsal surface forms the floor and sides of the food cavity (or mandibular molar plate cavity) which Snodgrass (1935) called cibarium. Immediately posterior to the hypopharynx is a transverse bar-like structure, from which two convergent short arms and two divergent long arms pass posteriorly. The two long divergent processes correspond to the hypopharyngeal branches of Imms (1906), the fulcrum of Snodgrass (1950) and Wolter (1963) and the posterior tentorial apodemes of Manton (1964). The fulcrum (fu) consists of two branches, one short and median and the other longer and lateral, approaching the lateral cranial walls. In this lateral branch there is a small notch, where a rod, attached to a ligament, is articulated.

This rod is commonly regarded as the cardo of the maxilla (Chaudonneret, 1973) to which the stipes articulates. The two short median branches of the fulcra are connected together by a muscular interfulcral formation (Hutasse-Jeannenot, 1974). Though it might seem to be only a skeleton supporting the hypopharynx, the fulcrum is an independent structure. Chaudonneret (1973) and Hutasse-Jeannenot (1974) have shown that the fulcrum is chitinous and ectodermal in origin. It forms the attachment for the maxillary and labial muscles. Snodgrass (1928) considered the same structure to be the primary element of the pterygote tentorium, resembling the lingual lora of Crustacea which according to Crampton (1921) are the precursors of portions of the tentorium of insects.

The two convergent short arms of the hypopharyngeal sclerite are known as the anterior arms of Folsom (anterior tentorial arms of Hoffmann, 1905, 1908; Denis, 1928; Manton, 1964 and superlingual apodemes of François, 1971). They are a characteristic of Collembola with chewing mouthparts. Hutasse-Jeannenot (1973) mentioned the presence of a point of articulation of the anterior part of the mandible with the anterior arm, which corresponds to the "mandibular boss" of Manton (1964) against which the mandible rotates. Hoffmann (1908) claimed that the hump on the mandibular stalk of Tomocerus longicornis articulated with this boss. Chaudonneret (1973) disputed the presence of any articulation for the anterior part of the mandible as this will interfere with the protraction of the mandibular tips. In the present work, there was neither a boss on the anterior arm, nor a hump on the mandibular stalk observed.

4. THE EXTERNAL FEATURES OF THE ORAL REGION

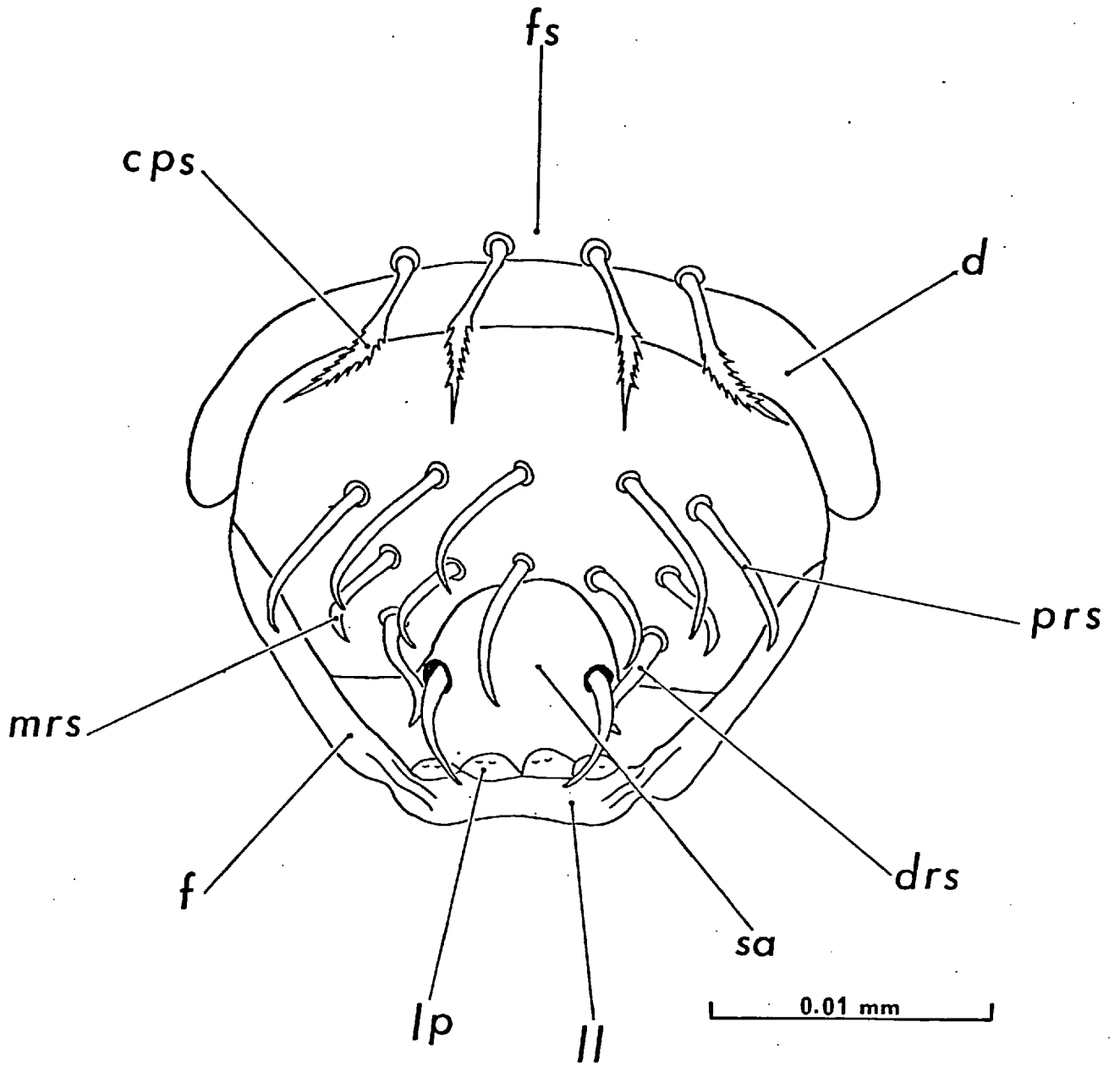
4.1 The labrum

The labrum (Plate 2C, D; Figure 4), which forms the dorsal boundary of the oral cone, is trapezoidal in shape, broader at the base than at the apex and with the postero-lateral angles rounded and protruding on both sides. It is separated from the prominent frontal sclerite (Chitinwulste of Hoffmann, 1905) by an invagination, referred to as the fronto-labral suture (Figure 4). Longitudinal sections show that this suture provides a rigid point of origin for the labral depressor and epipharyngeal elevator muscles (dilators of Hoffmann). As in some other Collembola (Goto, 1972) there is no obvious indication of a fronto-clypeal boundary.

The general armature of the labrum (Figure 4) resembles the standard type of labrum in Collembola (Yosii, 1976). The forwardly directed setae are arranged as 4/5, 5, 4, where the first row is not on the labrum itself, but directly behind on the frontal sclerite and hence named "prelabral setae" by Yosii (1963, 1976). These setae are distinctly barbed in all species studied. They arise from the perpendicular wall of the fronto-labral suture and project forwards and downwards on the base of the labrum. The sockets of the two inner setae lie closer together than to the other lateral ones. Following the prelabral setae are three transverse rows of smooth labral setae (p-, m- and d- row) of 5, 5 and 4 setae. This chaetal arrangement is without exception in members of the family Entomobryidae (Christiansen, 1958). However, during the present study, and on one occasion only, a specimen from the species

Figure 4 Dorsal view of labrum.

cps, ciliated pre-labral seta; d, fronto-labral suture; drs, distal row of setae; f, labral flap; fs, frontal sclerite; ll, lip of labrum; lp, labral papilla; mrs, median row of setae; prs, proximal row of setae; sa, smooth area.



E. albocincta showed six setae on the m- row (Plate 2D). No explanation could be given for this phenomenon, except that it was a very exceptional case. The setae of the d- row are shorter and more stout than the others and are slightly curved towards the median line. They were found to be mechanoreceptors, with a greater freedom of movement along the direction of their elongated sockets (i.e. in the direction of the secondary mouth-opening). The inner setae arise from oval sunken pits within the smooth area of the labrum.

The surface of the labrum, as in Entomobryomorpha in general, is very minutely granulated in accordance with the general tuberculation of the body cuticle (Noble-Nesbitt, 1963a, b; Ögel, 1965). The area along the anterior margin of the labrum is practically smooth and free of tuberculation and it broadens medially to form roughly a semi-circle (Plate 2E). The posterior part of the smooth area is free and slightly raised-up from the general surface of the labrum (Plate 2D).

The anterior margin of the labrum shows a row of four small papillae of various shapes in different species (Section 4.4.3). Each is furnished apically with some minute conical or grain-like tubercles, which were mistaken for setae by some authors (Christiansen, 1958; South, 1959, 1961; Stach, 1963). The transmission electron microscopy studies (Section 7.1), revealed the cuticular nature of the labral papillae and their non-sensory function. As Goto (1972) has reported in Folsomia candida, no structures were found that could be comparable to the Chitinhaken

described by Hoffmann (1905) arising on the epipharynx near to the tip of the labrum.

A pair of labral muscles originates from the fronto-labral suture and inserts on the distal end of the labrum (Figure 10lm). These muscles help to depress the labrum. No elevator muscles were found and the fronto-labral suture forms a ridge of thick elastic cuticle, which provides sufficient elasticity to allow the return of the labrum to its original position.

4.2 Outer lobe of maxilla

On either side of the secondary mouth-opening lies a structure variously known as the outer lobe of maxilla (Goto, 1972), galea (Olfers, 1911), palp or palpifer (Folsom, 1899; Manton, 1964; Singh, 1969; Massoud et al., 1975) and Maxillartaster (Stummer-Traunfels, 1891; Schaller, 1970). It arises from the distal end of the stipes of the maxilla, above and lateral to the maxilla head. The outer lobe in Entomobrya (Figure 5; Plate 3) is more or less rectangular, with the distal part narrower and more directed inwards towards the median line of the head. A narrow strip around the margins is devoid of any tuberculation. This area widens at the distal part with an obvious boundary. The dorsal side bears six setae, the longer two arise from the apex and side of a large papilla which Massoud (1971) termed palpe sétigère (Figure 5 sp). The rest of the lobe (sometimes known as the external lobe proper) bears 3 recurring setae in a row and a small seta on the dorsal margin. The large setae on the setigerous palp were found to be mechanoreceptors, which seem to help in the manipulation and direction of the food into the

Figure 5 Dorsal view of outer lobe of maxilla.
nta, non-tuberculate area; olp, outer lobe
proper; php, position of hyaline plate;
sp, setigerous palp

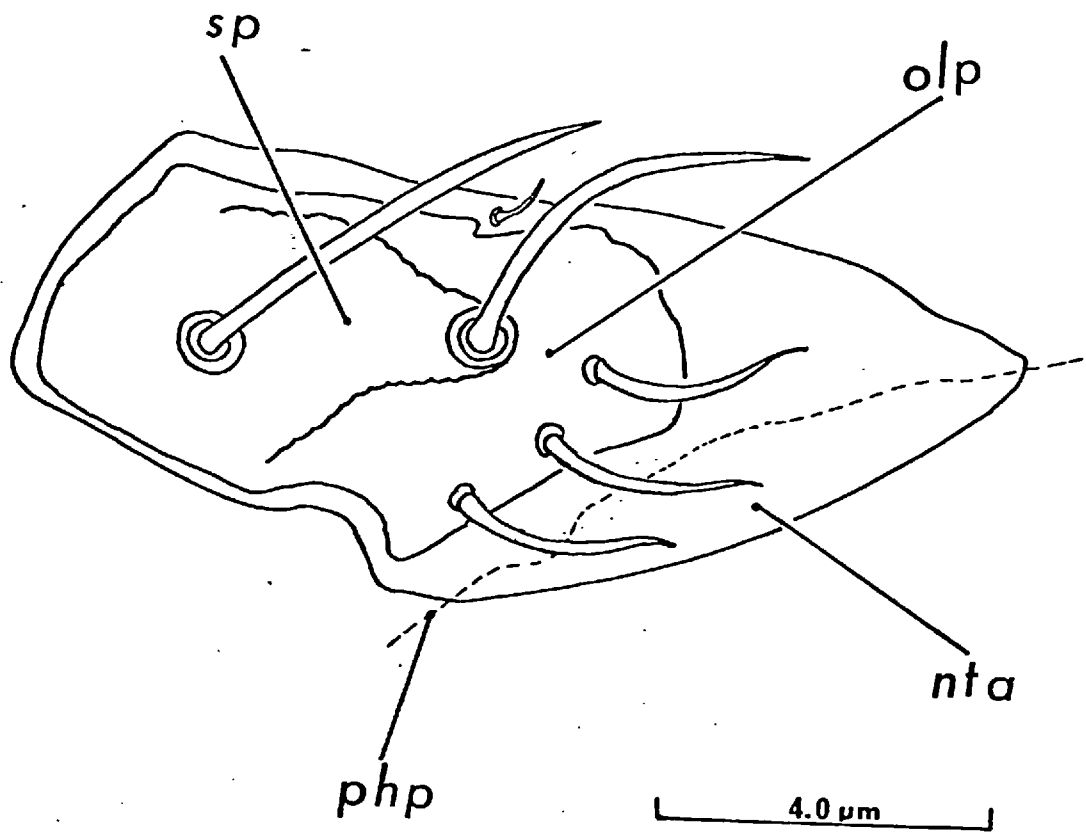


Plate 3: The Outer lobe of maxilla

- A. Lateral view of the outer lobe of maxilla, showing the non-granulated zone, the three anterior setae and the two mechanosensitive hair sensilla on the setigerous palp.

(E. intermedia X4,800)

- B. Outer lobe of maxilla partly covered with hyaline plate when the secondary mouth is closed.

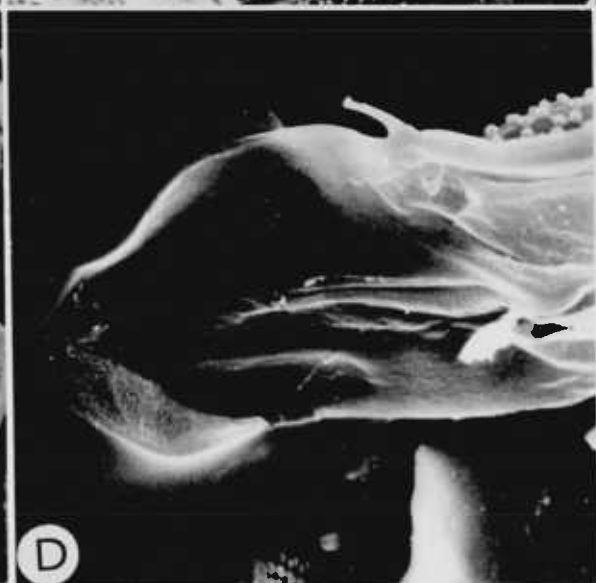
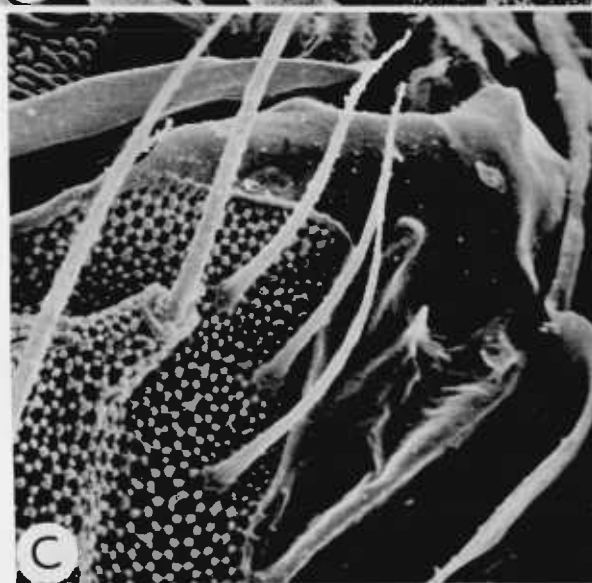
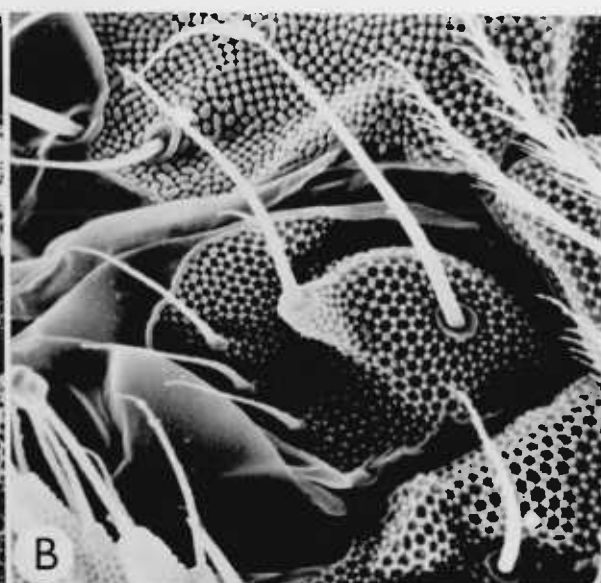
(E. albocincta X5,000)

- C. Tip of outer lobe of maxilla, showing spines on the non-tuberclate zone.

(E. corticalis X9,000)

- D. Inner view of outer lobe of maxilla, showing grooves and membraneous structures.

(E. nivalis X4,800)



pre-oral cavity.

The inner (medial) surface of the outer lobe (Plate 5D) bears various membranous and chitinous structures reminiscent of those found on the epipharynx (Section 5.4). There is a median raised-up membrane with a groove running in the centre, which terminates some distance from the distal tip of the outer lobe. At the ventral side, opposite the setigerous palp, the median membrane becomes swollen and it bears a flaplike structure with a fringed edge.

4.3 The labium

Massoud et al. (1975) in accordance with Folsom (1899), divided the labium of *Arthropleona* into three parts: An anterior part or palp, a median part or mentum and a posterior part or submentum. The three parts are easily recognizable in the labium of *Entomobrya*, as they are bounded by well-defined furrows (Figure 5; Plate 4A).

The anterior lobe (sl), known also as Klauenteil (Hoffmann, 1908) and partie sétigère (Denis, 1928) bears five groups of forwardly and medially directed papillae, from the apices and sides of which project twenty three setae (Plate 4B, C). The setae are arranged in two rows. The proximal row consists of two simple papillae, each surmounted by a subulate seta. Three setae in a row arise at the base of the papillae and two arise proximal to the first three, near the posterior boundary of the setigerous lobe. The distal row bears three groups of complex papillae (Plate 4D), each with one apical seta and several side ones. The most inner and median one carries

Figure 6 Ventral view of labium.

as, apical seta; ct, cuticular thickening;
ds, differentiated seta; hp, hyaline plate;
m, mentum; sg, salivary groove; sl, setigerous
lobe; sm, submentum; ss, side seta.

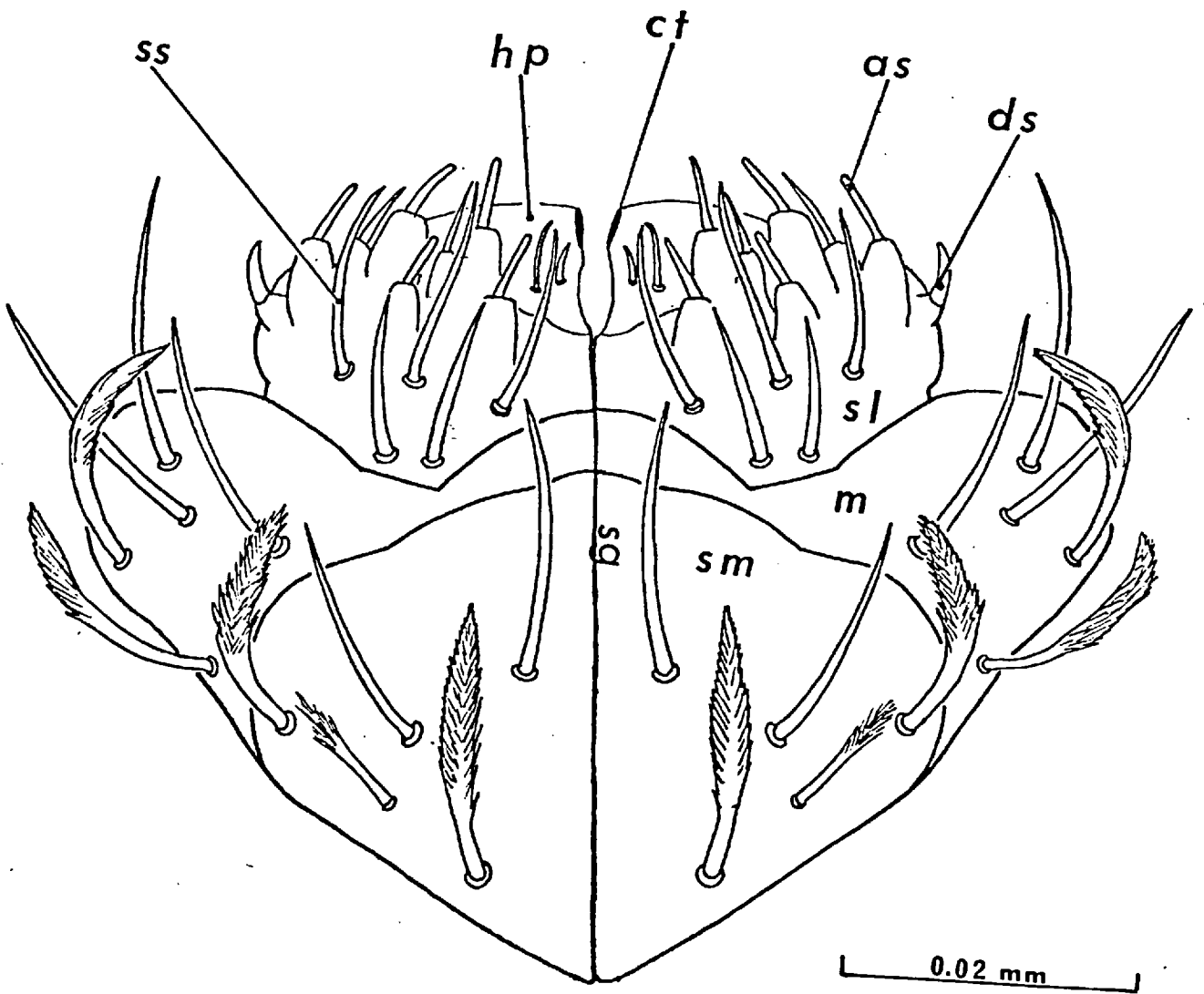
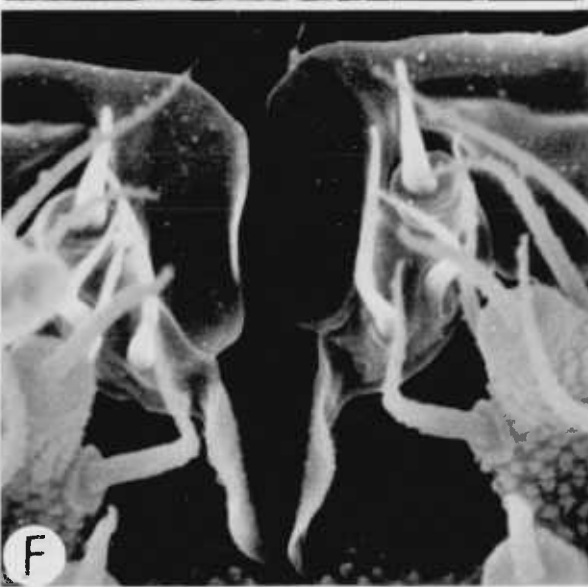
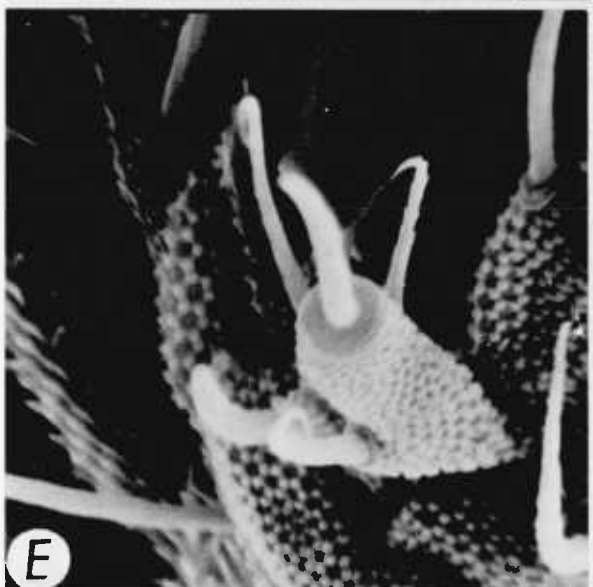
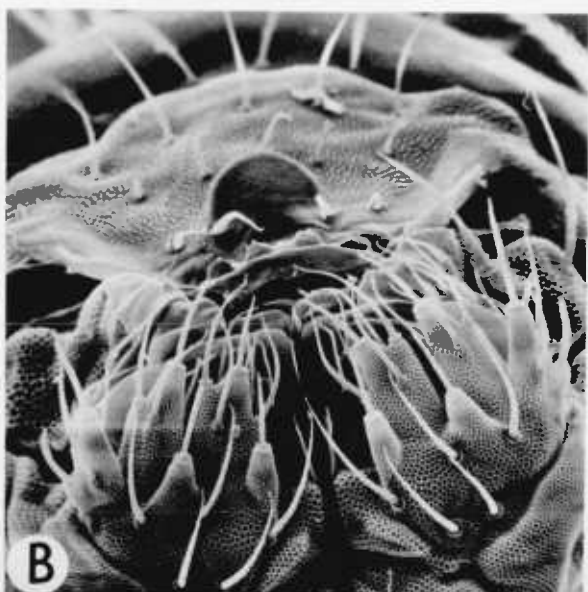
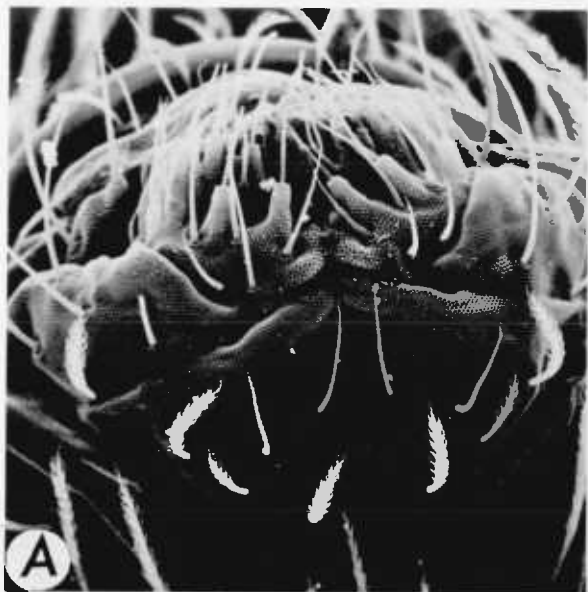


Plate 4: The labium.

- A. Ventral view of labium, showing the well-defined furrows between setigerous palp, mentum and submentum. (E. multifasciata X2,000)
- B. Facial view of the oral region, showing the two labial setigerous lobes bearing papillae and setae. (E. intermedia X4,000)
- C. Labial setigerous lobe. (E. intermedia X6,000)
- D. Dorsal view of distal row of labial papillae. The hyaline plate lies in front of the papillae. (E. multifasciata X6,800)
- E. Outer labial papilla. Note the terminal pores in both the apical and differentiated setae. (E. nivalis X10,800)
- F. Antero-median angles of the labium. Note the cuticular thickening and the three small sensilla on the hyaline plate.

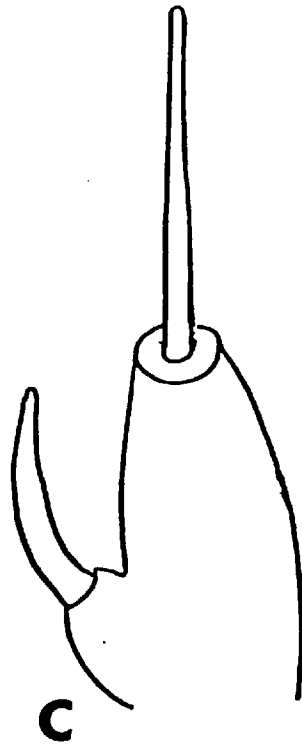
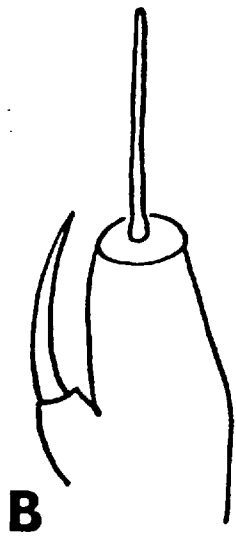
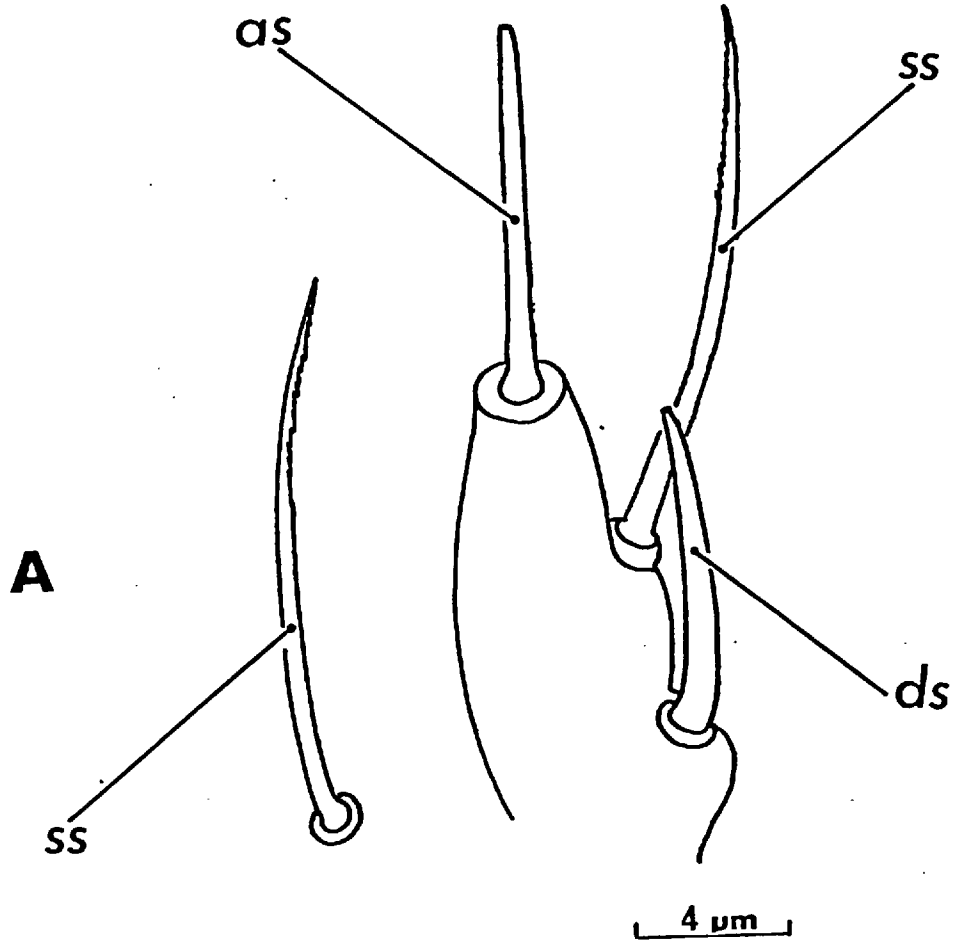


five side setae beside the apical one. The next group bears four side setae. The outer group carries a stout and slightly curved side seta, which Christiansen (1958) termed the "external differentiated seta" (Figure 7ds; Plate 4D) and to which he attributed some taxonomic significance. The differentiated seta may correspond to the outer lateral papilla described by Goto (1972) in Folsomia candida. All terminal setae and the differentiated seta terminate in a pore (Plate 4E). They act as gustatory contact chemoreceptors, while all the other setae on the setigerous lobe act as chemoreceptors.

The labium is divided along its median line into two halves. The separation is clear at the distal part, where the two halves diverge from one another. At the posterior end of the furrow between the two labial halves, there is a salivary channel leading to the exterior from the gnathal pouch, into which opens the ducts of the labial salivary glands (Manton, 1964). At its distal part and just anterior to the setigerous lobes, the labium bears a zone of non-tuberculate cuticle, described by Hoffmann (1908) as the hyaline Platte (Figure 6 hp; Plate 4D). When at rest (i.e. when the mouth is kept closed), the hyaline plate projects beyond the frontal edge of the labrum and covers the lower parts of the outer lobes of maxillae (Figure 5 php). The antero-median angles of the hyaline plate are particularly well sclerotized and their flat circular edges face inwards towards each other (Plate 4F). Both Hoffmann (1908) and Goto (1972) showed similar thickenings, but they gave no explanation of their function. In the present work (Section 10), it has been suggested that these median thickenings, when pressed

Figure 7 Labial setae.

- A. General shape of the different labial setae.
as, apical seta; ds, differentiated seta,
ss, side seta.
- B. External differentiated seta and papilla of
first instar Entomobrya nivalis.
- C. External differentiated seta and papilla of
adult Entomobrya nivalis.



against each other, render the lower part of the secondary mouth-opening water-tight during fluid feeding. On the hyaline plate and just lateral to each thickening, there are three short setae, one of which is more stout and is surmounted on a short papilla (Plate 4F).

The median part of the labium, or the mentum (m), lies posterior to, and to some extent lateral to, the setigerous lobes. This part was probably what Willem (1900) referred to erroneously as the palpe. In Entomobrya, the mentum bears five setae on each lobe, of which the two basal ones are barbed. The submentum (sm) lies posterior to the mentum and also bears five setae with three basal barbed ones. The five basal barbed setae of the mentum and submentum lie on the outer lateral boundary of the labium.

The labial musculature resembles that described by Hoffmann (1908) and Manton (1964). The labial muscles (Figure 10 lbm) originate from the posterior short arms of the fulcra (posterior tentorial apodemes) and insert at various parts of the labium. Possibly, the effect produced by these muscles results in the curving of the labium to form a concave inner labial surface, which will help in the scooping of food into the pre-oral cavity.

4.4 Variations in the external morphology of the oral region

During the course of this study, great similarity between the different species of the genus Entomobrya was observed as far as the external morphology of the mouthparts is concerned. The few variations observed are given below.

4.4.1 The general shape and chaetotaxy of the labrum

The general shape of the labrum in all species studied is, more or less, similar to the one shown in Figure 4. However, some specimens were observed to have more anteriorly narrower labra. This was not found to be specific to all specimens of the species concerned. The lengths of the various labral setae vary considerably within the age of the individual within the same species and hence are not useful in the taxonomy of the genus.

4.4.2 Shape of the smooth area on the labrum

The posterior margin of the labral smooth area in all the species examined, with one exception, was more or less rounded to form roughly a semicircle (Figure 8A). In E. corticalis, the margin is flattened (Figure 8B).

4.4.3 Form of the labral papillae

The use of the labral papillae in the taxonomy of the genus Entomobrya has been controversial (Christiansen, 1958; South, 1959; 1961). In the present investigation, however, although the form of these papillae was found to vary within the same species, yet some boundary could be drawn between broad general forms for different species. This broad general form in every species is definitely specific. A representative form of the labral papillae in each of the seven species examined is given in Plate 5. The description of each form is given below:

- (a) E. albocincta: The papillae are hemispherical and not well defined. There are always two tubercles on each of the outer papillae and three on the inner ones (Plate 5A).

- Figure 8 Variation in the shape of the smooth area of
the labrum.
- A. The more general shape.
- B. Shape of the smooth area in Entomobrya corticalis.

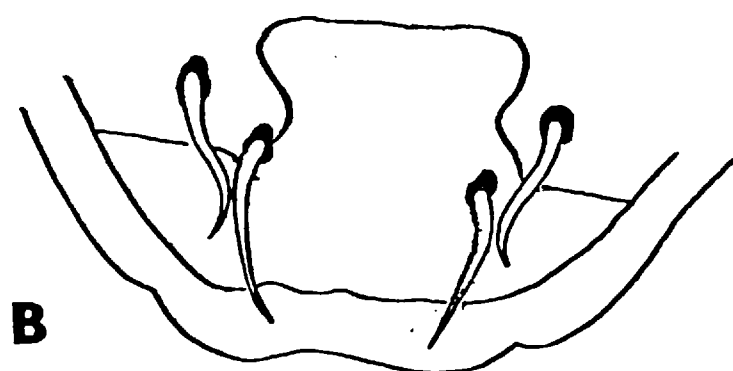
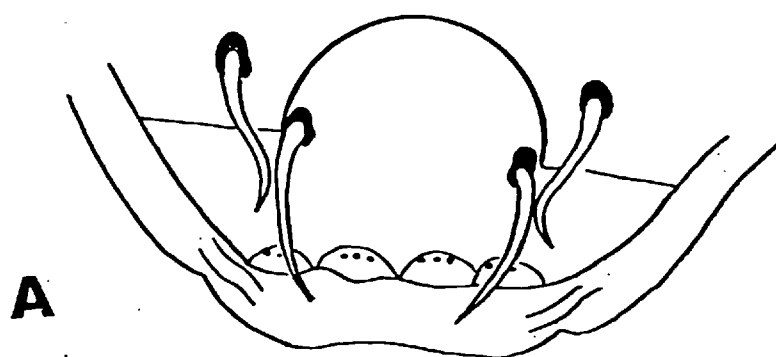
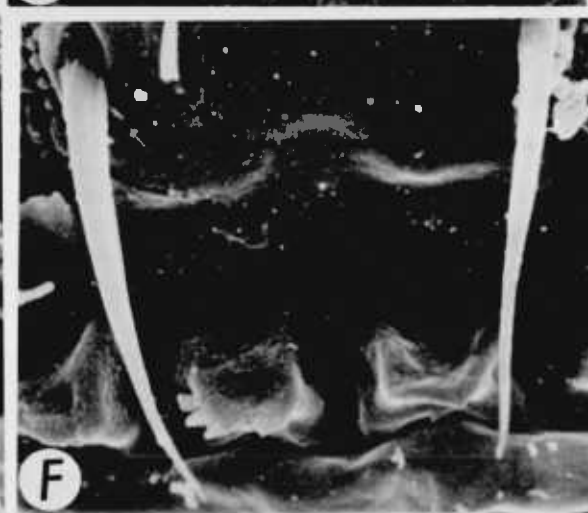
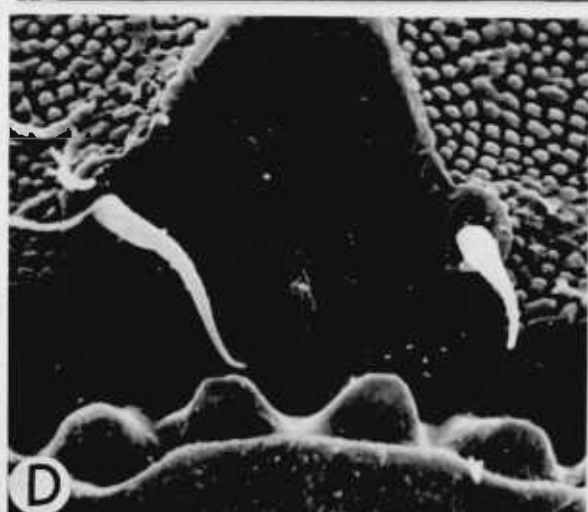
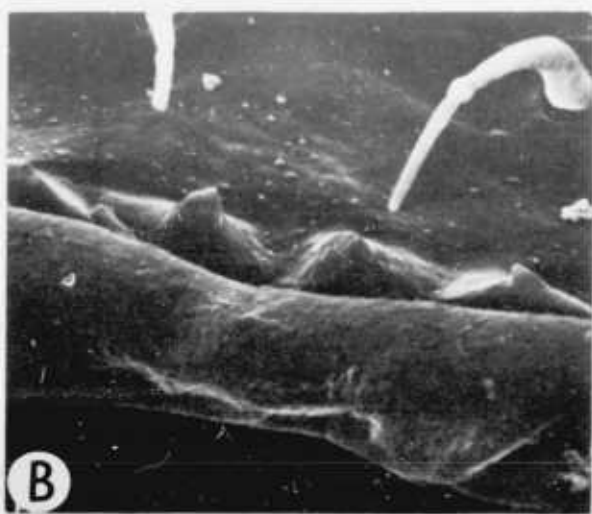
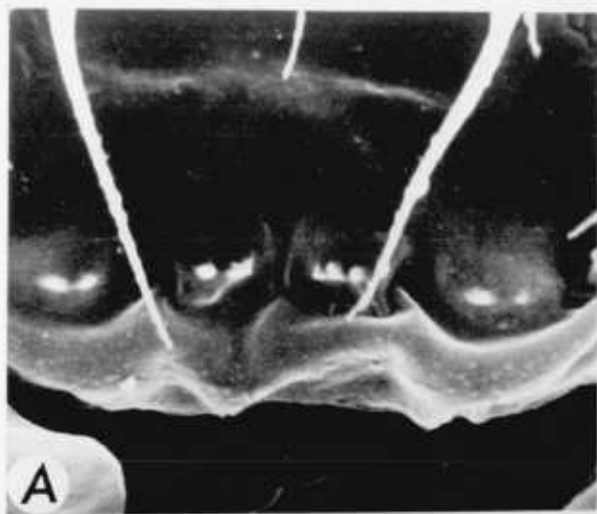


Plate 5: Variation in the shape of the labral papillae.

- A. E. albocincta (X5,6000)
- B. E. corticalis (X10,000)
- C. E. intermedia (X6,000)
- D. E. maritima (X10,000)
- E. E. multifasciata (X9,400)
- F. E. nicoleti (X10,000)
- G. E. nivalis (X4,000)



- (b) E. corticalis: The papillae are cone-like tapering apically in pointed ridges (Plate 5B).
- (c) E. intermedia: More truncate papillae, separated completely from each other and each of the inner ones terminating in at least one sharply pointed tubercle (Plate 5C).
- (d) E. maritima: The papillae are broad, erect rounded at the top without any tubercles (Plate 5D).
- (e) E. multifasciata: The papillae are similar to E. albocincta; but they are more clearly defined and the tubercles are more elongated. The lateral papillae bear two to three of these tubercles while the inner ones carry invariably two tubercles each (Plate 5E).
- (f) E. nicoleti: The papillae are broad and truncate at the top with only one of the inner papillae bearing three pointed small teeth laterally (Plate 5F).
- (g) E. nivalis: The papillae form almost a continuous ridge with two to three tubercles on each of the outer papillae and four to five on the outer ones (Plate 5G).

4.4.4 The chaetotaxy of the labium

There is little variation between species in the general chaetotaxy of the labium as given in Figure 6. The apical sensilla of the setigerous lobe vary in length in different specimens. This may be attributed to differences in the age of the specimens or to the ability of the animal to protrude and retract the sensilla to a

limited extent (Goto, 1972). On the other hand, the differentiated seta of the most external labial papilla was used by Christiansen (1958) in his revision of the genus Entomobrya. In the present investigation, the differentiated seta was found to extend beyond the tip of the papilla in the first instar of all the species examined (Figure 7 B), with the exception of only E. maritima where the seta does not reach the apex of the papilla. In the succeeding instars, the relative length of the papilla increases until ultimately in the mature instars the differentiated seta does not reach to the apex of the papilla (Figure 7C). Only in E. albocincta does the seta extend beyond the papilla apex in mature insects. The form of the differentiated seta was also found to vary with the age of the specimen. It is more curved in the early instars than in later instars. This phenomenon of differentiation and change of shape in organs was mentioned by Christiansen on the same paper (1958, p 445). The validity of this character in the taxonomy of the genus Entomobrya, is therefore much doubted.

5. THE ENCLOSED COMPONENTS OF THE MOUTHPARTS

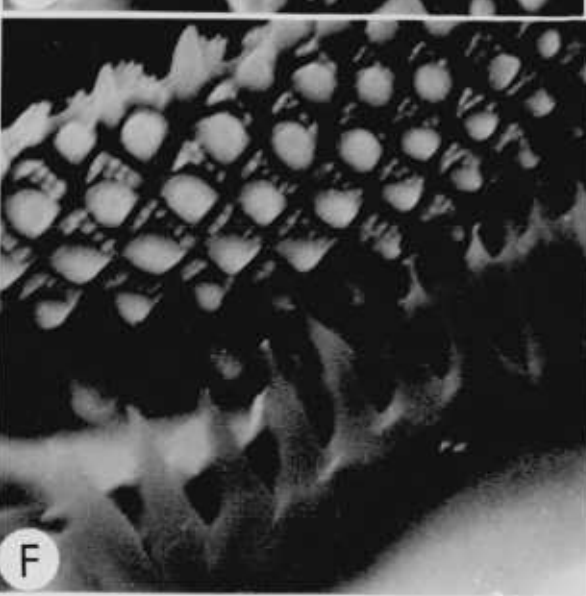
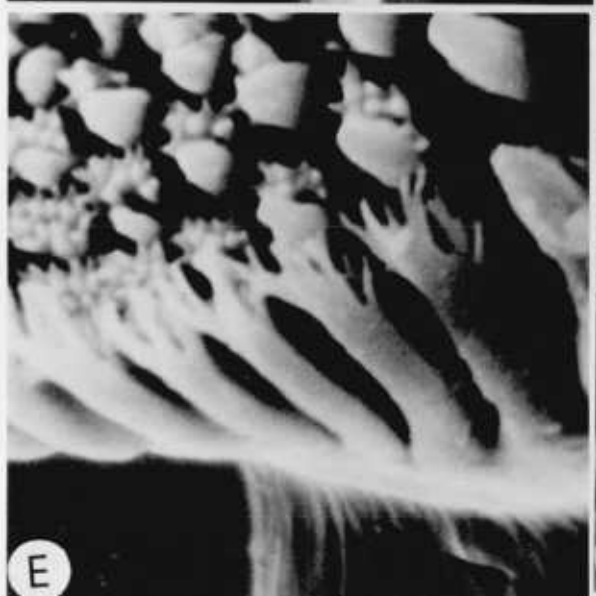
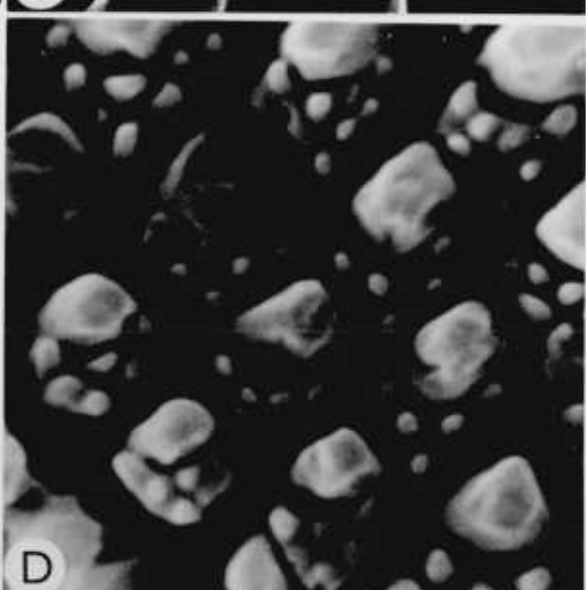
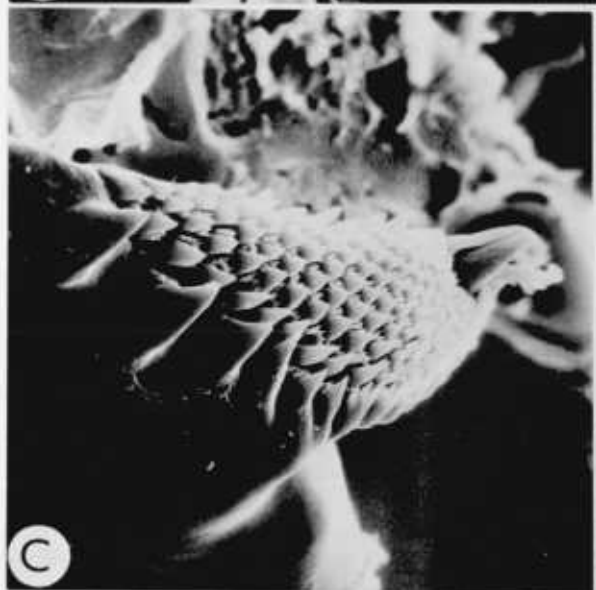
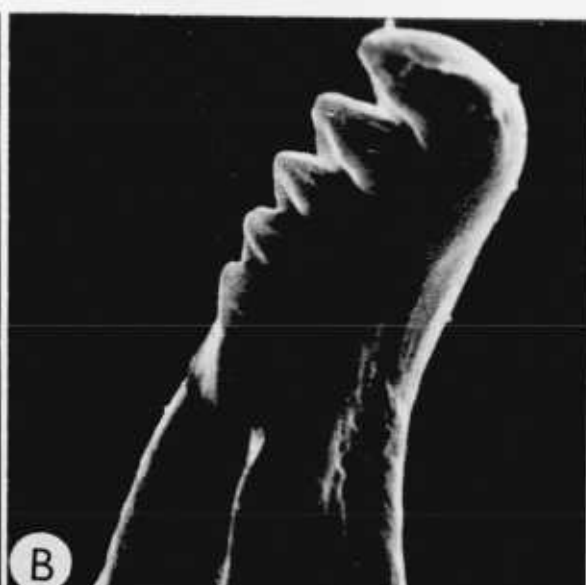
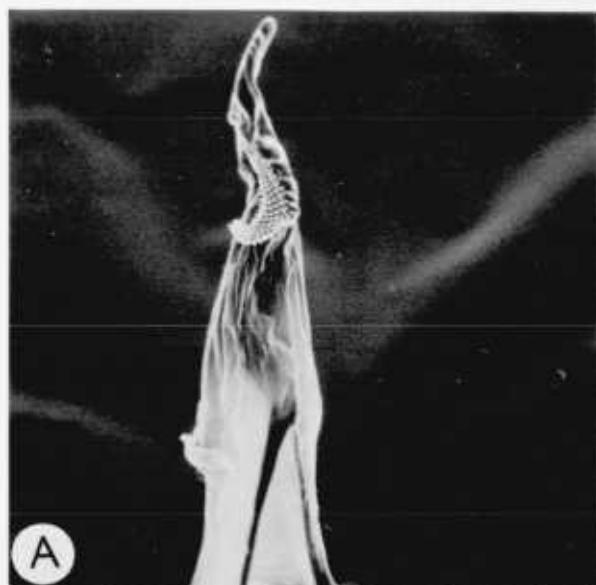
5.1 The mandible

The general form of the mandible is very similar to those described by other workers (Hoffmann, 1908; Manton, 1964; Goto, 1972). It is an elongated chitinous structure, stout at the base and narrow at the apex (Figure 3; Plate 6A). Functionally, it can be divided into two parts: An anterior part, which lies free in the pre-oral cavity and is directly concerned with the manipulation of the food and a posterior part (sometimes termed the shaft) to which are inserted the mandibular muscles. The anterior part carries both the apical teeth (Plate 6B) and the molar region (Plate 6C). The mandibular apical teeth are strong, well defined and the apical region is slightly bent to make an angle with the long axis of the mandible, while its outer surface is convex to fit into the curvature of the epipharynx (Plate 10A). The right mandible has always five apical teeth and lies slightly ventral and posterior to the left mandible, which has got only four apical teeth. This relative positioning of the mandibles may be essential to enable the interdigitation of the teeth, which together with the curvature of the molar plate towards the ventral side, may contribute to the rotary cutting movements of the mandibles (Manton, 1964, 1977).

The molar region (Plate 6C) is essentially as was extensively described by Goto (1972) for Folsomia candida. It consists of the crescentric molar plate bounded ventrally by a row of the large odontoid molar cusps. These are always six in Entomobrya and

Plate 6: The mandible.

- A. Left mandible, showing large triangular cavity on the shaft where the muscles insert. Note the bend of the anterior region in an S-shape. (E. corticalis X1,000)
- B. Mandibular apical teeth of the right mandible. (E. albocincta X10,000)
- C. Mandibular molar plate, showing large odontoid teeth on edge and the mandibular heel at the posterior end. (E. nivalis X6,800)
- D. Molar plate cusps and multi-cusped rods.
(E. albocincta X20,000)
- E. Buttress rods of the mandibular molar plate.
(E. nivalis X20,000)
- F. The position of the mandibular molar plate in respect to the superlingual filaments.
(E. multifasciata X10,000)



decrease gradually from the distal to the proximal one. The odontoid cusps are actually an outer row of the molar plate cusps forming the main body of the molar plate (Goto, 1972). They are replaced at the proximal end with what are known as the buttress rods (Plate 6E). These are thirteen in number in Entomobrya and run back up to the posterior angle of the molar plate. Each rod is divided into several finger-like processes at its tip but never separated along its entire length as was observed by Goto (1972). The buttress rods seem to be fairly flexible and they bend freely when they are pressed against members of the opposite mandible (Plate 10A).

The main "pile" of the molar plate is composed of regular rows of molar plate cusps with 5 - 7 finger-like multicusped molar rods inbetween (Plate 6D). The molar plate cusps are short and rigid and seem to form a relatively efficient grinding surface unlike that described by Goto (1972) in Folsomia candida. The variation in the form of the molar plate cusps in different specimens (Plate 6 C, D) was brought about by the difference in age of the different specimens.

At the extreme posterior tip of the molar plate, lies what is variously known as the mandibular heel or hook (Fersenteil of Hoffmann) (Plate 6C). It is composed of two curved projections rounded at the ends surrounding a strong stout chitinous rod which points medially. The rod seems to be inserted in a sort of socket in the molar plate, as in some examined specimens, the rod was found to be missing altogether.

The basal regions of the mandible, or shaft, is partly hollow with a large triangular mandibular cavity (Figure 3 mc), which opens to allow for mandibular muscle insertion. Both the mandibular hump (Höcker of Hoffmann) and the dorso-lateral mandibular ridge (Dachförmige Rampe) described by Manton (1964) are not clearly visible on the shaft of the mandible in Entomobrya. The mandibular shaft on the other hand, was found to be distorted by the action of lactic acid. It does not appear to be well sclerotized as would be expected from its role in muscle insertion.

The musculature and movement of the mandibles in Collembola were given in detail in the classic works of Manton (1964, 1977) on the mandibular mechanisms in Arthropoda. The mandibles in Collembola have two types of movements: protraction, which is accompanied by counter-rotation and retraction combined with rotation. Rotation is the movement when the molar surface of the mandible moves towards the median line and upwards, while in counter-rotation it moves laterally and downwards. The mandibular muscles originate either from the lateral walls of the head or the short arms of the fulcra (the anterior arms of Folsom) and insert in different parts of the mandibular shaft. The mandible seems to articulate in only a single point with the head capsule. No sign of an anterior articulation with the anterior arms of Folsom (the boss of Manton) or the Höcker of Hoffmann. Chaudonneret (1973) has suggested that such an articulation would interfere with the protraction of the mandible. The heel of the mandible may help to prevent excessive counter rotatory movement of the mandible.

5.2 The maxilla

The maxillae lie ventral to the mandibles, with their heads on either side of the hypopharynx (Figure 3; Plate 9A). The framework of the body of the maxilla is formed by the two rod-like branches of the stipes (s), which are reinforced by a transverse connecting bar at the middle. The apices of the maxillae converge anteriorly towards each other, while the stipes towards the posterior end are divergent. Anteriorly the stipe bears the head (mh) or capitulum and basally it joins the short cardo (c), which articulates with the basal end of the fulcrum by a ligamentous joint (the cardo-posterior tentorial membrane). The outer lobe of maxilla (described above) also articulates with the stipes.

The maxilla head (Plate 7A, B, C), variously known as lacinia (Folsom, 1899), Klauenteil (Hoffmann, 1908), Maxillen Kopf (Börner, 1908) and inner lobe (Singh, 1969) is not as complex in structure as has been reported in other Collembola e.g. Tomocerus longicornis and Folsomia candida. It is, more or less, similar to the basic maxilla head described by Börner (1908) in Tetrodontophora Reuter, 1882; but with fewer lamellae. The maxilla head in Entomobrya can be divided into two parts: An outer dorsal part, the ungulum or the galea and an inner ventral part, the lacinia. The ungulum is well developed, with three strong teeth or cusps of equal sizes which are curved medially. The whole of the inner ventral face of the maxilla head is occupied by a median appendage covered with filament-like projections arranged in rows. This probably corresponds to what Goto(1972) referred to as the subsidiary appendage (basaler Nebenanhang of Börner, 1908).

Plate 7: The head of maxilla.

- A. Maxilla head in ventral view. The unguar teeth, median subsidiary appendage and the two lateral lamellae (l_1 , l_2) are shown.

(E. nivalis X6,800)

- B. Maxilla head in median view. Note absence of the two lamellae on the ventral side.

(E. nivalis X6,800)

- C. Maxilla head in dorso-lateral view. Note the thick apical lamella between the ungulum and the median appendage.

(E. corticalis X7,600)

- D. Lateral lamella (l_2) and part of median appendage.

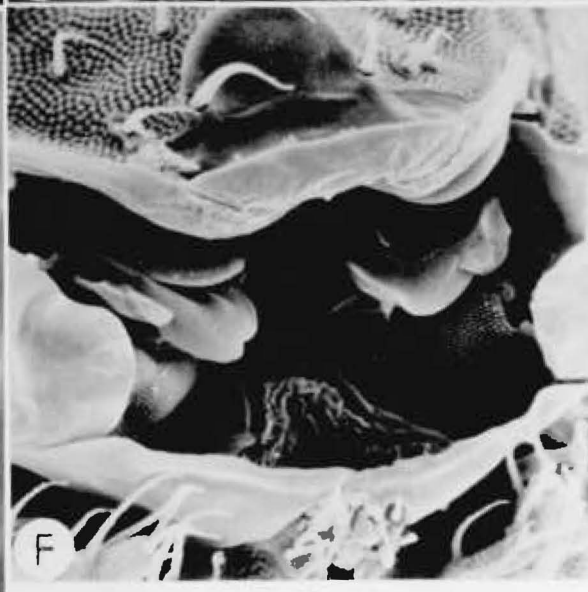
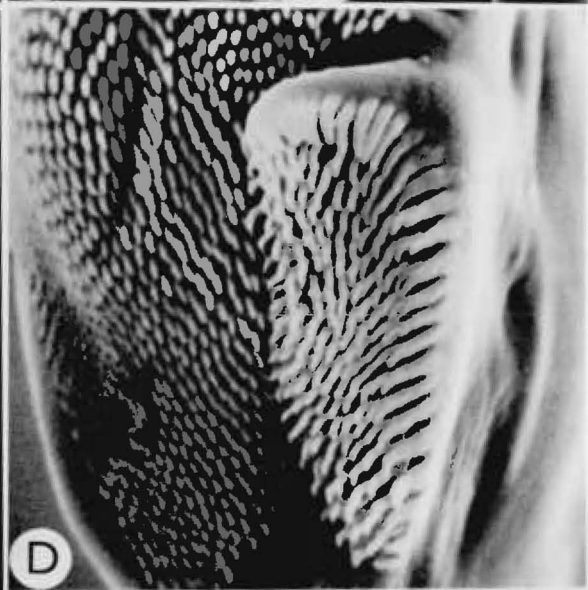
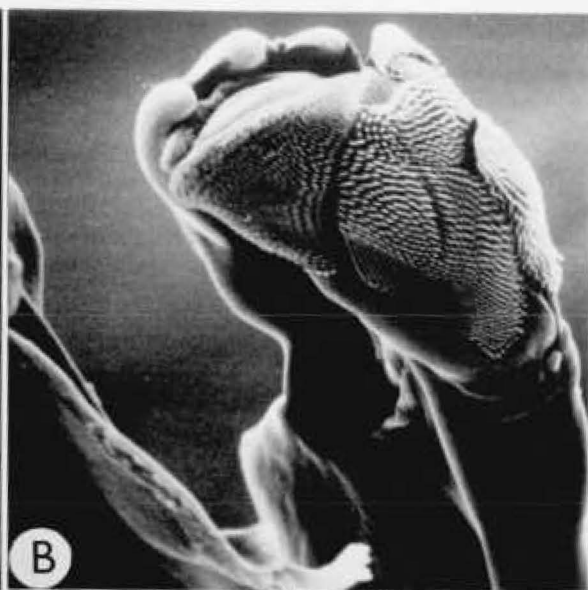
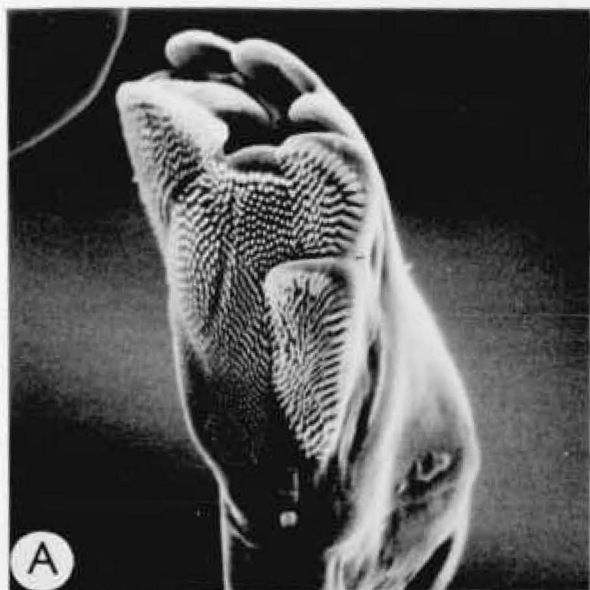
(E. nivalis X16,000)

- E. Protracted maxilla heads, The two lateral lamellae are dorsal to the median appendage while the apical lamellae lie ventral.

(E. nivalis X4,000)

- F. Opening of pre-oral cavity showing apices of mandibles, superlinguae, and maxilla heads in situ. The mandibles lie dorsal to the superlingual arms, while the maxilla heads lie in cavities between lingua and superlinguae.

(E. nivalis X4,000)



This part is convex in shape and is divided into three zones by strips free from projections. Lying on only the dorsal side of the median appendage is a pair of concave lamellae, undoubtedly corresponds to lamellae l_1 and l_2 of Börner. The lamellae on the ventral side are completely lost. Lamella l_2 is longer than l_1 , and it arises at the base of the median appendage near the junction of the stipes with the maxilla head. It is fringed along its entire periphery by finger-like, tapering filaments, distinctly longer and stouter than those lining the median appendage (Plate 7D). Lamella l_1 is only fringed on the anterior and lateral margins. The concavity in both lamellae is covered with filaments less stout than the ones on the margins. There are no structures similar to the apical rake or the basal shaft (Goto, 1972); but a short thick chitinous projection lies anterior to the median appendage and is capable of some restricted lateral movement within a cavity ventral to the ungular teeth (Plate 7C). The antero-ventral face of this projection is furnished with filaments. The components of the maxilla head i.e. ungulum and lacinia, are immovably fused together at their bases, where they form a stout, heavily sclerotized ring or sleeve (plaque chitineuse of Wolter, 1963 and prestipes of Adams and Salmon, 1972), which articulates with the stipes. The presence of this part was considered by Wolter (1963) to be a feature of the Collembola with chewing mouthparts.

The main movements of the maxilla are those of protraction accompanied by abduction and retraction associated with adduction. The maxilla head can be protruded through the opening of the pre-oral cavity (Plate 7E). During retraction, lamellae l_1 and l_2 lie dorsally

and the two median appendages are brought together. When completely retracted, the two heads of maxillae fit into cavities formed by the hypopharynx and the superlinguae (Plate 7F). The movement of the whole maxilla head is restricted to the lateral plane. This movement is brought about by muscles originating from the stipes and are inserted into the maxillary head apodeme, which articulates with the maxilla head. Individual movements of the lamellae are not directly controlled by muscles. Cuticular elasticity of the highly sclerotized parts probably accounts for the recovery movements and orientation of these structures (Manton, 1964). The muscles concerned with the main movements of protraction-abduction and retraction-adduction originate from the posterior lateral wall of the head and the short arms of the fulcrum (posterior tentorial apodemes) and they insert on the inner lateral wall of the stipes and dorsal inner wall of the cardo.

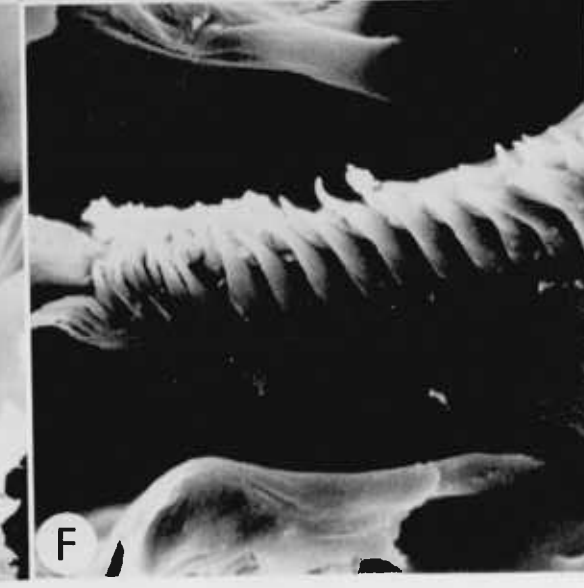
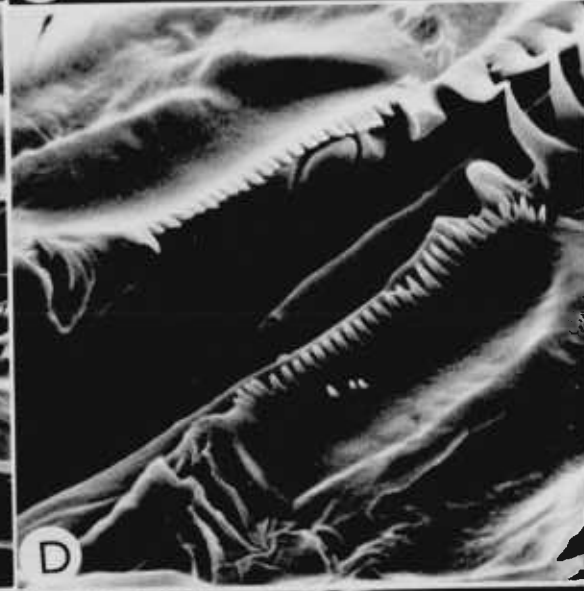
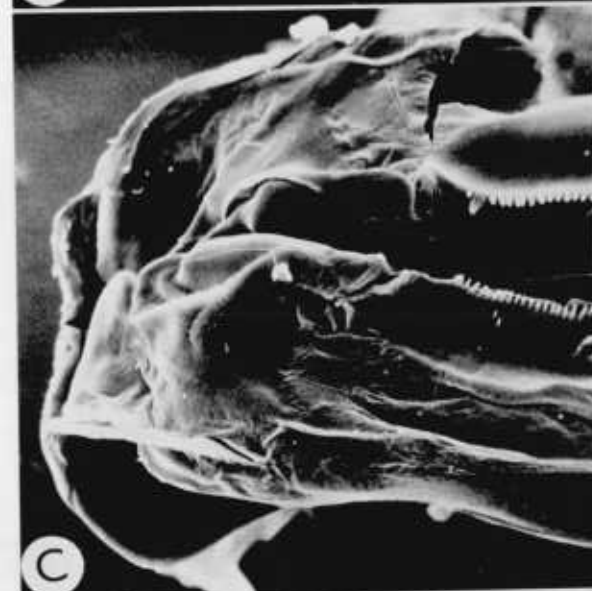
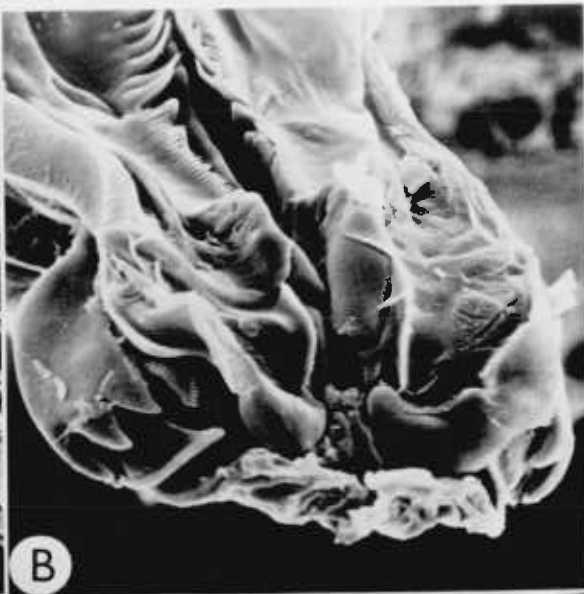
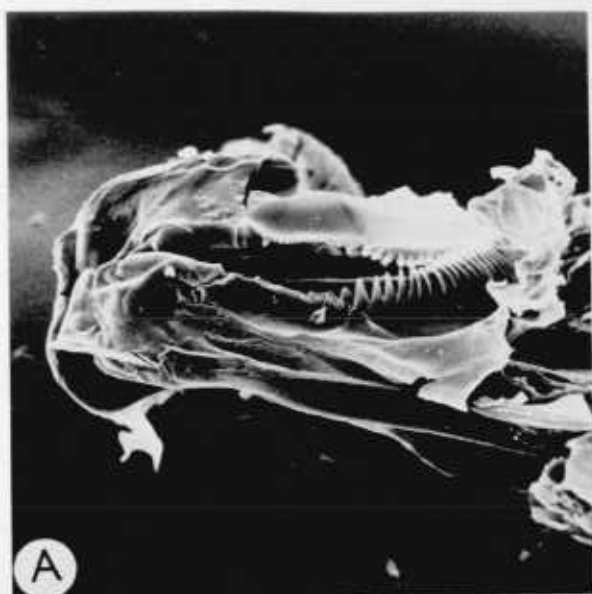
5.3 The hypopharynx

The hypopharynx in Collembola is a well developed organ, which, as indicated by its comparatively large size and complexity of structure, plays a more important role in feeding than in other groups of insects. However, due to its very delicate nature, attempts by previous workers (Goto, 1972) to remove it intact for more detailed examination were unsuccessful and the hypopharynx suffered considerable distortion and damage during those trials. In the present study, it was found more convenient to dissect the hypopharynx from previously dehydrated heads fixed to Stereoscan stubs by silver "dag" as described in the Materials and Methods.

The hypopharynx (Plate 8A, B, C), a rectangular tongue-like structure, occupies a median position in the pre-oral cavity immediately behind the labium. Posteriorly, its dorsal surface forms the floor of the gnathal pouch. It is composed of two parts: The median hypopharynx, also known as the glossa (Folsom, 1899; Hoffmann, 1908) and the lingua (Imms, 1906; Denis, 1928) overlaid by a pair of lateral lobes termed paraglossae or superlinguae (auctt.), which have been homologised by Crampton (1921) with the paragnaths of Crustacea. As mentioned before, the maxilla heads, or capitula, fit in a cavity lying on either side between the free dorso-lateral part of the superlingua and the lingua (Plate 9A, C). It is for this reason that the maxilla head is seen separating both structures in longitudinal sections (Plate 12A). From these sections, it is also clear that only the base of the lingua is fused with the ventral part of the labium and the apical region is supported on the terminal parts of the lingual stalks. On the other hand, the posterior part of each superlingua is fused ventrally to the postero-dorsal surface of the lingua and hence the superlinguae have no power of independent movement. However, the whole complex is capable of protraction and retraction through the movement of the fulcrum, which is joined anteriorly by a transverse bar to the body of the hypopharynx. As a result of this movement, the anterior part of the hypopharynx can be protracted through the opening of the pre-oral cavity. In the present study, and contrary to all previous reports (Wolter, 1963; Singh, 1969), the lingua was found to have intrinsic longitudinal muscles, which make it capable of some movement of its own. The importance of this movement will be discussed later in the Feeding Mechanism section.

Plate 8: Dorsal side of hypopharynx.

- A. Lateral view of dissected hypopharynx showing the curvature of the dorsal surface of the superlinguae. Note short and long filaments on the median sides of the superlinguae.
(E. nivalis X2,000)
- B. Facial view of hypopharynx with maxilla heads on both sides. (E. nivalis X2,800)
- C. Anterior part of superlinguae. Note the fine bristles on the head of the superlingual arms. (E. nivalis X4,000)
- D. Small filaments at the median margins of the superlinguae. The anterior part of the median ridge lies in the median groove between superlinguae. (E. nivalis X8,000)
- E. Long filaments of the superlinguae pointing towards one another with two thick rods anterior to them. (E. nivalis X7,600)
- F. Postero-median zone of superlinguae with long filaments closely set over the median ridge. (E. nivalis X7,600)



The lateral parts of the superlinguae are strengthened by being thick and more rigid. Their dorsal surface is modified according to the nature of food (Wolter, 1963). In solid feeders (fungal spores and hyphae) like Entomobrya, the dorso-median margins of the superlinguae are beset with a complete series of bristles and processes (Plate 8). At the anterior part of the large superlingual teeth, there are three rows of fine bristles (Plate 8C). Posterior to these, there are two rows of small uniform filaments. These are about thirty in number on each side and three filaments arise separately, some distance from the row (Plate 8D). Stout, blunt, short rods arise just posterior to the short filaments, which are followed by two rows of long, strong medially directed filaments (Plate 8E). At the posterior end of the row, the filaments become much finer, more numerous and closely set (Plate 8F). They converge and interlock together over a median, elongate ridge which runs backwards in the elliptical groove between the two rows of filaments (Plate 8D). A projection on the posterior part, brought about by a curvature of the edge of the superlingua, forms the ventral part of the triangular oral valve which lies in front of the pharynx (12 B). The concavity of the superlinguae matches the convexity of the mandibular molar surface, and the filaments on the superlinguae lie between the two molar surfaces (Plate 13B, D). The median hypopharynx or lingua (Plate 9) is a somewhat membraneous, elongate, unpaired, chitinous organ, though the median conspicuous furrow on the ventral side (Plate 9B), probably indicates its derivation from a paired condition. It is formed of three parts: An anterior free portion, an intermediate portion where it is fused to the superlinguae

Plate 9: Ventral side of the hypopharynx.

- A. Dissection showing ventral view of hypopharynx and maxilla in situ. Note fulcral arms at the postero-lateral angles of lingua.

(E. nivalis X1,300)

- B. Ventral view of lingua, showing hemispherical organ and the posterior tube passing in the furrow between the two halves of the lingua.

(E. nivalis X3,600)

- C. Ventral view of lingua with the prominent lingual structures: the anterior regulating valve, cylindrical body and hemispherical organ.

(E. nivalis X4,000)

- D. Same as C. Note membranous structure of the anterior part of lingua.

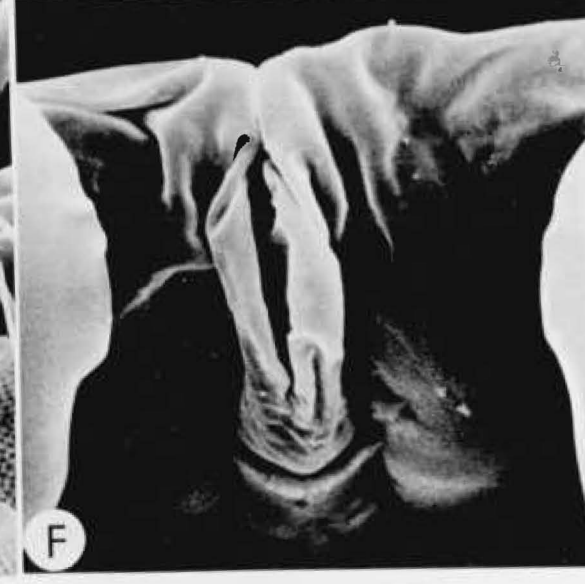
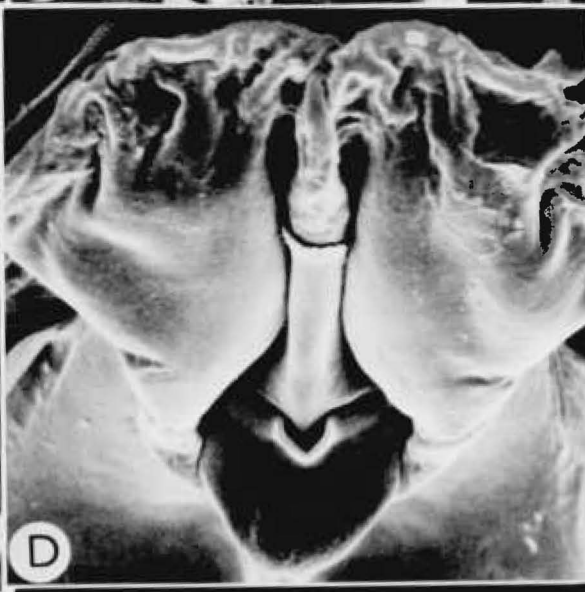
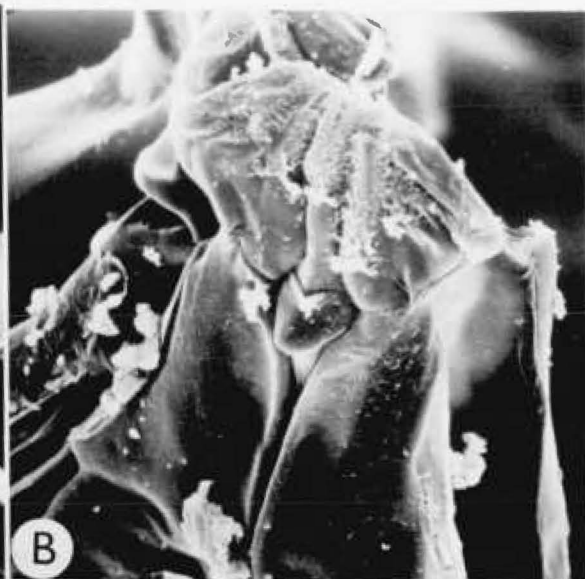
(E. nivalis X6,600)

- E. Protracted hypopharynx in situ.

(E. nivalis X4,800)

- F. Opening of lingual regulating valve guarded by two folds.

(E. nivalis X12,000)



and a posterior portion where it joins the stalks of the fulcrum. The anterior free part (Plate 9C, D) is formed from a strongly concave membraneous structure, which is folded along its anterior margin. This may form the subterminal dorsal fold of Folsom (1899) or the distal margin notch of Adam and Salmon (1972) which interlocks into the recurved boarder of the labrum (Plate 19A). The lingua becomes narrower in the middle part and the posterior part widens again to form a broad base, where it joins the fulcral arms.

The median part of the lingua is occupied by a peculiar structure, which terminates anteriorly in an elongate slit-like opening guarded by two membraneous folds on both sides (Plate 9E, F). This terminal structure resembles some sort of valve which can be closed by the guarding folds (Plate 9C, D). Leading to the mentioned opening is a cylindrical body with a hemispherical organ at its base (Plate 9B, C, D). The anterior edges of the hemispherical organ do not fuse with the proximal end of the cylindrical body and a triangular opening seems to lead into the interior of the hemispherical organ. A tube runs posteriorly from the hemispherical organ into a deep median furrow between the two halves of the lingua (Plate 9B). Both the cylindrical body and the hemispherical organ are invaginations to protect the delicate structures underneath. There is no mention of the lingual structures in the literature. Sections (Plate 13D), and electron transmission micrograph (Plate 18A) have not revealed much about their structure. However, the whole complex indicate a conducting function of some sort. It is suggested here

that the lingual spherical organ resembles a modification of the salivarium of the generalised biting insect, which Snodgrass (1935) has defined as the pocket between the hypopharynx and the labium into which opens the salivary duct; in higher insects converted into a salivary pump or a spinning apparatus. In Collembola, salivary juice is produced from labial segmental organs lodged in the posterior part of the head (Manton, 1964). This juice, discharged into a median posterior diverticulum of the preoral cavity by a pair of salivary ducts, can then flow either to the posterior side of the hypopharynx or directly out through the median gap in the labium, according to the pressure maintained by the two halves of the labium at this point. The other part of the juice can flow further anteriorly via the median tube between the two halves of the lingua to the hemispherical organ, where it is either stored or discharged through the anterior lingual orifice and so reaches the food surface. The outflow of the salivary juice from the hemispherical organ is regulated by the opening and closing of the guarding folds of the lingual orifice, while the inflow of saliva into the hemispherical organ is controlled by the transverse hypopharyngeal muscles. Contraction of these muscles enlarges the hemispherical organ's cavity and saliva is sucked into the organ from the posterior part of hypopharynx. The sucking action of the hemispherical organ cannot be eliminated as a factor helping in fluid feeding.

5.4 The epipharynx

The labrum continues backwards and inwards as the epipharynx, which forms the anterior wall of the pre-oral cavity. This structure has received little attention of the previous workers and their descriptions were mainly based on the reconstruction of serial sections, which made them inaccurate and in some instances erroneous.

The epipharynx (Plate 10A, Figure 9) can be divided into two regions: An anterior region and a posterior region. The anterior region appears as wedge-shaped in sections (Plate 13 C, D). It bears two rows of strong anteriorly-directed teeth on both sides of the very anterior part (Plate 10B). These teeth may correspond to the Chitinhaken described by Hoffmann (1908) as arising near the tip of the labrum at the point of insertion of the labrum depressor muscles. The two rows of teeth are kept in a cavity within the wall of the epipharynx. On the median gap between the two rows, there is a group of three to four small rounded papillae (Plate 10B). Posterior to the two rows of teeth, the epipharynx bears a flap-like structure with its margin fringed into three folds, each ending in a number of filaments. This structure is unpaired and lies only on the right side of the epipharynx. On the opposite side, there is a crescentric saw-like membrane with many filaments. The median part of the epipharynx is occupied by a chitinous raised-up structure (Plate 10C, D) which runs posteriorly to the cibarium (the mandibular molar plate cavity) and thus divides the pre-oral cavity into two lateral channels. On both sides of the median structure, the curved mandibles fit quite

Plate 10: Inner aspect of the epipharynx.

- A. Dissection showing the two mandibles in situ and the epipharyngeal structures.

The right mandible is dorsal and posterior to the left one. Note the convex shape of the apical region of mandibles following the curved shape of epipharynx.

(E. nivalis X3,600)

- B. Anterior part of epipharynx showing the two rows of teeth, the flap-like structure and part of the median membrane. Note the four small rounded papillae at the median line posterior to the two rows of teeth.

(E. nivalis X9,200)

- C. Posterior part of epipharynx showing the swollen median membrane, the lateral folds and the crescentric lateral membranes fringed with filaments.

(E. nivalis X14,000)

- D. Same as C. Swollen part of median membrane showing concave ventral surface which indicates its flexibility.

(E. nivalis X9,200)

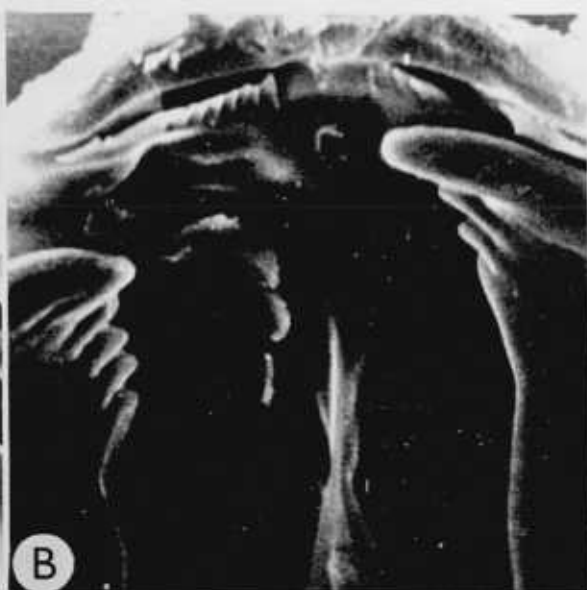
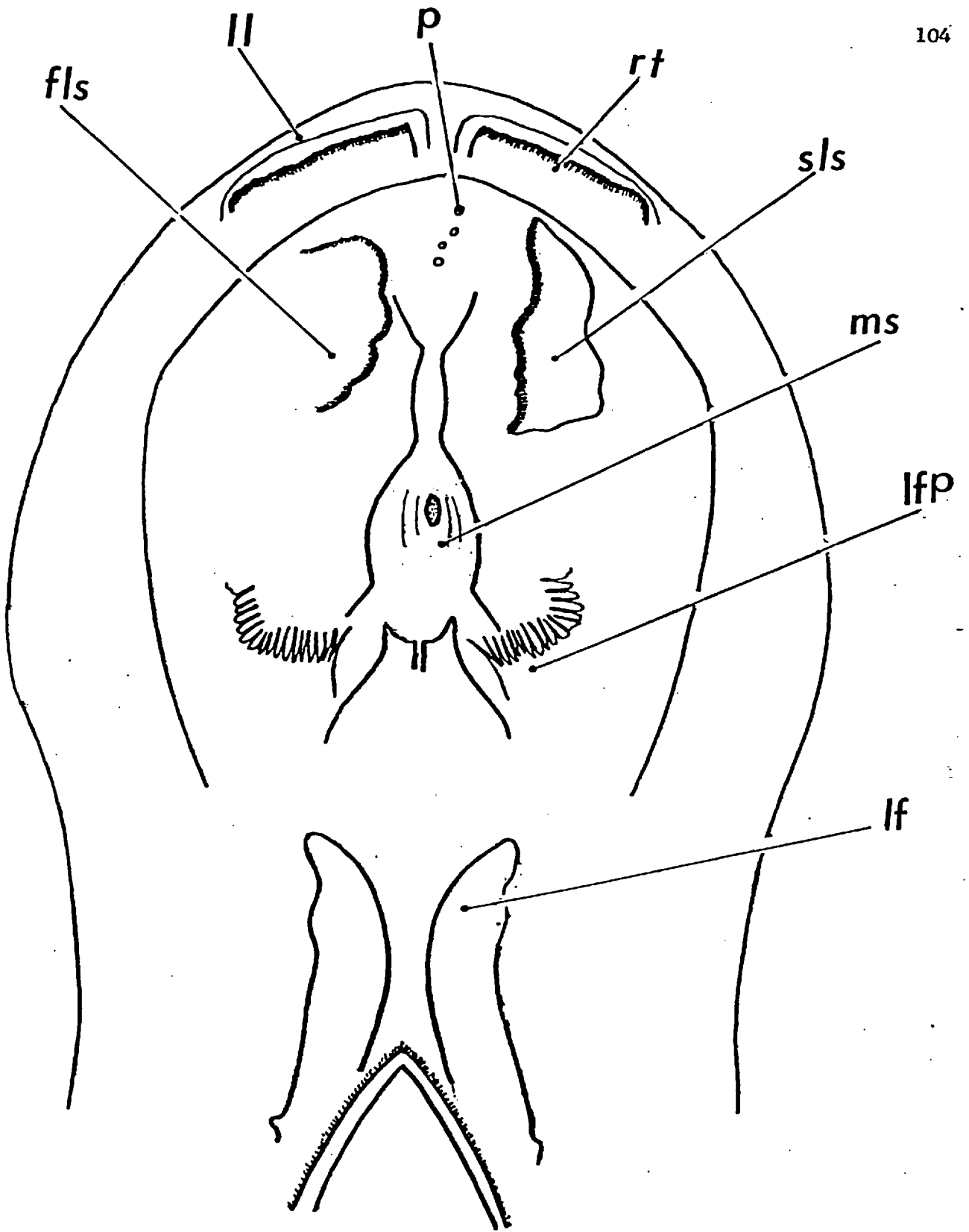


Figure 9 Ventral view of the epipharynx.

fls, flap-like structure; lf, lateral fold;
lfp, lateral flap; ll, lip of labrum;
ms, median structure; p, papilla; pom, position
of mandible; rt, row of teeth; sls, saw-like
structure.



comfortably (Plate 10A). The median structure widens posteriorly and forms a swollen part which seems to be divided into two lateral parts and a median part with some filaments at its apex. The lateral parts terminate in the form of a broad base fixed to the wall of the epipharynx. The median body, on the other hand, divides posteriorly and forms two free flaps which form the food retention valve with corresponding protrusions on the dorsal side of the superlinguae. The free flaps lie anterior to two divergent chitinous folds which are directed anteriorly. The folds have their margins fringed and their base is supported by two arms arising from the wall of the epipharynx. On either side of the two folds, there is a membrane with long filaments arising from its margin. Posterior to the folds and at the area of the mandibular molar plates, the epipharynx gradually becomes concave to form the dorsal part of the cibarium (Figure 10, Plate 12A, B, D). At the back of the cibarium and before the pharynx, lies the oral valve, formed by a long lateral fold arising from the epipharynx and the raised part on the dorsal side of the superlinguae, posterior to the filament area (Figure 10 ov; Plates 12B; 17A, B). This valve prevents the regurgitation of food from the pharynx into the cibarium.

There are two pairs of epipharyngeal muscles, which insert on the epipharynx and originate from the fronto-labral suture (Figure 10 epd; Plate 12A, B). The action of these muscles creates a negative pressure inside the pre-oral cavity. The median structure, on the other hand, presses down on the food in the food channel and helps to keep it confined to that channel.

The food retaining valve prevents the anterior movement of the food away from the region of the mandibular molar plates. Posterior to the oral valve, the epipharynx gradually merges into the roof of the pharynx and there is no sharp line of demarcation between the two.

6. HISTOLOGY AND HISTOCHEMISTRY

6.1 General histology

The thin plastic sections from tissue blocks originally prepared for electron microscopy gave crisp cytological detail.

A number of the general stains employed for these sections have proven to be valuable stains and exhibited in varying measures the property of staining differentially several cytoplasmic structures.

The crystal violet stain was especially useful for the demonstration of specific granules which stained an intense dark blue. The alkalized methylene blue with basic fuchsin gave a good colour contrast between cellular and connective tissue elements. Cell nuclei stained purple while mitochondria had a more intense red hue. Heidenhain's iron haematoxylin had proven particularly useful in the identification of striated muscles which stain dark blue to black with the mitochondria having a red to orange colour. Chlorazol black E, on the other hand, gave intense differential colouring to sections (Plate 11A).

Of all the general stains, toluidine blue was found to be the most convenient and easier to use. After counter staining and differentiation with basic fuchsin, it gave a greater over-all clarity and better display of the cytoplasmic components and showed much more contrast of cytoplasm, nuclei and connective tissue (Plate 11B) than that seen in other general stains. Although in alkaline solution toluidine blue functioned as a general stain, yet it became specific

Plate 11: Histochemical stains.

A. Transverse section of the head of

E. nivalis stained with chlorazol black E

(General stain). The cuticular parts

e.g. mandible; maxillae and postero-lateral angles of hypopharynx, show an intense colour.

Note prominent frontal sclerite. X230

B. Longitudinal section of head of E. nivalis

stained with toluidine blue. Nuclei,

mitochondria and muscles show good contrast.

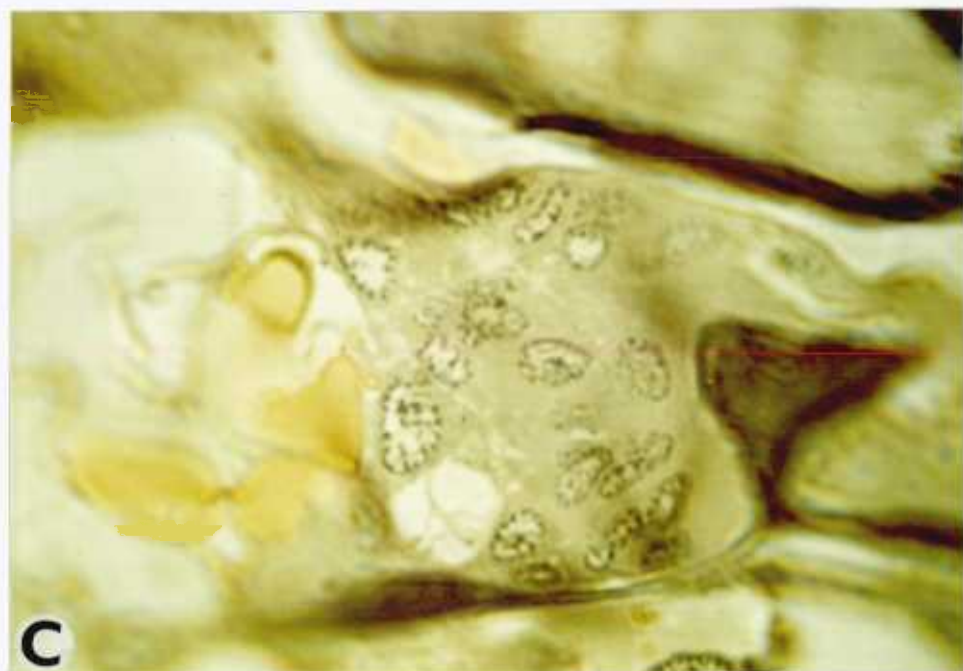
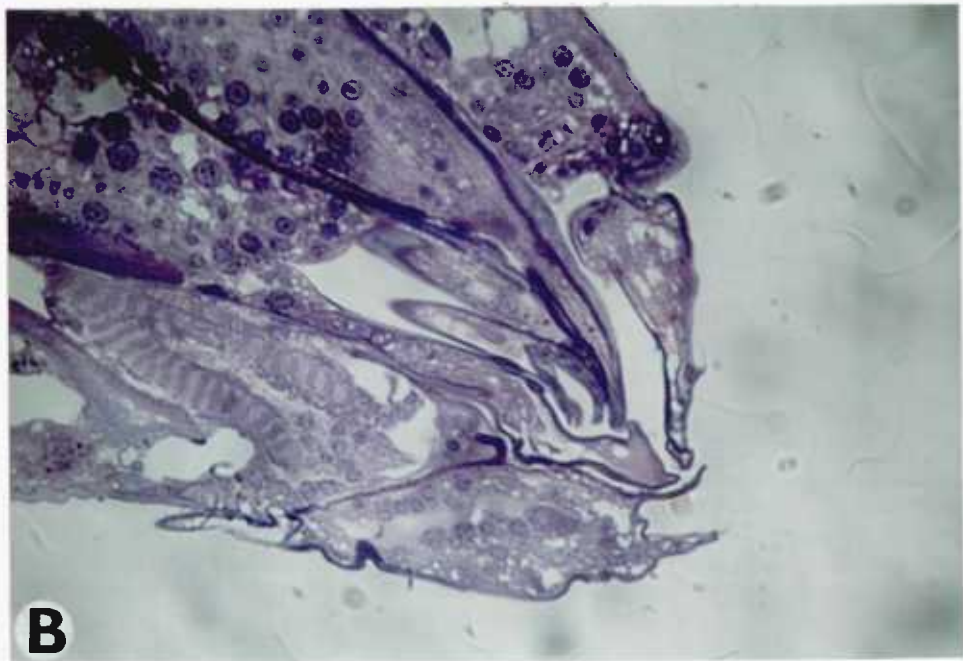
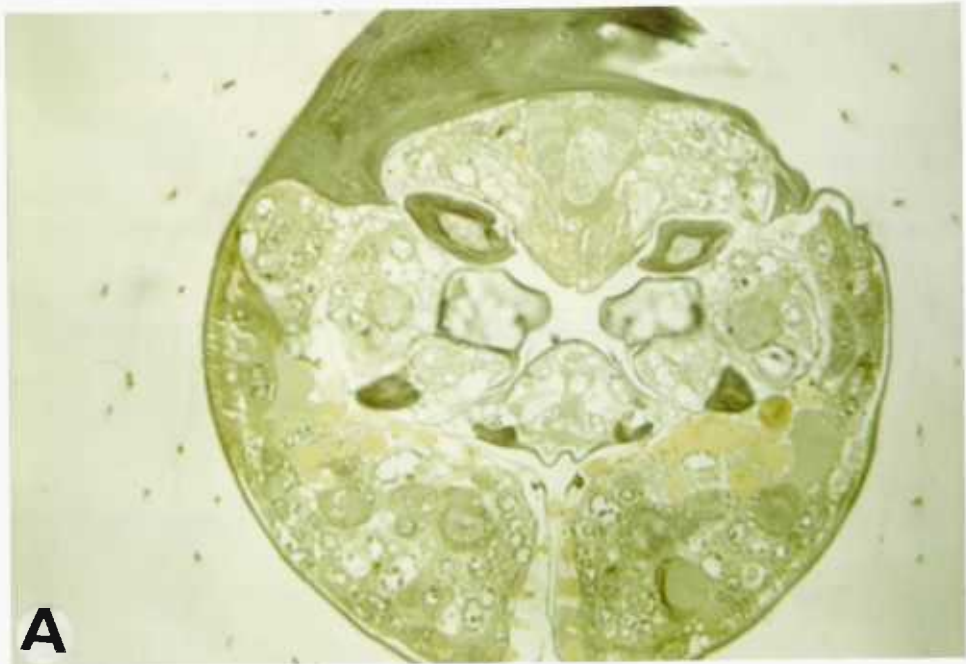
X460

C. Posterior part of hypopharynx stained with

Nile blue. Lipid inclusions are brown in

colour, nuclei dark green.

X2,300



for nuclei at pH6. For these reasons, it was employed in the staining of serial sections (Plates 12, 13) for the study of the interrelationships of the different mouthparts and their associated musculature, of which a short account is given below.

Longitudinal sections (Figure 10; Plate 12) show that the oral cone is bounded dorsally by the labrum and clypeus and ventrally by the labium and hence it encloses the buccal cavity. The labrum is broader at the base than at the apex and the labium is similarly drawn out beyond the head to form a kind of snout with the labrum. At its junction with the frontal sclerite (fs), the labrum becomes invaginated to form a transverse ridge of thick cuticle, referred to as the fronto-labral suture (fls). The suture stains dark with toluidine blue and most of the general stains, while the frontal sclerite shows a concentration of large nuclei. The clypeus slopes upwards and posteriorly meets the epicranium of the head. The dorsal surface of the labrum bears the smooth area (sa) which is formed of thick cuticle and has a raised up posterior margin (Plate 12D). The distal margin of the tip of the labrum is provided ventrally with a notch in which the tips of the superlinguae can lock (Plate 12B, D). Two pairs of epipharyngeal dilator muscles (ed) originate from the fronto-labral suture and insert on the epipharynx (Plate 12B).

The epipharynx (ep), which forms the roof of the buccal cavity, is wedge-shaped (Plate 13C, D). It bears two lateral folds (lf), which extend ventrally and divide the cibarium into two lateral pouches that enclose the mandibular molar plates (mmp).

Figure 10 Reconstruction of a longitudinal half of the head viewed from the middle line.

ci, cibarium; d, fronto-labral suture;
ep, epipharynx; epd, epipharyngeal dilator
muscles; fs, frontal sclerite; hp, hyaline
plate; lb, labium; lbm, labial muscle;
lbp, labial papilla; lm, labral muscles;
ls, labral seta; mh, maxilla head;
mmp, mandibular molar plate; ov, oral valve;
poc, preoral cavity; sa, smooth area;
sf, superlingual filaments.

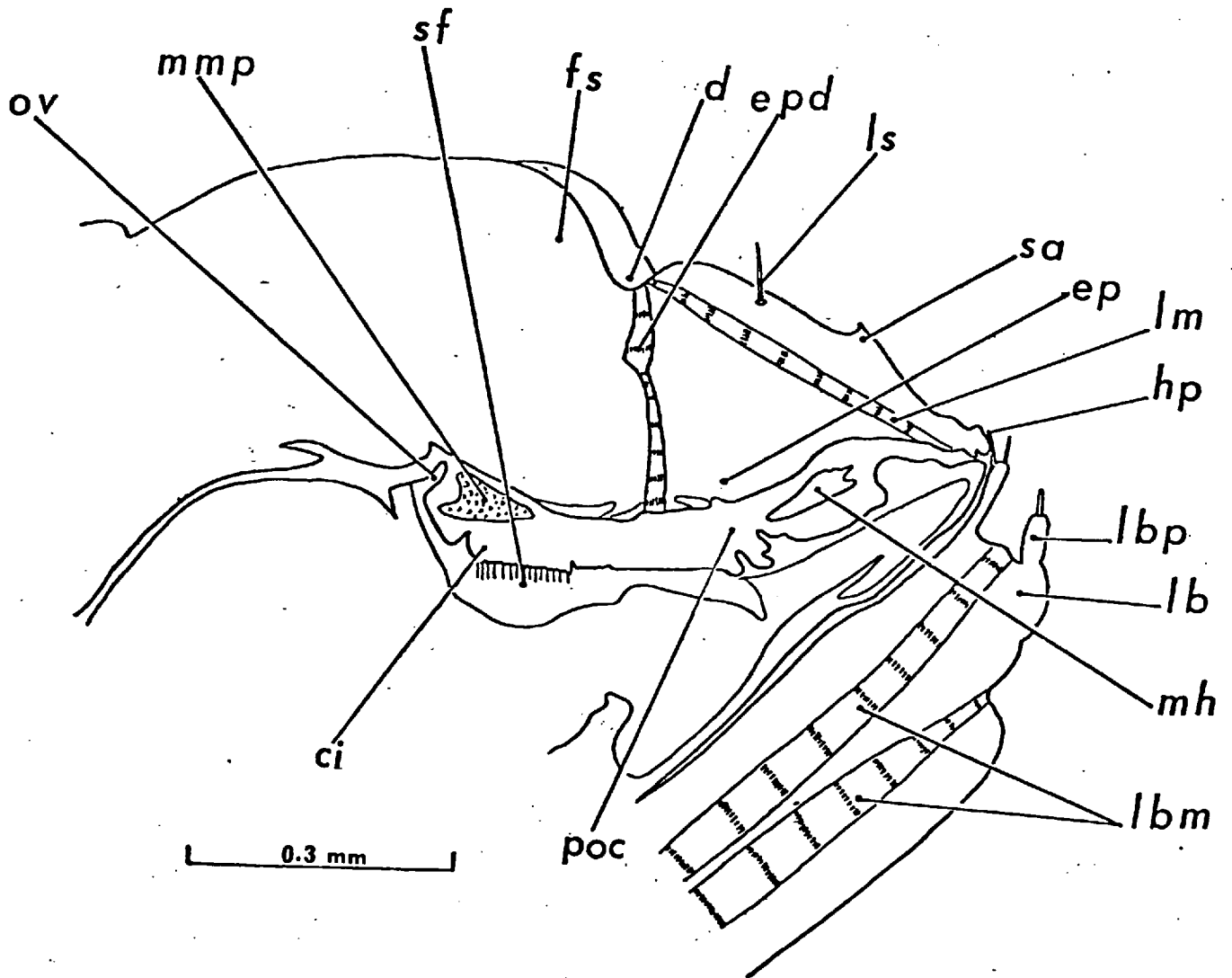


Plate 12: Longitudinal sections of the mouthparts of E. nivalis at different levels, viewed from the lateral side.
(All sections stained with toluidine blue X475).

A. Section at the level of the lateral side of the hypopharynx. The maxilla head (c) lies inbetween superlingua (sli) and lingua (li).

fls, fronto-labral suture; fs, frontal sclerite;
hp, hyaline plate; m, mandible; vc, ventral chamber of gnathal pouch.

B. Section at the level of the oral valve (ov) formed by a protrusion of the posterior part of the superlingua (sli).

ed, epipharyngeal dilator muscle; f, superlingual filaments; fs, frontal sclerite; li, lingua;
ph, pharynx; vc, ventral chamber.

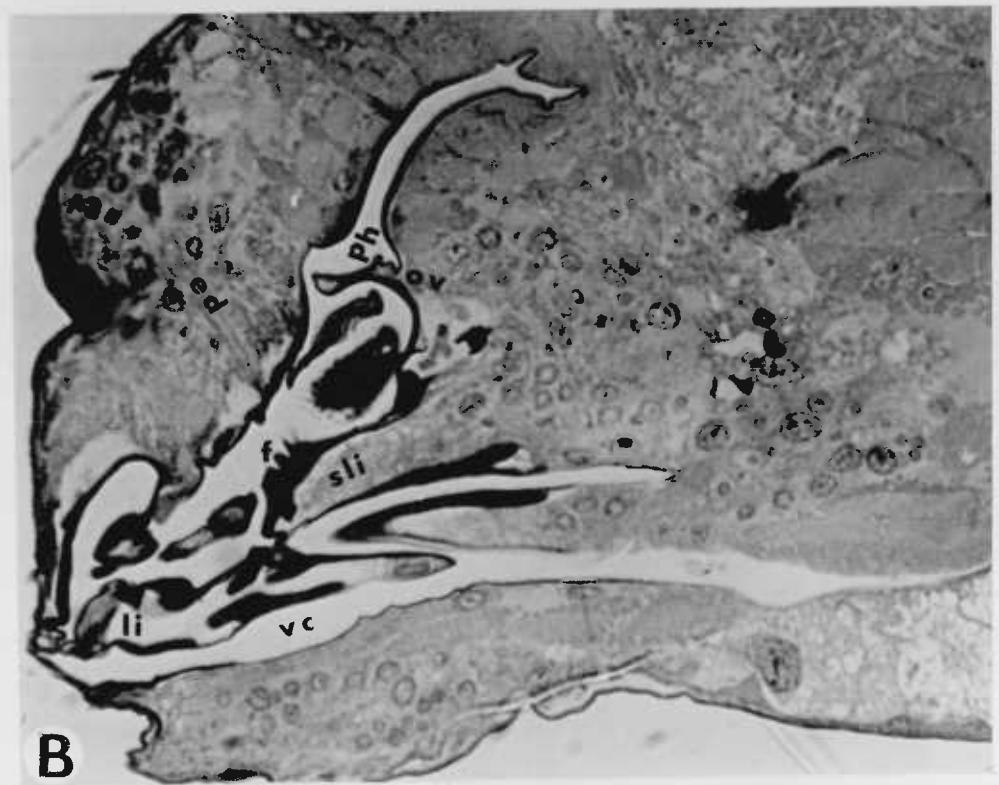


Plate 12: Longitudinal sections of the mouthparts of E. nivalis
at different levels, viewed from the lateral side.

(Continued)

- C. Section at the level of the median line of the mandible (m). The ventral chamber of the gnathal pouch (vc) opens to the outside through the salivary channel.

lm, lingual muscles; od, oesophageal dilator muscles.

- D. Section at the median line of the head.

c, maxilla head; fls, fronto-labral suture;
fs, frontal sclerite; hp, hyaline plate;
lb, labium; li, lingua; lm, labial muscles;
m, mandible; od, oesophageal dilator muscles;
sa, smooth area of labrum.

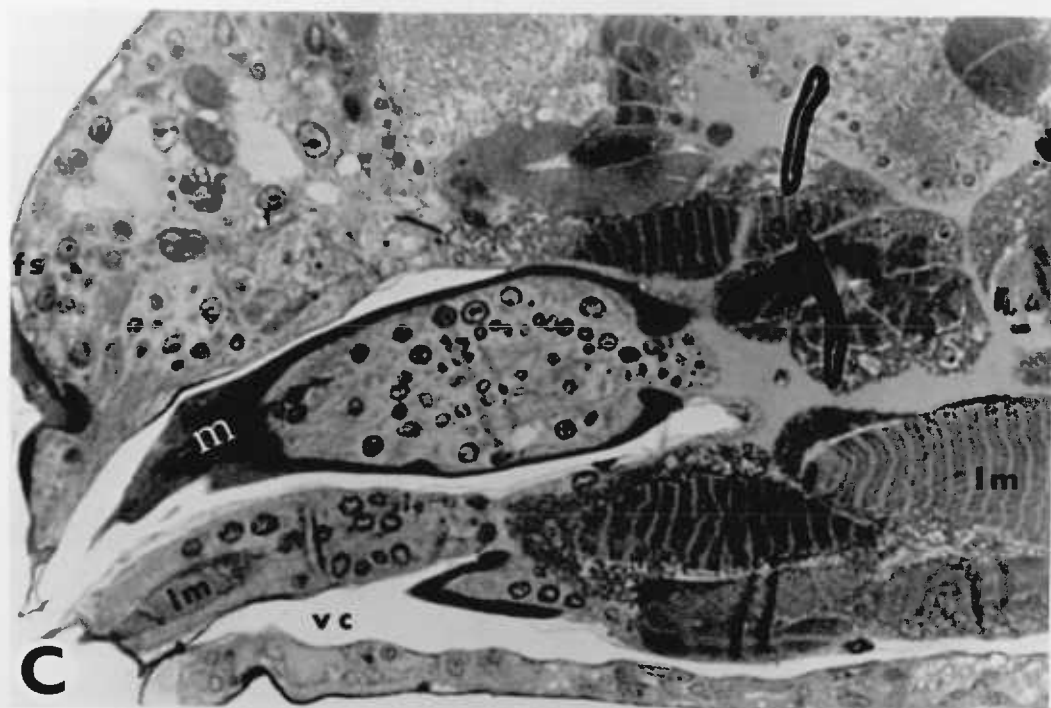


Plate 13: Longitudinal sections of the mouthparts of
E. nivalis viewed from above at progressively
lower levels.

A. Section at the level of the oesophagus,
showing the oesophageal valve (opv).
a, antenna; od, oesophageal dilator muscles;
oc, oesophageal constrictor muscles. X500

B. High magnification micrograph of section at
the level of the mandibular molar plates which
fit in cavities between superlingual arms and
the lateral folds (lf) of the epipharynx (ep).
Note the superlingual filaments (f) lying
inbetween the two molar plates.
li, lingua; m, mandible. X2500

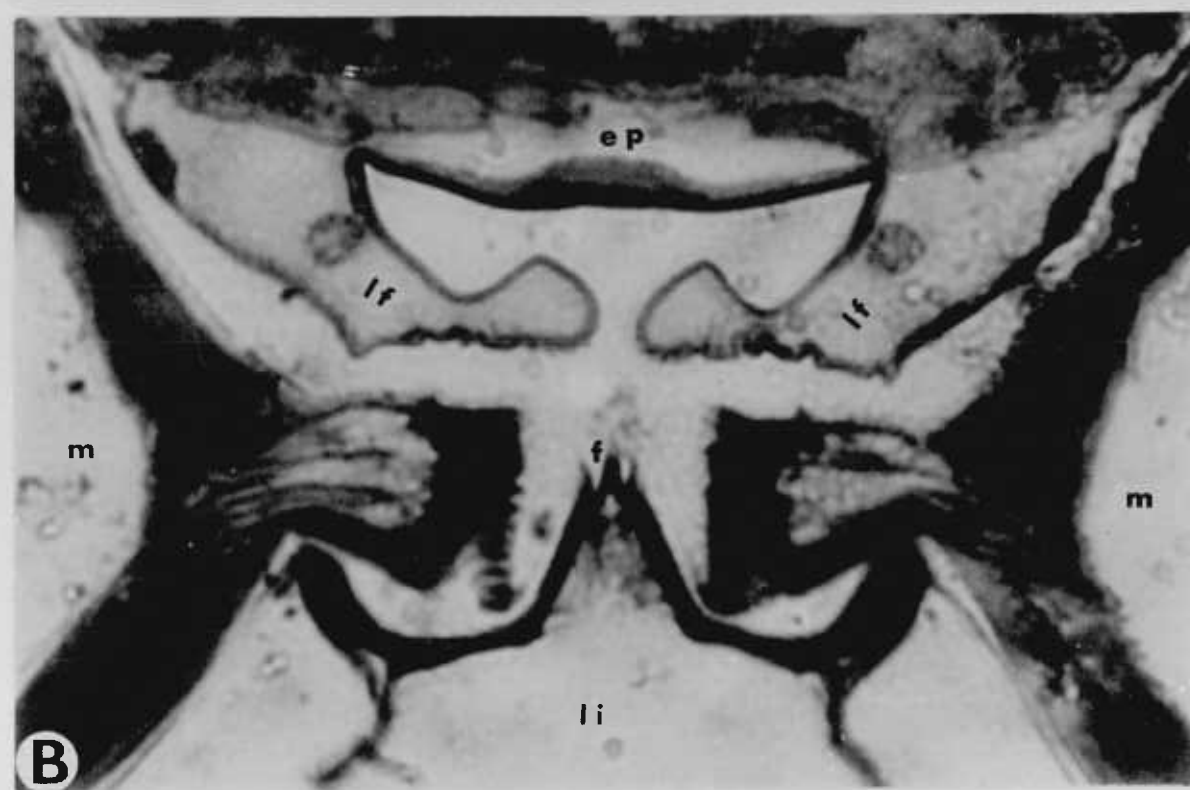


Plate 13: Longitudinal sections of the mouthparts of
E. nivalis viewed from above at progressively
 lower levels. (Continued)

C. Section at the level of the mandibular molar
 plates. Note maxilla heads (c) on both sides
 of lingua and the superlingual filaments lying
 inbetween the molar plates. The epipharynx (ep)
 shows the two lateral folds. X780
 fs, frontal sclerite; sli, superlingua

D. Section through the whole lingua (li),
 showing the two superlinguae (sli) and the
 cylindrical body (cb) inbetween. Note the
 fulcral arms (fu) at the postero-lateral
 angles of the lingua and the cuticular
 thickening of the lateral sides where the
 two maxilla heads (c) fit. X780
 ep, epipharynx; m, mandible



The epipharyngeal lateral folds also form the food retaining valve with the raised-up median part of the superlinguae (Plate 13C, D). The mandibles (m), which are free anteriorly and loosely articulated with the head, lie in pockets within the gnathal pouch (Plate 12A, C, D). The mandibular shaft shows numerous large nuclei (Plate 12C, D).

The large hypopharynx, formed by the ventral lingua (li) and the two lateral superlinguae (sli), occupy the central part of the oral cavity. A transverse union through the base of the hypopharynx links the anterior ends of the pair of fulcral arms (fu) and is continuous with the lateral hypopharyngeal thickenings (Plate 13F). The posterior part of the hypopharynx bears a cluster of large oval nuclei (Plates 11C; 12B, D). The dorsal surface of the superlinguae bears the median rows of filaments (f), while a protrusion at the posterior part forms the oral valve (ov) which guards the aperture to the pharynx (Plate 12 B). The pharynx (ph) slopes at first gently upwards, then curves down and is continued posteriorly as the oesophagus. The oesophageal muscles consist of the oesophageal dilators (od) and the oesophageal constrictors (oc) (Plates 12C; 13A). At the posterior end of the oesophagus, a protrusion forms the oesophageal valve (opv) which constitutes the junction between the foregut and the midgut (Plate 13A).

When retracted into the gnathal pouch, the two maxillary heads or capitula (c) lie in cavities on both sides of the lingua (Plate 13C, D). It is for this reason that they appear to lie between the lingua and superlinguae in longitudinal sections (Plate 12A). Ventral to the hypopharynx is the ventral chamber (vc) of the gnathal pouch, which opens ventrally through the salivary channel (sc)

into the median groove between the two halves of the labium (Plates 11B; 12C, D). The labial muscles (lm) originate from the posterior tentorial apodemes (or fulcral arms) and insert at the labium. The hyaline plate (hp) of the labium extends beyond the anterior margin of the labrum and thus keeps the secondary mouth opening tightly closed (Plate 12B, D).

6.2 Histochemistry

6.2.1 Polysaccharides and mucopolysaccharides

All the chitinous structures i.e. mandibles, maxillae, lateral parts of hypopharynx and the outer body cuticle, gave a positive reaction (= a purplish red colour) with the PAS test. The posterior part of the hypopharynx was weakly PAS-positive. This reaction was not affected by incubation with diastase, suggesting that the positive PAS reaction was not due to the presence of glycogen.

The tribasic stain failed to indicate the presence of mucopolysaccharides although it produced various shades of magenta with the chitinous structures of the mouthparts.

6.2.2 Lipids

The epon plastic coloured intensely with Sudan black to give a bright blue colour, against which the nuclei appeared as clear white objects. Lipid granules became intensely blue-black. These granules were demonstrated on the frontal sclerite, labium and the posterior part of the hypopharynx.

With Nile blue, the majority of the elements of the cells were stained in different shades of bluish green. The lipid inclusions at the posterior region of the hypopharynx were larger than those shown by Sudan black and they had a brown colour (Plate 11C).

6.2.3 Proteins

Alcoholic bromphenol blue gave completely negative results. It could be concluded, therefore, that no histochemically detectable proteins were present.

6.2.4 Nucleic acids

Araldite sections of the mouthparts stained distinctly with methyl green-pyronin. The nuclei varied in staining from intensely red to light pink, indicating the presence of DNA. On the other hand, the cytoplasm of certain parts also showed various shades of red, most probably depending on the concentration of RNA, as in the shafts of the mandibles and the posterior region of the hypopharynx. The latter was distinctly pyroninophilic.

6.2.6 Enzymes

Samples of the dissected enclosed mouthparts which were treated for succinic dehydrogenase, revealed a strong activity in the mandibular muscles and the posterior part of the hypopharynx. The use of iodoacetate as inhibitor, completely suppressed the dehydrogenase activity, as did heating in boiling water.

The overall activity of the non-specific esterase was rather poor and it was only on prolonged incubation that a weak reaction was obtained. This activity was inhibited by either eserine sulphate or heating.

In summary, the posterior region of the hypopharynx appears to bear cells which appear to be glandular in nature, and hence the mandibular molar plate cavity or cibarium may be the site of some enzymic activity.

7. TRANSMISSION ELECTRON MICROSCOPY OF SOME FEATURES OF THE MOUTHPARTS

7.1 The Labrum

The surface of the labrum and most of the body is covered by a soft, thin, transparent cuticle. The basic structure of the cuticle and underlying epidermis (Plate 14C) agrees with that reported for other species of Collembola (Imms, 1906; Ogel, 1958, 1965; Lower, 1958; Noble-Nesbitt, 1963; Barra, 1977) and for the pterygote insects (Richards, 1951; Wigglesworth, 1953). In general, two major cuticular layers are present: An endocuticle (enc), which consists of lamellae lying one above the other and is bound on its outer side by the very thin, electron-dense epicuticle (ep). A layer of mesocuticle is only present in localized areas of thick cuticle (Noble-Nesbitt, 1963). The "lamellated" appearance of the endocuticle results from cuticular canals, which contain multiple filaments (wax canal filaments) in the distal half and axial filaments in the proximal half (Barra, 1977). The epicuticle corresponds to the cuticulin layer, which may be considered as the basic epicuticular layer.

The whole of the epicuticle is thrown into minute tubercles (Plate 14D) which are approximately 0.2 μ m in diameter, 0.3 μ m high and 0.5 to 0.8 μ m apart. They give in surface view a granular, sculptured, hexagonal pattern to the general surface of the cuticle (Section 4.1). These tubercles were referred to as "minor tubercles" and "epicuticular tubercles" by Noble-Nesbitt (1963) and Ogel (1965), respectively. No major tubercles as were reported by Noble-Nesbitt (1963) from Podura aquatica Linnaeus, 1758 are found in Entomobrya. Pore canals (pc) pass up through

Plate 14: Ultra structure of the labrum.

A. Low magnification micrograph of a large field of a longitudinal section through labrum, showing a setal insertion. The smooth area (sa) at the anterior part is greatly thickened posteriorly. X6,000
enc, endocuticle; hcs, socket;
ls, labral seta; sa, smooth area.

B. Articulation of a labral mechanoreceptor.

X18,000

ds, dendrite sheath; exc, extracellular cavity; hcs, socket; hs, hair shaft;
p, pivot; ss, socket septum;
tb, tubular body.

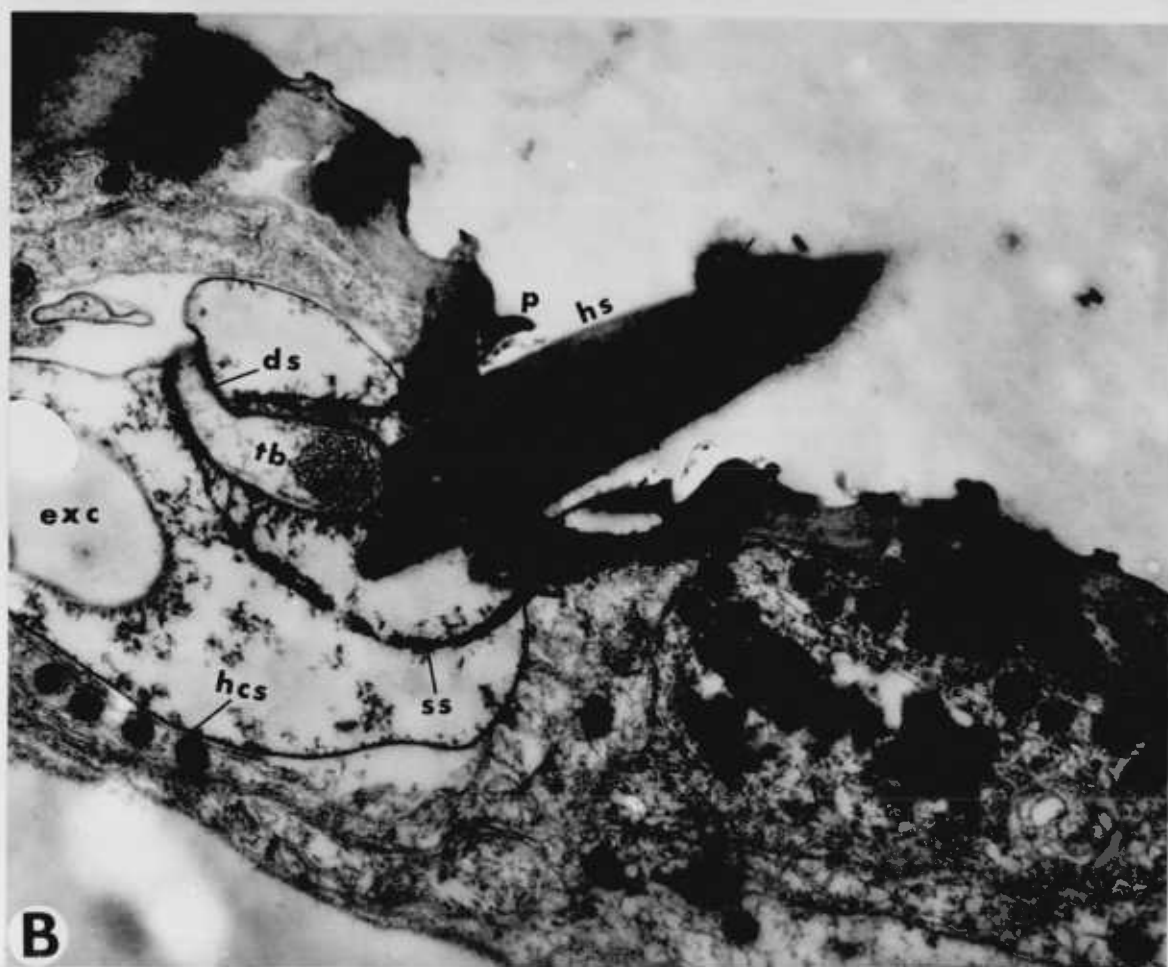
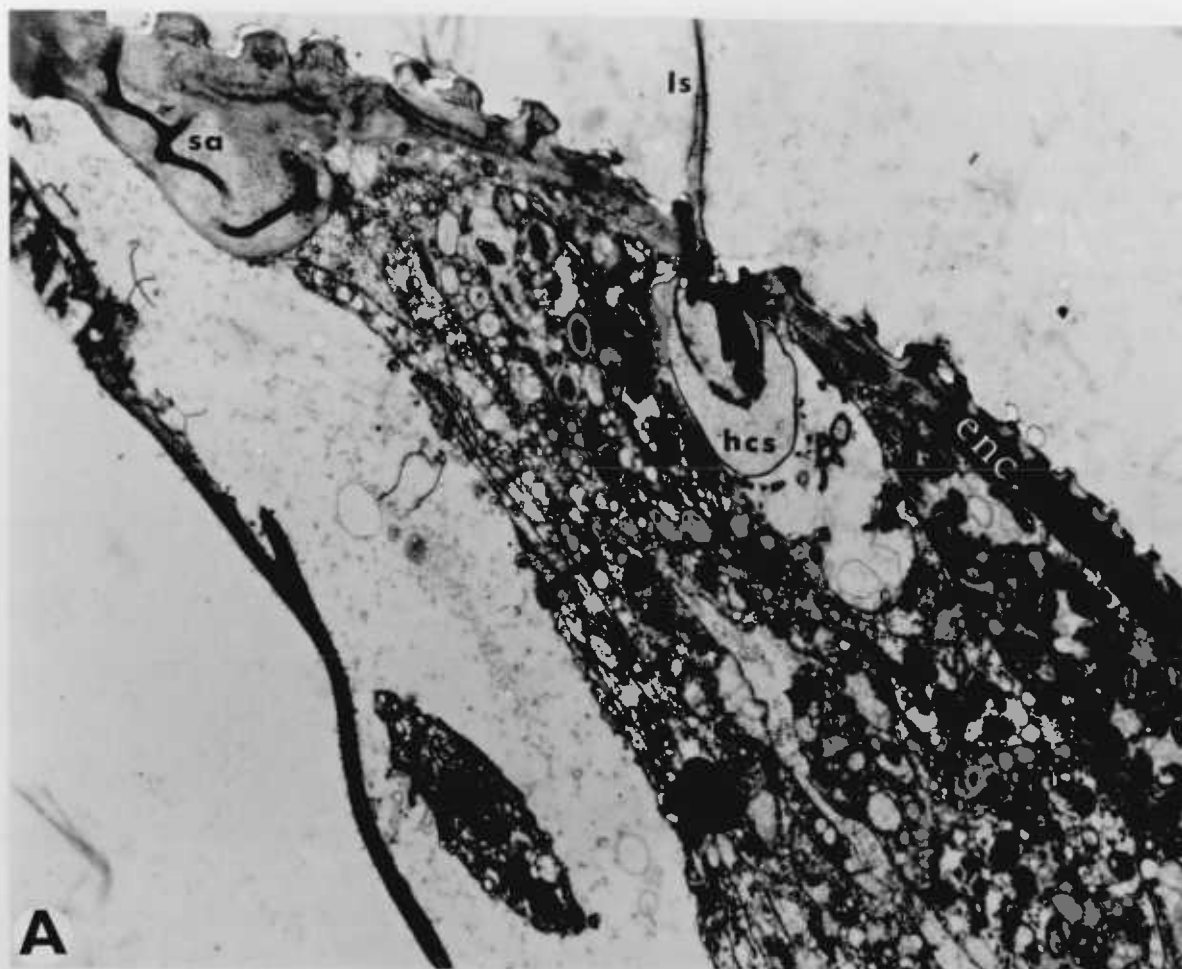
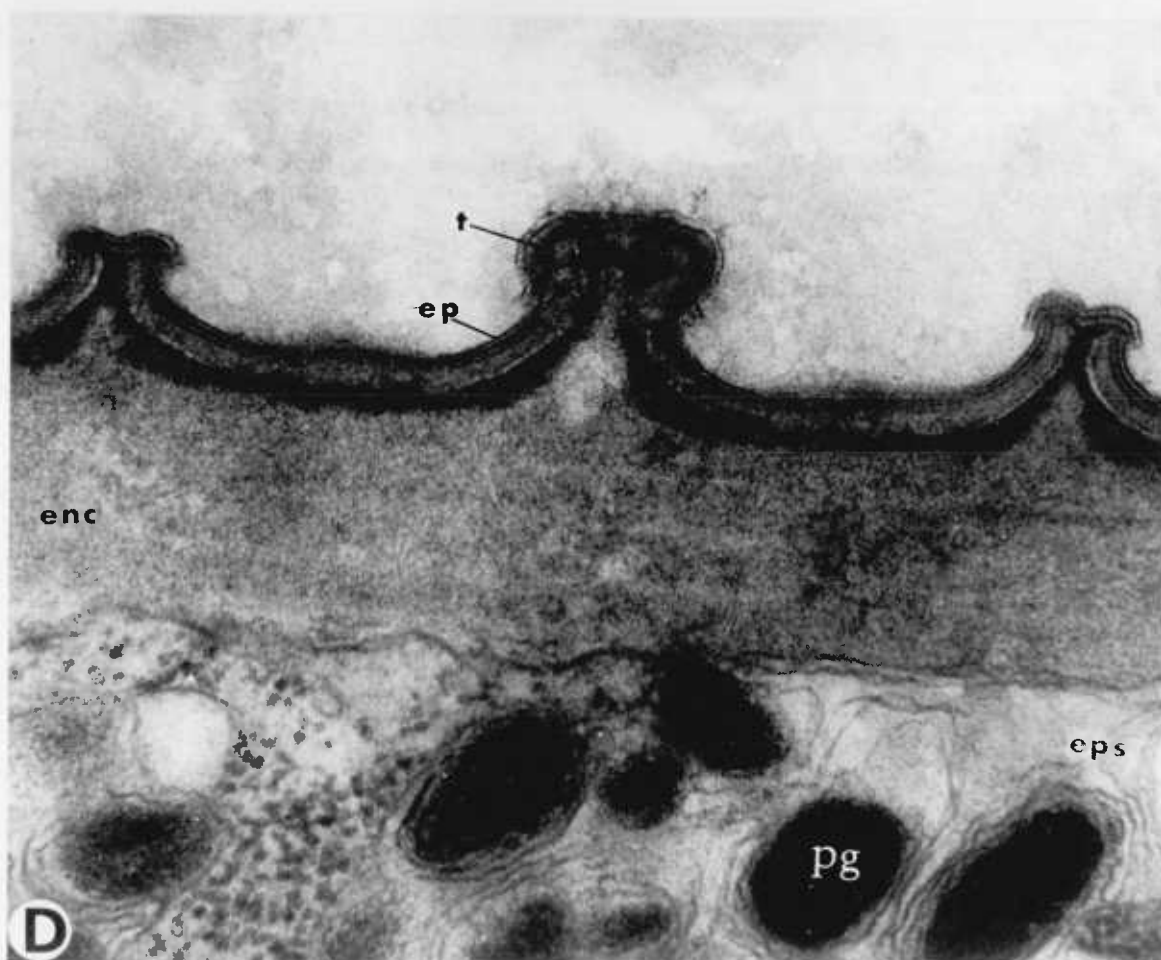
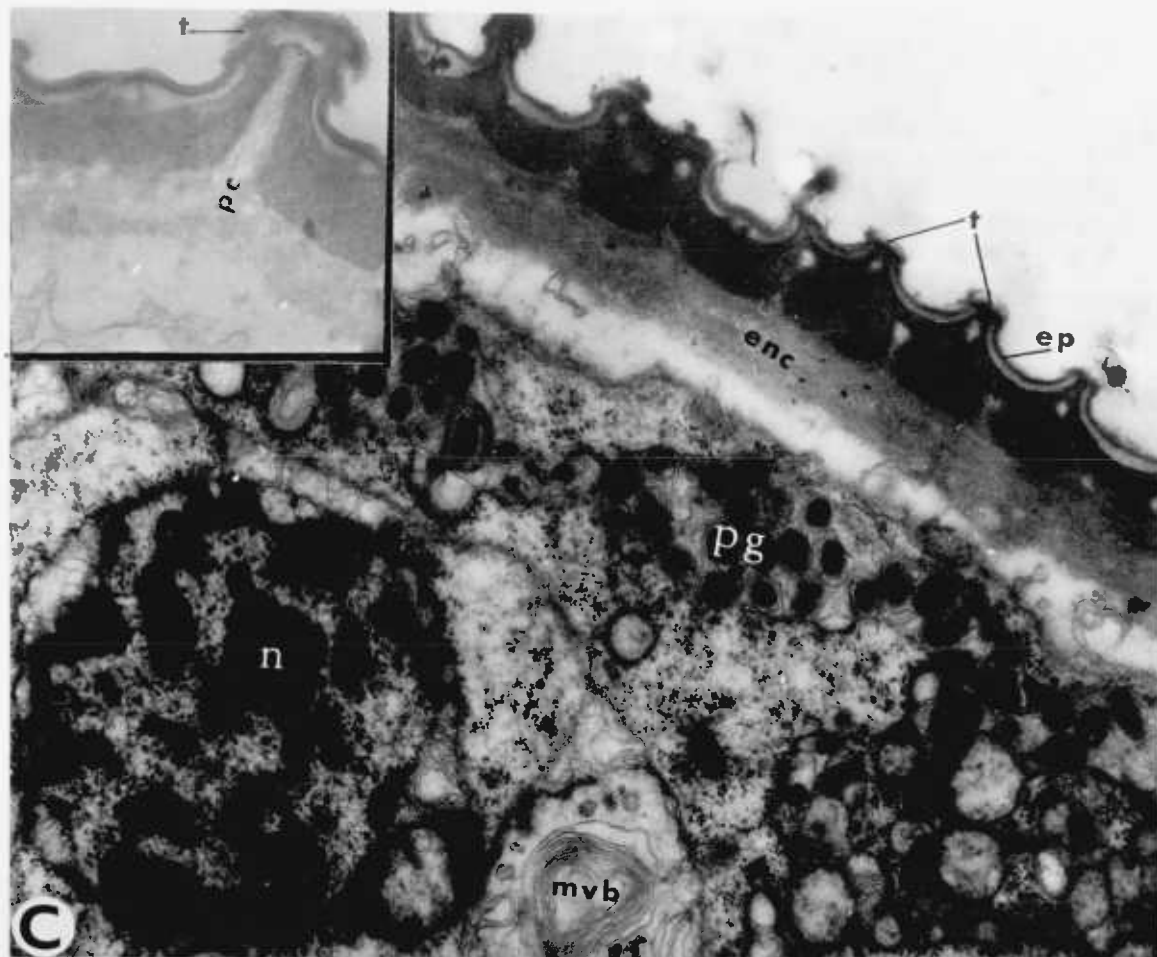


Plate 14: Ultrastructure of the labrum (continued).

- C. Electron micrograph of section through epidermis and cuticle. Inset shows enlargement of a tubercle (t) and the pore canal (pc) passing inside. X24,000
enc, endocuticle; ep, epicuticle;
mvb, multivesicular body; n, nucleus.
- D. Section through cuticle and part of epidermis showing the substructure of the two major cuticular layers: the endocuticle (enc) and the epicuticle (ep). X84,000
eps, epidermis; pg, pigment granule;
t, tubercle.



the cuticle to the tips of the epicuticular tubercles, but without penetrating the continuous layer of the epicuticle.

The epidermis contains many pigment granules (pg) which impart the body colour. The nuclei (n) are very unevenly distributed through the epidermis, leaving large parts of it enucleate. The ultrastructure of the epidermal cells varies with their cycles of secretory activity (Locke, 1969), but they have a microvillate surface and contain numerous mitochondria, Golgi vesicles and cisternae of smooth-surfaced endoplasmic reticulum as well as cytoskeletal structures in the form of oriented microfibres and microtubules. A basement membrane is apparently absent. This agrees with results reported for some Collembola (Lower, 1958; Ogel, 1958, 1965; Noble-Nesbitt, 1963), but differs from the position in pterygote insects (Wigglesworth, 1953) and other Collembola (Imms, 1906).

Of the setae on the labrum, only the two anterior-most ones are modified as sensilla. These arise within the smooth area of the labrum from elongate rather than circular sockets (Plate 2E). The articulation of these sensilla (Plate 14B), which act as tactile mechanoreceptors, is similar to that of the labial mechanoreceptors. A detailed description of the ultrastructure of this type of sensilla is given in Section 7.4. All the remaining labral setae have the same type of insertion (Plate 14A). The seta inserts into a socket, which is differentiated from the rest of the cuticle. The socket is lined at its base by a ring of the epicuticular articular membrane which is produced by the tormogen cell. The seta itself is produced by a single trichogen cell.

The labral papillae at the anterior margin of the labrum were found to be solid cuticular processes in longitudinal sections. They are rigidly connected with the cuticle and form an integral part of its endocuticle. The smooth area free of tubercles which lies posterior to the labral papillae does not show any special features to relate to its true function. The posterior margin of this area is a bit raised from the general surface of the labrum and a limited area of thick cuticle (4 μ m thick) is observed under that part, which tapers anteriorly into the thinner general labral cuticle of only 1 μ m thickness.

7.2 The epipharynx

The epipharynx (Plates 15, 16, 17) appears as a wedge-shaped structure in longitudinal sections, bounded dorsally by the cuticle of the labrum (lc) and ventrally by the two lateral folds (lf). There is a layer of endocuticle (enc) running along the ventral margin, which becomes thicker on both lateral sides. The tip of the epipharynx (Plate 15B), which lies ventral and anterior to the superlingual arms of the hypopharynx, has two chitinous rods (cr) which converge at the median line. These rods probably resemble the lateral folds after they have merged with the epipharynx. Deposits of electron-dense material (ed) are also present at the epipharyngeal tip.

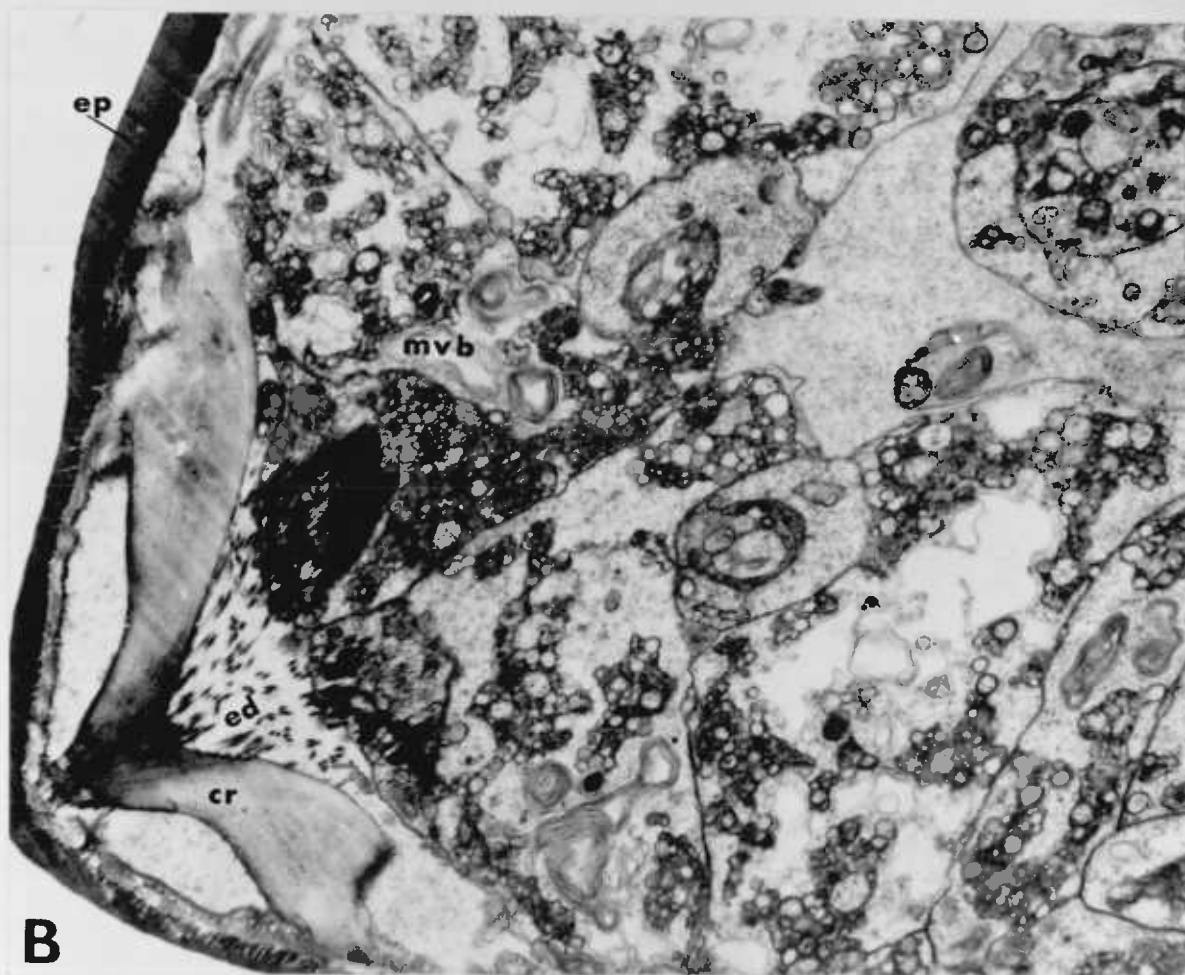
Despite some variations in size, shape and electron density, the cells of the epipharynx exhibit a fairly uniform structural organization with several multivesicular bodies (mvb) in the prenuclear and supranuclear regions of the cytoplasm (Plate 15B). Multivesicular (or multilamellar) bodies are frequently observed

Plate 15: Relative position of the epipharynx and the ultrastructure of its ventral tip.

A. Electron micrograph showing the relative position of the epipharynx and the superlingual arms (sla). At both sides of the epipharyngeal tip, two chitinous rods (cr) which resemble the lateral folds after merging with epipharynx. Note the heavy sclerotization of the tips of superlingual arms. Part of the lingual cylindrical structure (cb) can also be seen. X10,000

B. Ventral tip of the epipharynx. The margins of the tip are hard and epicuticular in structure (ep). The cellular matrix is made of components of an ordinary epidermal cell with many multivesicular bodies (mvb).

X13,200



in the vicinity of the Golgi complex and a typical multivesicular body consists of a single limiting membrane which encloses a number of small vesicles, surrounded by a matrix of variable electron density. They are about $1\mu\text{m}$ in diameter and are generally spherical with concentric lamellae (Plate 15B), although oblong or dumb-bell forms are also encountered.

Plate 16A shows a micrograph of the dorsal part of the epipharynx, next to the labral surface. The labral cuticle consists of the thin electron-dense layer of epicuticle (ep) and the thick layer of lamellate endocuticle (enc), $2.5\mu\text{m}$ thick. A variety of vesicles (ve) of variable size and luminal content are present in the cytoplasm. They have a smooth membrane and contain materials of different electron density. The vesicles increase in size towards the dorsal end of the epipharynx. They are profusely surrounded by a large number of mitochondria (m). When seen in longitudinal section, the mitochondria appear as elongated structures or columns. Their internal structure is characterized by a large number of cristae. These show an orientation transverse to the long axis of the mitochondrion.

In the ventral part of the epipharynx (Plate 16B), the most conspicuous structures are the two bands of muscle fibres (sm). These undoubtedly comprise part of the epipharyngeal dilator muscles. The fibres appear roughly as rectangular structures, complementarily packed with each other. They show the characteristics of striated muscles with myofibrils showing a very regular laminal organization. The sarcomeres, though lack the precise transverse alignment shown

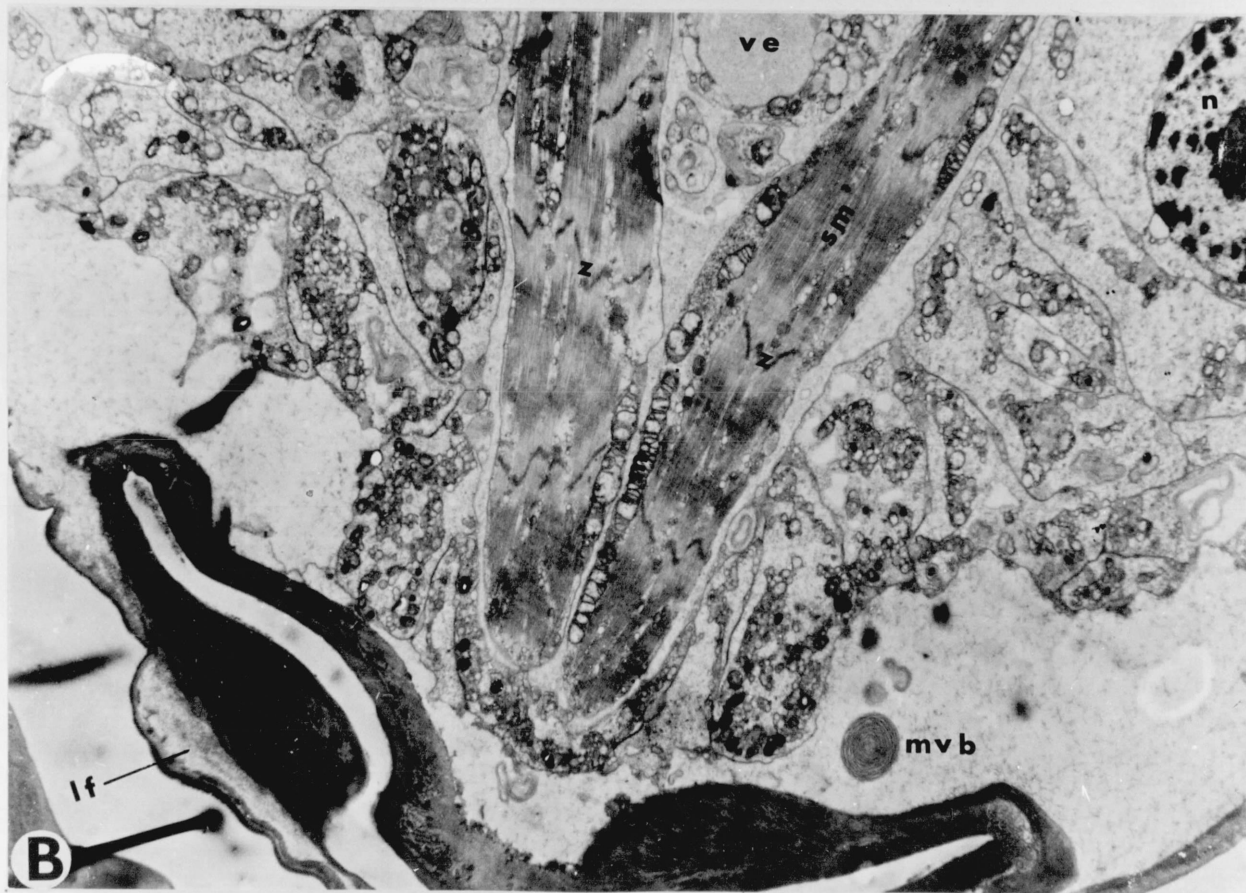
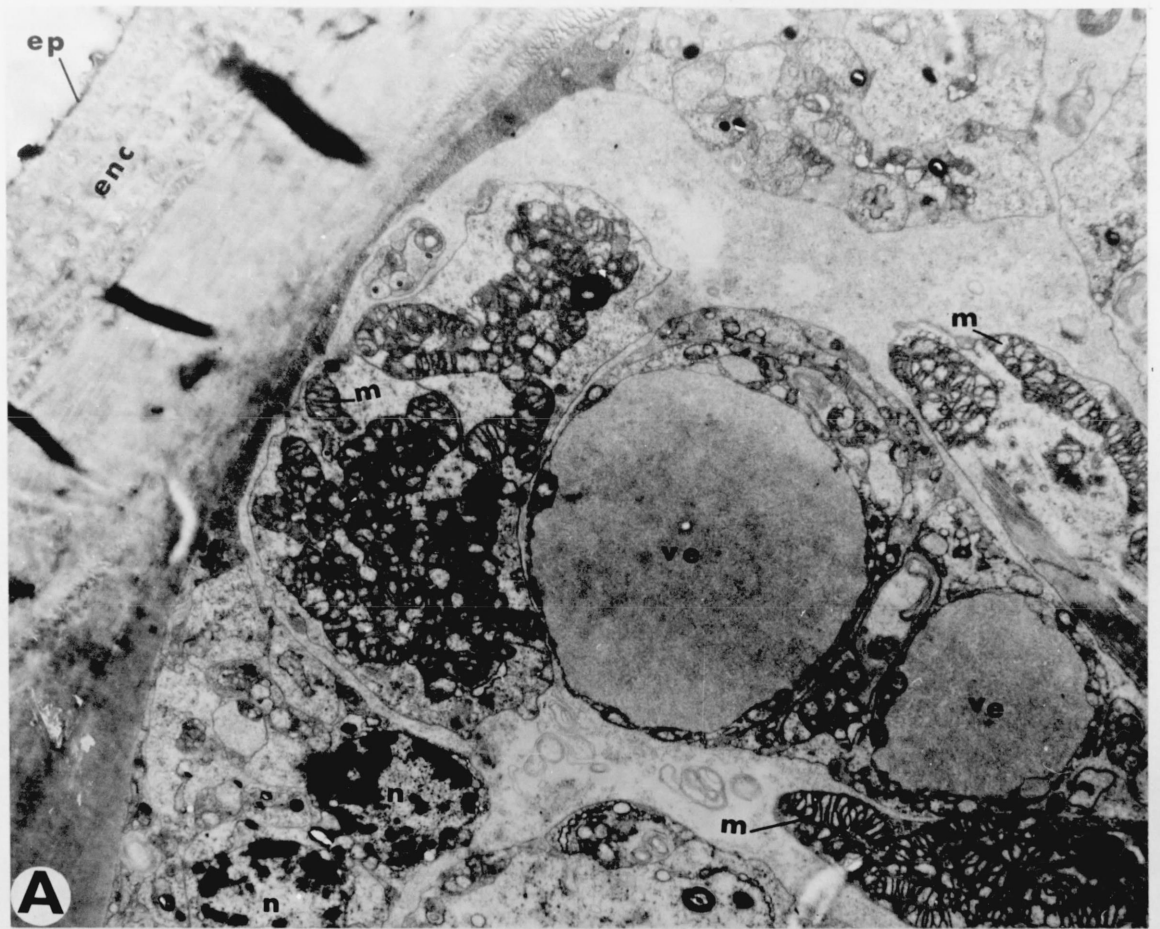
Plate 16: Ultrastructure of the epipharynx.

- A. Electron micrograph showing dorsal part of the epipharynx next to the labral surface, where the epicuticle (ep) and the lamellate endocuticle (enc) are clear. Large vesicles (ve) filled with a compound of unknown origin are profusely surrounded by mitochondria (m). The field shown also includes the nuclei (n).

X10,000

- B. The ventral part of the epipharynx. Two conspicuous bands of striated muscles (sm) comprise part of the epipharyngeal dilator muscles. The sarcomeres lack the precise transverse alignment shown by insect flight muscles, but contain dense Z-bands (z), A-bands and wide I-bands. Along the sides lie small mitochondria (m). The multivesicular body (mvb) contains concentrically arranged pairs of membranes. The lateral fold (lf) is separated from the rest of the epipharynx by thick hard cuticle.

X10,000



by insect flight muscles (Elder, 1975), yet the classical banding of the myofibrils is well evident. The sarcomeres have dense irregular Z-discs (Z), A-bands and I-bands. It has to be noted, however, that no M-lines can be recognized in the fibrils. At a resting length, the sarcomeres have a mean length of about 5 μ m, the A-band is about 3 μ m and the half I-band is about 1 μ m.

There is an almost continuous system of elongate mitochondria (m) on both sides of the sarcomeres. Part of the cell nucleus with electron-dense material, several multivesicular bodies (mnb) and vesicles (ve) constitute the most important components of the cell in the micrograph (Plate 16B). At the extreme ventral end of the epipharynx are the lateral folds (lf). These as well as the epipharyngeal tip are heavily chitinized. The lateral folds form the oral valve (ov) (Plate 17A, B) with a corresponding protrusion on the posterior end of the superlinguae (sli) of the hypopharynx.

7.3 The hypopharynx

The electron micrographs in Plate 18A, B, show longitudinal sections of parts of the lingua and superlinguae which form the hypopharynx. The anterior part is lined with two layers of cuticle; an outer electron-dense hard layer of epicuticle (ep), which is thickest at the tips of the superlingual arms (sla), and an inner layer of less dense pliable endocuticle (enc). On the lateral sides, the outer layer of hard cuticle is missing and the lingua is bound by a broad layer (3.5 μ m) of conspicuously lamellate

Plate 17: The structure of the oral valve.

- A. Micrograph of the oral valve at the posterior end of the cibarium (c) formed by the lateral fold (lf) of the epipharynx (ep) and the protrusion on the posterior end of the superlinguae. Arrow shows path of food to the pharynx. X10,000

- B. Enlargement of A showing the superlingual filaments anterior to the oral valve where the molar plates are situated. X15,000

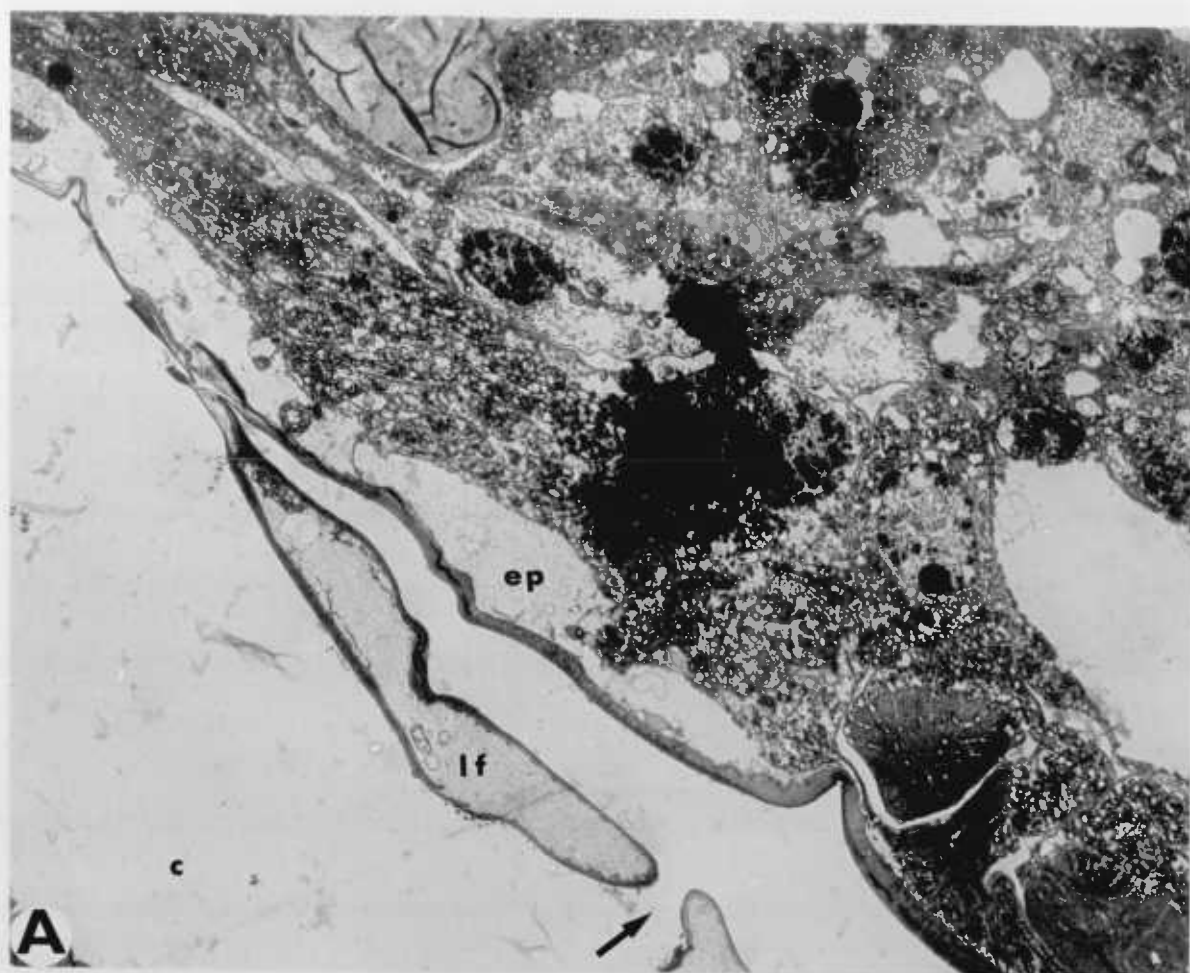


Plate 18: Ultrastructure of the hypopharynx.

- A. Electron micrograph of the hypopharynx at the level of the lingual cylindrical body (cb) which lumen shows cellular continuity with the cellular matrix of the lingua.

This is made of the components of an ordinary epidermal cell, with several multivesicular bodies (mvb). X10,000

- B. Lateral part of the hypopharynx showing lamellate cuticular thickening (enc) lining the cavity where the maxilla head (mh) fits when retracted. X10,000



endocuticle. The cuticular layer is broadest at the middle part where the lingua constricts to form the two lateral cavities in which the two maxilla heads (mh) fit when retracted inside the oral cavity (Plate 18 B). The stalks of the fulcrum attach themselves to the postero-lateral angles of the lingua through two cuticular rods which extend for some distance within the body of the lingua.

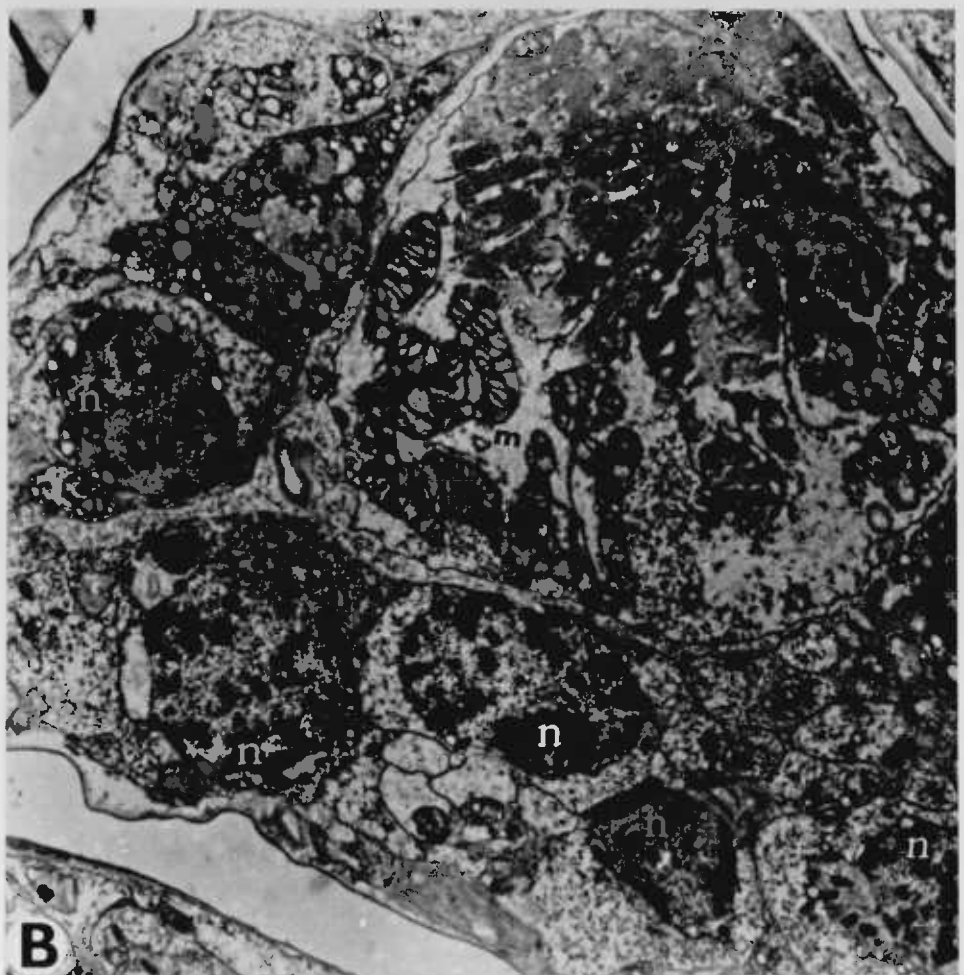
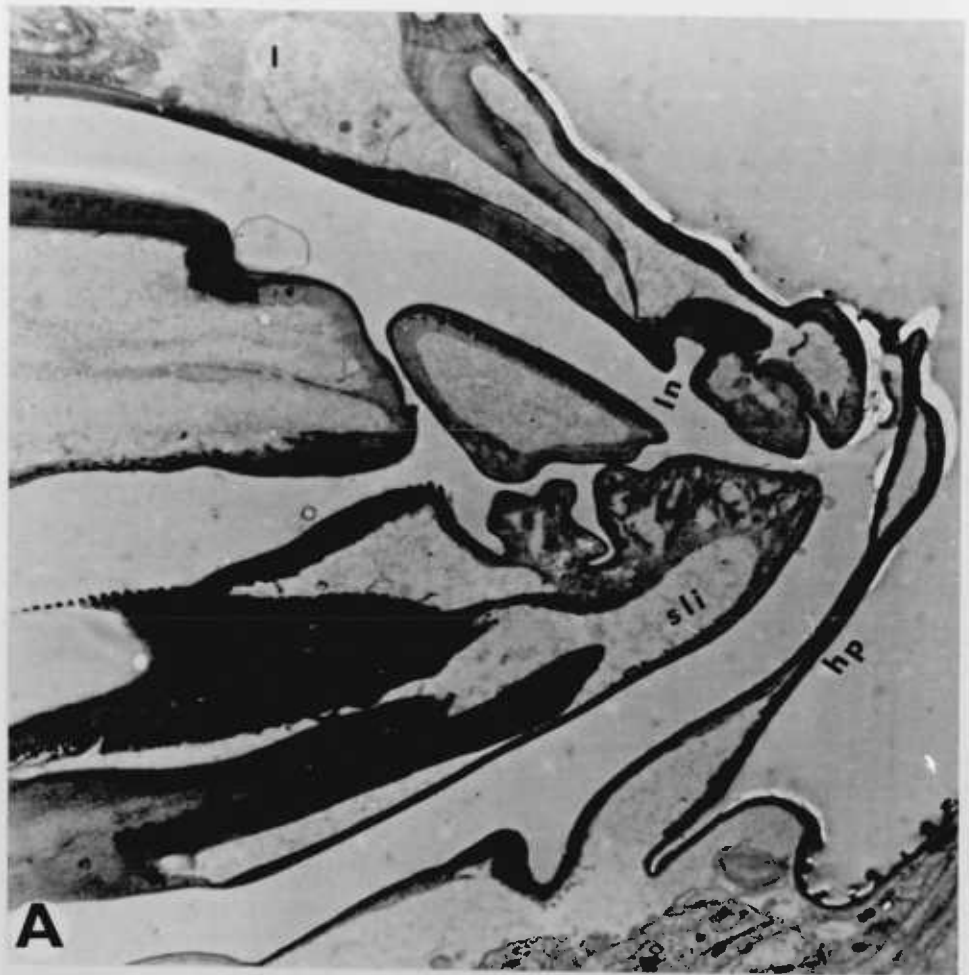
The general cellular organization of the lingua is similar to that of the epipharynx. The cytoplasm contains several multivesicular bodies (mvb), each with a single limiting membrane enclosing a number of vesicles. The anterior-most part of the lingua and superlinguae is devoid of the cellular components. The nuclei are unevenly distributed through the mass of the lingua, the anterior part being scarcely nucleated, while in the posterior part, the spherical nuclei (3 - 4 μ m in diameter) are found in clusters (Plate 19B). This zone of nuclear concentration at the posterior part of the hypopharynx corresponds to the regions of high enzymic activity revealed through histochemical analysis (Section 6).

At the antero -median part of the lingua and between the two superlingual arms (Plate 18A), there is an oval-shaped structure (cb), which constitutes a longitudinal section through the cylindrical body on the ventral side of the lingua (Plate 9C). The outer margin is bound by the two cuticular layers of epicuticle and endocuticle. The outer layer of epicuticle is thick on the lateral sides, but becomes narrower and forms an invagination at the tip of the

Plate 19: Anterior part of the oral region and ultrastructure of the posterior part of lingua.

- A. Electron micrograph of a longitudinal section through anterior part of the oral region. Note the labral notch (ln) where the anterior tip of the superlingua (sli) interlocks. The anterior part of the superlingua shows extensive vacuolation revealing a less rigid structure as expected. The hyaline plate (hp) covers part of the labrum (l). X3,000

- B. Ultra structure of the posterior part of the hypopharynx showing a cluster of nuclei (n) and numerous mitochondria (m). X6,000



structure. The inner layer of endocuticle extends posteriorly to form two arms or strands which delimit the gap to the rest of the lingua. The lumen of the hemispherical organ, like the anterior part of the lingua, is devoid of any cellular components and it forms a continuous surface with the general matrix.

The anterior part of the superlingua shows in longitudinal section (Plate 19A) a characteristic structure with extensive vacuolation of cytoplasm and dilation of intercellular spaces, reminiscent of the cortical layer found in the cuticle of soft bodied animals e.g. nematodes. From this structure, it seems that this part is not as rigid as it looks in scanning electron micrographs (Plate 8C). The posterior stalks are electron dense and seem to be fairly sclerotized and rigid.

The electron micrograph in Plate 20 reveals for the first time the presence of both longitudinal (lm) and transverse muscle (tm) in the hypopharynx of Collembola. A characteristic feature of the muscle fibres comprising the muscular layer is that each consists of a single myofibril. Three bands are distinct: A-, I- and Z-bands. Other bands are not discerned. The myofilament configuration within the A-band is very different from that described in insect flight and locomotory muscles (Ashhurst, 1968; Pringle, 1966). It has been noted in the visceral muscles of insects (Miller, 1975). The Z-bands characteristically follow an irregular course, each band comprising a number of electron-dense zones. Each myofibril contains only one nucleus (n), located laterally in the muscle cell. The sarcomere length is about 2.5 μ m and the I-band is

Plate 20: Electron micrograph of a longitudinal section
of the lingua, showing both transverse (tm)
and longitudinal muscles (lm) organization.

X9,000



relatively narrow (about 0.5 μ m wide) and is not so obvious as in skeletal muscles. It is possible that the features are correlated with the comparatively slow-acting properties of these muscles. The significance of the movement of the hypopharynx will be discussed in detail in Section 10 on the feeding mechanism.

7.4 The labial receptors

The labium bears a number of sensory hairs, or sensilla trichoidea, with a variety of forms. Three structurally different groups were recognized on the labial setigerous lobe (Figure 7A), namely, the apical setae, the differentiated setae and the side setae. There are three short setae on the fronto-median part of the hyaline plate which resemble the apical setae in their form. From a functional point of view, the main difference between different types of sensilla would seem to be their association with only one or with a number of neurones. It is generally assumed that sensilla with a single neuron function as mechanoreceptors, while those with more than one neuron are chemoreceptors (Lewis, 1970). No sensilla with porous cuticle, generally assumed to be a prerequisite for olfactory function, have been observed on the labium.

7.4.1 The mechanoreceptors

A sensillum is derived from one epidermal cell which divides to form the trichogen, tormogen, neurilemma, and nerve cell. The fully differentiated sensillum consists of (a) cuticular components,

(b) sensory neuron, and (c) sheath cells. The mechanosensitive sensilla will be described in this order.

The mechanosensitive hair sensilla on the labium are the long, slender, acuminate side setae with serrated margins. In these sensilla, the wall of the hair is thick and the lumen, which is filled with an amorphous electron-dense material, is restricted (Plate 21A; Figure 11). The hair is drawn to a sharp tip without a pore at the top. At the point of articulation, the cuticle immediately surrounding the socket (hcs) is electron-dense, suggesting that it is very hard. This same hard cuticle appears to form the suspending rods, which are common to all the labial hair sensilla. A constraint on the movement of the seta is imposed by a pivot (p) formed by a tip of the socket. This seems to indicate a restriction of movement of the hair in the direction of the secondary mouth opening.

The distal nerve fibres of the dendrite are enclosed within a thin electron-dense dendritic sheath (ds) (also known as scolopoid sheath or scolopale) as they proceed from the neuron to the setal base. The sheath (Plate 21A) is a cuticular tube that is coextensive with the thick-walled lumen of the hair (Peters, 1965) and it forms the connection between the movable hair shaft and the dendritic terminal. It appears similar to that enclosing the nerve fibres of other insect receptors in being very thin and of irregular outline for the greater part of its length (Larsen, 1963; Thurm, 1964, 1968; McIver, 1975; Gnatzy and Tautz, 1977). Sturckow (1964) suggested that the dendritic sheath may serve as mechanical and physiological protection for the

Plate 21: Ultrastructure of the labial mechanosensitive hairs.

A. Articulation of a labial mechanoreceptor showing the insertion of the tubular body (tb) in the hair shaft (hs) by a slender cuticular rod. X6,000
ds, dendrite sheath; enc, endo-cuticle;
ep, epicuticle; exc, extracellular cavity;
hcs, socket; p, pivot; ss, socket septum.

B. Enlargement of the sensory ending of a labial mechanoreceptor. Note the periodicity of the tubular body (tb) and the invagination on the dendrite sheath (arrow). X30,000
ec, electron-lucid cap; exc, extracellular cavity; ss, socket septum.

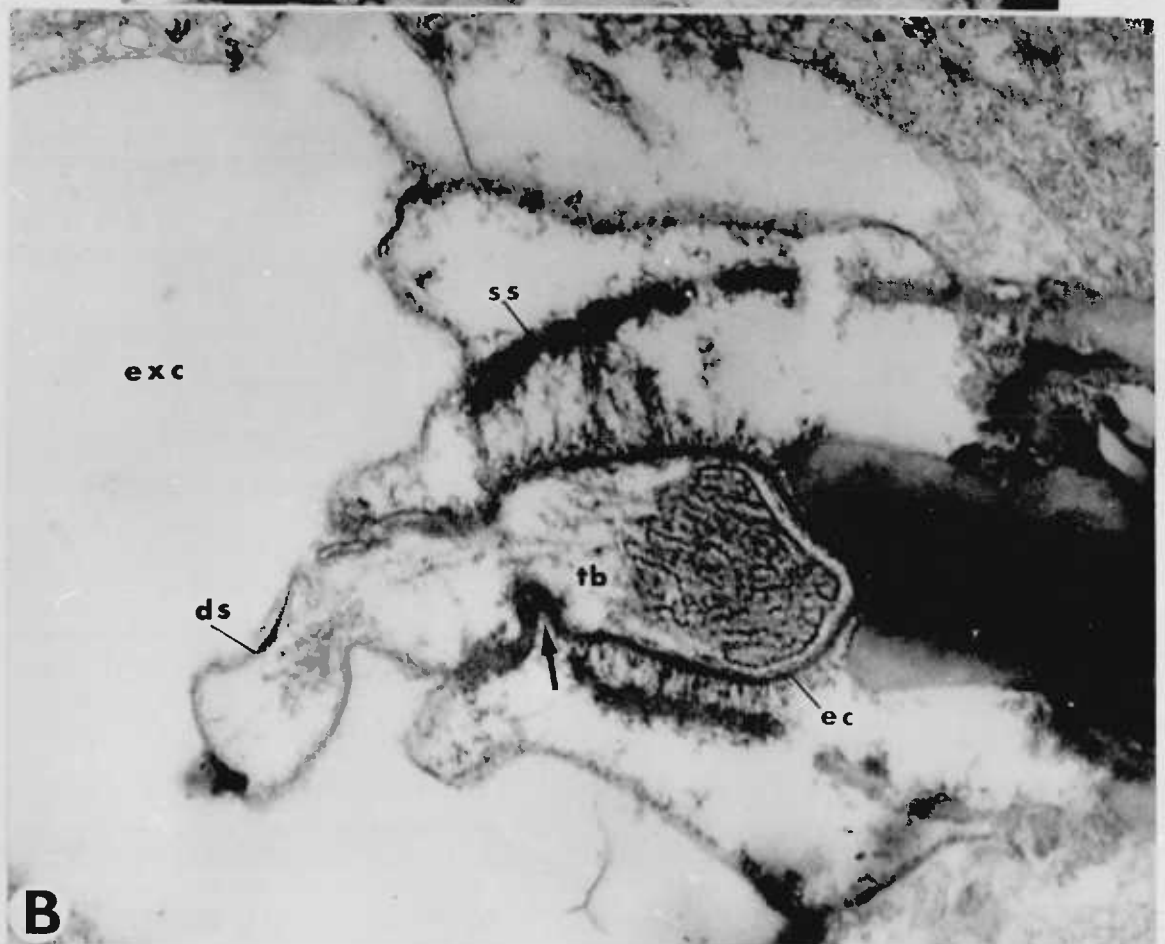
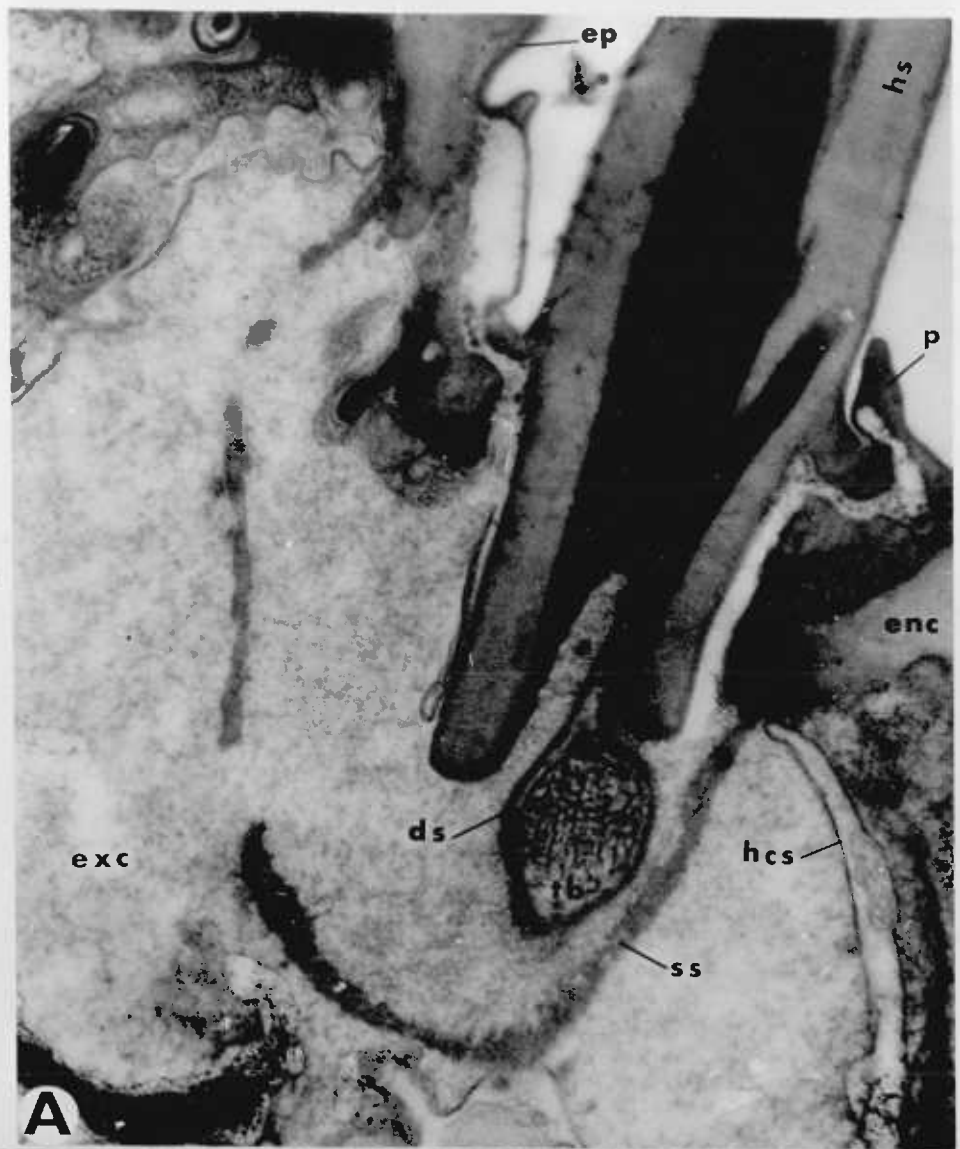


Figure 11 Articulation of the two types of labial sensilla.

A. Articulation of a mechanosensitive hair sensillum.

B. Articulation of a chemosensitive hair sensillum.

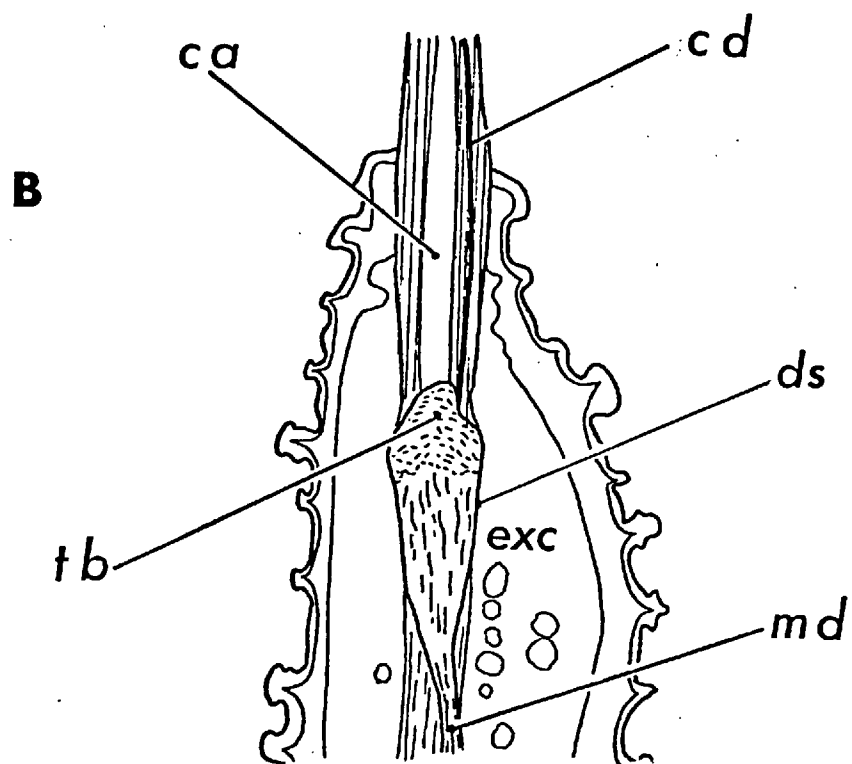
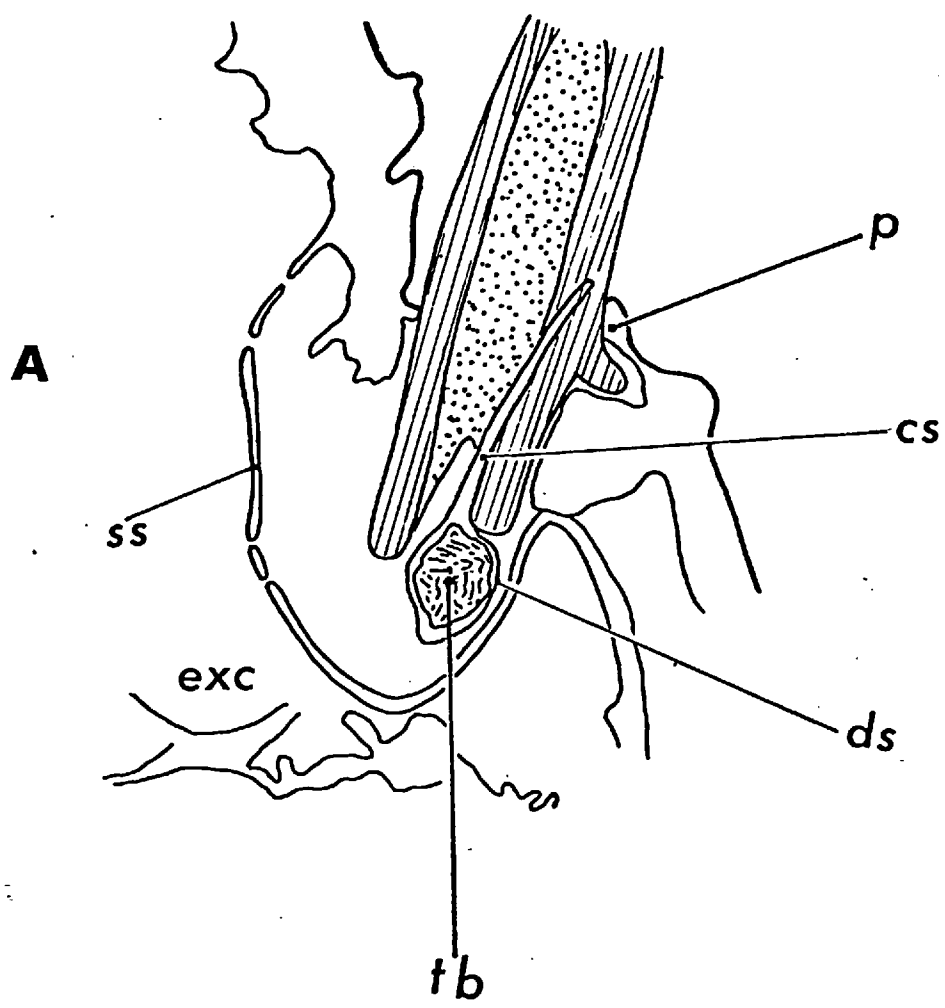
ca, hair shaft lumen; cd, chemoreceptor dendrite;

cs, chitinous shaft; ds, dendrite sheath;

exc, extra cellular cavity; md, mechanoreceptor

dendrite; p, pivot; ss, socket septum;

tb, tubular body.



nerve fibres. The scolopale invaginates into the wall of the hair by a slender shaft of cuticle some distance from the base (Plate 21A). A similar insertion was reported in Gryllus Linnaeus, 1758 (Gnatzy and Schmidt, 1971) and Periplaneta Burmeister, 1838 (Gnatzy, 1976). An extracellular cavity or vacuole (exc), which is surrounded by the trichogen cell, evidently corresponds to the "receptorlymph cavity" of Nicklaus et al. (1967). Hanging into the cavity from the cuticle of the socket is an electron-dense curtain or septum (ss) of what is believed to be cuticle. This "socket septum" (Gaffal et al., 1975) attaches closely to the dendritic sheath (Plate 21B). It is, presumably secreted by the tormogen cell as it withdraws from the socket, according to Blaney and Chapman (1969).

The sensory neurone is composed of an axon, cell body and dendrite. The cell body is typical of bipolar neurones, with a large, round nucleus lying rather far from the sensilla insertion site, and a relatively small margin of cytoplasm. The dendrite passes from the neuron cell, up the scolopale to end at the base of the hair. It undergoes, as in other sensilla (Dallai, 1971), a constriction of ciliary structure soon after it leaves the cell body. This constriction marks the region of transition between the relatively broad inner (proximal, Sinnesfortsatz) segment and the finer outer (distal, Sinnescilium) segment (Zacharuk et al., 1971).

At the distal end of the outer dendritic segment and just below the hair base, there is a characteristic terminal structure consisting of parallel neurotubules closely packed together in an electron-dense matrix (Plate 21B). This enlarged tubular body

(tb) is characteristic of the ends of mechanoreceptive dendrites associated with tactile hairs (Thurm, 1964, 1965). It governs the elastic properties of the terminal process, which in turn contributes to the directional sensitivity of the sensillum. In the tubular body of labial mechanoreceptors, far more than 100 microtubules are interconnected by dense material and surrounded by a smooth membrane. There is always an electron-lucid gap of $0.1\mu\text{m}$ between the dendrite membrane and the tubular body. Proximal to the tubular body, a sharp invagination of the cuticular sheath projects into the dendrite. This invagination could prevent a downward movement of the dendrite during the stimulation of the sensory hair (Slifer, 1961).

The neuron cell is surrounded by a neurilemma cell (thecogen cell of Steinbrecht and Muller, 1976) which lies within the trichogen cell, which in turn lies within the tormogen cell (Dethier, 1977). The nucleus of the tormogen cell is commonly near the basement membrane of the epidermis, while that of the trichogen cell is near the base of the dendritic sheath. Proximally, the neuron cell gives rise to the axon, which extends uninterrupted to the suboesophageal ganglion.

The characteristics of the articulation and innervation, such as the thick walls of the hair shaft and the presence of the tubular body, suggest strongly a mechanoreceptive function of this type of sensilla. On the other hand, it has been shown during the course of this study through electrophysiological techniques (Section 9.2) that this type of hair acts as tactile mechanoreceptors.

7.4.2 The contact chemoreceptors

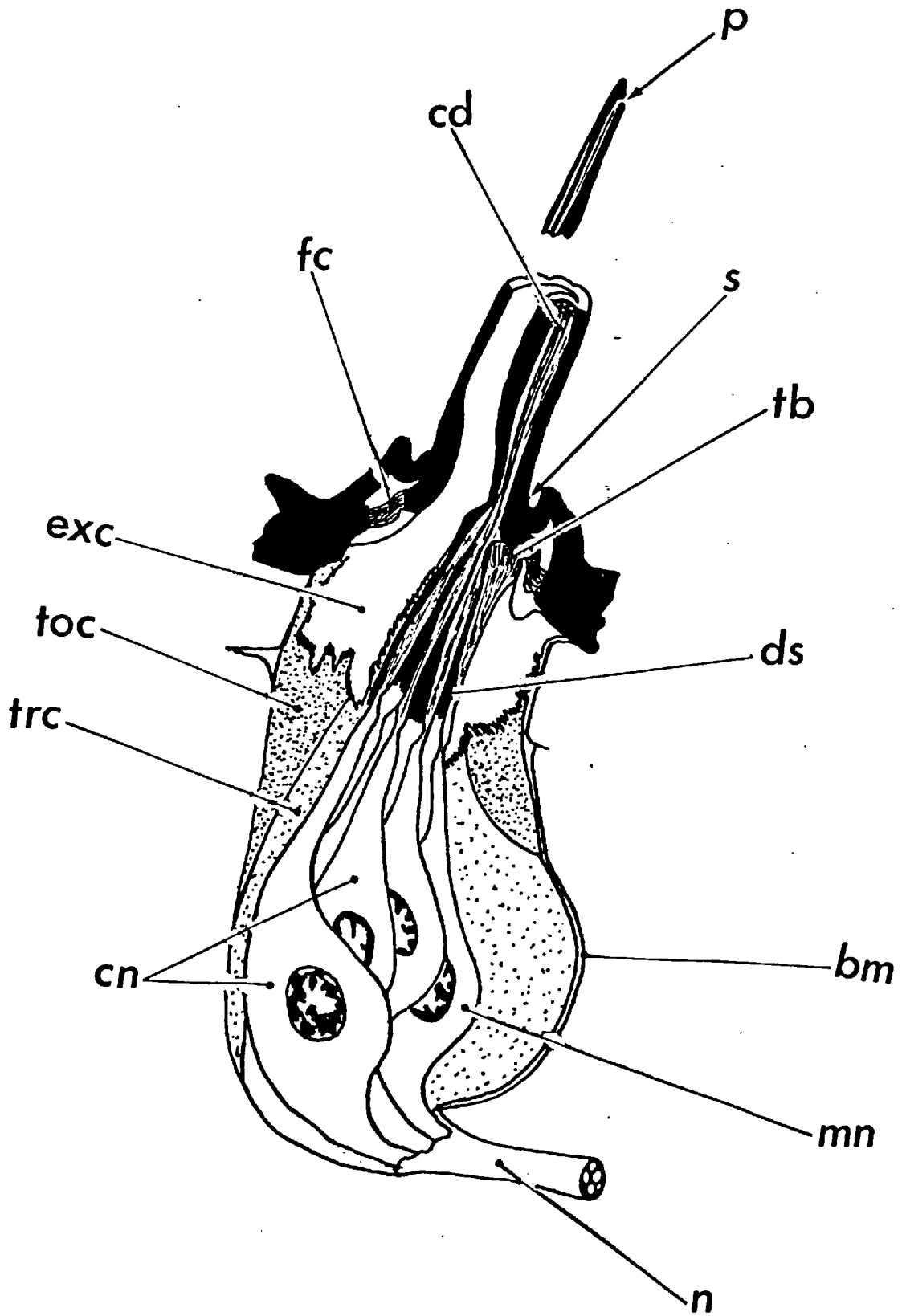
The chemosensitive hair sensilla on the labium include the apical and differentiated setae of the setigerous lobe and the setae on the hyaline plate. Compared to the tactile hair sensilla, they are relatively short (about 10 μ m) and they insert deep in the cuticle. Each is attached to the cuticle by a thin flexible cuticular collar and has no obviously oriented articulation. The surface of the hair is smooth or with regularly parallel annulations (Plate 4E). Typically, the hair has a blunt tip, perforated by a single large pore and the lumen is divided longitudinally into two compartments. The terminal pore closely resembles that in chemosensory hairs in other insects, e.g. Adams et al. (1965) and Sturckow et al. (1967, 1973).

Figure 12 shows a composite picture from serial sections for the details of the structure of a labial chemosensory hair. The sensillum is innervated by four sensory cells, which lie some distance from the hair base. All four neurons together with the trichogen and tormogen constitute a subhypodermal sac hanging below the hair. The sac is lined with a membrane that is continuous with the basement membrane of the epidermis and the neurilemma surrounding the neurons. The dendrites, each separated by a membrane, form a tight bundle, held together by septate desmosomes. They run through an extracellular space which is bound by a thin layer of electron-dense material and similar to that found in the mechanoreceptive sensilla. The dendrites then run into the papilla body in a canal of 0.7 μ m diameter delimited by the thin

Figure 12 Details of the structure of a chemosensory hair

(Redrawn from Dethier, 1977).

bm, basement membrane; cd, chemosensory dendrite;
cn, chemoreceptor neurons; ds, dendrite sheath;
exc, extracellular cavity; fc, fibrous cap;
mn, mechanoreceptor neuron; n, neurilemma;
p, pore; s, socket; toc, tormogen cell;
trc, trichogen cell.



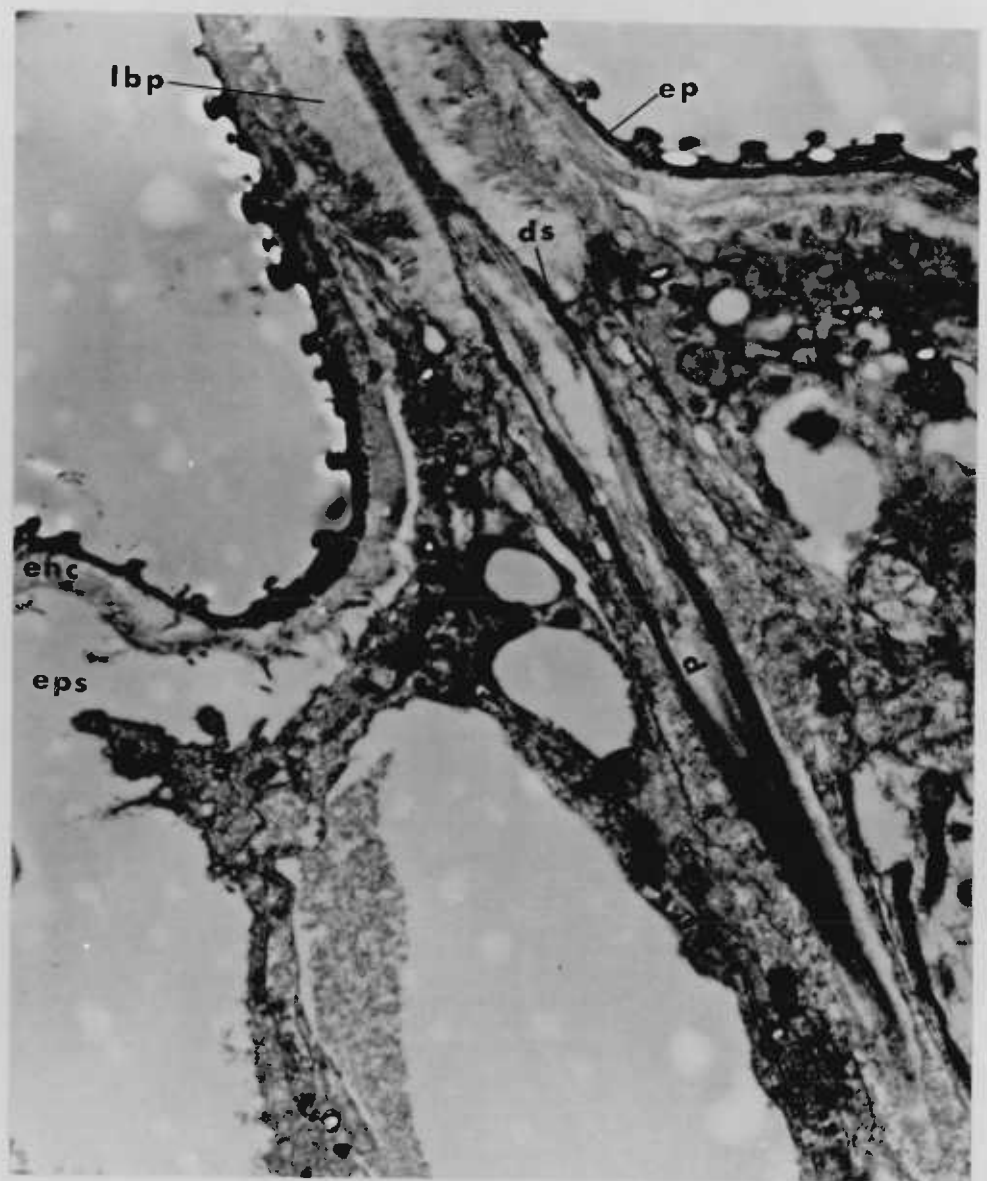
cuticular scolopoid sheath (Plate 22). Before entering the scolopoid sheath, the dendrites constrict, as in mechanoreceptive sensilla, to form a ciliary region.

As the base of the hair is approached, one of the dendrites becomes enlarged with densely packed microtubules and form a conspicuous tubular body (tb) characteristic of the ends of mechanoreceptive dendrites. This dendrite terminates in a complex electron-dense structure close to the internal wall of the hair shaft. The remaining three dendrites enter the hair circular cavity, where the dendritic sheath gradually merges with the thick-walled lumen in the shaft and the dendrites proceed up the hair, where they eventually terminate immediately below the apical pore. Although the apical opening of the sensillum has been resolved by the scanning electron microscope, yet the actual exposure of the dendrites to the environment could not be detected. No "viscous substance" was observed extruding from the tips of the hairs as has been noted by Sturkow (1967) in Stomoxys Geoffroy, 1762.

The general account of the ultrastructure of this type of sensilla agrees with the descriptions of contact chemoreceptors of other insects given by other workers (Adams et al., 1965; Blaney and Chapman, 1969; Slifer, 1970; Eisenbeis, 1976; Dethier, 1977). Electrophysiological recordings from single hairs of this type (Section 9) provide the evidence that these sensilla act both as mechanoreceptors and contact chemoreceptors.

Plate 22: Electron micrograph of a longitudinal section of a labial papilla (lbp), showing the microtubule bundle (d) derived from the cilium fibres, as it penetrates the body of the papilla. (X18,000)

ds, dendrite sheath; enc, endocuticle;
ep, epicuticle; eps, epidermis.



8. BEHAVIOURAL RESPONSE TO CHEMICALS

The response of individuals of Entomobrya nivalis to contact chemical stimuli was purely orthokinetic, which was indicated by the cessation of random movement when favourable chemicals were encountered, with the result that individuals would tend to aggregate in the area presenting optimal conditions. Because of the absence of a suitable method for estimating the feeding response, the behavioural reaction to various chemicals was measured in terms of the effect of the test substances on the distribution of a population of Collembola.

The distribution of the animals in the first multi-choice experiment of six test substances is given in Table 1 and the means are represented graphically in Figure 13. The results disclose that sodium chloride solution had a significant effect ($p < 0.01$) on the orientation behaviour of E. nivalis. When the animals, moving at random in the arena, ultimately came in contact with the discs impregnated with sodium chloride solution, they ceased all locomotory activity. Thus, over a period of time, aggregations were formed. No attractant properties were shown by the sodium chloride discs, as the animals did not move directly toward the source of stimulation; but having arrived there by chance, they stopped. By eliciting this kind of response, sodium chloride solution can be called an "arrestant" according to the definition of Dethier and Kuch (1971). The highest response occurred in the first hours of the experiments, with the number of animals arrested decreasing progressively with

Table 1. Total numbers of E. nivalis aggregated on filter paper discs impregnated with various substances during a period of 16 hours as compared to water. (Experiment repeated twice).

R e p l i c a t e s

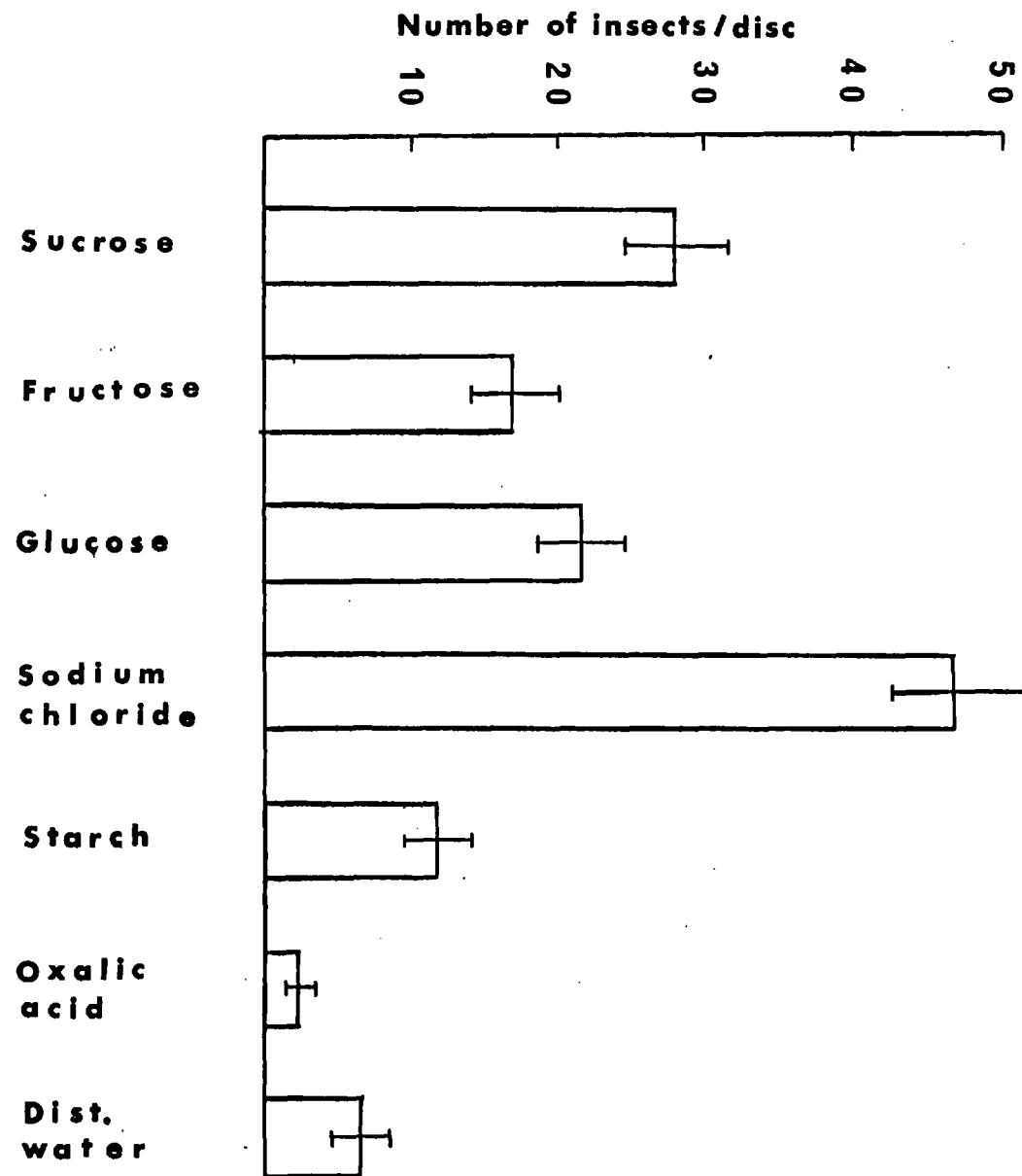
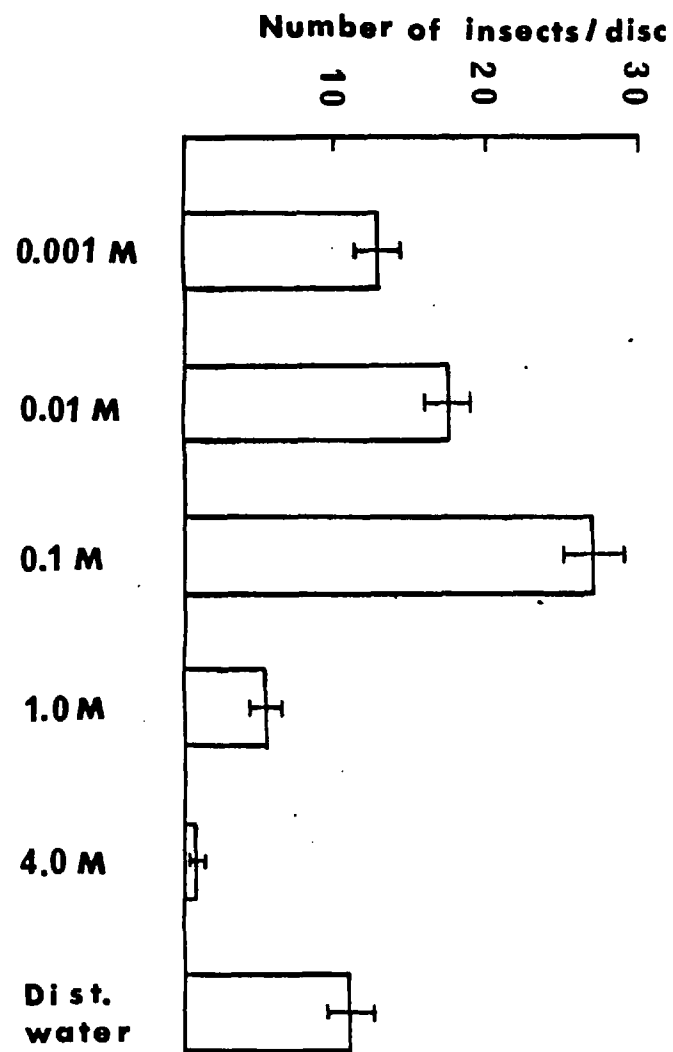
Test substance	1	2	3	4	5	6	7	8	9	10	Mean ± S.E.
Sucrose (0.2M)	34	21	23	21	37	27	23	37	31	22	27.80* ±0.61
	31	27	45	18	31	24	22	38	17	27	
Fructose (0.2M)	13	25	9	15	21	22	9	14	19	13	17.10* ±0.55
	25	24	12	10	20	22	23	6	24	16	
Glucose (0.2M)	25	14	24	29	19	32	16	11	23	18	22.35* ±0.58
	28	31	13	21	30	13	26	19	27	28	
Sodium chloride (0.2M)	39	52	51	49	51	35	48	50	53	49	46.75* ±0.68
	40	29	42	52	56	37	41	42	46	73	
Starch (0.2M)	5	10	17	15	17	10	16	5	3	17	11.85** ±0.50
	12	13	18	16	13	10	15	6	16	4	
Oxalic acid (0.2M)	3	3	1	2	0	2	2	3	4	3	2.45** ±0.29
	4	0	4	7	1	0	3	2	4	1	
Distilled water	10	7	5	6	13	7	4	11	6	5	6.70 ±0.38
	6	8	6	4	5	3	8	12	5	3	

* $p < 0.01$

** Not significant

Figure 13 Response of Entomobrya nivalis to various substances including carbohydrates.

Figure 14 Response of Entomobrya nivalis to different concentrations of sodium chloride.



time. This may be an evidence of the adaptation of the insects, correlated with a rising threshold to the chemostimulation. During their stay on the discs, the animals did not appear to attempt imbibing the test solution through the mouth; yet the vesicles of the ventral tube in some animals were everted sufficiently to bring them in contact with the moistened filter paper.

The three tested sugars, namely, sucrose, glucose and fructose, elicited a significant response ($p < 0.01$) compared to distilled water, as well as showing significant variation ($p < 0.01$) between each other. Sucrose evoked the highest response, followed by glucose and then fructose. On the other hand, although starch impregnated discs seemed to draw out some response later on during the experiment, they did not show a significant difference in aggregation from the control discs. Very few animals occurred on discs treated with oxalic acid, but again the negative response of the animals was not significant.

The second behaviour experiment revealed that the aggregation response increased with increasing sodium chloride concentration from 0.001M to 0.1M and declined between 1.0M and 4.0M (Table 2; Figure 14). The p -values (based on Chi-square test) show that in all but two concentrations (0.1M; 4.0M), the differences were not significant i.e., the variation in choice was so great that any differences between the means were negated.

In the third multi-choice experiment, when the insects were offered five different salts of the same concentration, they showed a positive response towards all of them (Table 3; Figure 15).

Table 2. Total numbers of *E. nivalis* aggregated on filter paper discs impregnated with different concentrations of sodium chloride solution during a period of 16 hours as compared to water. (Experiment repeated twice.)

R e p l i c a t e s

Test substance	1	2	3	4	5	6	7	8	9	10	Mean ±S.E.
0.001M Sodium chloride	11	15	9	13	11	9	17	13	17	10	13.10** ±0.40
	13	12	15	18	9	19	13	10	17	11	
0.01M Sodium chloride	17	15	18	21	23	18	19	21	24	20	17.65** ±0.41
	12	13	16	21	17	19	18	13	13	15	
0.1M Sodium chloride	31	25	32	21	33	29	28	25	26	35	27.20* ±0.47
	27	31	23	19	23	28	29	21	26	32	
1 M Sodium chloride	7	5	7	4	6	5	7	5	8	7	5.70** ±0.27
	6	4	7	5	6	3	6	8	5	3	
4 M Sodium chloride	0	0	1	0	1	2	0	1	0	1	0.85* ±0.21
	1	1	1	2	1	1	0	2	1	1	
Distilled water	16	9	11	10	7	14	13	18	8	11	11.25 ±0.39
	15	10	11	7	11	11	8	9	14	12	

* $p < 0.05$

** Not significant

Table 3. Total numbers of E. nivalis aggregated on filter paper discs impregnated with different salts solutions during a period of 16 hours as compared to water.
(Experiment repeated twice.)

R e p l i c a t e s

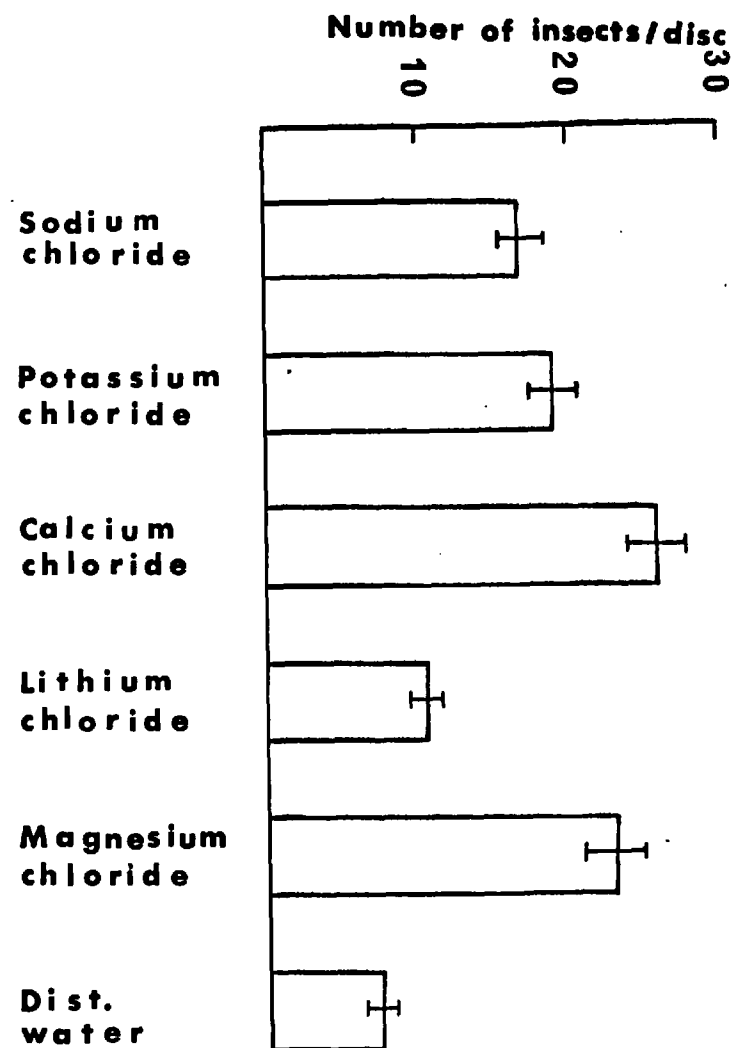
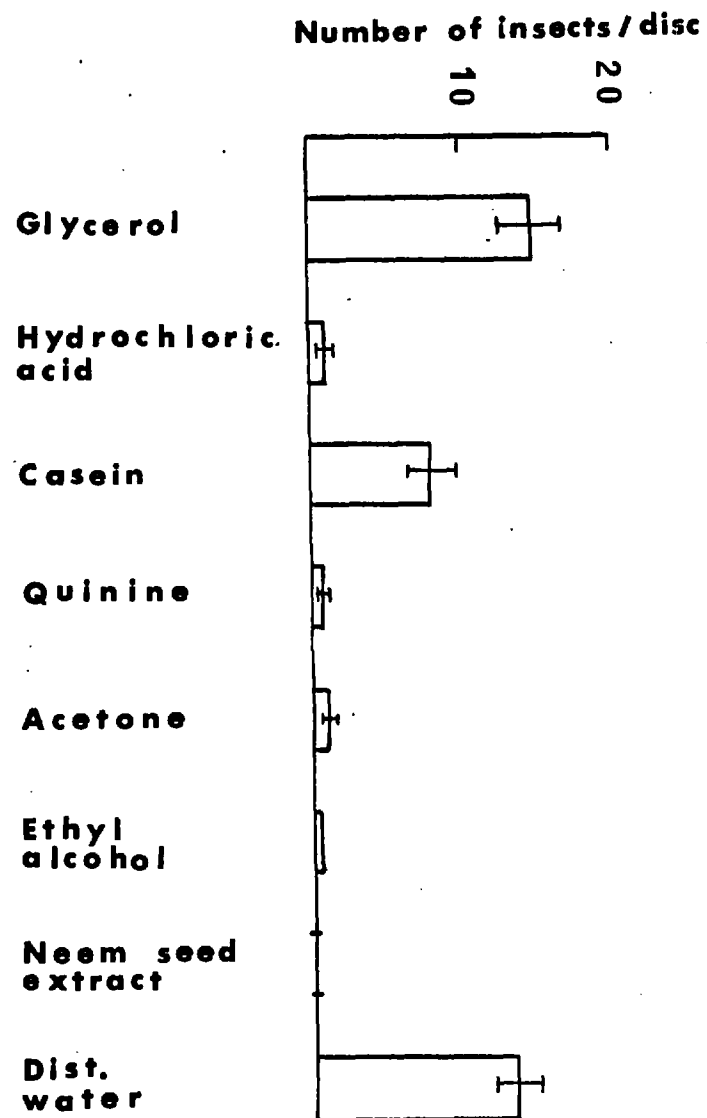
Test substance	1	2	3	4	5	6	7	8	9	10	Mean ±S.E.
Sodium chloride (0.2M)	15	19	18	21	14	21	18	16	13	20	16.85* ±0.42
	13	15	14	18	22	17	15	10	14	24	
Potassium chloride (0.2M)	20	15	26	16	20	23	19	13	16	20	19.85* ±0.43
	19	25	15	21	16	21	24	17	18	23	
Calcium chloride (0.2M)	29	27	26	30	27	21	30	19	23	29	25.85* ±0.46
	26	26	25	28	25	27	31	18	18	32	
Lithium chloride (0.2M)	11	15	7	8	11	13	14	10	9	15	10.90** ±0.34
	9	11	8	13	10	12	10	11	11	10	
Magnesium chloride (0.2M)	21	15	19	20	31	17	29	21	24	27	23.00* ±0.49
	23	19	26	33	24	18	24	19	26	24	
Distilled water	7	8	6	10	9	7	5	7	8	11	7.45 ±0.35
	10	3	11	4	10	5	7	4	8	9	

* $p < 0.05$

** Not significant

Figure 15 Response of Entomobrya nivalis to different salts.

Figure 16 Response of Entomobrya nivalis to various substances including a deterrent.



Calcium chloride and magnesium chloride elicited the highest response followed by potassium chloride, sodium chloride and then lithium chloride.

Of the seven various substances tested in the fourth experiment, only glycerol evoked a slightly positive response, with the animals showing variable degrees of avoidance to the other substances (Table 4; Figure 16). The neem seed extract was completely avoided by the animals.

In all experiments, there was no evidence that the insects imparted to the discs any substance that might have influenced the visits by other animals. Furthermore, there was no indication to whether the animals arrested on a certain disc were the ones which had been there before. The sex ratio of the test animals did not seem to affect the orientation response.

Table 4. Total numbers of E. nivalis aggregated on filter paper discs impregnated with various substances during a period of 16 hours as compared to water. (Experiment repeated twice)

R e p l i c a t e s

Test substance	1	2	3	4	5	6	7	8	9	10	Mean ±S.E.
Glycerol (0.2M)	19	18	21	17	14	13	9	16	21	18	14.45**
	14	7	8	16	18	10	15	14	10	11	±0.46
Hydrochloric acid (0.2M)	0	0	1	2	1	1	2	2	1	2	1.30*
	2	2	1	1	1	2	1	1	1	2	±0.18
Casein (0.2M)	5	12	6	14	5	9	6	16	13	7	8.35**
	5	6	11	6	12	7	3	9	6	9	±0.42
Quinine hydro- chloride (0.2M)	1	0	0	1	0	1	0	1	2	0	0.40*
	0	1	0	0	1	0	0	0	0	0	±0.17
Acetone (0.2M)	0	1	1	1	1	0	1	2	1	1	0.95*
	1	2	1	0	1	1	0	1	2	1	±0.17
Ethyl alcohol (0.2M)	0	0	1	0	0	0	1	1	0	1	0.45*
	1	0	0	0	1	0	1	1	0	1	±0.16
Neem seed extract (1%)	0	0	0	0	0	0	0	0	0	0	0*
	0	0	0	0	0	0	0	0	0	0	
Distilled water	15	8	16	13	9	11	14	17	16	8	13.30
	11	9	19	17	16	15	16	13	11	12	±0.41

* $p < 0.05$

** Not significant

9. ELECTROPHYSIOLOGICAL RESPONSES OF THE LABIAL CHEMORECEPTORS

9.1 General considerations

Neural responses to chemical stimulation were clearly detected in the apical and differentiated setae of the labial setigerous lobe. On the other hand, no such responses could be obtained from any of the side setae.

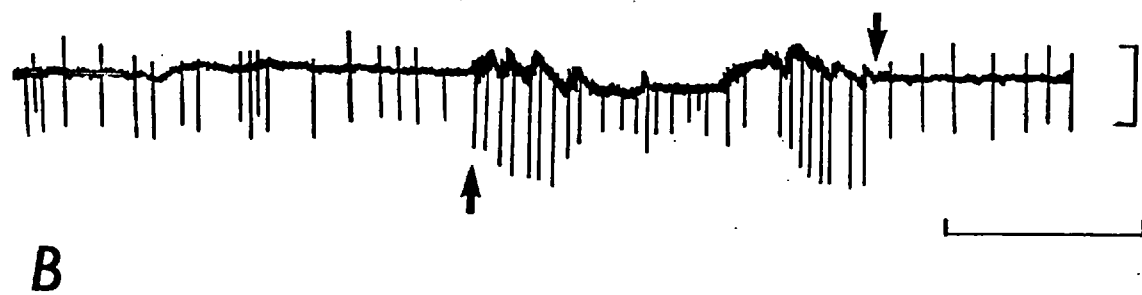
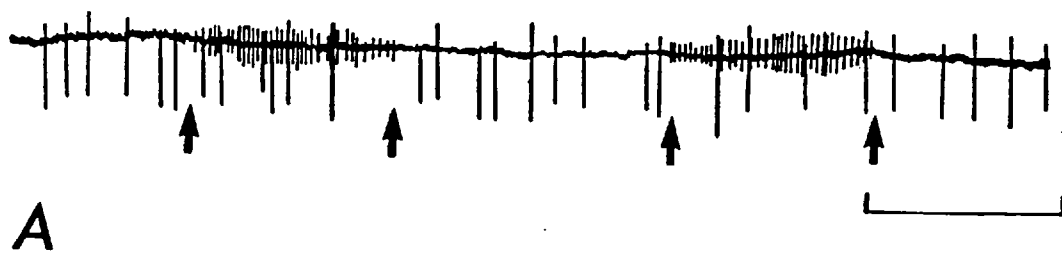
In some instances, when the stimulating electrode was placed over a sensillum, no spikes were recorded. However, after very gentle manipulation of the sensillum with the tip of the capillary, the sensillum starts to respond. This response was not a mechanoreceptor response, since distinct mechanoreceptor responses (Figure 17) were only produced by more vigorous movement of the sensillum and were of greater amplitude.

A frequent difficulty encountered during electrical recording was caused by the attempted movements by the insect, which were accompanied by volleys of impulses due to motor neurons firing. This produced a burst of low amplitude spikes (Figure 17) and a recognizable sound on the audio monitor. When this occurred, the record was not used for analysis and any insect having a tendency to be active was discarded.

In stimulus-response studies, the number of impulses in the first second was used to avoid the adaptation effect shown by the sensilla. Counts of impulses were made after 0.1 second from the onset of chemical stimulation as the exact time which the stimulus solution touched the chemoreceptor was always marked by an artifact known as the "on" artifact (Hodgson, 1957).

Figure 17

- A. Recording of attempted movements by the insect (between arrows) superimposed on the response by the sensillum to 0.1 M sodium chloride. Horizontal bar shows 0.5 second.
- B. Trace of response of a labial apical sensillum to mechanical stimulation (bending to 45°). Horizontal bar shows 0.5 second; vertical bar 0.1 m volt.



9.2 Mechanoreception

In order to record from the mechanoreceptor neuron whose dendrite terminates at the base of the hair (Section 7.4.2), sensilla which were already being stimulated by 0.1M solution of sodium chloride were displaced laterally by the stimulating capillary. The response consisted of a burst of impulses of the phasic type with a considerable greater amplitude than that of impulses caused by chemical stimulation (Figure 17). Bending the seta to about 45° from resting position, gives the highest rate of firing and if the sensillum is kept in this position, an adaptation rapidly occurs, followed by a steady phase which continues in some setae for some time. Other mechanoreceptors adapted completely to zero in less than a few seconds.

9.3 Response to 0.1M sodium chloride

When labial apical sensilla were stimulated with a 0.1M sodium chloride solution, they showed a phasic-tonic response typical of insect chemoreceptors (Figure 18). Usually there would be a volley of spikes generated within a few milliseconds, followed by a rapid decay in frequency for a fraction of a second, until a relatively stable plateau would be reached. Although the spike size and frequency varied somewhat with each stimulation (Table 5), the general characteristics of the discharge were the same.

Figure 18 Representative responses of labial apical sensilla
to stimulation with 0.1 M sodium chloride.
Horizontal bar shows 0.5 second; vertical
bar 0.1 m volt.

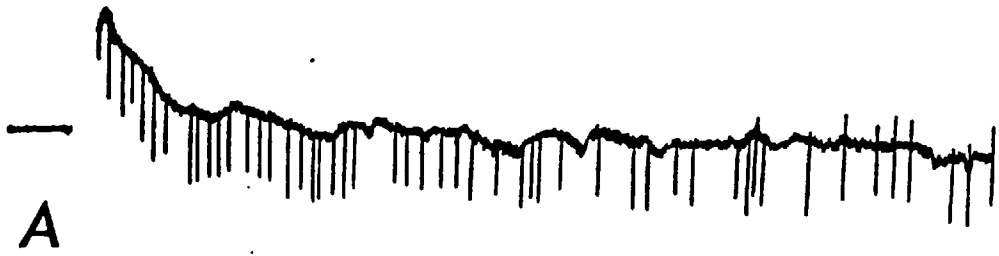


Table 5: Variability of response of five sensilla to stimulation by 0.1M sodium chloride solution in the first five seconds.

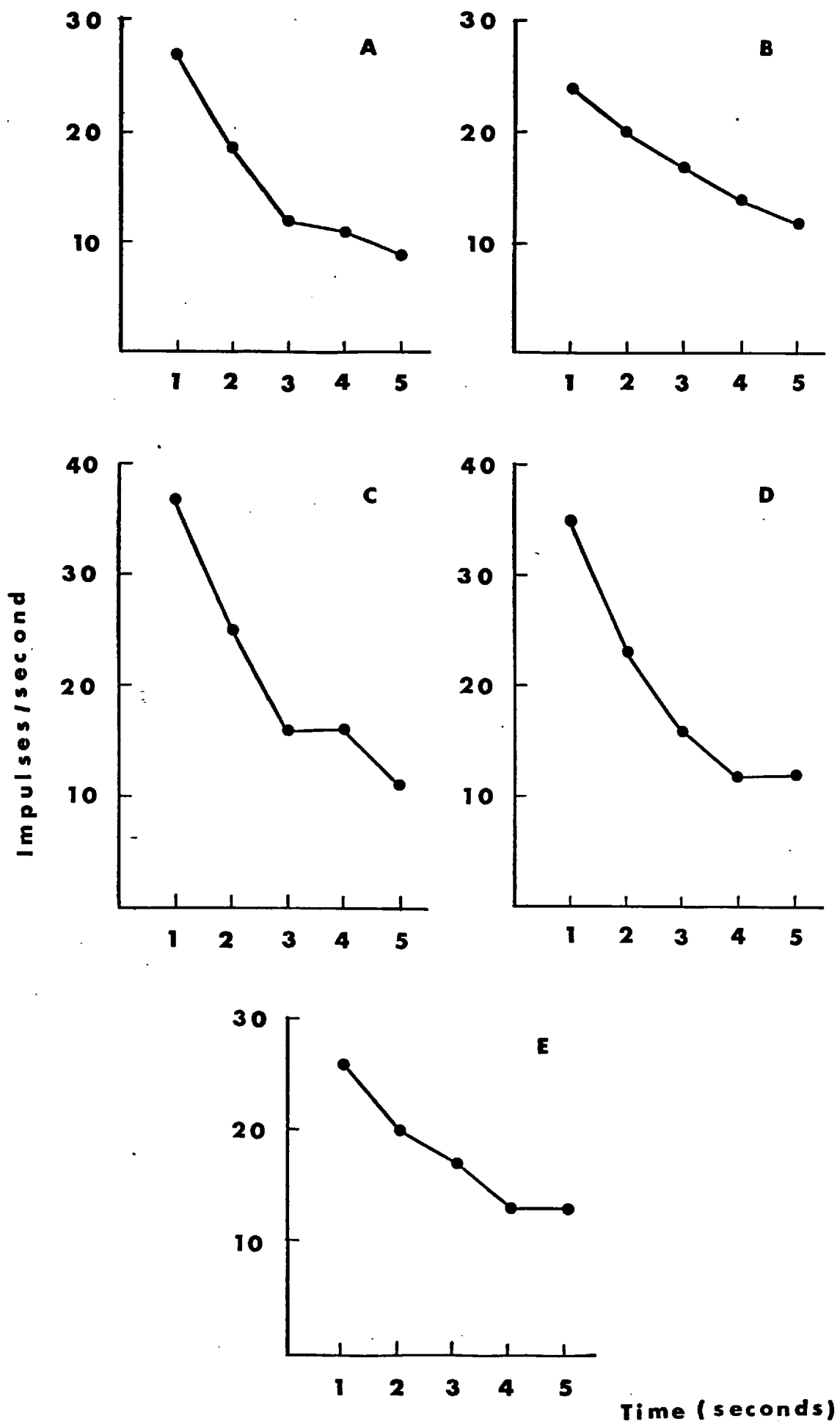
Sensillum	Number of seconds				
	1	2	3	4	5
1	27	18	12	11	9
2	24	20	17	14	12
3	37	25	16	16	11
4	35	23	16	12	12
5	26	20	17	13	13

Receptor cells in the sensilla tested exhibited rapid adaptation, dropping from a high frequency discharge to a much lower steady discharge frequency within 3 to 4 seconds after the onset of chemical stimulation (Figure 19). More than one amplitude category of spikes was recognizable, each series clearly originating in a single neuron. Unusually large impulses occurring infrequently appear to result from consequent summation of two or more small impulses (Blaney, 1974). Due to the great variability in the amplitude of spikes, it was not possible to assign the impulses to distinct classes and hence to individual neurons.

9.4 Responses to different concentrations of sodium chloride

Four different concentrations of sodium chloride aqueous solution (0.05M, 0.1M, 0.5M and 1.0M) were tested on individual

Figure 19 Adaptation curves for the first five seconds of response shown by five sensilla to stimulation by 0.1 M sodium chloride. The first three curves illustrate responses shown in Figure 18.



labial sensilla. The application of 0.05M solution failed to produce a response, indicating a subthreshold concentration. With the other concentrations, considerable variability was noted; but in general, there was a tendency for firing rate to increase as the concentration of the salt increased (Figure 20). At higher concentrations above 1.0M, the response levelled out. The concentration-response curve (Figure 21) is linear revealing that the tested response was proportional to the logarithm of concentration except very low concentrations. The intersection of the curve with the x-axis puts the threshold for sodium chloride at 0.01M, which is below the threshold during the experiments. This indicates a possible non-linearity of response to threshold concentrations. As before, the response showed rapid adaptation and the impulses were of more than one amplitude.

9.5 Responses to different salts

Application of supra-threshold concentrations of two salt solutions i.e. lithium chloride and potassium chloride, elicited responses very similar to that produced by the same concentrations of sodium chloride solution. Although the firing rates were slightly higher for potassium chloride than the two other salts, all monovalent salts probably act alike.

9.6 Response to water

According to Dethier (1977), only the water neuron was stimulated at electrolyte concentrations below 0.1M. In the

Figure 20 Responses of the labial apical sensilla to stimulation with different concentrations of sodium chloride.

A. Response to 0.1 M sodium chloride.

B. Response to 0.5 M sodium chloride.

C. Response to 1 M sodium chloride.

Horizontal bar shows 0.5 second, vertical bar 0.1 m volt.

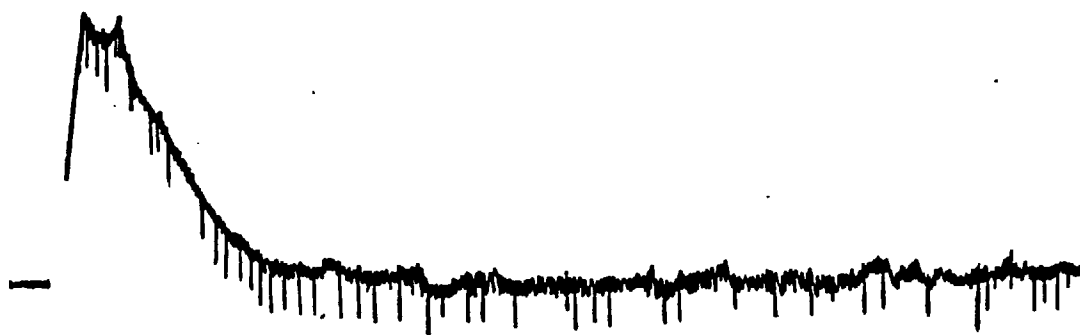
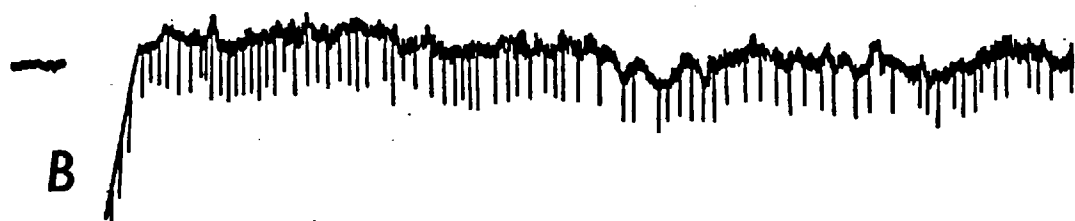
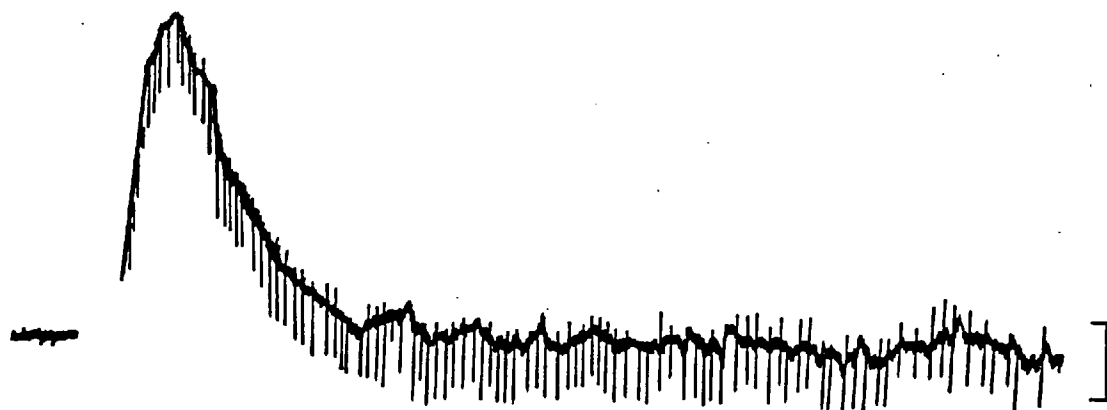
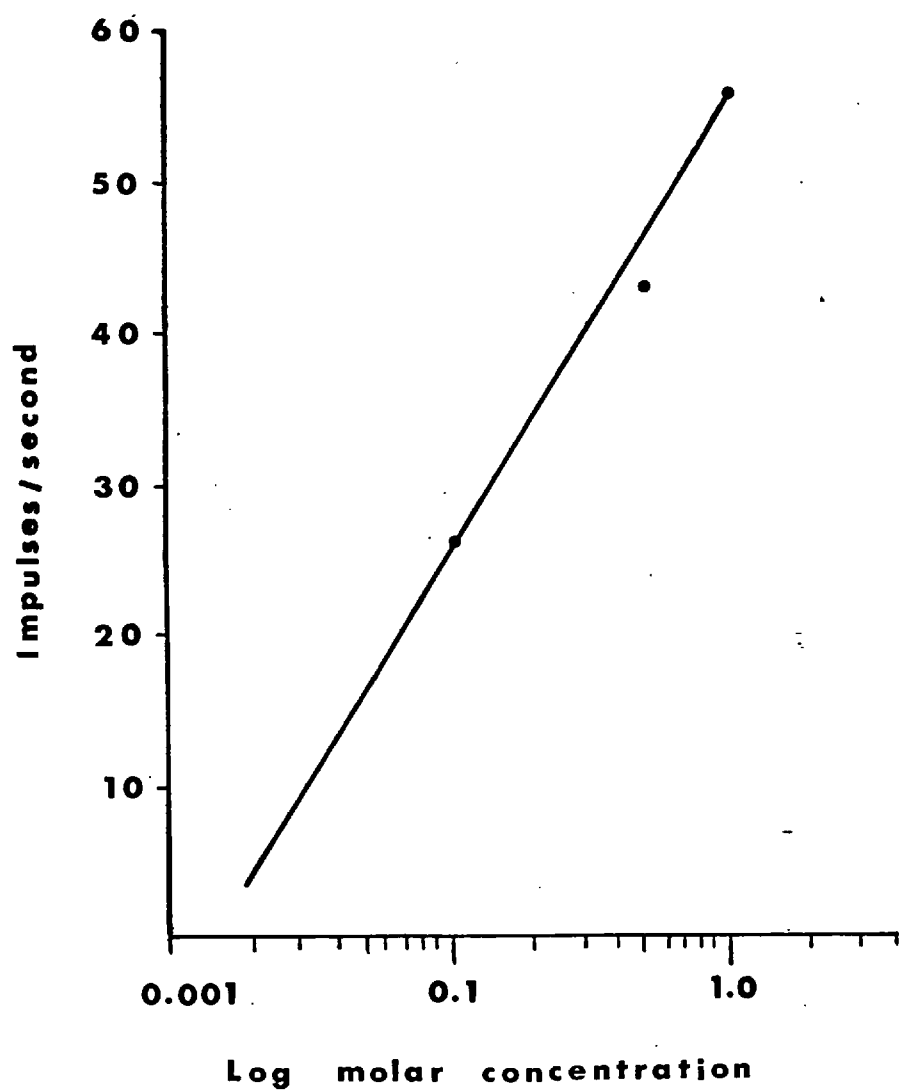
**A****B****C**

Figure 21 Concentration - response curve of labial apical sensilla to sodium chloride. Points indicate concentrations tested.



present investigation, dilute solutions of both sodium chloride and lithium chloride (0.05M) failed to stimulate spike activity in any of the labial sensilla tested. It seems, therefore, that these sensilla are unresponsive to water stimulation.

9.7 Responses to different compounds

The responses of the labial chemoreceptors to three compounds, namely, sucrose, neem (Melia azadirachta) seed extract and fungus (Cladosporium herbarum) extract were tested. The concentrations of these compounds were chosen to coincide with that in behavioural experiments. All the compounds were presented in 0.1M sodium chloride solution to ensure adequate electrical conductivity of the stimulating solution and the responses were compared with responses to stimulation with 0.1M sodium chloride alone.

Sucrose was selected for testing as it is the most commonly encountered sugar in nature and as it gave the highest behavioural response. Application of 0.2M sucrose solution resulted in a typical discharge of a train of action potentials in the form of small spikes, somewhat adapting but maintaining a fairly high frequency, higher than that obtained with the salt solutions (Figures 22, 23).

An electrode containing 1% extract of neem seed in 0.1M sodium chloride solution applied to the apical sensilla, produced a high frequency discharge of large spikes (Figure 22) reminiscent of the bursting discharge or volleying response obtained

Figure 22 Responses of individual labial apical sensilla to stimulation with various substances.

A. 0.2 M sucrose.

B. 1% neem seed extract.

C. 10% fungus extract.

Horizontal bar shows 0.5 second, vertical bar
0.1 m volt.

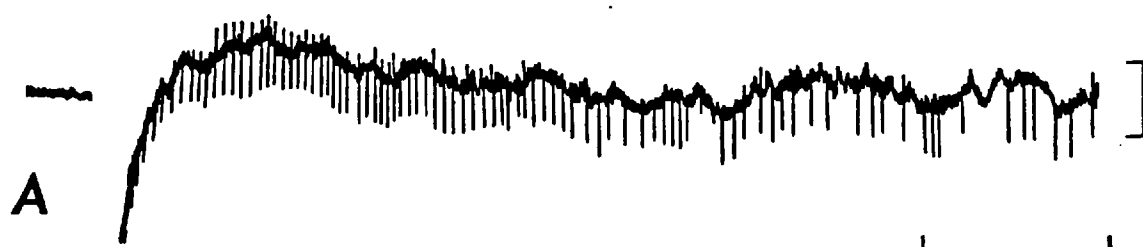
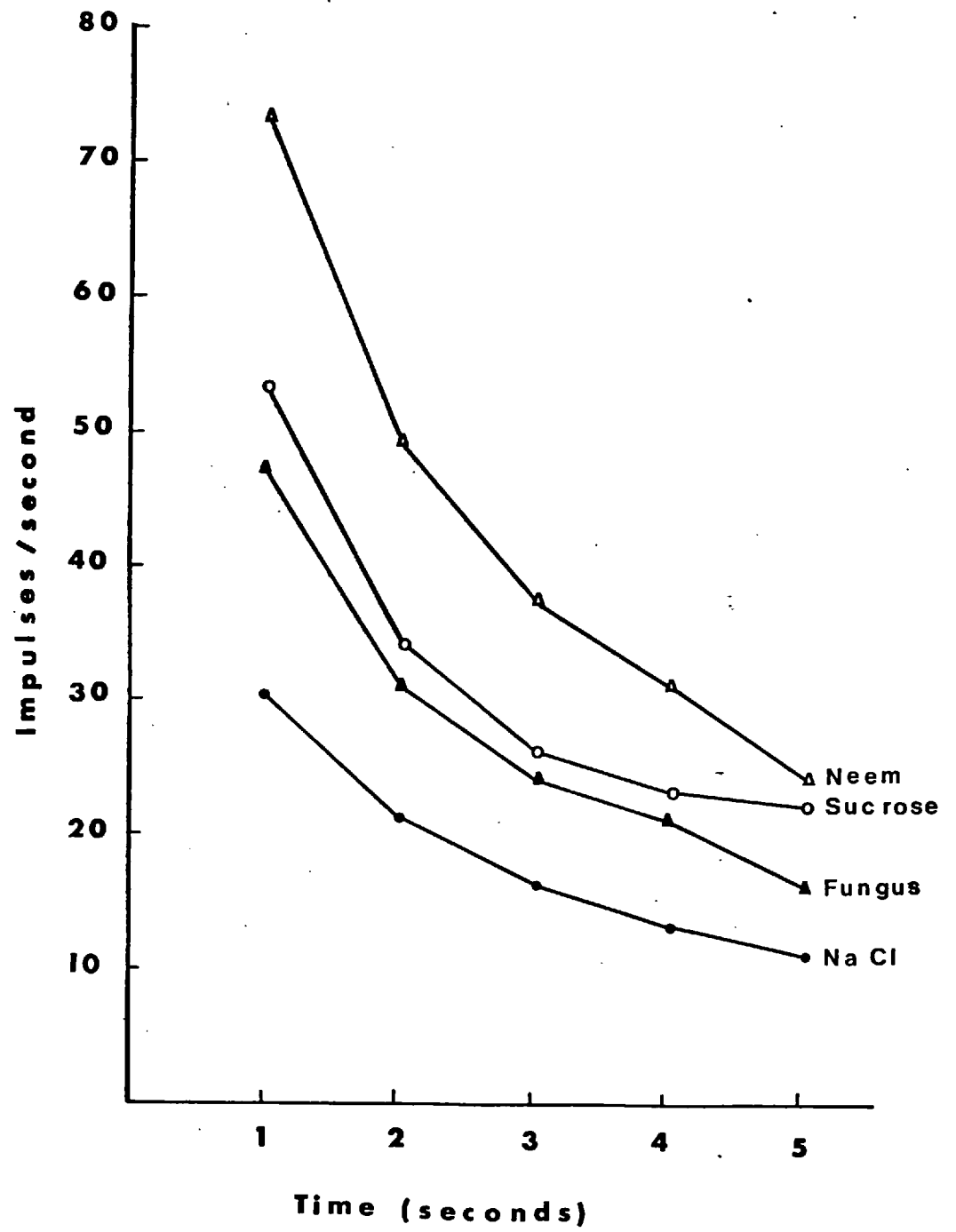


Figure 23 Response curves for the first five seconds of stimulation with 0.2 M sucrose, 1% neem seed extract and 10% fungal extract as compared to response to stimulation by 0.1 M sodium chloride.



from other insect chemoreceptors when stimulated with a deterrent substance (Haskell and Schoonhoven, 1969). A high level of background "noise" accompanied the recordings of the response. The initial discharge showed fairly rapid adaptation, followed by a steady low frequency discharge similar to the salt spike discharge described before.

The apical sensilla responded to stimulation by compounds present in the fungus extract by producing a discharge (Figure 22 C) not very different from that obtained from stimulation with a high concentration of sodium chloride (Figure 20.C). The small number of sensilla tested with the fungus extract in the present study may account for the failure to obtain a more pronounced response.

The response of individual neurons within the sensillum could not be identified with certainty by inspecting the amplitude pattern of impulses on traces. It followed, therefore, that firm conclusions regarding the number of cells responding at any time could not be drawn. However, it was fairly certain that three was the maximum number of neurons observed firing at one time.

On the other hand, the variability of responses obtained from different sensilla as well as from the same sensillum following subsequent stimulations with the same stimulant suggested that each single neuron responded with differential sensitivity to a range of stimuli. It seems more likely that the concept of specialized neurons described for the blowflies and other insects (Whitehead and Larsen, 1976; Dethier, 1977) was not applicable in the case of Entomobrya nivalis.

10. FEEDING HABITS AND MECHANISM

On the basis of the study of the detailed anatomy of the different components of the mouthparts and the knowledge of the musculature and the possible movements of these parts, together with the close observations made on live specimens both in cultures and confined in cavity microscope slides, an account of the feeding habits and methods of feeding in the genus Entomobrya is given below.

The majority of the species in the genus are suited to a sedentary or semi-sedentary life in shrubs, under bark of trees and among the litter on the soil, where food in the form of hyphae and spores of a wide range of fungal species is available (South, 1959). Before feeding can begin, possible food must be located and preliminary testing carried out. It seems that food location in Entomobrya involves searching until a suitable food substance is encountered by chance probing, when feeding supervenes. The live specimens kept under observation were found moving continually about, stopping frequently to examine the fungus which was provided as food. The examination was done by lowering the head and tapping the substrate rapidly and lightly by the antennae. This seemed to be a sampling procedure, whereby the antennal tips were applied to the substrate in front and to the sides of the animal. When food was encountered, the animal would stop and commence feeding. The antennae were kept moving rapidly during feeding, indicating the presence of certain chemoreceptors, which in addition to the labial receptors possibly served in the location and selection of food.

It was difficult to know whether olfactory as well as gustatory stimuli were used for testing before eating. It was observed that some individuals in cultures did not react at all to the presence of food and stayed away from it. This phenomenon may correspond to the inactive phase reported by Singh (1964) which occurs immediately after a moult, possibly to allow the cuticular structures of the mouthparts and the lining of the alimentary canal to harden.

with

On coming in contact with food, the active animals made rapid movements of the mouthparts, accompanied by frequent upward lifts of the head. The latter movements appeared to help in the scooping of the fungal hyphae and spores from the surface of the substrate by the heavily chitinated forward margin of the labium and the scooplike structure of the hyaline plate. The tips of the mandibles and maxillae were then protruded through the opening of the preoral cavity which was widened by lowering the labium and moving the two outer lobes of maxilla laterally (Plate 7E). The hypopharynx was also slightly protruded and in order to allow this, the two lobes of the labium moved some distance apart. The mandibles, being more capable of protraction than the maxillae, seized the food first by the incisor teeth, bringing the short distal series of interdigitating cusps together in the middle line. The large first apical tooth might then pierce the food particle and the remaining incisors with their tips facing ventro-medially, were ready to scrape its surface on the rotation-retraction movement of the mandibles. The maxilla heads, lying ventro-lateral to the

mandibles, would grip the food more firmly against the two median appendages, while the lamellae lay dorsal in position. The food was then brought to the opening of the preoral cavity, where the hypopharynx and labium were pressed against it ventrally and the labrum, aided by the distal row of setae, was pressed against it dorsally. The labrum was depressed or curved to be more convex and both the labral papillae and the anterior smooth area, which became more vertical, formed a barrier to prevent the food particles from drifting to the posterior part of the labrum. At this point, the apical sensilla of the labium was further stimulated by the new chemostimuli released as a result of piercing the food particle and the food was either rejected or taken into the preoral cavity. In the latter case, it was first mixed with the copious salivary juice which flowed either to the posterior side of the hypopharynx and so to the food surface via the slit-like opening of the lingua or directly out through the median gap in the labium. The mandibles were brought closer together as they moved further back, bringing the scraped food into the preoral cavity. Following the retraction of the mandibles, the maxilla heads began to move medially and backward to their position in the cavities on either side of the hypopharynx. As they did so, and on passing the fine bristles on the antero-dorsal side of the superlinguae, the food particles were brushed off the median appendages of the maxilla heads to the dorsal surface of the superlinguae. At the same time the lateral lamellae swept the food particles backwards and prevented stray fungal particles from going into the lateral cavities on both sides of the hypopharynx.

The retraction movement of the hypopharynx, together with the action of the bristles and filaments of the superlinguae, brought about by the action of the longitudinal intrinsic muscles in the lingua, would direct the food towards the concave posterior end of the superlinguae.

When the mandibles, maxillae and hypopharynx were completely retracted inside the preoral cavity, the external opening was closed and the hyaline plate of the labium was brought over the anterior edge of the labrum and covered parts of the outer lobes of maxilla. As a result of the lowering of the labrum, the wedge-shaped epipharynx hanged vertically above the median part of the superlinguae and thus the gnathal pouch was divided longitudinally into two lateral halves. The anterior rows of teeth and the flaplike structure of the epipharynx helped in moving any remaining food particles posteriorly along the dorso-lateral surface of the superlinguae and also prevented the food from escaping dorsally between the superlingua and epipharynx. The two maxilla heads moved laterally within the cavity between the lingua and the superlinguae and were brought together along the median cavity between the superlinguae, where they started grinding the food particles by the two median appendages. At the same time, the ground up food was continuously moved posteriorly towards the cibarium, i.e. the mandibular molar plate cavity. The two folds on the epipharynx forming the food retaining valve prevented the food from moving to the front, away from the grinding surfaces of the mandibular molar plate and the filaments on both sides of the epipharyngeal folds prevented any ground up food escaping dorso-laterally.

When the partially ground up food reached the posterior part

of the superlinguae it was picked up between the grinding faces of the rotating mandibular molar plates during their retraction - rotation movement, helped by the rows of superlingual filaments. The buttress rods on the posterior periphery of the molar plate acted as a ledge to keep the food particles under the multicusped rods for more thorough grinding and they also brushed off the ground up food from the surfaces of the molar plates during their protraction - counter - rotation movement. The mandibular heel, on the other hand, prevented the mandibles from excessive counter-rotation. By their fine and precise movements, the mandibles were thus able to cut into the soft food particles, followed by grinding as in all animals using the rolling movement. The finely ground up food was then passed through the oral valve at the posterior end of the cibarium and into the oesophagus by the negative pressure created by the oesophageal muscles.

Although the usual food of members of Entomobrya is of a solid or semi-solid nature, they can also feed on fluid food. There have been reports about species of the genus drinking condensation droplets on the surface of the culture vessels (South, 1959). In the present study, it was observed that insects in dried-up cultures moved irregularly with great excitement to the filter paper when it was watered, and started sucking the water from the moistened part.

During the uptake of fluid food (or water) the oral cavity formed a functional water-tight sucking tube by sealing the sides i.e. by bringing the hyaline plate over parts of the outer lobes of maxillae which themselves covered the lateral margins of the labrum,

and also by pressing the labial lobes together, and bringing the cuticular thickenings firmly against each other. The fluid food was then sucked up by the action of the epipharyngeal and oesophageal dilator muscles producing a negative pressure in the oral cavity. The up-flow of the the fluid food was helped by the lapping movements of the hypopharynx, which was produced into a concave scooping shape by the contraction of the lingual transverse muscles. The mandibles and maxillae were kept immobile during fluid feeding.

11. DISCUSSION

The Discussion will be organised around four main points:-

11.1 The functional morphology of the mouthparts

Many morphological features have been found to be associated either with special performances and abilities or with particular habits. Collembolan mouthparts are no exception to this rule. Wolter (1963) has expressed the opinion that the basic structure of the mouthparts of Collembola in general is very uniform. The structure of the mouthparts of Entomobrya supports this view showing a close similarity to the generalised form although several variations in structure exist.

The type of labrum found in Entomobrya is the most typical and standard type in Collembola, where the surface is coarsely and equally granulate and with setae arranged as 4/5, 5, 4 (Yosii, 1976). This chaetal arrangement has been found without exception in Entomobryidae (Christiansen, 1958). This shows that the Entomobrya albocincta specimen observed in the present study to have 6 setae in the m-row was just an exceptional case. The marginal structures of the labrum are varied in different genera of Entomobryidae. Similar structures to the labral papillae of Entomobrya are found in other genera and these vary from few recurving spinules in Xenylla Tullberg, 1869, and Pogonognathellus to bristles in Tomocerus and incised ridges in Isotomurus Börner, 1903. The presence of these structures in various forms confirms the function attributed to the labral

papillae in this study as a barrier for food particles not to drift backwards along the surface of the labrum. Another feature of the labrum of Entomobrya, the smooth area, is found in various families in Arthropleona (Hypogastruridae, Onychiuridae, Entomobryidae) as well as in Symphypleona (Neelidae). In some of the genera in Entomobryidae the smooth area may be intruded medially in a narrow and wedge-like way as in Lepidocyrtus (Szeptycki, 1967).

From Manton's work (1964, 1977), the efficiency of the mandibles as grinding organs has been well explained. However, Goto (1972) has disputed the grinding function usually attributed to the molar plate in the light of the flexible nature of that plate as observed in Folsomia candida. In the present study, the molar plate has shown an efficient grinding surface, yet the efficiency of the chewing type of mouthparts in the breakdown of food is still questioned. The gut content studies carried out on various species of Collembola (Poole, 1959; South, 1959; Singh, 1969) have revealed a large percentage of intact viable fungi, reaching more than 40% of the gut content in some cases, and which pass unharmed through the gut. One explanation for this phenomenon may be that an appreciable proportion of the food ingested escapes laterally through the cavities on both sides of the hypopharynx and thus avoids the grinding surfaces of the molar plates. On the other hand, some of the mandibular features such as the dorso-lateral ridge and the mandibular hump reported by earlier workers were not observed in Entomobrya. These structures have been mainly described from species of Tomocerus and Orchesella which were extensively studied

mainly due to their comparatively large size (Folsom, 1899; Hoffmann, 1905, 1908; Manton, 1964; Singh, 1969). In works on other species of Collembola, these structures have either not been mentioned (Mukerji, 1932; Tuxen, 1959) or their presence speculated (Goto, 1972). It follows that these structures are not universal in all species of Collembola.

The great variation shown in the structure of the maxilla head seems to point to a variation in the nature of the food taken by different Collembola. The compact structure of the maxilla head observed in the present study as opposed to the more complex structure shown by other Collembola (Goto, 1972; Massoud and Ellis, 1974) may indicate a more dependence on fungal spores and hyphae for food. Other soil Collembola like Folsomia and Tomocerus may depend on a large portion of their diet in the form of soil litter and humus (Knight and Angel, 1967).

The hypopharynx is a substantial organ in Collembola playing a more important role in feeding than in other insects. The present study has revealed an even greater complexity of structure of this part than was previously suspected and has thrown more light on its function. The transverse and longitudinal muscles, shown to be present in the lingua, allow the hypopharynx to play a more essential role in the feeding of Collembola through the independent movements of this organ. Consequently, the hypopharynx can be utilized more successfully in fluid feeding. On the other hand, the lingual structures which have been reported for the first time indicate a role in the conducting the salivary juice. According to Manton (1964) the copious salivary juice discharged into a median

posterior diverticulum of the preoral cavity, can then flow to the posterior side of the hypopharynx and so to the food surface. However, the route taken by the juice to the anterior part of the hypopharynx has not been indicated. Snodgrass (1935) has given the name salivarium to the organ between the base of the hypopharynx and the labium into which opens the salivary duct in the biting insects. The salivarium is converted into a salivary pump in the higher insects (e.g. Diptera and Heteroptera). It seems that the lingual hemispherical organ resembles a modification of the salivarium. A subsidiary role of the salivarium in cockroaches is to store water, which is drawn on when the insect is desiccated (Sutherland and Chillseyzn, 1968). It appears quite possible that the hemispherical organ plays the same role in Collembola owing to the marked difference in resistance to drying shown by different species, which may reach a hundredfold in some cases (Choudhuri, 1963).

The posterior part of the hypopharynx has been shown to be a site of enzymic activity through histochemical tests. The presence of succinic dehydrogenase in a tissue is known to be an index of its capacity for oxidative reactions (George and Berger, 1968). This can be correlated in the hypopharynx with the potential of that organ for generation of energy. If this is added to the fact that the base of the hypopharynx has shown several ultrastructural and histochemical distinct features like a concentration of large oval nuclei, numerous mitochondria and large lipid globules, the possibility of the existence of a lingual gland becomes more eminent. The secretions of this gland, which are probably mainly

enzymic in action, are discharged into the cibarium, where mixed with the finely ground food there, assist its digestion.

11.2 Chemical basis of food selection

In analysing the selection of food by phytophagous insects, Dethier (1953) found it convenient to consider the stimuli involved under three categories, those which orientate the insect to the plant, those causing it to bite and those which maintain feeding. It thus follows that the preference of insects for food plants depends on the presence of common specific substances which provide olfactory or gustatory stimuli, without which feeding fails to occur. Thorsteinson (1960 quoted by Dadd, 1963) however, suggests that while feeding is primarily stimulated by universally distributed food substances, including both nutrients and water, various degrees of oligophagy result from the presence of factors which inhibit feeding. Thus food preference involves a balance between acceptance and rejection.

Many authors (South, 1959; Knight and Angel, 1967; Singh, 1969; McMillan, 1976) have shown that Collembola exhibit preferences in its choice of food. They have also shown that the most preferred food varies with the species of Collembola under consideration. In the green plant, chemicals which elicit a feeding response are primary plant chemicals, including sugars, amino acids, lipids, steroids and certain vitamins. Although certain chemical constituents of a few yeasts and fungi are known, the relationship between chemicals and food selection by microphytophages is largely unknown. No attempt to isolate the chemical factors involved in

collembolan food preferences has been made. It has only been possible to correlate the physical factors of the food with its palatability (McMillan, 1976). Among fungi, size and shape of spores and hyphae may influence their ingestion. In the present study and because of the absence of suitable method for estimating the feeding response, it was not possible to assess the feeding responses separately from the orientating responses. As a result, the technique finally evolved measured a combination of the orientation responses and the feeding responses. The rationale behind the experimental procedure is that measurements based upon the reactions of an entire animal may be used to estimate the sensitivity of the animal's receptors (Hodgson, 1953). Thus it appears that the behavioural response of a lower organism to an applied stimulus is a fair measure of the comparative stimulating ability of that stimulus.

The general preference for sugars is not surprising as they are ideal sources of instant energy and are generally abundant and ubiquitous (Dethier, 1977). On the other hand, carbohydrates are the main components of the fungal cell wall (Bartnicki - Garcia, 1968) and it therefore seems likely that they induce feeding in *Collembola*.

The results from the behavioural tests with sodium chloride are difficult to explain since sodium chloride is widely known as a repellent (Dethier, 1955). The interesting observation of the eversion of the ventral tube vesicles to bring them into contact with the impregnated filter paper discs may throw some light on the

explanation of this phenomenon. Noble-Nesbitt (1963b) has shown that uptake of water can occur across the ventral tube vesicles and that most of the exchange of sodium between insect and medium occurred over the same organs. On the other hand, Houlihan (1976) has observed Petrobius brevistylis (Lepismatidae) everting its ventral abdominal sacs when placed on filter paper discs containing sodium chloride solution and that the strongest solution which a sufficient number of animals would evert their abdominal sacs on had a concentration of 0.2M. Those animals that would evert their sacs on this solution behaved in the same way as with the uptake from distilled water. It therefore seems likely that Collembola aggregating on sodium chloride discs were absorbing sodium ions by the ventral tube vesicles. As the test animals for the experiments were collected from the wild populations in the field, this may reflect that the sodium content in the natural food of these animals, i.e. fungal spores and hyphae, is so low that the availability of this ion could be a limiting factor in the diet of Collembola. As Eisenbeis (1976) has reported the presence of chemoreceptors in the transport epithelium of the ventral tube of Tomocerus and Orchesella, it appears that these sensilla are used in the detection of the various ions. It would be interesting to test these sensilla electrophysiologically for their response towards different salts.

Aqueous extracts of the leaves or seeds of several species of Meliaceae, particularly the Indian neem tree, Melia azadirachta, act as a feeding repellent at very low concentrations to many species

of Orthoptera (Sinha and Gulati, 1965). Extracts of compounds present in seed kernels demonstrated outstanding deterrence to feeding by at least nine insect species highly destructive to fruit, vegetable and horticultural crops in the United States when applied to the leaves of host plants at 0.25 - 1% in the greenhouse and field. In multi-choice experiments, discs impregnated with 1% neem seed extract were completely avoided by E. nivalis, indicating a strong repellency towards Collembola. Although other experiments using oxalic acid, hydrochloric acid and quinine hydrochloride revealed a negative response by test animals, they were not sufficiently clear cut to characterise a particular response to "acid" or "bitter" stimuli in Collembola.

11.3 The mouthpart receptors in relation to feeding

Feeding behaviour can be said to begin with the insect approaching the food substance; as regards Collembola, Boyd (1967) demonstrated the ability of Entomobrya purpurascens Packard, 1873 to detect at a distance the odour of decaying potato which releases up-wind anemotactic behaviour resulting in movement towards the odour source. On the other hand, thin walled receptors perforated by a large number of small pores (which are characteristic features of olfactory sensilla according to Slifer, 1970) have been found on the antenna (Altner and Thies, 1972) and postantennal organ (Altner et al., 1970; Altner et al., 1971; Dallai, 1971; Karuhize, 1971) of Collembola. During the present investigation, the antennae of feeding animals were observed to be in continuous motion. These

are probably the chief organs for olfactory perception of chemicals associated with food.

Having arrived at a possible food, testing followed by acceptance or rejection takes place (Haskell and Schoonhoven, 1969). The receptors on the labial setigerous lobe certainly act as a first line of testing and selection where exploration of the food takes place prior to any ingestion. It is surprising that hitherto little attention has been paid to the detailed behaviour involved in feeding or the physiological basis of the function of the mouthpart receptors. McMillan (1976) showed that the morphological features of the food such as the size and shape of fungal spores and hyphae seemed to affect the choice of food in Collembola. It is possible that these morphological features of the food could be determined by the side setae on the setigerous lobe when they are testing the food.

When the food particle has been scraped by the action of the apical teeth of the mandible, new chemostimuli may be released and the apical contact chemoreceptors will then be evolved. Furthermore, it is probable that olfactory organs, perhaps especially those situated near the secondary mouth opening (i.e. those on the postantennal organ), are stimulated by new odours.

Since the setigerous lobe of the labium contains most sense organs which are not found elsewhere on the mouthparts, and which are definitely the most important in connection of feeding once the food has been located, it was decided to study the ultra-structure of these receptors in detail as well as to carry out an electrophysiological investigation about their responses to various

chemicals. The main finding of the study of the structure of these sensilla is the observation that both mechanoreceptors as well as chemoreceptors are found. A single neurone innervates each tactile hair sensillum while the chemoreceptors are innervated by four neurones, one of which acts as a mechanoreceptor. A tubular body has been found so consistently in mechanoreceptive sensilla of arthropods that it is now considered to be a diagnostic feature for mechanoreceptors. Extensive comparative discussions on the structure of tubular bodies have been given in some recent papers (Altner et al., 1970; Gaffal and Hansen, 1972). Generally, a tubular body consists of densely packed microtubules and an interspersed electron-dense substance that is mostly arranged in periodical layers. However, there is a large degree of structural variation and consequently some authors use the term "tubular body" where others do not. Perhaps a clearer and more generalized definition of the term "tubular body" as has been suggested by Foelix and Chu-Wang (1973) would eliminate this problem. They defined a tubular body as any dense arrangement of microtubules which is restricted to a dendritic terminal, regardless of the amount of electron-dense substance present. Little is known about the function of tubular bodies. Thurm (1965) conjectures them to be the transducer region for mechanical stimuli, whereas Schmidt (1969) assumes that they serve only a passive resistance function during deformation of the dendritic terminals.

The morphological features of the chemosensitive hair sensilla of Collembola provide indirect evidence that they function

as contact chemoreceptors. Despite their relatively shorter length (approximately 10 μ m), compared to the tactile side sensilla (15 μ m in length), the chemosensitive apical sensilla reach the same level as the side sensilla arise from a lower point on the papilla and are a bit curved. Thus contact with the substrate can be made at the same time as with the tactile hair sensilla, or sometimes even earlier. The chemosensitive hair sensilla of *Collembola* possess structural properties which are common among known contact chemoreceptors of other arthropods (Lewis, 1970; Slifer, 1970): a) a blunt tip equipped with a pore; b) a thick cuticle wall; c) a double lumen within the hair shaft; d) a multiple innervation which consists of a mechanoreceptor ending at the hair base and three chemoreceptors entering the hair lumen; e) the chemoreceptive dendrites are restricted to one lumen, do not branch, and are exposed to the outside at the distal pore. No evidence for the existence of a "closing mechanism" of the pore opening as has been attributed to some contact chemoreceptors of insects (Stürckow et al., 1967; Blaney et al., 1971) or the extruding fluid "Sensillen-liquor" (Stürckow, 1967) has been found.

The electrophysiological analysis carried out on the apical chemoreceptive sensilla confirms the morphological evidence as to the function of these receptors as gustatory chemoreceptors responding to a wide range of chemical classes. It is also clear that, in some cases at least, individual neurones are capable of responding to more than one type of compound. This characteristic,

together with the variability of results following stimulation with a constant stimulus, suggests that these sensilla do not respond in the manner commonly accepted for chemoreceptors. The intriguing correlation of four neurones in the blowfly chemoreceptor with four different taste modalities (Dethier, 1963, 1977; Hodgson, 1968) is apparently not applicable to contact chemoreceptors in Collembola. Other reports of compounds stimulating more than one cell have been given by Schoonhoven (1969) and by Dethier and Kuch (1971). The specificity as exists in these systems, appears to be more at the level of the sensilla than at the level of neurones. As a result of this, some sensilla have a notably vigorous overall response to salt, some to water, some to sugar depending on the presence in these sensilla of one or more neurone having their differential sensitivity particularly biased towards a specific modality (Blaney, 1974). There does not, for instance, appear to be a specific sugar-sensitive cell, though some cells may respond more vigorously to sugars than to other substances. It is likely that discrimination of different chemicals occurs within the central nervous system rather than at the level of the receptors.

The variability in amplitude and nerve impulses elicited upon stimulation with the same stimulus has also been reported by Haskell and Schoonhoven (1968) and Blaney (1974) in the sensilla of Locusta migratoria (L.). They have given the suggestion that this variability may be related to changes in dendritic membrane resistance, which is in series with the recording electrode. Alternatively, Morita and Takeda (1957) and Stürckow (1971) suggested that these changes may

be related to different amounts of viscous material present at the tip of the sensillum. This material was not observed in sensilla of Entomobrya and the changes in response were observed to occur too rapidly to be accounted for by the varying amount of the exuding material. However, this variability in response can be explained by the process of opening and closing of the distal pore of the sensilla which Blaney and Chapman (1969) and Stürckow et al., (1967) have described in Schistocerca and Phormia, respectively. Bernays et al. (1972) have shown that the resistance across tips of sensilla varies inversely with the number of open sensilla. This same phenomenon of opening and closing of the distal pore of the sensilla may explain the observation that gentle manipulation of the tips of non-responsive sensilla causes them to respond.

No special response was elicited by the stimulation with the sucrose solution as might have been predicted from the behaviour experiments. Some quantitative discrepancies between the behavioural and electrophysiological results do exist (Hodgson, 1957). Thus, it is not always possible to interpret behavioural responses solely in terms of the spike potentials in the electrophysiological experiments. This is not surprising in view of certain necessary differences in the technique as behavioural experiments are determined by applying chemicals in aqueous solutions, while electrophysiological measurements have to be made with some electrolyte also in solution. This is the main disadvantage of the latter technique as there is always the possibility of an interaction between the effects of the electrolyte

and the test solution. For this reason, although the electrophysiological experiments give direct access to many types of information otherwise difficult to obtain, their maximum usefulness in many cases will be realised when they are employed in combination with behavioural tests.

The tested apical sensilla responded to stimulation by the neem seed extract with a discharge resembling the specialized "bursting" response shown for chemoreceptors by other insects to the preferred food (Haskell and Schoonhoven, 1969). These results support the idea that a range of information, both on the acceptable and non-acceptable components present in food, is passed to central nervous systems by a range of receptors and the final choice depends on nervous filtering and integration of a wide spectrum of data (Dethier, 1966). It follows that a specialized response of this nature to a deterrent substance would be a powerful aid to selection and discrimination.

On the other hand, it is surprising that there was not a more distinct response to the fungus Cladosporium herbarum extract which is a common food of the genus (South, 1959). This cannot, however, be regarded as the typical response elicited by a "desirable" substance, as other such substances have a markedly different effect. The grass extract, for example, evoked a typical "bursting" pattern in mouthparts sensilla of Schistocerca and Locusta (Haskell and Schoonhoven, 1969). Blaney (1974) has suggested that sensitivity to a particular compound is not universally distributed among sensilla and that some sensilla have a notably vigorous overall response to

specific modalities. The small number of sensilla tested with the fungus extract may account for the failure to encounter one of the fungus sensitive sensilla and hence the resulting failure to obtain a reliable response. It is obvious that an electrophysiological analysis using a larger number of sensilla is very much needed. In the meanwhile, the response to fungus extract must be regarded as uncertain.

The procedures and results described in the present study establish a basis for further electrophysiological studies of the specificity and sensitivity of chemosensitive sensilla in Collembola. Of particular interest will be studies of the possible role of these receptors in the perception of different substances in fungal extracts, and whether these sensilla are only involved in contact chemoreception (gustation) or perhaps in olfaction as well. It has been shown in other insects that some of the typical contact chemoreceptors also respond to strong odours (Hopkins, 1964).

11.4 Methods of feeding in Collembola

In the light of the study on the feeding mechanism in Entomobrya, it seems that the view of Macnamara (1924), which is held widely, of simply dividing the collembolan mouthparts into chewing or sucking types according to the presence or absence of the mandibular molar plates, should now be revised. Wolter (1963) has agreed with such a division and he subdivided the sucking type into rasping-sucking and piercing-sucking. He stated that the basic structure in both types is very uniform and that the main functional

change is the narrowing of the buccal cone to form a sucking tube. Sharma and Kevan (1965) have further subdivided the sucking type to include what they termed as anomalous group without giving a clear reason for this new subdivision.

On the other hand, Adams and Salmon (1972) have suggested that a sucking type of feeding does not exist among Collembola. Instead, they have suggested the term "suspension feeding" for Collembola lacking mandibular molar plates, in which case fluids are taken in mainly as a medium to carry food in suspension. In Entomobrya, although the principal food of the genus is fungal material, both spores and mycelia, of saprophytic Fungi Imperfecti common in their habitat (South, 1959), yet in the present study, it was found that members of the genus are also capable of taking up fluid food (or water). The well modified molar plates, compact maxilla heads and the toothed hypopharynx seem to contribute to the grinding function of the mouthparts of the genus, while the well organised hypopharyngeal and pharyngeal dilator muscles contribute to the sucking function. The feeding mechanism in Entomobrya is thus essentially a rasping (or piercing) sucking and chewing process. It seems probable that a number of other Collembola may belong to this group.

12. SUMMARY

1. The head capsule shows no definite sign of segmentation. The arrangements of the mouthparts has been given in outline and is found to be similar to that described by previous workers.
2. The labrum is of the standard type in Collembola, where the surface is coarsely and equally granulate and with setae arranged as 4/5, 5, 4. Only the two anterior setae are modified as mechanoreceptors. The labral papillae on the anterior margin are specific to different species.
3. The outer lobe of maxilla, which articulates with the stipes, bears six setae, the longer two arising from the apex and side of a large papilla and are both modified as mechanoreceptors. The ventral surface bears various membranous and chitinous structures.
4. The labium, which is divided medially into two halves, is differentiated into an anterior setigerous lobe, a median mentum and a posterior submentum. The setigerous lobe bears five groups of papillae with apical and side sensilla. The relative lengths of sensilla to associated papillae seems to have no taxonomical significance. Barbed and smooth setae arise from the mentum and submentum.
5. The anterior part of the mandible carries four and five cusps in the left and right mandibles, respectively. The molar plate

resembles a strong grinding surface with odontoid molar cusps, buttress rods and molar plate cusps. No mandibular hump or dorso-lateral ridge are observed. The arrangement of the mandibular muscles is similar to that described previously.

6. The maxilla is formed of a maxilla head, a pair of rod-like stipes and a short cardo which articulates with the fulcrum. The head or capitulum is less complex and more compact than in other Collembola and comprises an unglum with three strong teeth, a median appendage and two pairs of lamellae fringed by filaments.

7. The hypopharynx, occupying a median position in the preoral cavity, is well developed and seems to play a more essential part in feeding. It is formed of a median somewhat membranous lingua and a pair of lateral chitinous superlinguae. The dorso-median margins of the superlinguae are beset with a series of filaments which lies between the two molar surfaces. The lingua has intrinsic transverse and longitudinal muscles, making it capable of some independent movement. A hemispherical organ on the lingual ventral side resembles the salivarium in other insects.

8. The complex epipharynx is wedge-shaped and forms the roof of the buccal cavity. It bears rows of teeth and flaplike structures fringed with filaments laterally and a chitinous raised-up structure medially. These structures help in keeping the food confined to the food meatus. Two lateral folds form the dorsal part of the oral valve which prevents the regurgitation of food from the pharynx.

9. The thin plastic sections from tissue blocks originally prepared for electron microscopy gave crisp cytological detail and a number of stains employed for these sections have proven to be valuable stains. The interrelationships between different mouthparts have been provided with the aid of these sections. The posterior part of the hypopharynx has been shown histochemically as a site of enzymic action and a probably glandular function is suggested for it.

10. The ultrastructure of the cuticle reveals a similar structure to that of other Collembola with a lamellate endocuticle and a thin epicuticle thrown into minute tubercles through which pass the pore canals. Both the epipharynx and hypopharynx exhibit a fairly uniform ultrastructural organization with several multivesicular bodies, variable sized vesicles and numerous mitochondria. The epipharyngeal dilator muscles show the characteristics of striated muscles, while the hypopharyngeal muscles which consist of a single myofibril, show irregular Z-bands.

11. The labial sensilla are divided into two types according to their innervation. The tactile mechanoreceptors are innervated by one bipolar neuron which dendrites end proximally at the hair base. Each dendritic terminal exhibits a tubular body, a dense array of microtubules typical for mechanoreceptive sensilla. A dendritic sheath encloses the outer dendritic segments which are connected to the inner segments by a ciliary structure. The dendritic processes are surrounded by three cells: an envelope

cell, a trichogen and a tormogen. Chemoreceptive hair sensilla occur as labial terminal setae. Their morphological characteristics are: two lumina within the hair shaft, a blunt tip with a pore opening and a multiple innervation of four bipolar neurons per sensillum. One mechanoreceptive dendrite terminates with a characteristic tubular body at the hair base. The three chemoreceptive dendrites enter the hair lumen and traverse unbranched to the apical pore, where they are exposed to the outside. These sensilla act both as tactile and gustatory receptors.

12. The response of individuals of Entomobrya nivalis to contact chemical stimuli is purely orthokinetic. They show an affinity to both sugars and salts; but evade neem seed extract, quinine and acids. The salt is taken in ionic form by the ventral tube vesicles, showing a probable deficiency in the natural diet of Collembola.

13. Electrophysiological recordings from single labial chemosensitive sensilla confirm the presence of several chemosensory neural units as well as a mechanosensory unit. Variability of response to a constant stimulus is appreciable both between and within sensilla. This has been suggested to result from the opening and closing of the apical pore of the sensillum. The sensilla responded to a range of compounds indicating a non-specialization of individual neurones to the different chemical classes. The specificity is more shown by the sensilla rather than by individual neurones. Neem seed extract

elicited a "bursting" response shown for preferred food in other insects, while the fungal extract failed to evoke a reliable response.

14. The principal food for Entomobra is solid food in the form of fungal hyphae and spores; but individuals are also capable of taking fluid food. After being transferred to the preoral cavity by the mandibular incisors and maxillae heads, the solid food particles are swept along the dorsal surface of the superlinguae by the aid of the maxillary head lamellae and superlingual filaments. The food is then picked-up by the grinding molar plates and transferred to the pharynx. In fluid feeding, the food is sucked-up by the action of the epipharyngeal and oesophageal dilator muscles, producing a negative pressure in the oral cavity. The feeding mechanism is essentially a rasping, sucking and chewing process which probably exist in a number of other Collembola.

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