The Integumentary Mucous Secretions of the Earth Worm
*Megascolex mauritii*

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A histochemical study of the epidermal gland secretions in earth worm *Megascolex mauritii* has been made. The secretions of the aluminous gland is a glyco or mucoprotein and that of the mucous gland cells is an acid mucopolysaccharide. Two types of mucous glands are present in the epidermis, the small and large mucous glands, secreting hyaluronic acid and highly acidic sulphated mucopolysaccharide, respectively.

The functions of the secretions from these glands are investigated. The albuminous and small gland cells secrete mucin under normal conditions and this mucin keeps the body surface moist and serves as lubricant during locomotion. The large mucous gland secretes only after irritation and its secretion may be an alarm pheromone.

The epidermal glands of most soft bodied invertebrates secrete several chemically distinct mucosubstances each related to a particular function. The epidermal mucin of earth worms serves several functions, such as keeping the body surface moist to facilitate respiration and acting as lubricant during locomotion. It is also produced in response to noxious stimuli. But no study has so far been made to find if the mucin of earth worms has chemically distinct mucosubstances related to these various functions. Stephenson and Bahl observed that the epidermal mucin is secreted by two kinds of glands, the albuminous and mucous glands. Ewer and Hanson suggested that the secretions of these two types of glands may be chemically distinct. But no attempt has so far been made to study the chemistry of the secretions of these glands. Hence the present investigation was undertaken to study the histochemistry of the glands and the chemistry of the mucin in relation to the biology of the worm.

### Material and Methods

A culture of *Megascolex mauritii* was maintained in the laboratory as described elsewhere. For histological studies of epidermal glands, specimens were narcotised with dilute alcohol before fixation to minimize the loss of mucin. Subsequently the body wall was cut open and the same fixed in Saez after removing the viscera. 6 to 8 µ thick paraffin sections were stained with Mallory's triple stain and Ehrlich's haematoxylin-eosin. Paraffin and frozen sections were used to perform histochemical tests for proteins and polysaccharides. For the detection of proteins the bromophenol blue as recommended by Mazia et al. the Morel-Sisley diazotization with 1-amino-8-naphthol-4-sulphonic acid as outlined by Pearse and the Baker's modification of Millon's test were employed. In addition biuret and xanthoproteic tests were also proved useful for this purpose. For polysaccharides, sections were stained with periodic acid—Schiff (PAS) reagent after McManus and Hotchkiss. The acid mucopolysaccharides were visualised after staining with alcin blue (BCX) in phosphate buffer, pH 3.0 as recommended by Mowry and toluidine blue according to the method of Kramer.

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7. D. W. Ewer and J. Hanson, J. Roy. microsoc. Sci. 65, 40 [1945].
15. R. D. Hotchkiss, Arch. Biochem. 16, 131 [1948].
and Windrum. In latter method some of the sections were incubated before staining to hyaluronidase in phosphate buffer (pH 6.0) for 18 h at 37 °C to enable identification of hyaluronic acid if present by its liability to this enzyme. The methylene blue extinction (MBE) point method was employed according to Pearse for the detection of acid mucopolysaccharides containing sulphate groups. The xiphisternum of mouse and the cock’s comb which are known to contain sulphated mucopolysaccharides and hyaluronic acid respectively were used as controls for comparison with the test material.

For the study of chemistry, the mucin from the general body surface under normal conditions was collected and the same was precipitated and purified by the following procedure. The precipitation was done by acidification with dilute acetic acid and dialysed for 48 h against distilled water in a refrigerator to remove low molecular weight substances. The dialysed precipitate was then dissolved in water (pH adjusted to 7.0) and reprecipitated with 8 volumes of ethanol followed by washing with acetone and drying in a vacuum desiccator. This material was used for the chromatographic analysis for sugars and amino acids and electrophoretic analysis of acid mucopolysaccharides.

After hydrolysis with 6 N HCl for 6 h at 100 °C, the aminoacid and sugar constituents were studied by paper chromatography using butanol : acetic acid : water (4:1:5) as irrigating solvents. The amino acids and sugars were identified by their Rf values and by comparison with standards.

Electrophoretic analysis of the mucin was carried out in a horizontal paper electrophoretic apparatus using phosphate buffer (pH 5.6; ionic strength 0.005). Samples were spotted on 2 cm wide Whatman No. 1 filter paper strips and a current of 200 V was applied for 48 h. Dried strips were stained with toluidine blue as described elsewhere.

**Histological and Histochemical Observations**

1. **The albuminous gland cells**

These cells are columnar measuring 8 to 10 μ in width. The nucleus is basal in position and the cytoplasm shows the presence of fine secretory granules (Fig. 1) distributed uniformly as described by Bahl.

The results of the histochemical tests on the albuminous gland cells are summarized in Table 1. The granules are intensely positive to PAS reagent but are negative to all stains employed for acid mucopolysaccharides. Substances reactive to PAS are glycogen, glyco or mucoprotein and neutral mucopolysaccharides. Certain lipids and proteins are also reactive to PAS reagent. The granules give positive reaction after pyridine extraction to remove lipids and deamination followed by methylation to block the amino and carboxyl groups. Specific blockage of reactive moieties of polysaccharides by acetylation resulted in the loss of reactivity to PAS reagent. The PAS staining is not abolished by treatment with diastase indicating that the staining is not due to glycogen. The positive reactions with Millon’s, bromophenol blue and Morel-Sisley diazotization tests indicate the presence of protein. The above observations may suggest that the secretions of the albuminous gland cells may contain a neutral mucopolysaccharide and a protein probably occurring as glyco or mucoprotein.

2. **Mucous gland cells**

These are distinguished from the albuminous gland cells by their basophilia. They are found in large numbers all over the body in two different sizes, small and large (Fig. 1 u. 2 A).

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**a) Small mucous gland cells**

These gland cells have distended apical part and measure 10 to 12 μ in width (Fig. 2 A). The cytoplasm contains large and round secretory granules which stain light blue with alcian blue. This property of the granules may suggest that the secretions of this gland cell may be an acid mucopolysaccharide 22. The metachromatic staining with toluidine blue (Table 1) may characterize the substance as an acid mucopolysaccharide containing uronic acid or sulphate groups 23. Since pretreatment with hyaluronidase affected staining with toluidine blue, the substance may be hyaluronic acid.

<table>
<thead>
<tr>
<th>Name of the test</th>
<th>Albinous gland</th>
<th>Mucous gland</th>
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<tbody>
<tr>
<td></td>
<td>Small mucous gland</td>
<td>Large mucous gland</td>
</tr>
<tr>
<td>1. PAS</td>
<td>+</td>
<td>–</td>
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<tr>
<td>2. PAS-after pyridine</td>
<td>+</td>
<td>–</td>
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<tr>
<td>3. PAS-after methylation</td>
<td>+</td>
<td>–</td>
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<tr>
<td>4. PAS-after diastase</td>
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<td>–</td>
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<tr>
<td>5. PAS-after acetylation</td>
<td>+</td>
<td>–</td>
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<tr>
<td>6. Toluidine blue –</td>
<td>–</td>
<td>+</td>
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<tr>
<td>metachromasia</td>
<td>(β)</td>
<td>(γ')</td>
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<td>7. Toluidine blue –</td>
<td>–</td>
<td>+</td>
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<td>metachromasia after 95%</td>
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<tr>
<td>alcohol</td>
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<tr>
<td>8. Toluidine blue –</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>after hyaluronidase</td>
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<td></td>
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<tr>
<td>treatment</td>
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<tr>
<td>9. Methylene blue –</td>
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<td>extinction point</td>
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<td>10. MILLON’s test</td>
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<tr>
<td>11. Bromophenol blue</td>
<td>+</td>
<td>–</td>
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<tr>
<td>12. MOREL-SISLEY diazo­</td>
<td>+</td>
<td>–</td>
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<td>titzation method</td>
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<td></td>
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<tr>
<td>13. Alcian blue</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 1. Results of the histochemical reactions of the epidermal glands of earth worm *Megascoleæ mauritii*. Key: + positive; ± weak reaction; − negative reaction; 0 not observed.

**b) The large mucous gland cells**

This type of glands are larger and roughly round (Fig. 2 A) measuring 20 to 25 μ in diameter. They are fewer in number than the small mucous gland cells. The entire cell is filled with intensely basophil granular inclusions which stain deep blue with alcian blue and red with toluidine blue. The γ-metachro-

matic staining with toluidine blue may indicate presence of a highly acidic mucopolysaccharide. The metachromasia was not removed by washing with 95% ethanol indicating presence of esters of sulphuric acid 17. Methylene blue stained the gland at pH 2.6 and the extinction point lies between pH 1.0 and 1.4. The capacity to bind methylene blue below pH 4.0 is considered indicative of presence of sulphate groups. These observations may suggest that the mucin secreted by the small mucous gland cells is a sulphated mucopolysaccharide. Bromophenol blue stains these glands faintly while the MILLON’s and the MOREL-SISLEY diazotization tests are non-reactive. These reactions suggest that the protein if any, is unlike that in the first type of mucous gland cells.

After irritation with ether these gland cells become vacuolated and contain very little cytoplasm around nucleus indicating that the contents are ejected out (Fig. 2 B).

**Observations on the Properties of the Mucin**

**a) Mucin collected under normal conditions**

Tests performed on the mucin secreted on the surface of earth worms placed directly on a glass slide showed histochemical reactions similar to those observed in the small mucous gland cells. These secretions in addition stained with PAS reagent indicating the presence of glyco or mucoprotein. It also reacted positively with the MILLON’s test. The presence of protein was confirmed by chromatography which revealed the presence of 14 amino acids, viz. alanine, arginine, aspartic acid, glycine, leucine, iso leucine, glutamic acid, lysine, histidine, serine, proline, tyrosine, valine and phenylalanine. The Elson-Morgan reaction was positive both before and after hydrolysis suggesting the presence of amino sugars 24. Chromatographic analysis of the mucin revealed presence of two sugar components which were identified as glucosamine and uronic acid. The foregoing observations indicate that the mucin secreted under normal conditions contain an acid mucopolysaccharide which is likely to be hyaluronic acid and a glyco or mucoprotein. It is suggested that under normal conditions only the albinous and small mucous gland cells contribute to the mucin found at the surface.

22 H. F. STEEDMAN, Quart. J. microscop. Sci. 91, 477 [1950].
The histological studies reported earlier suggest that the large mucous gland cells secreted only after irritation. The mucin secreted after irritation was analysed chromatographically and electrophoretically for a comparison with the mucin secreted under normal conditions.

Chromatographic analysis for carbohydrates showed the presence of galactosamine in addition to glucosamine and uronic acid. Electrophoretic patterns of the acid mucopolysaccharide of the mucin secreted under normal conditions as well as after irritation are given in (Fig. 3). The mucin secreted under normal conditions formed one band moving towards the anode to a distance of 4 cms from the origin. This fraction stained purple with toluidine blue similar to the inclusions of the small mucous gland cells. On the other hand the mucin secreted under irritant conditions resolved into two anodic fractions of which the one with fast anodic mobility migrated to a distance of 4 cms and the other 1.5 cms from the origin. These fractions stained purple and red respectively with toluidine blue indicating the presence of two different types of acid mucopolysaccharides in concordance with the histochemical results.

Discussion

Ewer and Hanson\(^7\) reported that the mucous gland cells of earth worms stained with certain dyes like mucicarmine and were metachromatic with thionine, while the albuminous gland cells did not react suggesting that the mucous gland cells contain acid mucopolysaccharides which may not be present in the albuminous gland cells. The results reported in the present study show that the secretions from the albuminous gland cells is a glyco or mucoprotein. The mucous gland cells on the other hand secrete acid mucopolysaccharides. These gland cells are of two types each secreting a different type of acid mucopolysaccharide. The common type, a small basophil gland cells, occurs in large numbers all over the epidermis and differs markedly from the other type which is larger, intensely basophil and less frequent. The acid mucopolysaccharide secreted by the large mucous gland cells is sulphated and more acidic than the secretions of the small mucous gland cells, which is of the nature of hyaluronic acid.

The biological attribute to hyaluronic acid and mucoprotein is water retention to keep a surface moist and to serve as lubricant\(^2\). In view of the role of epidermal mucin in earthworms as a lubricant during locomotion as well as a substance keeping the body surface moist, it is interesting to note the presence of hyaluronic acid and mucoprotein in the mucin; contributed by the small mucous gland cells and albuminous gland cells respectively.

Ressler et al.\(^{25}\) reported that the mucin secreted by undisturbed earth worms was different from the mucin secreted in response to irritation and the latter was disliked by other worms of the species, suggesting the presence of some alarm pheromones in it. It is interesting to note that the large mucous gland cells secrete a highly acidic sulphated mucopolysaccharide in response to irritation. It may be noted in this context that ophiuroids under conditions of irritation secrete a substance which is a highly acidic mucopolysaccharide\(^2\). In the light of the present study it is suggested that the pheromones referred to by Ressler et al.\(^{25}\) in earth worms may be the highly acidic mucopolysaccharide secreted by the large mucous gland cells.

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