THE OCCURRENCE OF MUCOID SUBSTANCES IN INSECTS

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Summary

Three histochemical tests, which demonstrate mucoid substances of vertebrate origin, have been applied to a variety of insect tissues. Mucoid materials seem to be absent from the contents of the insect midgut, but a positive reaction may be given by the striated border of the epithelium. Goblet cells of the larval midgut of Lepidoptera and rectal glands of all of the insects studied give a negative reaction, but the salivary glands of the cockroach, grasshopper, larval calliphorids, and worker honeybee all contain mucoid substances. In general, these materials seem to be of less frequent occurrence in insects than they are in most other animal phyla. The significance of the observed distribution of mucoid substances in insects is discussed, particularly in relation to the functions of the peritrophic membrane and the salivary glands.

I. Introduction

There are many references to the occurrence of mucins in insects, but few of them are based on good evidence. Platania (1938) considered that the midgut of *Reticulitermes* produces a mucin which is incorporated in the lamellae of the peritrophic membrane, and refers to the statements, all published over 40 years ago, of six authors who reported "mucus" in the gut of a variety of insects. Ichikawa (1931) described a mucus layer in the gut of Collembola, and Weil (1936) also considered the peritrophic membrane of bees and wasps to have mucus incorporated in it. Hodge (1936) described a thin film of "mucous material" on the surface of the midgut epithelium of *Melanoplus;* the goblet cells in the midgut of larval Lepidoptera have been thought to secrete mucus (Frenzel 1886), and certain large cells of the *Psychoda* larval gut have been called mucous cells by Haseman (1910). But von Dehn (1933), Wigglesworth (1948), and others have maintained that mucins are absent from the insect gut. The current theory of the function of the peritrophic membrane in insects, namely that it serves to protect the midgut epithelium (Wigglesworth 1939), assumes that it replaces the mucins, which perform the protective function in many other groups of animals.

The rectal pads have been thought to secrete mucus (Sayce 1899; Marshall 1948); and the central cells of the cockroach salivary glands have been called mucous cells by Lebedeff (1899).

The contradictory conclusions in the majority of the above reports, and the uncertainty of the techniques employed (only mucicarmine and toluidine blue in most of the work) suggested the need to investigate the occurrence of mucoid substances in insects. The small quantity of material available from

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insect tissues precluded the detection of mucoid substances by any of the chemical tests that have been developed (see Burnet 1948), and so several histochemical methods, which give satisfactory results with vertebrate mucins, were employed.

Since the chemistry of none of the insect products has been studied it is not possible to classify them according to any modern scheme (for example, that of Meyer 1948), and for this reason the term "mucoid substance" has been used rather than the more specific mucin, mucopolysaccharide, mucoprotein, etc. The term mucoid substance will thus include all naturally occurring polysaccharides and protein-polysaccharide complexes in which at least part of the sugar moiety is a hexosamine.

II. Methods

Tissues were fixed in Carnoy's, Helly's, and alcoholic Bouin's fluids. Carnoy's alcohol-acetic acid mixture was found to be the best of these and was used in all later work. Although aqueous fluids were avoided when possible, no improvement in the preparations resulted from spreading the paraffin sections on non-aqueous liquids. A number of mucin stains (mucihaematin, mucicarmine, thionine) were used, but later replaced by the following three histochemical tests:

(1) The Gomori (1946) test for glycogen and mucin. Glycogen can be easily removed by ptyalin digestion and the test appears to be fairly specific; however, over-staining can result in a loss of specificity.

(2) The Bismarck brown method (B) of Leach (1947), a technique resulting in a much improved specificity for water-labile mucoproteins.

(3) The toluidine blue method, which gives a characteristic metachromasia with most mucoid substances, which Lison (1936) believes is practically a specific microchemical test.

Although none of these tests can be said to be absolutely specific, positive reactions with all of them provide a strong indication of the presence of mucoid substances.

III. Observations

An experiment was performed to determine which of the staining procedures, if any, gave the most easily detectable reaction with insect mucins. If any of the claims referred to above were justified it was to be expected that a positive reaction would be given by salivary glands, midgut goblet cells, and/or rectal pads. Sections of *Periplaneta* salivary glands and rectal pads and transverse sections of *Ephestia* larvae were fixed in Carnoy's, Helly's, and alcoholic Bouin's fluids and stained in toluidine blue, Gomori's methenamine, and Bismarck brown. The results are presented in Table 1.

From these data it is presumed that the *Periplaneta* salivary glands do include mucoid-containing cells, but that the rectal glands or goblet cells apparently do not. It was also clear that there were reactions in some other

tissues towards these histochemical tests. Thus the chitinous intima of the *Periplaneta* oesophagus, the cuticle of the *Ephestia* larvae, and the contents of the silk glands of *Ephestia* all gave positive results in some tests. Also from this experiment it appeared that the combination of Carnoy's fixative and Bismarck brown gave the best results. An attempt was then made to determine what tissues of a variety of insects gave this test for mucoid substances. Tissues showing a positive reaction were later checked by the other methods. Serial sections of several individuals of each of the following species were examined: *Ctenolepisma longicaudata* Esch., *Periplaneta americana* (L.), (embryos and adults), *Locusta migratoria* (L.), *Nasutitermes exitiosus* (Hill) (soldiers), *Coptotermes lacteus* (Froggatt) (soldiers), *Tenebrio molitor* L. (larvae and adults), *Ephestia kuhniella* Zeller (larvae), *Tineola biselliella* Hum. (larvae and adults), *Gnorimoschema operculella* Zeller (larvae), *Pieris rapae* (L.), (larvae), *Musca domestica* L. (larvae), *Lucilia cuprina* Wied. (larvae and adults), and *Apis mellifica* L. (workers).

TABLE 1

REACTIONS OF VARIOUS INSECT TISSUES TO TESTS FOR MUCOID SUBSTANCES

Tissue / Test	*Periplaneta* Salivary Glands			*Periplaneta* Rectal Pads			*Ephestia* Larval Midgut Goblet Cells		
	Carnoy	Helly	Alc. Bouin	Carnoy	Helly	Alc. Bouin	Carnoy	Helly	Alc. Bouin
Gomori's methenamine	+	±	+	−	−	−	−	−	−
Bismarck brown	+	±	+	−	−	−	−	−	−
Toluidine blue	±	+	±	−	−	−	−	−	−

+ indicates positive reaction; − indicates no characteristic stain observed; ± indicates probable positive reaction.

Of all these species positive reactions for mucoid substances were found in the following tissues.

(1) Cuticle — for example, in *Ctenolepisma,* and in larvae of *Ephestia, Gnorimoschema, Lucilia.*

(2) Chitinous intima — for example, in the foregut of *Periplaneta, Locusta, Lucilia.*

(3) Striated border of midgut epithelium — *Ctenolepisma;* caeca and midgut of *Locusta;* midgut, but not crypts, of adult *Tenebrio;* midgut of *Pieris* and *Tineola;* and cells at the base of the crypts only, in *Apis.* This latter case was very specific and the reaction was clearly restricted to a few cells only in each crypt.

(4) Striated border of only a few malpighian tubules of *Tenebrio.*

(5) Fat body — some inclusions of larval fat body of *Lucilia* and the same tissue when found in recently emerged adults, and a diffuse reaction in the fat body of larvae of *Tenebrio* and of *Tineola.*

(6) Peritrophic membrane — as in larvae of *Lucilia* and *Ephestia,* and in *Periplaneta.*

(7) Connective tissue — as in the midgut of *Periplaneta.*

(8) Salivary glands – *Periplaneta, Locusta, Apis,* larvae of *Lucilia* and *Musca.*

Of these examples a secretion of mucoid substances was observed only in the salivary glands. In the remainder the reaction was confined to formed cellular elements. In the cockroach salivary glands Lebedeff (1899) described acini containing two cell types. The peripheral cells were thought to produce digestive enzymes, whereas the central cells, which undergo a conspicuous cycle of secretory activity, were thought to produce mucin. The designations are not appropriate since "central cells" frequently occur on the periphery of the acini. But it is certainly these which produce the mucoid substance. However, not all the "central cells" give a positive reaction and it is evident that they do so only at certain periods of this secretory cycle. In *Periplaneta* starved for 14 days the "central cells" containing mucoid materials are greatly increased in number; in a *Periplaneta* fed only starch for 14 days the mucoid substance is very greatly depleted, and the cytoplasm of most of the central cells is markedly vacuolate. It is noteworthy that Day and Powning (1949) reported that the salivary glands of cockroaches similarly treated contained much less amylase than normal. Apparently mucin and amylase are secreted together. Preparations stained for polysaccharides by the method of McManus (1946) indicated that the cells giving the mucin reactions contained no stainable polysaccharide, although the "peripheral cells" were stained by this method.

In *Locusta* the "zymogenic cells" of Beams and King (1932) again give the positive reaction.

In *Apis* the "salivary glands" are well developed and are of several types (Kratky 1931). Only the cells of the lobes of pharyngeal glands give a positive reaction, and all cells appear to react with equal intensity.

In larvae of *Musca* and *Lucilia* the contents of the salivary glands give a strong positive reaction but the cells themselves do not, and in *Lucilia* at least this reaction is greatly enhanced in the prepupae at which time the glands become greatly swollen with a secretion of unknown function. The salivary glands of adult *Lucilia* gave a negative reaction. In the lepidopterous larvae examined the contents of the silk glands were weakly positive, but the cells of the glands were negative.

A positive reaction for mucoid substances was especially looked for in the goblet cells of the lepidopterous larval midgut in the region of peritrophic membrane formation in many species, in the head secretion of soldier termites, in

the reproductive tracts of all species studied, and in the embryonic tissues and imaginal buds, but the reaction was negative in all these examples.

It is likely that other tissues in other species might give positive reactions. For example, Glasgow (1936) describes dorsal cephalic glands of the larva of *Hydropsyche* and considers that they probably secrete mucus, but the above examples are sufficient to indicate the most usual sites of mucoid substances.

IV. Discussion

(1) Before considering the distribution of positive reactions for mucoid substances in insect tissues it is appropriate to refer briefly to the types of materials included in this category. A recent review by Kurt Meyer (1948) includes three main types; the mucopolysaccharides, the mucoproteins, and the glycoproteins. Among the neutral mucopolysaccharides are examples giving on degradation residues of acetylglucosamine only — and chitins are typical of such materials. It is obvious, therefore, that the synthetic ability for mucopolysaccharide formation is highly developed in insects, and this, of course, explains the positive reaction of some cuticles to histochemical tests for mucoid substances. Also hyaluronic acid is an example of the acid mucopolysaccharides and, although it has not been proved, it is likely that this cementing substance occurs in insects as well as in vertebrates. Thus, cells of the midgut epithelium fall apart when soaked in a solution of hyaluronidase (Day and Powning 1949), and hyaluronidase itself has been extracted from a variety of insects (Duran-Reynolds 1939).

In view of the above it is all the more remarkable that other types of mucoid substances occur so infrequently in insects in comparison with their occurrence in vertebrates and some invertebrates (cf. Ewer and Hanson 1945; Kruidenier 1948).

(2) The absence of mucins in the lumen of the insect midgut (except their occasional presence in the peritrophic membrane) lends weight to the hypothesis that one of the principal functions of the latter is the protection of the midgut epithelium.

The fact that the striated border of some species gives a positive reaction (confirming the observation of Hodge 1936) does not weaken this argument; and it is interesting that Gersh (1948) found the striated borders of several vertebrate tissues also gave a positive reaction for glycoprotein.

(3) A similar parallel is not found in the goblet cells. In the vertebrate stomach and large intestine these are essentially producers of mucus. They must serve another function in the gut of lepidopterous larvae; none of the functions suggested for the goblet cells seem satisfactory.

(4) A comparison between the distribution of mucoid substances in vertebrates and insects is of interest. Dempsey and Wislocki (1946) and Wislocki *et al.* (1948) consider a number of locations of such substances in vertebrates. Of these, comparable sites occur in insects only in the stroma of

growing tissues, in some tissues which undergo repeated growth cycles (e.g. the midgut epithelium), in intracellular mucus, and in the secretion of certain glands. In the cockroach embryo, imaginal buds of larvae, and in the insect midgut no mucoid substances were found, and mucous glands appear to be much less frequent than in vertebrates. The salivary glands of insects are the only glands regularly found secreting mucoid materials.

(5) In vertebrates it has been reported that the sites of mucin formation also often give a reaction for alkaline phosphatase, and Leach (1947) has suggested that phosphatase may be a mucoprotein. No correlation between the locations of the two substances is found in insects (cf. Day 1949), indicating that none of the alkaline phosphatases found in insects are mucoproteins.

(6) The frequent occurrence of mucin in insect salivary glands suggests that it functions as a lubricant or to overcome harmful drying of the mouth-parts, which in the cockroach are moistened with salivary secretion during feeding (Wigglesworth 1939). This is substantiated by the absence of mucoid substances in some salivary glands whose function has been modified – as in lepidopterous larval silk glands. However, even in the silk gland of the webworm, *Hyphantria,* Kinney (1926) claims that a mucoid material surrounds the silk in the gland.

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VI. References

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