Observations on the Cornicles of Aphids.

Gail Garwes Wynn

Louisiana State University and Agricultural & Mechanical College

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in

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by

Gail Garwes Wynn
M.S., Louisiana State University, 1962
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ABSTRACT

The cornicle area of eighteen species of aphids was studied histologically. In Cinara spp. and Longistigma caryae (Harris) (Lachnini) a multicellular sac, whose wall is a layer of flat cells with compressed nuclei, extends into the cornicle. The sac is surrounded by multivacuolated fat cells, and a mass of lipid is found in the sac. In Monellia costalis (Fitch) (Callaphidini) a syncytial gland apparently of epidermal origin extends into the minute cornicle. Stegophylla sp., also in this tribe, has a structure resembling that of the Lachnini. In the Aphidini (Aphis, Dactynotus, Macrosiphum, Cachryphora, Hyalopterus, Amorphophora) large fat cells collect in the cornicle and the subcornicle area, some with a central nucleus and many fat vacuoles, others with a large central vacuole and a peripheral nucleus in a ring of vesicular cytoplasm. There is no sac in this group. Large granular cells are located near the cornicle valve in Neophyllaphis podocarpi Takahashi (Thelaxini). There are many smaller cells with central nuclei in the subcornicle area.

The presence of lipid in the large vacuoles of Aphidini and in the cornicle sacs of Lachnini was
demonstrated with staining of frozen sections with Sudan Black B.

All species studied possess a muscle originating on the venter below the cornicle and inserting on the cornicle valve. Its position is such that in addition to opening the valve, it may elevate the cornicle in species possessing a long movable cornicle. A dorsoventral muscle immediately anterior to the cornicle but not attached to it may also aid in elevation of the cornicle. This muscle, however, may be more useful, when it works in association with the dorsoventral (lateral) muscles, to compress the body contents, thereby causing emission of a cornicle droplet.

The cornicle droplets consist of haemolymph, haemocytes, and lipid from dissociated fat cells. Although the nature of the lipid is unknown, there is evidence that it may be fatty alcohol or triglyceride.

Aphids fed various concentrations of sucrose in water produce varying amounts of fat depending upon the sugar concentration in the diet.

It is suggested that the primary function of cornicle droplet emission in aphids is as a means of eliminating excess fat metabolized from a high carbohydrate diet. In the "mealy" or "woolly" aphids examined, the presence of
lipid over the general body surface is correlated with the absence of large central vacuoles in the cornicle fat cells. Thus, in these forms much of the excess fat is eliminated through the body surface.

In aphids with long movable cornicles the cornicle droplet may have become adapted secondarily as a defensive mechanism, as suggested by other investigators, but even in these aphids cornicle droplets are emitted spontaneously in the absence of predators.
INTRODUCTION AND REVIEW OF LITERATURE

The cornicles (nectaries, siphunculi, rückenröhren) of aphids have been a matter for conjecture and study by many workers. The position of the cornicles, the external form, the embryology, the internal histological structure, and the origin, production, method of release, histology, and chemical nature of the cornicle droplet, and its function have been the subjects of studies, the results of which have led to little agreement.

The purposes of this study were as follows:
1. To re-examine the internal histological structure of the cornicle and the subcornicle area of a number of species of aphids in several tribes using techniques which had not been used previously.
2. To make observations of the cornicle droplet and to study it from a histological and chemical standpoint.
3. To observe the effect of a change of the aphid's diet on the internal histological structure of the cornicle and the subcornicle area.

Position and External Form

Lüstner (1904) made the observation that aphids on the exposed parts of plants usually had long cornicles
while those that lived in galls and on the roots which tended to be hairy or waxy had short rudimentary cornicles.

In his observations, Gillette (1908) always found the cornicles located either between the fifth and sixth segments of the abdomen or upon the sixth segment. He found that they were not always illustrated in these positions by other workers.

Hottes (1928) noted that the cornicles were located on the dorsum of the sixth abdominal segment. He recognized five structural types. The procornicicular form, difficult to discern as a cornicle, was found in the genus Monellia. Members of the tribe Lachnini possessed the tuberculate type which was situated on a tubercle of the body. The truncate form found in Chaitophorini and Callaphidini (excluding Monellia) was trunk-like in shape and it was no longer than wide at its base. Species of Aphidini had the cylindrical form of cornicle. The fifth type was pore-like. Regardless of the type, Hottes found an opening either at or near the apex of the cornicle. This opening was a semicircular slit which was opened or closed by a valve. The valve was attached around the rim of the cornicle for a short distance by a flexible hinge.

An anomaly was found in a colony of Megoura viciae Kalt. (Zirnits, 1930). Each cornicle of one aphid was
divided into two branches; another individual had this characteristic on one cornicle. The base of the cornicle in each case made a common trunk. The outer branch appeared to be the normal functioning one while the inner branch was narrower, smaller, and presumably nonfunctional.

Remaudiere (1964, 1966) noted that the cornicles have a constant position in a given genus. In many aphids they are situated on the fifth tergite of the abdomen and are borne on the posterior edge of this tergite thus appearing to be located between the fifth and sixth segments. In some other species of aphids the cornicles are located on the sixth abdominal tergite. Remaudiere found a species of *Aphis* with eleven individuals having supernumery cornicles. The normal pair of cornicles was situated on the posterior border of tergite five, while the extra cornicles were located on the posterior part of tergite six. Some of these extra cornicles had valves. He considered these extra cornicles due to mutations since tergite six is a normal position for cornicles in some aphids but not in the genus *Aphis*. Two-thirds of his collected specimens had this anomaly.

**Histology of the Cornicle and Subcornicle Area**

Mordwilko (1895), probably the first to study the
cornicle area of an aphid, regarded the sac seen in
*Lachnus viminalis* Boyer as a wax sac.

Henneguy (1904) noted that the cornicles were cuticular tubes which communicated with a unicellular hypodermal gland.

Probably the most extensive work on cornicles was that of Hottes (1928). He studied twenty species histologically. In *Lachnus coloradensis* Gillette he noted within the cornicle, a large nucleated glandular mass which consisted of two kinds of cells. Hottes denoted these "A" cells and "B" cells. The "A" cells were found close to the valve, had no cell boundaries, contained large granular nuclei scattered randomly throughout the cytoplasm, and possessed coarsely vacuolated cytoplasm. The vacuoles were of variable size. The "B" cells were of a more delicate nature, surrounded the glandular mass of "A" cells, and filled the greater portion of the cornicle cavity. The limits of these cells could be distinguished even though the cell membranes were not present. The cytoplasm was greatly vacuolated with small and uniform vacuoles. A cavity was seen at the extreme inner part of the tubercle which Hottes thought to be an artifact produced by the shrinking of the interior cells leaving a membrane-like residue. Hottes found the
cornicles of *Longistigma caryae* (Harris) to be very similar to those of *Lachnus*. The glandular part was considerably larger than that of *Lachnus*, and the cells of the "A" type were not as numerous. The internal structure of the cornicles of *Eulachnus agilis* (Koch) departed widely from that of the genus *Lachnus*. However, Hottes did not describe the nature of the departure. In *Monellia caryella* (Fitch), the internal structure of the cornicle consisted of a sac-like, glandular tissue in which the cytoplasm was differentiated into three regions. Next to the mouth of the cornicle there was an open, sac-like area which in life, Hottes thought, was filled with the secretory substance. This region was surrounded by a deeply staining mass of vacuolated cytoplasm containing nuclei. Peripheral to this region was a layer of less-deeply-stained cytoplasm. No cell membranes were distinguished. In *Symydobius americanus* Baker, Hottes observed the valve located about half way down the cornicle. He described a large glandular sac, the walls of which consisted of a single layer of vacuoles surrounded by a homogeneous, deeply staining cytoplasm in which deeply staining nuclei were situated. In several individuals, Hottes noted smaller, glandular, spherical sacs similar to those he had seen in the embryo. He noted that the large sac did not join the opening of
the cornicle directly, even though it extends up into the cornicle cavity. He concluded that since some individuals showed smaller sacs in addition to the larger one, the cornicles of this species were in a transitional stage between the cornicles of the *Myzocallis* type (tribe, Callaphidini) and the cornicles of the genus *Aphis*. Hottes found the histology of the cornicle area of *Neothomasia populicola* (Thomas) and *Neothomasia abditus* similar to the structure of *Symydobius americanus*. *Callipterinella betulaecolens* (Fitch) was also similar to the former, but did not have the small, spherical sacs in addition to the larger one. *Myzocallis bellus* (Walsh) was the same, and *Calaphis betulella* (Walsh) occasionally showed the small spheres. The histological structure of the cornicle area of *Anoecia oenotherae* Wilson was found to be more like that of *Neothomasia* than of *Lachnus*, and Hottes suggested that *Anoecia* may not be as closely related to the Lachnini as had been thought. In his study of *Drepanaphis acerifoiiii* (Thomas), Hottes believed that he had found the "phylogenetic goal" toward which the internal structure of the cornicle had been directed. In the body cavity in the vicinity of the cornicle, and in the cornicle itself, he noted numerous hollow and partially hollow spheres. Each of these had one nucleus, and when the
sphere was hollow, the outer surface was only one vacuole in thickness. If the spheres were not hollow, they appeared to be partially filled with vacuoles separated from each other by a network of cytoplasm. The spheres were tightly compacted in the cornicle, but more loosely arranged in the body cavity. To Hottes, these resembled the loose spheres found in the genus *Symydobius*. He thought that the solid spheres showed a resemblance to fat cells, but that they were not fat cells because of a nuclear difference and because of their cytoplasm which he believed to be more evenly vacuolated and more darkly staining than the cytoplasm of the fat cells. Both *Melanoxantherium salicus* (Linnaeus) and *Melanoxantherium smithiae* (Monell) represented the type of structure found in *Drepanaphis*. In the genera, *Acyrthosiphon*, *Microsiphum*, * Macrosiphoniella*, *Catamergus*, and *Tritogenaphis*, Hottes observed that the internal structure of the cornicle was similar to *Drepanaphis* with minor differences. The number of spheres varied greatly. Sometimes they extended across the posterior end of the body, and at other times they were more limited in number. The cells of the cornicle area of *Thargelia albipes* (Oestlund) differed from those of the former genera. They suggested oenocytes to Hottes but were smaller in size, had evenly vacuolated
cytoplasm, and stained differently.

**Embryology**

The external portion of the cornicle was derived from ectoderm (Hottes, 1928); the internal part, from mesoderm (Witlaczil, 1882; Hottes, 1928).

Hottes (1928) studied the embryology of the cornicle in four species of aphids. In studying the embryo of *Lachnus coloradensis*, Hottes noted a finely granulated nucleus in a mass of vacuolated tissue in the region where the cornicle would later appear. This was intimately associated with fat cells. The vacuoles, Hottes suggested, were now empty spaces but were once filled with secretory substance. In *Monellia caryella*, Hottes noted two hollow ball-like structures in the posterio-lateral portion of the abdomen. These resembled hollow rings, and were the first indication of the developing cornicles. Nuclei were seen within the bands while the central area was clear, indicating to Hottes that at this stage in the embryo, it was already filled with secretory substance. None of Hottes' sections showed the opening of the cornicle or the muscle of the valve. He did not find this surprising since in the adult the external part of the cornicle was difficult to find. The embryo of *Symydobius americanus*
had a hollow structure much like that in Monellia. Some of the embryos had one or two smaller glandular masses near the larger ones, but they were of the same nature. Hottes found the first indications of the developing cornicle in rather mature embryos of Drepanaphis acerifolii. In the posterio-lateral margin of the abdomen were noted a number of hollow spheres consisting of vacuoles surrounded by cytoplasm. Each of these spheres consisted of one cell as only one nucleus was seen. None of these embryos showed the external part of the cornicle.

Muscular Arrangement

Flügel (1904) showed a muscle attached to the anterior base of the cornicle plus the valve muscle, which ran into the cornicle.

Hottes (1928) could not find the muscle illustrated by Flügel which attached to the base of the cornicle. However, in Lachnus coloradensis, Monellia caryella, and Symydobius americanus, he found a muscle attached near the medial free portion of the valve. This valve muscle had its origin on the ventral part of the body wall. He concluded that this muscle performed a double function: that of opening the valve and that of moving the cornicle which he observed to be slightly movable in some aphids.
In histological studies of the cornicle of *Aphis gossypii* Glover, Yu-swu (1956) found two bundles of muscles associated with the cornicle. One of these muscles, designated by her as the "valvular muscle," originated on the ventral side of the sixth abdominal segment and went obliquely through the segment and up to the upper part of the cornicle where it inserted on the inner surface of the valve. The other muscle, called the "tube basal muscle," arose on the dorsum (sic) of the sixth abdominal segment and had its insertion on the base of the cornicle.

Edwards (1966) found the cornicle valve muscle originating on the dorsal (sic) part of the segment posterior to the cornicle and inserting on the cornicle tip.

**Origin of Cornicle Droplet**

De la Torre Bueno (1907) expressed the opinion that the cornicles of aphids were the excretory canals of wax-producing glands, differentiated in a special manner.

Weber (1930) observed that the blood was the origin of the cornicle droplet. He noted that at the apex of the cornicle there were ostioles which were opened by special muscles allowing wax-laden blood cells to escape.
Edwards (1966) thought that the cornicle wax arose from cells which might be modified oenocytes. He stated in his work that the secretion was stored in large globules encased by a membrane. Sometimes residual cytoplasm and a nucleus were present and sometimes these were not seen. Edwards studied three species, *Aphis fabae* (Scopoli), *Macrosiphum rudbeckiae* (Fitch), and *Myzus persicae* (Sulzer). He observed the globules in the cornicle stalk and in the body cavity below the cornicle. He also noted haemocytes in the cornicle lumen in his histological sections.

Strong (1967) noted that the cornicle droplet originated in the dorso-lateral area of the abdomen in the region of the cornicle attachment. He saw globules measuring 8 to 10 micra through the body wall in freshly mounted specimens of *Myzus persicae*. These globules could be seen to stream upward and bubble through the cornicle tip when the droplet was being produced. He noted that the globules coalesced and formed a homogeneous droplet within 2 to 4 seconds upon reaching the exterior. Strong made the observation that the globules in the abdomen which formed the cornicle droplet were separated from the haemolymph because no insect blood was extruded through the cornicle. However, he was unable to find a membrane separating the globules from the blood sinus.
He studied *Myzus persicae*, *Acyrthosiphon pisum* (Harris), and *Chaitophorus* sp. In his sections of these species, hollow, circular bodies which lacked a nucleus were stained, but lipid was not demonstrated in these bodies by the methods he used. When the aphids were dissected and stained *in situ* with Sudan Black B (a lipid dye), the globules at the bases of the cornicles hardened and stained black. In *Chaitophorus* sp. granules were noted in the globules in histological preparations. Strong thought that these might be glandular.

**Production and Method of Release of Cornicle Droplet**

Weber (1930) noted that the emergence of the cornicle droplet was voluntarily regulated by the valve-like lip of the aphid's cornicle. When the valve was opened, the cells from the cornicle emerged and exploded, forming the droplet.

Edwards (1966) observed that the release of the cornicle droplet was under reflex control. When he touched one side of the aphid or the cornicle on that side, the wax was given off on the same side. The release of the droplet was achieved by retraction of the cornicle muscle. The actual expulsion of the droplet, he thought, was accomplished by abdominal turgor since he saw no
contractile structure at the base of the cornicle.

Strong (1967) noted that aphids of all instars were capable of producing the cornicle droplets. He stated that the production was partly under direct nervous control, as a mild mechanical stimulation such as a gentle touch on any of the thoracic tergites with a fine point resulted in the elevation of the cornicles and this was generally followed by the production of the cornicle droplet. If the stimulation was applied to the right side of the aphid, then the right cornicle produced a droplet and the left cornicle remained in the depressed position. Strong found that by gently blowing on the appropriate area with a fine stream of air the response was elicited. The same response was obtained when the aphid was anesthetized with carbon dioxide, but no response was obtained when the insect was anesthetized with ether (a nerve anesthesia). When he repeatedly stimulated aphids, they produced up to six droplets in succession. He found that the first droplet was always the largest. *Myzus persicae* and *Chaitophorus* sp. produced droplets 98 per cent of the time after stimulation while 60 to 70 per cent of the pea aphids (*Acyrthosiphon pisum*) responded. In *Acyrthosiphon* the production of the droplet seemed to be correlated with the temperature. At low temperatures there was a lowering
of the frequency of response. There also seemed to be a correlation with the body water content, as 52 per cent produced droplets when removed from the plant for 20 minutes and 6 per cent produced droplets when they were removed from the plant for an hour and then stimulated to elicit the response. In the pea aphid Strong observed that in the normal feeding position, the movable cornicles were extended straight back in a plane approximately parallel to the longitudinal axis of the body. When the aphid was stimulated, the cornicles were raised and extended through an arc of about 110 degrees, and the droplet was produced only after the cornicles were so raised. Strong was unable to discover the mechanism which forced the droplet from within the body to the outside. He thought that the cornicle valve muscle could not contract to open the valve when the cornicle was in the depressed position.

Histology of Cornicle Droplet

Weber (1930) saw cells which intensely refracted light in the aphid's cornicles. These cells which formed the droplet and stemmed from the blood were modified blood cells and contained wax.

The cornicle droplet was studied by smear technique
using Wright's stain by Yu-swu (1956). The same cellular elements as those present in the haemolymph were found.
She stated that the cornicle fluid was constituted of proleucocytes, macronucleocytes, granular leucocytes, spherule cells, phagocytes, adipoleucocytes, oenocytes, and plasma.

Chemical and Physical Nature of Cornicle Droplet

Blüsgen (1891) noted that the chemical composition of the cornicle droplet was "wax-like." It hardened rapidly upon exposure to air.

De la Torre Bueno (1907) noted that the tiny droplet which appeared when an aphid was picked up, was colored and that it was a waxy substance.

When Gillette (1908) pinched an aphid between his thumb and forefinger in order to examine the genitalia, he observed that a waxy droplet of white, yellow, brown, red, or deepest black fluid usually was expelled from the tip of the cornicles. He noted that these sticky drops accumulated at the end of the cornicle and in some cases ran down the cornicle, but they did not often free themselves from the cornicle.

Edwards (1966) observed that the cornicle droplet was composed of lipid droplets in a water vehicle. These
droplets coalesced and when they came into contact with a solid surface, they rapidly crystallized to form a hard waxy plaque. Since dehydrated aphids did not emit the droplets, he concluded that the aphid was releasing material from the haemococele when the valve was open, and he analogized this to reflex bleeding in coccinellids. The large open cells which he pictured in the cornicle cavity and below it in the body contained wax, part of which was fixed by osmium. Edwards observed that an immersed droplet retained in a small, inverted, clean glass cup remained clear and liquid for many hours before crystallization occurred. When it contacted air or a solid surface, rapid crystallization resulted. He was unable to detect a solvent in the cornicle droplet when it was analyzed by gas chromatography. A solvent, Edwards thought, would account for the liquid state of the drop within the aphid's body and the solid state without. Droplets of liquid cornicle wax floating on water and dehydrating agents remained fluid only under clean conditions and without vibrations. Edwards concluded that there might be two explanations for the rapid crystallization of the cornicle droplet. In the aphid's body the material was in a stable, liquid-crystalline state and it changed to the solid-crystal phase on contact with
a seeding nucleus. Alternatively, he surmised, that the cornicle material was super-cooled and foreign matter provided the seeding nucleus. Since the melting points of the droplets of Macrosiphum rudbeckiae, Myzus persicae, and Aphis fabae were 48°C, 42°C, and 37.5°C, respectively, the waxes of these three species should crystallize on seeding at normal summer temperatures.

In the three species studied by Strong (1967), the size of the cornicle droplet ranged from 30 to 275 micra. This depended on the size of the aphid and whether the droplet was the first or the last one produced. The droplets of Chaitophorus sp. were hyaline and became pearly white upon hardening; those of Acyrthosiphon pisum and Myzus persicae were tinged yellow and became semi-transparent, yellow balls when hard. The droplets shrunk when they hardened. Strong noted a weight loss in the aphids after the production of cornicle droplets. When he performed solubility tests on the droplets of Myzus persicae and Chaitophorus sp., Strong found that the droplets were insoluble in water, tertiary butyl alcohol and methanol, slightly soluble in acetone, ethanol, petroleum ether, and glacial acetic acid, and very soluble in chloroform, methyl ethyl ketone, and pyridine. The pH of the droplets ranged from 6 to 7 and the melting
point of the droplet of *Myzus persicae* was 45°C to 49°C. Strong noted that the relative humidity influenced the hardening of the droplet indicating that water was present in the droplet. He found no correlation between the hardening time and the size of the droplet. His explanation for the hardening of the droplet involved the coalescing of the globules, their becoming miscible with the aqueous medium, and with sufficient evaporation of water to supersaturate the solution, hardening of the droplet resulted. In chemical analyses of the cornicle droplets of these three species, Strong found traces of six amino acids in the droplets of *Myzus persicae* and *Chaitophorus* sp.; none was detected in the droplet of *Acyrthosiphon pisum*. A faint trace of trehalose was found in the droplets of *Myzus persicae*. The droplets of *Acyrthosiphon pisum* contained three triglycerides and a small amount of hydrocarbon. There were no free sterols, free fatty acids, monoglycerides, diglycerides, or sterol esters. In *Myzus persicae* all of the lipids were represented by two spots which he identified as triglycerides. There were no free fatty acids. The fatty acid composition of these two triglycerides resembled the total fatty acid composition of *Myzus persicae*. 
Function of Cornicle

The functions attributed to the cornicles have been diverse. Some of the first workers to observe the cornicle droplet thought that it was honeydew (Bonnet, 1745; Morren, 1836; Linnaeus, 1758; Witlaczil, 1882). Bonnet and Witlaczil later thought the cornicles were urinary organs.

Reaumur (1738) thought that the dark colored fluid which came from the cornicles was analogous to the feces of other animals.

That the function of the cornicles was excretory was held by others including Buckton (1876). He helped to perpetuate the idea that honeydew came from the cornicles by illustrating an ant collecting a droplet from the tip of the cornicle.

Hottes (1928) believed in the excretory nature of the cornicles. He thought that volatile substances ingested from the plant escaped through them. Certain sap products may not have been assimilated by the aphid after entering its blood and were removed by the glandular internal structure of the cornicle.

Yu-swu (1956) also attributed an excretory function to the cornicles. She noted a reduction in longevity, reproductive period, and fecundity in Aphis gossypii when
the cornicles were cut or sealed. She stated that in the absence of Malpighian tubules, the cornicles might serve as excretory organs. It should be noted that it has been demonstrated that the intestines of aphids function as urinary organs (Kowalevsky, 1889). Gersch (1942) showed that when fluorescein was injected into aphids, it was excreted by the walls of the gut and discharged into the rectal pouch. The cornicle droplets did not show any uric acid reactions (de la Torre Bueno, 1907). Furthermore, Strong (1967) demonstrated that blocking the cornicles of *Acyrthosiphon pisum* had no adverse effects on the adult aphids. The production of young and the life span were not affected.

Because of the high metabolic rate of aphids, a respiratory function was attributed to the cornicles (Kaltenbach, 1843; Kyber, 1815). Kyber, as an alternative, suggested that the cornicles might serve as levers by which aphids could raise the hind part of the abdomen.

Perhaps the most prevalent idea among investigators of this problem was that the cornicles served some protective or defensive function. The production of the cornicle droplet was thought to defend the aphid when attacked by a parasite or predator (Büsgen, 1891; Henneguy, 1904; Horvath, 1905; de la Torre Bueno, 1907). Dixon
(1958) using the nettle aphid, Microlophium evansi (Theob.) and the larva of the ten-spot ladybird beetle, observed that when a larva seized a leg or an antenna of an aphid larger than itself, the aphid often escaped by pulling the part free. However, when the aphid and the larva were the same size, the aphid could not pull itself free and a droplet appeared at the tip of one or both of the cornicles. The cornicle nearest the seized appendage swung over and touched the coccinellid larva. By this method the mouth parts of the larva were "waxed" and the aphid frequently escaped. The larva was observed to be immobilized for 42 to 94 minutes by the cornicle droplet. Dixon noted that when the aphid was smaller than the larva, the aphid rarely escaped. Edwards (1966) observed in the laboratory that when small predators or parasites of aphids received the droplets, they were fixed in a cast. Among several thousands of aphids collected in the field, he found two that had the shriveled bodies of Aphidius sp. fixed to their backs in a plaque of cornicle wax. Reed (1966) stated that a volatile pheromone was released by the aphid at the time of extrusion of the droplet and that this produced an intra-colony alarm response. Both Gillette (1908) and Strong (1967) doubted the protective function attributed to the cornicles and the droplets, but they
offered no alternative explanation for the production of the droplet. Gillette thought that the droplet was ineffective as a protective device since in his observations, it was seldom seen to free itself from the cornicle.

**Fat Body of Aphids (Aphidinae)**

Several investigators have studied the fat body of aphids. Wheeler (1892) noted oenocytes scattered throughout the fat body.

Cockbain (1961) in his study of tethered flight in aphids, found a decrease in the fat cells in the thorax with increased exercise. He illustrated fat cells located in the thorax and in the posterio-lateral abdominal areas.

Working with the English grain aphid, *Macrosiphum granarium* (Kirby), Rutschky and Campbell (1964) found no difference in the cytology of the fat body cells in viruliferous and nonviruliferous aphids. Stellate nuclei appeared in the fat cells of both aphids and became increasingly numerous as the nymphs matured.

Wigglesworth (1965) noted that the mycetome in aphids was formed by strips or masses of modified fat cells.

**Lipids in Aphids (Aphidinae)**

Barlow (1963) in studies with gas chromatography, found that five members of the Aphididae had a proportion
of myristic acid much greater than any of thirty other species of insects studied.

In a study of the total lipid composition of aphids, Strong (1963a) found that 79 per cent of the fatty acids of Myzus persicae was myristic acid. Eighty per cent of the crude extract of Macrosiphum barri Essig was recovered as triglyceride. He found that the fatty acid composition changed with the stage of development in some aphids but not in others. In Myzus persicae the alates had more C_{16} and less C_{14} than nymphs and apterous adults, while in Rhopalosiphum fitchii (Sanderson) the alates had more C_{14} and less C_{16} than apterous individuals.

Strong (1963b) in a study of the ability of Myzus persicae to synthesize fatty acids, found that this species did not make fatty acids that contained less than fourteen carbon atoms. When fed a diet of 18 per cent sucrose with uniformly labelled glucose-C^{14}, the aphids incorporated the labelled carbon in three fatty acids primarily: palmitoleic, steric, and oleic. Small amounts were incorporated in myristic, linoleic, and linolenic.

Aphins

The natural yellow pigment of aphid haemolymph, protoaphin, is water soluble and assumes a deep magenta
color in alkaline solutions. It is converted into yellow, orange, and red derivatives (Duewell et al., 1948).

Cromartie (1959) noted that the stable end product in aphid conversion was a symmetrical multiring structure with the formula $C_{30}H_{22}O_8$.

**Lipids in Eriosomatinae**

In sections of a newly born woolly aphid, *Proci-philus tessellatus* (Fitch), the whole body cavity was found to be filled with a reticulate fat body (Wheeler, 1892). The nuclei measured 3.5 micra. Oenocytes were scattered randomly throughout the reticulum. These were seen as large, brownish, opaque cells with sharp contours, distributed in the meshes of the fat body.

Baker (1915) noted the wax glands in *Eriosoma lanigera* (Hausmann). The secretion of these glands was exuded in a powdery and a filamentous condition. In the latter case it was discharged through plates composed of a ring or an aggregate of several large cells. Each cell contained a central excavation, or wax chamber, within which the secretion accumulated.

The product of the numerous hypodermal glands of *Eriosoma lanigera* was shown to be the glyceride of saturated fatty acids, therefore, a fat and not a wax (Schultz,
Maloeuf (1938) stated that no pores were found in *Eriosoma*, and it was thought that the "wax" exited in a liquid state.

Wax from *Pemphigus xylostei* (DeGeer) resembled that of the cochineal insect which was composed of n-triacontanoic acid ($C_{30}H_{60}O_2$) and a corresponding ketoacid and a keto-alcohol (Schultz and Becker, 1931; Chibnall *et al*., 1934).

Maloeuf (1938) noted that the wax of *Pemphigus xylostei* was secreted by all stages after the first instar. The wax glands were unicellular, ectodermal glands and the cuticle bounding them was very thin.

Related Observations

Other insects demonstrate morphological and physiological characteristics similar to aphids.

**Lipids in the Coccidae:**

Comstock (1916) thought that certain organs in the coccids were homologous to the cornicles of aphids. A pair of openings on the sixth abdominal segment of *Dactylopius* sp. exuded two drops of clear fluid when the insect was gently rubbed.

Sulc (quoted by Hottes, 1928) described cells in the
mealy bug, *Pseudococcus farinosus* DeGeer which were suggestive of the internal structure of the cornicles of certain species of aphids. He thought that these cells were degenerate fat cells which functioned in the defense of the organism.

In the coccid, *Orthezia* sp. wax was discharged at the apex of a hollow hair by the rupture of the tip (Maloeuf, 1938).

**Reflex Bleeding:**

Cuenot (1896) described reflex bleeding in certain beetles as *Coccinella*, *Timarcha*, and meloids and in the orthopterans *Eugaster* and *Ephippiger*. The fluid which escaped was actually blood. It appeared around the mouth, limb joints, and the base of the elytra. The blood was strongly compressed by contraction of the abdomen and escaped by the rupture of the exoskeleton at points of least resistance. In all of these forms the blood contained cantharidin or other caustic or repellent substances. There was evidence that this function was of some value as a protection from predators.

In some insects, it was noted that preformed holes existed in the form of pores or ostioles. (These might have marked the site of glands.) In the tenthredinid larvae *Cimbex* and *Trichiosoma*, these pores were closed
by valves when the blood pressure was relaxed (Hollande, 1926).

On the back of *Pseudococcus* and at the apex of the cornicles of aphids were ostioles, opened by special muscles which allowed wax laden blood cells to escape (Weber, 1930; Wigglesworth, 1965).

In some insects, as the squash beetle, *Epilachna borealis* Fabricius, the fluid which escaped was actually the product of a special group of dermal glands. The fluid ejected from the tibio-femoral articulation was a bitter yellow fluid (McIndoo, 1916).

Happ and Eisner (1961) also described reflex bleeding in a coccinellid, *Epilachna varivestis* Mulsant, noting that the blood which issued from ruptured bristles of the larvae and from the tibio-femoral joints of the legs of the adults, was repellent to ants.

In an acridiid, *Dictyophorus* sp. it was noted that the blood was mixed with air to form a brownish foam as it escaped. It was thought that both the tracheae and the body wall were perforated (Grasse, 1936).

Carpenter and Eltringham (1938) observed that some moths produced a "nauseous froth" which was largely blood and air. They thought that the noxious properties might be due to glandular secretions. Maloeuf (1939) noted
that some grasshopper nymphs could eject the fluid as much as two inches.
CHAPTER I

MATERIALS AND METHODS

In this investigation eighteen species of aphids belonging to five tribes of the family Aphididae were studied (Table I).

Throughout the course of the investigation stock colonies of Dactynotus verbesinae and Aphis gossypii were maintained in the laboratory. A colony of Neophyllaphis podocarpi was maintained for several months in a temperature chamber at 15.6°C. For several weeks colonies of Dactynotus nigrotuberculatus, Dactynotus tissoti, Aphis fabae, and Cachryphora serotinae were kept in the laboratory on their respective host plants. Dactynotus verbesinae was reared on potted plants of crown beard, Verbesina virginica Linnaeus, Aphis gossypii, on detached cotton cotyledons in a pan of water-soaked absorbent cotton, Aphis fabae on potted dock, Rumex crispus Linnaeus and Dactynotus nigrotuberculatus, Dactynotus tissoti, and Cachryphora serotinae on goldenrod, Solidago hirsutissima Mill. Neophyllaphis podocarpi was reared on Chinese yew (Podocarpus macrophylla) seedlings. The cotton was rooted
in vermiculite in the laboratory.

The aphids were maintained under a photoperiod of 14 hours of light provided by two 90 watt fluorescent lamps and 10 hours of darkness. All experiments were carried out at room temperature (24 - 30.6°C).

Other species of aphids used in the study were collected in the field and brought alive to the laboratory where they were studied.

All eighteen species of aphids were studied by routine histological methods which included fixation for twelve to eighteen hours in alcoholic Bouin's solution, dehydration in a graded ethyl alcohol series, clearing in xylene or in methyl salicylate, infiltration for one hour with Paraplast (56 - 57°C) in a vacuum oven at 58 - 60°C, and subsequent embedding in Paraplast. Blocks were made and were cut at 7 micra with an American Optical microtome. Serial sections were mounted on cleaned, albuminized glass slides, allowed to air dry, and were stained with Harris' hematoxylin and eosin. Slides were covered with Permount and #1 coverslips.

Several species of aphids were fixed in Regaud's fluid, run up, infiltrated, and embedded in the routine manner. These were cut at 5 micra and were subsequently stained with Novelli's stain for mitochondria.
These same species were fixed in cold Gendre's fluid. The slides prepared from these specimens were stained with absolute alcohol saturated with iodine for demonstration of glycogen. Control slides were treated with diastase for thirty minutes at room temperature.

A number of species were fixed in cold calcium-formalin, postchromed in 3 per cent potassium dichromate, and routinely treated for paraffin embedding. Other calcium-formalin fixed specimens were cut at 10 micra on a cryostat at -30°C. Alternatively, fresh frozen individuals of these species were cut on the cryostat. Some of these were postfixed in 40 per cent formaldehyde fumes. All of these were subsequently stained with Sudan Black B for lipids and were covered with glycerine jelly. Control specimens were prepared for the lipid studies by refluxing the aphids in 50 ml of methanol:chloroform (3:1) at 60°C for 12 hours (Barka and Anderson, 1963). These were then processed as the experimental specimens.

In preparing for cytological observations of the cornicle droplet, the droplet was allowed to flow from the aphid's cornicle onto a few drops of saline (0.85 per cent sodium chloride) or onto saline-methylene blue on a slide. A coverslip was then applied. Herxheimer's solution was allowed to flow under the coverslip for
demonstration of lipids. In some cases smears were made and were stained with Wright's solution.

For further characterization of the cornicle droplet by thin layer chromatography, droplets were collected in microcapillaries. These were made by drawing out glass capillary tubes (0.9-1.11 mm x 100 mm). The diameter obtained was such that the droplet flowed into the microcapillary without solidifying. The microcapillary was dropped into a ground glass stoppered tube containing 0.5 ml of re-distilled hexane or methylene chloride. The microcapillaries were crushed within the solvent. This material was spotted onto glass plates coated with silica gel G (250 micra) impregnated with Rhodamine 6 G. The plates were developed in petroleum ether-diethyl ether-glacial acetic acid (84:15:1) in chromatography jars (Lambremont et al., 1966). The control spots consisted of a mixed lipid standard prepared commercially (oleic acid, triolein, methyl oleate, and cholesterol oleate). For comparison, material obtained when whole aphids were extracted with hexane or methylene chloride was spotted on these plates.

Some experiments were performed in which cotton aphids were fed on artificial diets, consisting of distilled water, or 1 per cent, 5 per cent, 10 per cent,
20 per cent, 40 per cent or 60 per cent sucrose solutions. Reagent grade sucrose was dissolved in distilled water and refrigerated in 50 ml polyethylene dropper bottles. Before use the solutions were examined microscopically for mold growth. For feeding the aphids, small glass bottles were used (2 cm in diameter and 4.5 cm in height). A film of Parafilm "M" was stretched over the mouth of the bottle. Several drops of solution were sandwiched between this film and a second film applied over the first. Third instar aphids were placed on this film with a camel hair brush. The aphids were caged with a polyethylene bottle stopper from which the top and bottom had been removed. The top was replaced with a nylon net. When prepared, these cages were inverted and illuminated with the same photoperiod as the stocks. Control aphids were placed on a cotton cotyledon disc (2 cm in diameter) and caged. The aphids were allowed to feed for 48 hours during which period molting occurred. They were then fixed in cold calcium-formalin and prepared for paraffin embedding or they were whole mounted directly in glycerine jelly after a quick immersion in acetone. Those mounted in glycerine jelly were immediately photographed.
Table I. Synopsis of Microtechnique

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<th>70% Alc. Par; H&amp;E</th>
<th>Ca-for. Par; H&amp;E</th>
<th>Ca-for. Par; Sudan</th>
<th>Frozen Sudan</th>
<th>Extracted Par; Sudan</th>
<th>Regaud's Par; Novelli</th>
<th>Gendre's Par</th>
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Table I. (Continued)

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<td><em>Melaphis rhois</em> Fitch</td>
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CHAPTER II

HISTOLOGY OF THE CORNICLE AREA

1. Nature of the Cells

Members of the tribe Lachnini which were fixed and embedded in paraffin for study, have a thin multinuclear membrane which extends from the cornicle valve into the subcornicle area of the body cavity. This single layer of cellular material with flattened nuclei forms a large sac. In adults of *Cinara watsoni* a few cells are noted within the sac. Each of these has granular cytoplasm and a large nucleus (Plates I and II). These cells are not found in nymphs of *Cinara watsoni* nor in adults of *Cinara carolina*. Here the sacs appear empty of cellular material (Plates IV and V). Frozen sections of *Longistigma caryae*, stained with Sudan Black B, demonstrate a large black mass filling the area where the sac is located in paraffin sections of this species and of *Cinara* spp. (Plate VI). Large fat cells surround the sac on all sides. The nucleus of the fat cell is centrally located and the cytoplasm is highly vacuolated (Plates I and II, A).

Two species of the tribe Callaphidini were studied.
They present different histological pictures in the cornicle area. In *Monellia costalis* a discrete "gland" is seen (Plate VII). The seemingly hollow central cavity (of paraffin sections) communicates with the exterior at the cornicle tip (when the valve is open). In examination of a series of sections, approximately four, large, highly granular nuclei are seen situated in a dark staining ring of cytoplasm peripheral to the cavity. This, in turn, is surrounded by vesicular cytoplasm. No cell boundaries can be distinguished with the exception of the external and internal limiting membranes. Dorsally (top of the illustration), this "gland" seems to be intimately associated with the ectoderm. In *Stegophylla* sp. a histological picture bearing striking resemblance to that of *Cinara watsoni* pictured in Plate IV occurs.

Members of the tribe Aphidini investigated have large fat cells filling the cornicle and the subcornicle area. In *Amphorophora sonchi* these take the form of large ring-shaped cells with a narrow peripheral band of vesicular cytoplasm. A large vacuole makes up the majority of the cell. The nucleus is compressed in the peripheral cytoplasm. Surrounding these cells and located throughout the body cavity, fat cells with centrally located nuclei and vacuolated cytoplasm are seen (Plates VIII and IX).
Members of the genus Aphis in paraffin sections have a similar cellular arrangement in the cornicle area as in *Amphorophora* (Plates X and XI). Frozen sections of *Aphis gossypii* which are stained with Sudan Black B show these cells filled with lipid. They appear as amorphous black bodies (Plate XII). *Cachryphora serotinae*, a species similar in size to *Aphis gossypii*, has numerous fat cells of the large vacuolated type completely packing the cornicle and the subcornicle area, so that paraffin prepared sections have a lace-like appearance (Plate XIV). Several rows of these cells can be seen in the cornicle in both sectioned material and in freshly prepared whole mounted aphids (Plate XV). The cells are more numerous and smaller than in *Aphis*. Members of the genus *Dactynotus* have a cellular make-up similar to that in *Aphis* (Plate XVII). Specimens which are stained for lipid have spherical black bodies in the cornicle area. All cellular detail is lost. The extracted lipid control specimens of *Dactynotus verbesinae* have completely lost their cellular integrity in the cornicle and subcornicle area (Plate XVIII). The cells in the cornicle and the subcornicle area in *Hyalopterus pruni* are the typical fat cells of aphids. Each is a large cell with a centrally located nucleus and a prominent nucleolus. The cytoplasm is
multivacuolated (Plate XX). The cells in the cornicle and adjacent area in *Macrosiphum euphorbiae* are similar to those of the other members of this tribe, having the large vacuole which makes up most of the cell, compressed ring of cytoplasm, and peripheral nucleus (Plate XXII).

*Neophyllaphis podocarpi*, a member of the Thelaxini, has large granular cells located adjacent to the cornicle valve. These form a "cap" in the cornicle area. Beneath these are seen many smaller cells with central nuclei. No globules can be seen through the body wall in the cornicle area of *Neophyllaphis* in whole mounted specimens as can be seen in members of the Aphidini. The smaller cells seen in this species resemble the fat cells in *Hyalopterus pruni* (Plates XXIII, XXIV, and XXV).

*Melaphis rhois*, an eriosomatine aphid without a cornicle, was studied. The specimens have highly vacuolated tissue located in the posterior abdominal region. Cytological detail is scant in the preparations. Aphids of this subfamily produce woolly lipid secretions over the body.

Mitochondria are present in the strands of cytoplasm and the peripheral ring of cytoplasm of the fat cells of *Aphis gossypii, Cachryphora serotinae, Dactynotus verbescinae*, and *Neophyllaphis podocarpi*, species which were
fixed and stained for mitochondria. These take the form of minute magenta specks when viewed with oil immersion.

Glycogen is not detected in the cytoplasm of these cells by means of the iodine method.

2. Nature of the Muscular Arrangement

The valve, located near or at the apex of the cornicle in all of the species considered in this investigation, has a muscle inserted on its inner border. This muscle runs through the cornicle nearer to the posterior edge than the anterior edge and is inserted on the ventral body wall. This arrangement is seen in Cinara watsoni (Plate III). The muscle is interrupted in the section illustrated. In the series of sections, the muscle is seen as a continuous strip. The plane of sectioning cut a piece of muscle from the middle. This appears in the preceding section which is not pictured.

In a series of sections of a nymph of Cinara watsoni a part of the valve muscle is seen (Plate IV). Where the muscle passes through the area of the sac, the sac has a multilocular appearance (Plate IV, A). This is also the situation in the adult. Dorsoventral muscles are present in the immediate vicinity of the sac.

In Stegophylla sp. this multilocular arrangement is
also present. In this species the dorsoventral muscles (lateral abdominal muscles) and the passage of the cornicle valve muscle cause this condition.

In longitudinal sections of *Monellia costalis* a muscle is seen inserting at the tip of the cornicle posterior to the "gland", centrally located, and originating, seemingly, on the ventral aspect of the segment of the cornicle (Plate VII). In cross sections, dorsoventral muscles are seen immediately adjacent to the "gland" on the medial side.

Cross sections of *Amphorophora sonchi* show the cornicle valve muscle with the usual origin and insertion (Plate IX). The three illustrations are sections cut in a series from the posterior pole to the anterior pole of the aphid. In Plate IX, C, an indication of the body wall is seen on the right side of the section. This is immediately anterior to the base of the cornicle. In preceding sections, a muscle is inserted here and originates on the ventral body wall. This species illustrates well the muscular arrangements in the cornicle and the cornicle area in the tribe Aphidini.

Longitudinal sections of *Aphis gossypii* show the cornicle valve muscle, in part, at the posterior internal surface of the cornicle (Plate XIII). Another muscle
attaches just anterior to the cornicle near its base (Plate XIII, B). The attachment is on the dorsal body wall. In the section pictured, this muscle superficially appears to be attached to the base of the cornicle. However, in close examination of the series with oil immersion, no muscle fibers are found here; they are inserted on the body wall itself.

In sections of the cornicle of Dactynotus nigrotuberculatus, the valve is seen near the apex of the cornicle with the valve muscle inserting on its inner border (Plate XVI).

All aphids of this study having the long cornicles have a muscle, as mentioned in Amorphophora sonchi and Aphis gossypii, immediately anterior to the base of the cornicle. It is seen quite clearly in Hyalopterus pruni to be a muscle of the body wall and not of the cornicle (Plate XXI, B). The cornicle valve muscle passes through the cornicle and makes a bend at the posterior border of the cornicle (Plate XXI, A). This condition is also seen in Dactynotus verbesinae (Plate XIX). Dissected specimens of Dactynotus verbesinae, stained with carmine and mounted, show these same muscle arrangements: the valve muscle, and the dorsoventral muscle located immediately anterior to the base of the cornicle.
The cornicle valve muscle of *Neophyllaphis podocarpi* is shown in Plate XXIV. As in the other species, it inserts on the valve and originates on the aphid's ventral body wall below the cornicle. The dorsoventral muscles which segregate the cornicle area are seen in the cross sections of this aphid (Plate XXV).
Plate I. Longitudinal Section through Cornicle of *Cinara watsoni* Tissot

(Calcium-Formalin Fixation; Paraffin Embedding; Hematoxylin and Eosin Stain; Magnification: 150x)
Plate II. Cross Section through Cinara watsoni Tissot in Area of Cornicle

(Calcium-Formalin; Paraffin; Hematoxylin and Eosin; Magnification: A, 150x; B, 500x)

A: Sac with cells
B: Enlargement of sac
Plate III. Cross Section through the Cornicle Showing Cornicle Muscle in *Cinara watsoni* Tissot

(Bouin's Fluid; Paraffin; Hematoxylin and Eosin; Magnification: 150x).
Plate IV. Cross Sections of Nymph of *Cinara watsoni* Tissot

(Calcium-Formalin; Paraffin; Hematoxylin and Eosin;
Magnification: 140x)

A, B, C: Serial sections, posterior to anterior progression, right cornicle visible in B and C
Plate V. Longitudinal Section through Cornicles of 
*Cinara carolina* Tissot

(70 Per Cent Alcohol; Paraffin; Hematoxylin 
and Eosin; 
Magnification: 270x)
Plate VI. Cross Sections through *Longistigma caryae* (Harris) in Area of Cornicle

(Calcium-Formalin; Cryostat; Sudan Black B; Magnification: 150x)

A, B: Serial sections, black stain represents lipid
Plate VII. Longitudinal Sections through *Monellia costalis* (Fitch) in Cornicle Area

(Calcium-Formalin; Paraffin; Hematoxylin and Eosin; Magnification: 570x)

A: Cornicle "gland" and valve muscle

B: Cornicle "gland" lumen-
Plate VIII. Cross Section through Subcornicle Area of *Amphorophora sonchi* (Oestlund)

(Bouin's Fluid; Paraffin; Hematoxylin and Eosin; Magnification: 570x)

Note large central vacuoles
Plate IX. Cross Sections of *Amphorophora sonchi* (Oestlund) in Area of Cornicles

(Bouin's Fluid; Paraffin; Hematoxylin and Eosin; Magnification: 140x)

A, B, C: Serial sections posterior to anterior progression
Plate X. Cross Section through Cornicle Area of *Aphis fabae* Scopoli

(Bouin's Fluid; Paraffin; Hematoxylin and Eosin; Magnification: 580x)

Note large vacuoles filling cells
Plate XI. Longitudinal Section through Cornicle and Subcornicle Area of *Aphis gossypii* Glover

(Bouin's Fluid; Paraffin; Hematoxylin and Eosin;
Magnification: 550x)
Plate XII. Cornicle and Subcornicle Area of *Aphis gossypii* Glover

(Cryostat; Postfixation in Formaldehyde Fumes; Sudan Black B; Magnification: 550x)

A: Fat cells in lumen of cornicle

B: Fat cells in subcornicle area
Plate XIII. Longitudinal Sections through *Aphis gossypii* Glover

(Calcium-Formalin; Paraffin; Hematoxylin and Eosin; Magnification: 210x)

A: Section of valve muscle in cornicle

B: Dorsoventral muscle anterior to cornicle
Plate XIV. Cross Section through Cornicle and Subcornicle Area of *Cachryphora serotinae* (Oestlund)

(Calcium-Formalin; Paraffin; Sudan Black B; Magnification: 620x)
Plate XV. Whole Mounted and Sectioned Specimens of *Cachryphora serotinae* (Oestlund)

(B, C: Calcium-Formalin; Paraffin; Sudan Black B;
Magnification: 320x)

A: Cornicle of aphid mounted alive in glycerine jelly

B: Diagonal section of cornicle

C: Sectioned posterior abdominal area
Plate XVI. Cornicle and Valve Muscle of *Dactynotus nigrotuberculatus* Olive

(Calcium-Formalin; Paraffin; Sudan Black B; Magnification: 650x)

A, B: Serial sections
Plate XVII. Cornicle and Subcornicle Area of Dactynotus verbesinae (Boudreaux)

(Calcium-Formalin; Paraffin; Sudan Black B;
Magnification: 170x)

A: Aphid mounted alive in glycerine jelly

B: Longitudinal section
Plate XVIII. Cornicle and Adjacent Area of
Dactynotus verbesinae (Boudreaux)

(A: Fresh Frozen; Cryostat; Sudan Black B;
B: Extracted; Calcium-Formalin; Paraffin;
Magnification: 170x)

A: Cross section through cornicle area

B: Longitudinal section of cornicle and adjacent area
Plate XIX. Section of Cornicle Muscle of Dactynotus verbesinae (Boudreaux)

(Calcium-Formalin; Paraffin; Hematoxylin and Eosin; Magnification: 200x)
Plate XX. Longitudinal Section through Cornicle and Subcornicle Area of *Hyalopterus pruni* (Fabricius)

(Bouin's Fluid; Paraffin; Hematoxylin and Eosin;
Magnification: 650x)

Note multivacuolated fat cells, absence of large central vacuoles
Plate XXI. Muscles in Area of Cornicle of
Hyalopterus pruni (Fabricius)

(Bouin's Fluid; Paraffin; Hematoxylin
and Eosin;
Magnification: 650x)

A: Section of cornicle muscle

B: Dorsoventral muscle anterior to cornicle
Plate XXII. Longitudinal Sections of Cornicle Area of *Macrosiphum euphorbiae* (Thomas)

(Bouin's Fluid; Paraffin; Hematoxylin and Eosin; Magnification: 600x)

A: Large vacuolated fat cells in cornicle and subcornicle area

B: Fat cells in subcornicle area
Plate XXIII. Cornicle and Subcornicle Area of *Neophyllaphis podocarpi* Takahashi

(B: Bouin's Fluid; Paraffin; Hematoxylin and Eosin; Magnification: A, 250x; B, 600x)

A: Aphid mounted alive in glycerine jelly

B: Cross section
Plate XXIV. Cornicle Valve Muscle in *Neophyllaphis podocarpi* Takahashi

(Bouin's Fluid; Paraffin; Hematoxylin and Eosin;
Magnification: 580x)

A, B: Serial sections
Plate XXV. Dorsoventral Muscles in Area of Cornicles in *Neophyllaphis podocarpi* Takahashi

(Bouin's Fluid; Paraffin; Hematoxylin and Eosin; Magnification: 580x)
Plate XXVI. Vacuolated Tissue in Posterior Abdominal Area of *Melaphis rhois* Fitch

(Bouin's Fluid; Paraffin; Hematoxylin and Eosin; Magnification: 850x)
CHAPTER III

THE CORNICLE DROPLET

1. Gross Observations

The cornicle droplets of several species of aphids were examined during formation under a binocular dissecting microscope. Grossly, the color of the droplet correlates with the color of the aphid and its haemolymph. *Aphis fabae*, a "black" aphid with dark red haemolymph, has a cornicle droplet with dark red components.

In the tribe Aphidini, as the droplet is formed, both the globules in the posterior abdominal area as well as smaller particles located here and along the sides of the aphid can be seen to move rapidly posteriorly into the cornicle. In the species of this tribe, the droplet as it forms has a clear peripheral area. The interior of the droplet is filled with pale yellowish or golden globules, depending on the species. Smaller colored granules are also seen on the interior of the droplet; the color of these corresponds to the gross coloration of the aphid.
In *Aphis gossypii*, the droplet when it first emerges from the cornicle is translucent and appears straw colored, pale yellow, or greenish. Droplets produced simultaneously from different cornicles of a single aphid may not be exactly the same color. The newly formed droplet glistens and reflects objects. It is fluid and will flow onto an object when touched. With time the droplet on the tip of the cornicle becomes opaque and pearly white or cream colored. If prodded with a minute blunt instrument, it shatters. The droplet becomes indented and misshapen as it hardens. In this species, the droplet always solidifies immediately when it rolls onto another object, whether it be a leaf, a dissecting instrument, an aphid's body part, a coccinellid larva, or a clean glass slide.

Two forms of *Dactynotus verbesinae* were observed as their cornicle droplet formed. One of these is a bright orange aphid, the other, dark brown. Grossly, the droplet is colored as is the aphid. The droplet of this species does not solidify rapidly when touched with an object. Occasionally, a droplet has been observed to run down the cornicle or touch the hind femur without immediately hardening. A glistening fluid droplet that has rolled from the cornicle tip is seen in Plate XXVII, A. Plate
XXVII, B shows dried droplets of different sizes; the smaller is quite misshapen.

Some observations of the cornicle droplet were made using *Aphis gossypii* feeding on cotton cotyledons in a petri dish of water-soaked absorbent cotton, and therefore easily observed under a dissecting microscope. Newly born first instar nymphs are capable of forming a cornicle droplet when gently touched in the region of the cornicles. Droplets from one or both cornicles can be elicited by touching the aphid on the head, the middle of the abdomen, a leg, or other body area as readily as touching in the cornicle area. (This is also true in *Dactynotus*.) All other instars are capable also of producing droplets. The response is not always elicited. Results are seen in Table II. The fourth instar nymphs tested were lethargic and nearing molt.

The length of time involved in hardening of the droplet was observed in apterous nymphs, alatoid nymphs, and apterous adults of *Aphis gossypii*. The results appear in Tables III, IV, and V. Alate individuals always get their wings glued in the droplet when stimulated to produce droplets. No consistency in the time of hardening of the droplet is noted. When both cornicles produce a droplet at the same time, the length of time of hardening
of each is often different. In some instances the smaller droplet hardens in less time; at times the reverse is true as seen in the tables. Several droplets that took a long time to harden were touched with a fine pointed instrument. They were no longer fluid but tacky in consistency and did not flow onto the point.

Other observations were made to determine what happens to the dried cornicle droplet. Aphids were gently touched to elicit the formation of the droplet. With a droplet dried at the tip of each cornicle, the aphid was transferred to a fresh cotton cotyledon and examination of the aphids was made at 24 or 36 hours. The results are summarized in Table VI. Adult apterous aphids of this species have been observed to lower the abdomen and touch the tip of the cornicle to the surface of the leaf. In this manner the dried droplet is dislodged from the tip of the cornicle. The nymph acts in a similar manner or the droplet is shed with the exuviae. As seen in Table VI, when compared with older individuals, first instar nymphs have a tendency to retain the dried droplet on the tip of the cornicle.

A newly molted apterous nymph with a wet droplet on one cornicle was observed to lower its abdomen. The droplet flowed onto the leaf and hardened. This individual
struggled for 10 minutes with the tip of the cornicle stuck to the surface of the leaf. An apterous adult, observed walking across the leaf with fluid cornicle droplets, lowered its abdomen on the left side and the droplet spread on the leaf and hardened. This individual pulled the cornicle free from the dried droplet and walked away repeating the process on the right side. It took 15 seconds to free the right cornicle. Other parts of the body may become stuck to the leaf or to another aphid. An alatoid nymph became glued to the leaf when the droplet rolled onto the leaf and stuck the left hind tarsus there. In another observation, two alatoid nymphs became stuck to each other, the right tarsus of one to the left hind tibia of the other. In most cases, however, the aphids easily freed themselves from dried droplets.

As noted, cotton aphids can walk around while the droplet remains fluid. They also have been noticed kicking away honeydew without disturbing the liquid nature of the droplet.

After a cornicle droplet has been formed, others can be produced. In some cases the first dried droplet is rubbed off. In some instances when the cornicles already have dried droplets, second droplets may be produced simply pushing the first aside. Individuals of the cotton
aphid never produced more than four droplets in succession in this study.

No consistency in the size of the droplets was noticed. The first droplet produced was not always the largest one. An adult aphid was seen to produce two small droplets when prodded. These hardened and were removed. With further stimulation, one large droplet was formed.

Cotton aphids set aside on leaves and undisturbed by anything except each other have been observed with dried droplets on their cornicles. In one case while looking at a colony in the laboratory, an aphid apart from the others, was seen to give a droplet spontaneously.

2. Microscopic Observations

The cornicle droplets of a number of species of aphids were observed under a compound microscope after the droplet was allowed to flow onto a clean glass slide. The droplets of *Dactynotus* spp., *Cinara* spp., *Longistigma caryae*, and *Neophyllaphis podocarpi* were observed in this manner before solidification of the droplet occurred. As solidification occurs, large crystals form as seen in Plate XXVIII. This shows crystallized components of the cornicle droplet of *Cinara watsoni*.

A photograph of a droplet of *Neophyllaphis podocarpi*
on a slide before solidification appears in Plate XXIX. A clear peripheral area is seen. The center of the preparation is packed with many spherical bodies containing dark red granules.

The smears or saline preparations of the droplets of *Dactynotus verbesinae* and *Cinara watsoni* reveal many spherical bodies filled with colored granules. Nuclei are not always visible in these, possibly obscured by the granules. However, the cellular nature of these bodies can be seen in Plate XXX. In methylene blue-saline preparations the nuclei become pale blue and the nucleoli bright blue. The granules in *Dactynotus verbesinae* are bright orange in the orange-red form of this aphid and brownish in the dark form. Many of the cells contain large yellow spheres in addition to the small colored granules. When Sudan III-IV in 70 per cent alcohol-acetone is introduced under the coverslip, the yellow spheres within the cells as well as the numerous, free, variously sized yellow globules scattered throughout the preparation, become orange-red. No color change occurs when the alcohol-acetone solution without the dye is introduced. When preparations are made using droplets obtained from a cut leg, an antenna stump, or from a small puncture in the aphid's abdomen, similar cells,
containing yellow spheres and colored granules are seen. Plate XXXI shows various cells and fragments seen in the saline-methylene blue preparation of the cornicle droplet of Cinara watsoni. The small refractive spheres are bright yellow.

3. Chemical Tests

The ability of the droplet to dissolve in various solvents was tested. The droplet was placed in the test solution by immersing the tip of the cornicle at the time of formation of the droplet. The solution was in a 1 ml beaker. Observations were made under a dissecting microscope. The results of these tests are seen in Table VII.

In water and 70 per cent alcohol the colored granular material diffuses into the solution. The golden globules float on the water surface and form a "string of beads" in 70 per cent alcohol and acetone. In the latter this gradually disappears. When Sudan III-IV is added to the 70 per cent alcohol the "string of beads" becomes red in the pink solution. In xylene, hexane, and methylene chloride an interface between the droplet and the solution is always present. The yellow-golden globules escape from within the droplet and dissolve. A residual streak remains in the beaker containing the colored
granules. When these granules are examined microscopically in * Macrosiphum euphorbiae*, they appear as spherical bodies filled with pink granules, similar to the cells seen in saline preparations of the cornicle droplet of *Dactynotus verbesinae*. The granules fade on standing in xylene, hexane, and methylene chloride.

Cornicle droplets of *Dactynotus nigrotuberculatus* and *Dactynotus verbesinae*, when analyzed by thin layer chromatography for lipids, produce the chromatogram pictured in Plate XXXII. Both the cornicle droplet and the total body lipid extract produce the same migration pattern. As can be seen, the two spots fall between oleic acid and triolein, components of the standard mixture.
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Table III. Hardening Time of Cornicle Droplets of Apterous Nymphs of *Aphis gossypii*

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</tr>
</tbody>
</table>

* Indicates Larger Droplet
Table IV. (A) Hardening Time of Cornicle Droplets of Alatoid Nymphs of *Aphis gossypii* (First Group)

<table>
<thead>
<tr>
<th>Aphid</th>
<th>Time (min)</th>
<th>Left Cornicle</th>
<th>Right Cornicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.1</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5.0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.16</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.07</td>
<td>0.16</td>
<td></td>
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</table>
Table IV.  (B) Hardening Time of Cornicle Droplets of Alatoid Nymphs of *Aphis gossypii* (Second Group)

<table>
<thead>
<tr>
<th>Aphid</th>
<th>Time (min)</th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cornicle</td>
<td>Cornicle</td>
<td></td>
</tr>
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<td>0.09</td>
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</tr>
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<td>5</td>
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<td>0.18</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.12</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.2</td>
<td>0.18</td>
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<tr>
<td>8</td>
<td>1.5</td>
<td>0.5*</td>
<td></td>
</tr>
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<td>9</td>
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<td>10</td>
<td>0.09</td>
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* Indicates Larger Droplet
Table V. (A) Hardening Time of Cornicle Droplets of Apterous Adults of *Aphis gossypii* (First Group)

<table>
<thead>
<tr>
<th>Aphid</th>
<th>Time (min)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Left Cornicle</td>
<td>Right Cornicle</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.4</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3.07</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.26</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.12</td>
<td>10.07</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>15.5</td>
<td>15.5</td>
<td></td>
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<td>7</td>
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<td>-</td>
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<td>10</td>
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</table>
Table V. (B) Hardening Time of Cornicle Droplets of Apterous Adults of *Aphis gossypii* (Second Group)

<table>
<thead>
<tr>
<th>Aphid</th>
<th>Time (min)</th>
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<th>Right Cornicle</th>
</tr>
</thead>
<tbody>
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<td>1</td>
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<td>0.75</td>
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<td>2</td>
<td>0.25</td>
<td>0.25*</td>
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<tr>
<td>3</td>
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<tr>
<td>4</td>
<td>0.22</td>
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<td>-</td>
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<tr>
<td>10</td>
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<td></td>
<td>0.12</td>
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</table>

* Indicates Larger Droplet
Table V. (C) Hardening Time of Cornicle Droplets of Apterous Adults of *Aphis gossypii* (Third Group)

<table>
<thead>
<tr>
<th>Aphid</th>
<th>Time (min)</th>
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</tr>
</thead>
<tbody>
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<td>1</td>
<td>-</td>
<td>2.25</td>
<td>2.25</td>
</tr>
<tr>
<td>2</td>
<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>3</td>
<td>0.07</td>
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<td>0.12*</td>
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<tr>
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<td>0.8</td>
</tr>
<tr>
<td>5</td>
<td>0.43</td>
<td>-</td>
<td>-</td>
</tr>
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<td>-</td>
<td>-</td>
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<tr>
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</tr>
<tr>
<td>8</td>
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<td>0.12</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

* Indicates Larger Droplet
<p>| Instar | Droplets on Leaf or Exuviae | Aphids with Unilateral Droplet | Aphids with Bilateral Droplets | Aphids Remaining on Cotyledon | Aphids Stimulated | Droplet Formation | Hours after Droplet Formation | Aphids Remaining on Cotyledon | Aphids with Unilateral Droplet | Aphids with Bilateral Droplets | Aphids Remaining on Cotyledon | Aphids Stimulated | Droplet Formation | Hours after Droplet Formation |
|--------|-----------------------------|---------------------------------|---------------------------------|---------------------------------|-------------------|------------------|-----------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|-------------------|------------------|-----------------------------|-----------------------------|
| 1st    |                             |                                 |                                 |                                 |                   |                  |                             |                                 |                                 |                                 |                                 |                   |                  |                             |                             |
| 2nd    |                             |                                 |                                 |                                 |                   |                  |                             |                                 |                                 |                                 |                                 |                   |                  |                             |                             |
| 3rd    |                             |                                 |                                 |                                 |                   |                  |                             |                                 |                                 |                                 |                                 |                   |                  |                             |                             |
| 4th    |                             |                                 |                                 |                                 |                   |                  |                             |                                 |                                 |                                 |                                 |                   |                  |                             |                             |
| 5th    |                             |                                 |                                 |                                 |                   |                  |                             |                                 |                                 |                                 |                                 |                   |                  |                             |                             |
| 6th    |                             |                                 |                                 |                                 |                   |                  |                             |                                 |                                 |                                 |                                 |                   |                  |                             |                             |
| 7th    |                             |                                 |                                 |                                 |                   |                  |                             |                                 |                                 |                                 |                                 |                   |                  |                             |                             |
| 8th    |                             |                                 |                                 |                                 |                   |                  |                             |                                 |                                 |                                 |                                 |                   |                  |                             |                             |
| 9th    |                             |                                 |                                 |                                 |                   |                  |                             |                                 |                                 |                                 |                                 |                   |                  |                             |                             |
| 10th   |                             |                                 |                                 |                                 |                   |                  |                             |                                 |                                 |                                 |                                 |                   |                  |                             |                             |
| 11th   |                             |                                 |                                 |                                 |                   |                  |                             |                                 |                                 |                                 |                                 |                   |                  |                             |                             |
| 12th   |                             |                                 |                                 |                                 |                   |                  |                             |                                 |                                 |                                 |                                 |                   |                  |                             |                             |</p>
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<tr>
<th>Solvent</th>
<th>Species*</th>
<th>Droplet-Solvent Interface</th>
<th>Yellow-Golden Globules</th>
<th>Small Colored Granules</th>
<th>Residual Streak in Beaker</th>
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<td>float</td>
<td>diffuse</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>no</td>
<td>float</td>
<td>-</td>
<td>no</td>
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<tr>
<td></td>
<td>3</td>
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<td>float</td>
<td>diffuse</td>
<td>no</td>
</tr>
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<td>float</td>
<td>-</td>
<td>no</td>
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<td>solidify</td>
<td>diffuse</td>
<td>no</td>
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<td></td>
<td>2</td>
<td>no</td>
<td>solidify</td>
<td>-</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>no</td>
<td>solidify</td>
<td>diffuse</td>
<td>no</td>
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<td>solidify</td>
<td>-</td>
<td>no</td>
</tr>
<tr>
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<td>dissolve</td>
<td>-</td>
<td>no</td>
</tr>
<tr>
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<td>dissolve</td>
<td>remain</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>yes</td>
<td>dissolve</td>
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<td>yes</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>yes</td>
<td>dissolve</td>
<td>remain</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>yes</td>
<td>dissolve</td>
<td>remain</td>
<td>yes</td>
</tr>
<tr>
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<td>dissolve</td>
<td>remain</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>yes</td>
<td>dissolve</td>
<td>remain</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>yes</td>
<td>dissolve</td>
<td>remain</td>
<td>yes</td>
</tr>
<tr>
<td>Methylene</td>
<td>2</td>
<td>yes</td>
<td>dissolve</td>
<td>remain</td>
<td>yes</td>
</tr>
<tr>
<td>Chloride</td>
<td>3</td>
<td>yes</td>
<td>dissolve</td>
<td>remain</td>
<td>yes</td>
</tr>
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</table>

* 1, *Aphis fabae*; 2, *Aphis gossypii*; 3, *Dactynotus verbesinae*; 4, *Macrosiphum euphorbiae*
Plate XXVII. Cornicle Droplets on Living Specimens of *Dactynotus verbesinae* (Boudreaux)

(Magnification: 45x)

A: Wet droplet on aphid's left cornicle

B: Solidified droplets
Plate XXVIII. Crystallized Cornicle Droplet of Cinara watsoni Tissot

(Magnification: 160x)
Plate XXIX. Cornicle Droplet of *Neophyllaphis podocarpi* Takahashi on Clean Glass Slide

(Magnification: 140x)
Plate XXX. Cells in Cornicle Droplet of *Dactynotus verbesinae* (Boudreaux)

(Methylene Blue-Saline Preparation; Magnification: 1550x)
Plate XXXI. Cells in Cornicle Droplet of *Cinara watsoni* Tissot

(Methylene Blue-Saline Preparation; Magnification: 650x)

A, B, C: Phase contrast of different fields
Plate XXXII. Lipid Chromatogram of Extracts of
*Dactynotus nigrotuberculatus* Olive

B: Body extract

C: Cornicle droplet

S: Standard (oleic acid, triolein, methyl oleate, cholesterol oleate)
CHAPTER IV

THE EFFECT OF DIETARY CHANGES ON THE CORNICLE AREA

*Aphis gossypii* was fed water and various sucrose solutions through an artificial membrane. After making histological preparations, serial sections were examined. Cells were counted in serial sections through the cornicle and subcornicle area. Comparable sections were examined and cells counted in each aphid. The cell count was started in the section where the cornicle first appeared and proceeded anteriorly. In measuring the diameter of the large vacuolated cell, the section used in all cases was located two sections anterior to the section where the cornicle last appeared. The slides were coded to avoid bias. The results of the counts appear in Table VIII and the sections are illustrated in Plates XXXIII, XXXIV, and XXXV. A large number of granular cells with large central nuclei appear in the sections of those aphids feeding on water and 1 per cent sucrose solutions. None of these cells are seen in the sections of those aphids feeding on higher concentrations of sucrose or on the control cotton cotyledon. The large vacuolated cells are
as numerous in aphids feeding on water and 1 per cent sucrose diets as in those on 40 per cent sucrose and the cotyledon, but these cells are about one half the diameter in the former. The large vacuolated cells are less numerous in aphids on the 5 per cent and 10 per cent sucrose diets than the lower or higher concentrations. The diameter of the vacuole, however, is greater than in those on water and 1 per cent sucrose. Multivacuolated cells appear in all sections through the cornicle area with the exception of those from aphids feeding on 40 per cent sucrose. These have only the large vacuolated cells.

In repetitions of these feeding experiments and examination of whole mounted aphids, the vacuolated cells can be seen through the body wall in the cornicle and the posterior abdominal area. A control aphid, which fed on a cotton cotyledon, is compared with an aphid fed on water in Plate XXXVI. The large vacuolated cells have disappeared in the aphid on water. In aphids on a 5 per cent sucrose diet, small vacuoles are noted. There are more of these in aphids on 10 per cent sucrose. This is approaching the control (normal) condition. (Plate XXXVII). Those aphids on 40 per cent and 60 per cent sucrose diets closely resemble the controls (Plate XXXVIII).
When old apterous female aphids, feeding on cotyledons, are examined after they have stopped reproducing, only a few small vacuoles are seen through the body wall in the cornicle and adjacent areas (Plate XXXIX).
Table VIII. The Effect of Feeding Sucrose Solutions on the Histology of the Cornicle Area

<table>
<thead>
<tr>
<th>Medium</th>
<th>Average Number Large Vacuolated Cells</th>
<th>Average Number Multi-Vacuolated Cells</th>
<th>Average Number Granular Cells</th>
<th>Average Diameter Large Vacuolated Cell (micra)</th>
<th>Number Aphids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>14</td>
<td>2</td>
<td>8</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>1 Per Cent Sucrose</td>
<td>13</td>
<td>2</td>
<td>3</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>5 Per Cent Sucrose</td>
<td>10</td>
<td>5</td>
<td>0</td>
<td>16.8</td>
<td>1</td>
</tr>
<tr>
<td>10 Per Cent Sucrose</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>21.6</td>
<td>2</td>
</tr>
<tr>
<td>40 Per Cent Sucrose</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>Cotton Cotyledon</td>
<td>15</td>
<td>1</td>
<td>0</td>
<td>21.6</td>
<td>2</td>
</tr>
</tbody>
</table>
Plate XXXIII. Cross Sections through the Cornicle Area of *Aphis gossypii* Glover

(Calcium-Formalin; Paraffin; Hematoxylin and Eosin; Magnification: 580x)

A: After feeding on distilled water

B: After feeding on 1 per cent sucrose
Plate XXXIV. Cross Sections through the Cornicle Area of *Aphis gossypii* Glover

(Calcium-Formalin; Paraffin; Hematoxylin and Eosin; Magnification: 580x)

A: After feeding on 5 per cent sucrose

B: After feeding on 10 per cent sucrose
Plate XXXV. Cross Sections through the Cornicle Area of *Aphis gossypii* Glover

(Calcium-Formalin; Paraffin; Hematoxylin and Eosin;
Magnification: 580x)

A: After feeding on 40 per cent sucrose
B: After feeding on cotton cotyledon
Plate XXXVI. Whole Mounted Specimens of *Aphis gossypii* Glover

(Glycerine Jelly; Magnification: 160x)

A: After feeding on cotton cotyledon

B: After feeding on water
Plate XXXVII. Whole Mounted Specimens of Aphis gossypii Glover

(Glycerine Jelly; Magnification: 160x)

A: After feeding on 5 per cent sucrose
B: After feeding on 10 per cent sucrose
Plate XXXVIII. Whole Mounted Specimens of Aphis gossypii Glover

(Glycerine Jelly;
Magnification: 160x)

A: After feeding on 40 per cent sucrose

B: After feeding on 60 per cent sucrose
Plate XXXIX. Whole Mounted, Old, Female of *Aphis gossypii* Glover (Postreproductive, Showing Scarcity of Lipid)

(Glycerine Jelly; Magnification: 160x)
DISCUSSION AND CONCLUSIONS

In his study of members of the tribe Lachnini, Hottes (1928) noted a cavity which he interpreted to be an artifact produced through the shrinkage of cells in the area of the cornicle. Mordwilko (1895), an earlier investigator, interpreted this cavity as a "wax" sac. In discussing his histological findings, Strong (1967) referred to their observations stating that he could find no such structure. However, he was studying species of the tribe Aphidini, in which no large sac is present. In my study, paraffin preparations of members of the Lachnini show this sac as an extremely thin walled, cellular structure. It is filled with lipid, as demonstrated in _Longistigma caryae_. I was unable to discern the "A" and "B" cells seen by Hottes. There are, however, a few scattered cells within the sacs of some of the specimens. During the fixation of aphids in alcoholic fixatives, such as 70 per cent alcohol and Bouin's fluid, sometimes expulsion of the droplet occurs. This may account for the small number of cells seen in the sac of the Lachnini.

A member of the Callaphidini, _Stegophylla_ sp.,
resembles species of the Lachnini in the histology of the cornicle area. However, *Monellia costalis* has an entirely different structure here. This appears similar to an epidermal gland. It appears in my sections exactly as drawn by Hottes from specimens of *Monellia caryella*.

The tribe Aphidini has been the most intensely studied by other investigators and by me. Hottes persisted in regarding the cells seen in the cornicle and the posterior abdominal areas as glandular sacs. Edwards (1966) regarded these as cells since he saw a nucleus and residual cytoplasm in some of them. Strong (1967) saw no nuclei in his histological preparations, although he studied one species, *Myzus persicae*, in common with Edwards. He called the cells in the cornicle and subcornicle area "globules" or "spherical bodies." In my study, the histological sections of species of this tribe show large, singly vacuolated cells in the cornicle and subcornicle area, except in *Hyalopterus pruni* where multivacuolated cells are present. Hottes found this latter condition in *Thargelia albipes* (= *Brevicoryne symphori-carp* (Thos.)). He described multivacuolated cells with centrally located nuclei in the cornicle and subcornicle area of this species. In my study, the large vacuolated cells are surrounded by multivacuolated cells and in one
specimen of *Dactynotus verbesinae*, fixed in Regaud's fluid, multivacuolated cells are present in the cornicle area as in *Hyalopterus pruni*. The large vacuolated cells may represent, therefore, the final stage in lipid cell maturation for maximum storage of lipid. At first the cell has a few vacuoles, then many, and finally, one tremendous vacuole takes up the cell pushing the nucleus to the periphery in a narrow ring of cytoplasm. Strong (1967) was unable to demonstrate lipid in these large vacuolated cells by his histological methods. In my study both *Aphis gossypii* and *Dactynotus verbesinae* have lipid filling these cells as demonstrated in the frozen preparations stained for lipids.

The one member of the Thelaxini studied, *Neophyllaphis podocarpi*, has a histological picture unlike those of species of the other tribes. In fact, the sections of the cornicle area resemble Hottes' description and drawings of the "A" and "B" cells of *Lachnus*. *Neophyllaphis podocarpi* has no sac.

*Melaphis rhois*, a species of the tribe Melaphidini of the subfamily Eriosomatinae, was not studied as thoroughly as the aphids which possess cornicles. The highly vacuolated tissue, pictured in the posterior and lateral abdominal areas, is similar to the description of fat cells
filling the body cavity of the woolly aphid, described by Wheeler (1892). The members of this subfamily have very poorly developed or no cornicles, but produce lipid "wool" secretions over the body surface.

Some confusion exists concerning the muscular arrangements in the cornicle and the subcornicle area of aphids with the cylindrical type of cornicle (Aphidini). It is my opinion that difficulty is encountered when judgments concerning the origins and insertions of muscles are made from sectioned specimens unless many sections, which have been cut in several planes, are examined. In sectioning, a muscle or a few fibers may slide from the natural position and contribute to the confusion. Flögel (1905) and Hottes' descriptions of the origin and insertion of the cornicle valve muscle in members of the Aphidini agree with my findings. The muscle originates on a ventral segment below the cornicle and inserts on the median part of the valve. Hottes stated, with reservations, that the origin appeared to be the ventral part of the segment following the cornicle segment. Yu-swu (1956) noted the origin and insertion of the valve muscle as described. Edwards, however, held that the valve muscle had its origin on the dorsum of the segment following the cornicle segment. My findings do not agree
with his findings.

In addition to the valve muscle, Yu-swu described a tube basal muscle which inserted on the posterior proximal part of the cornicle and originated on the dorsum of the following segment (sixth segment). I was unable to find this muscle in the species, *Aphis gossypii*, with which she worked.

In addition to the cornicle valve muscle, Flügel illustrated a muscle attached to the anterior base of the cornicle and originating on the ventral body wall in *Aphis ribis* Linnaeus. At this ventral point another muscle arose and inserted a short distance anterior to the cornicle. Hottes was unable to find these muscles in his sections. In my study these muscles are easily seen, but the muscle nearer to the cornicle does not insert on the anterior base of the cornicle although in some sections it appears to do this. The insertion of the muscle is on the dorsal body wall immediately anterior to the base of the cornicle. It may function as a levator of the cornicle. Flügel's illustrations appear similar to the section pictured in Plate XIII, B.

In his study of *Monellia caryella*, Hottes saw no muscle attached to the valve of the cornicle. In all of the specimens of *Monellia costalis* examined in this study,
a muscle inserting on the cornicle valve and originating on the ventral body wall has been seen.

Most of the investigators have thought that the contraction of the valve muscle opens the valve and raises the cornicle. Strong, however, stated that the valve muscle could not contract when the cornicle was in the depressed position. He reasoned from his observations that the expulsion of the droplets of the species he studied occurred only when the cornicles were raised. The manner in which the cornicle valve muscle curves around the posterior base of the cornicle when the cornicle is depressed, suggests that this muscle could, in fact, act to raise the cornicle.

The dorsoventral, lateral muscles noted in the cross-sections of all of the species studied and located in the immediate vicinity of the cornicle area are probably partly responsible for the expulsion of the cornicle droplet by increasing the internal pressure on contraction, together with other such muscles anteriorly.

Both Edwards and Weber (1930) expressed the opinion that material from the haemocoele was being released when the cornicle valve opened and the droplet formed. Strong, on the contrary, thought the globules he saw were separated from the blood sinus. He found no haemolymph in
the droplets, but he noted that water was present in the
droplet. Yu-swu in her study of *Aphis gossypii* noted
many cellular types in the droplet. I was unable to see
intact cells in the saline suspension preparations of the
droplets of this species. The lipid globules and scat-
tered nuclei were seen. As noted, the droplets of *Cinara*
spp., *Dactynotus* spp., and *Neophyllaphis podocarpi* contain
cellular elements and lipid globules. The lipid globules
are free in the preparation and also occur as smaller
spheres within the cells. In saline preparations many of
the cells burst, especially the large, vacuolated ones,
as indicated by the presence of the free nuclei, cellular
fragments, and free lipid globules. The difficulty
encountered by other investigators might have been over-
come by the use of large, brightly colored aphids. In
these the colored granules of the droplet are one of the
components whose cellular nature is discernible when
examined with a compound microscope. In pale green aphids,
such as *Myzus persicae*, these "pigment cells" may not be
present or may be overlooked. I encountered this diffi-
culty with *Aphis gossypii*, as noted.

Strong (1963a, 1967) in the analyses of the total
body extracts of members of the Aphidini and of the cor-
nicle droplets of species of this tribe, found striking
similarities between the two. In the total body extracts most of the lipid present was recovered as triglyceride composed of a high proportion of myristic acid. The cornicle droplets of the species he studied contained triglyceride, primarily; the droplets of *Myzus persicae* contained two triglycerides. Strong interpreted the chromatographic pattern of the cornicle droplet as triglyceride although the mobility was not the same as the triolein standard. He thought the delayed rate was caused by the high proportion of myristic acid he found in the triglycerides of the cornicle droplet. A chromatogram similar to that produced by droplets of *Myzus persicae* was obtained in my study using droplets of *Dactynotus nigrotuberculatus*. The total body extracts produced the same chromatographic pattern as the cornicle droplet. In part (aside from the histological findings), the basis for my interpretation of the cells in the cornicle and the subcornicle area of the Aphidini as fat cells is the agreement of my lipid thin layer chromatographic analyses with Strong's thin layer and gas chromatographic analyses and his interpretation of the lipid as triglyceride. However, Lambremont (personal communication) interprets Strong's and my chromatograms as representing fatty alcohols as suggested by the chromatograms of lipid classes
published by Malins (1966). The matter is open and under investigation.

In the lipid extracted specimens which were examined histologically all cellular detail is lost in the cornicle and the subcornicle area. The contents of the vacuoles are also lost in routine paraffin infiltration of specimens in which the temperature of infiltration is 58 - 60°C. By fixation in cold calcium-formalin and subsequently post-chroming, one might expect bound lipids to remain through the paraffin infiltration procedure. However, the contents of the vacuole are still lost. The contents are preserved only in fresh-frozen or fixed-frozen sections which are stained. The combination of methods strengthens the idea that the lipids are not bound to other components as phosphate or protein.

The nature of the lipid in the droplets of the tribes other than Aphidini is unknown. As previously stated, in the three tribes in which the droplets were examined microscopically, Lachnini (Cinara), Aphidini (Dactynotus), and Thelaxini (Neophyllaphis), both cells and lipid globules are noted. Since the cornicle droplets of the Lachnini contain these components which are similar to those of the Aphidini and the Thelaxini, tribes without a sac in the cornicle area, it is possible that this sac
merely partitions off part of the body cavity in the cor­
nicle area and may have been formed, in the evolutionary
process, by the coalescing of numerous fat cells.

Hottes concluded that phylogenetically there are
two types of cornicles, a "primitive" type in which the
glandular mass has a direct connection with the outside,
as in Monellia and Lachnus and a "modern" type in which
the glandular mass has an indirect connection with the
cornicle opening as in Aphidini and Symydobius.

From the fossil record nothing is known of the
evolution of the Lachnini or of its age. The Aphidini
is a young tribe whose evolution is associated with that
of the cohort of dicotyledons, Rosales. It is thought
that both Cinara (Lachnini) and Aphis (Aphidini) have
split into numerous species in recent times (Heie, 1967).

My studies lead me to conclude that the cornicle,
a monophyletic structure, was in past ages the opening of
an epidermal gland, a condition which exists in Monellia
today. The other species have evolved along separate
lines. There is no gland in the Aphidini. The cornicle
communicates with the haemocoele allowing haemolymph and
blood cells, including fat cells, to escape. The Thelax­
nini, a very old group, have a droplet composed of com­
ponents of the haemocoele, as noted. The cells in the
cornicle of this tribe need to be investigated further. The cap of large granular cells is unlike any seen in the other species. It would be enlightening to carry out histochemical tests on the cells here. My findings do not permit judgments of phylogenetic significance.

It is probable that both species differences and individual physiological differences are responsible for the various responses in formation of the droplets noted by other investigators. Strong (1967) obtained up to six droplets produced in succession and the first droplet formed was always the largest. In my study of *Aphis gossypii* no more than four droplets were produced in succession and the first droplet was not always the largest.

Gillette (1908) noted that the cornicle droplet was rarely dislodged from the tip of the cornicle. This is probably the case in young nymphs with short stubby cornicles. As observed in my study, most first instar nymphs of *Aphis gossypii* retain the droplet until it is molted with the exuviae. Older nymphs and adults remove the dried droplet by touching the tip of the cornicle to the surface of the leaf.

In my study, *Aphis gossypii* has been observed to produce cornicle droplets spontaneously. Dried droplets are often noted on the tips of the cornicles of a few
aphids in undisturbed colonies and, as stated, aphids can relieve themselves of the dried droplets with ease. (This may be why more droplets are not seen on aphids in the undisturbed colonies.) These findings suggest that the droplet is produced to rid the aphid of excess fat (an excretory function) and not as a defensive mechanism in response to a stimulation, as suggested by other investigators. The feeding experiments demonstrate that the nature of the fat cells is influenced by the diet. When starved or fed a low sucrose diet, the fat cells of *Aphis gossypii* decrease in size. In aphids fed higher concentrations of sucrose, the condition of the fat cells noted in aphids feeding on cotton cotyledons is approached. It is possible that the large store of fat in this area is needed to maintain the high reproductive rate. As noted, old females, no longer reproducing, have very few fat cells in the cornicle and the subcornicle area. On the other hand, reproductively senile aphids may also have their other physiological functions depressed. When fat becomes excessive, an avenue of exit is available through the cornicles. In the Eriosomatinae and other species with small cornicles, "mealy" or "woolly" secretions of the general epidermis may substitute for the cornicle secretion. *Hyalopterus pruni*, an aphidine, produces
a dense "meal" on the body surface, and apparently its fat cells in the cornicle area do not attain the final centrally vacuolated form of other aphidines.

Edwards suggested two possibilities for hardening of the droplet. Either it changed from a liquid-crystalline state to the solid-crystal phase on contact with a seeding nucleus, or it was supercooled and a seeding nucleus caused rapid crystallization. Another method of solidification of the droplets of the Aphidini, after the movement of the haemolymph and fat cells to the exterior, might involve the evaporation of the haemolymph with the subsequent rupture or shrinkage of the fat cells' envelopes. The lipid is thereby expelled and crystallizes on exposure to air as it is no longer intracellular. There is no evidence that the cornicle lipid is in a solvent which upon evaporation would allow crystallization.
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Gail Patricia Garwes was born in Savannah, Georgia, April 8, 1937. She graduated from Savannah High School in 1955 and from Armstrong Junior College in 1957. She received her Bachelor of Science degree in science in June, 1959 from Oglethorpe University, Atlanta, Georgia. She received the Master of Science degree in zoology in January, 1962 from Louisiana State University. In June, 1962 she married Robert A. Wynn. For the following three years she worked as a research assistant in the Department of Anatomy, Medical College of Georgia. She re-enrolled at Louisiana State University in September, 1965 and is a candidate for the degree Doctor of Philosophy in August, 1968.
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Title of Thesis: Observations on the Cornicles of Aphids

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