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The Colleterial Glands of Cockroaches¹

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ABSTRACT

The 19 species whose colleterial glands were studied represent three types of egg-laying behavior—those that abandon the oötheca shortly after its formation, those that carry it externally during embryogenesis, and those that carry it internally. The glands vary in size from species to species according to the size of the oötheca elaborated. In *Diploptera punctata* they are not required during the development of the embryos in the uterus of the female. β -glucosidase is present in the right colleterial gland of cockroaches representing all three types of egg-laying behavior, and also in that of a praying mantis. An unidentified glucoside (Gl-A) was demonstrated by paper

chromatography in extracts of the left colleterial glands of 19 species. This Gl-A occurs along with a glucoside of protocatechuic acid in seven species of the subfamily Blattinae; with a second unidentified glucoside (Gl-B) in two species, representing two genera, of Pseudomopinae. In nine other species, representing those that carry oöthecae either externally or internally during embryogenesis, Gl-A is apparently the only precursor of the tanning agent. The β -glucoside of protocatechuic acid was found also in extracts of left colleterial glands of praying mantids and in extracts of accessory glands of the two-lined grasshopper.

The eggs of cockroaches are enclosed in a covering or oötheca, the raw materials of which are produced by a pair of accessory reproductive structures known as colleterial glands (Kadyi 1879, Wheeler 1889, Duchamp 1878-79, Dufour 1841).

The biochemistry of the oötheca of the oriental cockroach, *Blatta orientalis*, was studied by Pryor (1940) and the substance responsible for the tanning of the oötheca was identified as protocatechuic acid or 3-4 dihydroxybenzoic acid (Pryor et al. 1946). Brunet and Kent (1955) and Kent and Brunet (1959) clarified the role of the colleterial glands of *B. orientalis* and *Periplaneta americana* in the tanning of the oötheca. These authors have shown that the left colleterial gland produces both the protein and the precursor of its tanning agent 3-hydroxy-4-O-(β -glucopyranosido)-benzoic acid. The right gland secretes a β -glucosidase which, when mixed with the secretion of the left gland, hydrolyzes the β -glucoside yielding protocatechuic acid and glucose. Then, presumably, the protocatechuic acid can be oxidized to quinone and tanning of the oötheca proceeds.

The oöthecae of *B. orientalis* and *P. americana*, characteristic of many oviparous cockroaches, become dark brown and hard a few days after they are abandoned.

Many genera of cockroaches, however, produce oöthecae distinctly different in form and color from those of *P. americana* and *B. orientalis* (Roth and Willis 1954). Hard, dark brown oöthecae which completely enclose the eggs are produced by the oviparous species which abandon their egg cases. Thinner and less darkly colored oöthecae are elaborated by those species which carry their eggs externally throughout embryogenesis. Those cockroaches which carry their oöthecae in a brood sac, produce, in general, thin, soft, lightly colored oöthecae which in some species only partially cover the eggs.

A comparative study of colleterial glands in several genera and species of cockroaches was made to determine whether the differences in type of oöthecae produced is reflected in the morphology and physiology of the colleterial glands. Colleterial glands were examined from cockroaches representative of three types of egg-laying behavior: those which abandon the oötheca shortly after it is formed, those which carry the egg case externally throughout embryogenesis, and those which carry the eggs internally. Preliminary observations were made on two species of mantids and a grasshopper.

MATERIALS AND METHODS

The cockroaches were reared on Purina Dog Chow Checkers. *Ectobius pallidus* and *Parcoblatta virginica*, collected in Plymouth and Natick, Massachusetts, respectively, were kept for short periods in the laboratory. *Parcoblatta pennsylvanica* was collected near Fort Royal, Virginia.

Colleterial glands were dissected under insect Ringer's solution and, in general, were from females about to produce oöthecae. A drop of 1% merthiolate was added to solutions in which left and right colleterial glands were incubated together or separately. Acetate buffer, 0.1M, pH 4.8, was used for incubating right glands with extract of left glands or with salicin. Citrate buffer, 0.05M, pH 5.5, was used for incubating extract of left glands with a β -glucosidase prepared from *Aspergillus luchuensis* QM 873. Phosphate buffers, 0.10M, were used as solvents in the determination of some of the ultraviolet absorption spectra.

Extracts of left colleterial glands were prepared by rinsing freshly dissected glands in distilled water and grinding them in a glass homogenizer in about 10 times their volume of 95% ethanol. Large quantities of left glands were homogenized in a Virtis "45" homogenizer. The ethanol extract was separated from the precipitate by centrifugation and decantation.

Paper chromatograms were run at room temperature on Whatman No. 1 paper using butanol: acetic

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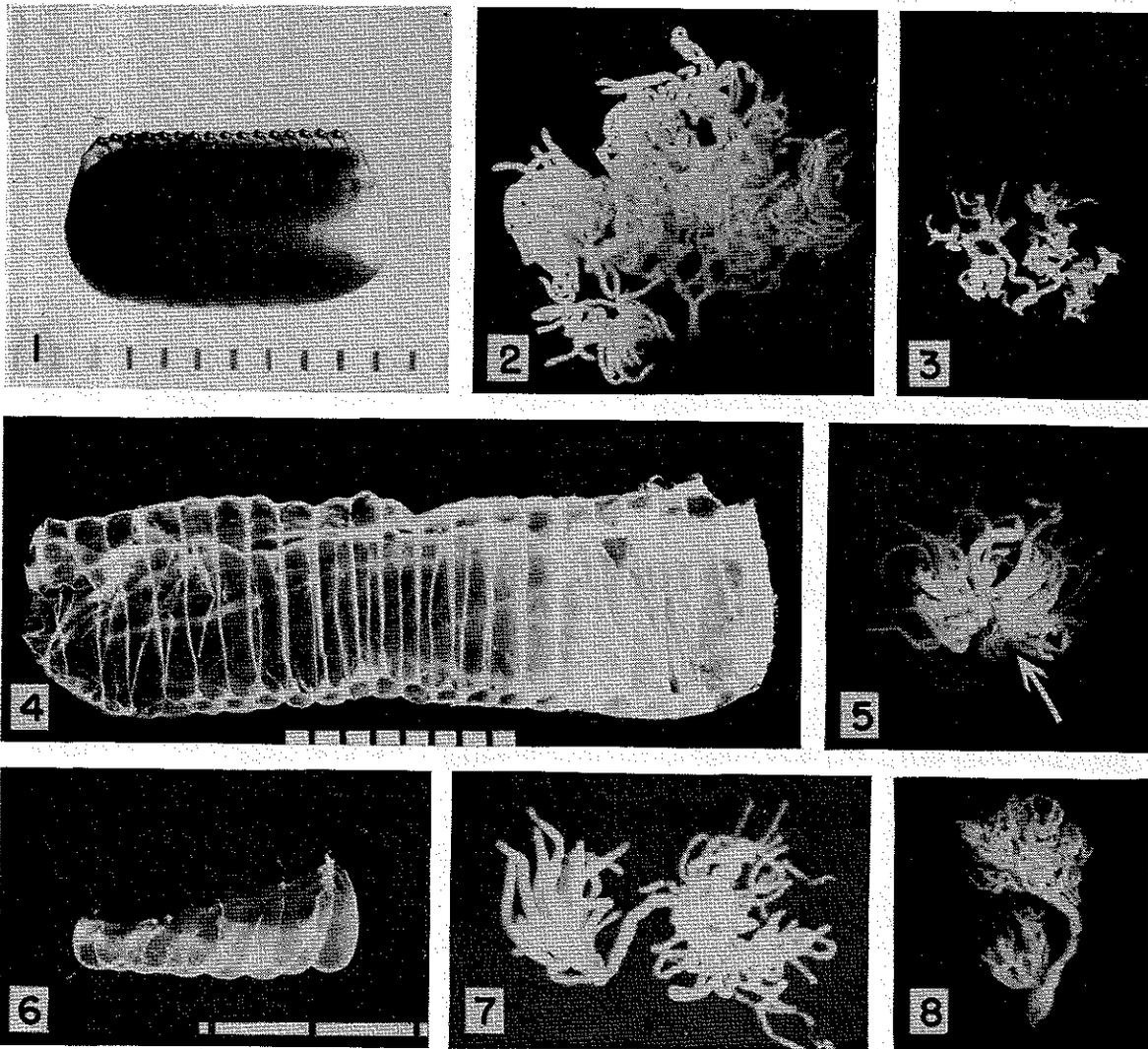
acid: water (4:1:5) or isopropyl alcohol: acetic acid: water (27:1:9) as descending solvents. The detecting reagents for phenolic substances were ferric chloride/potassium ferricyanide mixture (Barton et al. 1952) or diazotized *para*-nitroaniline (Snell and Snell 1953). Benzidine reagent (Horrocks 1949) was used to detect reducing sugars. Known substances were run on each chromatogram with unknowns.

EXPERIMENTAL RESULTS

Morphology of Colleterial Glands

A pair of colleterial glands composed of dichoto-

mously branched tubules was present in the 19 species examined (table 2). The left gland appears milky white, whereas the right gland is characteristically clear or yellowish as in *Blaberus craniifer*. The right gland is generally smaller than the left (fig. 3); in *Blattella vaga* the right gland has only two short branches. In contrast to other species, the right gland of *Diploptera punctata* is larger than the left (fig. 7). The size and structure of colleterial glands and oöthecae of three species is illustrated in figures 1 to 8. The oötheca of the false ovoviviparous *Leucophaea maderae* is large but thin, and the colleterial glands are small. The oötheca of *Periplaneta ameri-*



EXPLANATION OF PLATE 1

The colleterial glands are shown at the same magnification as the oötheca of the corresponding species. Scale in millimeters.

FIG. 1.—*Periplaneta americana*, oötheca. FIG. 2.—*P. americana*, left colleterial gland, fixed in 70% ethanol. FIG. 3.—*P. americana*, right colleterial gland, fixed in 70% ethanol. FIG. 4.—*Leucophaea maderae*, oötheca from which eggs have been removed. FIG. 5.—*L. maderae*, colleterial glands in Ringer's solution. The small right gland is indicated by an arrow. FIG. 6.—*Diploptera punctata*, oötheca from which eggs have been removed. FIG. 7.—*D. punctata*, left and right (on the right) colleterial glands from a female just before egg laying. FIG. 8.—*D. punctata*, inactive colleterial glands from a female during incubation of the oötheca.

cana is thick and dark, and the colleterial glands are large. The two pairs of glands of *Diploptera punctata* illustrate the difference in size of the glands at different periods of their secretory cycle (figs. 7, 8). As is characteristic for species which carry their oöthecae externally or internally during embryonic development, the colleterial glands are inactive and small while the oötheca is being carried (see Engelmann 1957 and 1959).

Effect of Removing Colleterial Glands

The large size of the right gland of *Diploptera punctata* led Hagan (1951) to propose that the right colleterial gland of *D. punctata* might function in the nourishment of the embryos. This hypothesis was tested by surgically removing, under carbon-dioxide anesthesia, either one or both colleterial glands from

Table 1.—Effect of removal of colleterial glands from *Diploptera punctata*.

Tissue removed	Number operated females	Number fertile females
Fat body (control)	15	5
Right gland	15	7
Left gland	15	3
Both glands	15	5

Chi square = 2.4 P > 10%.

females a few days after the oötheca was deposited in the brood sac. Some of the females aborted the oötheca after the operation but were able to deposit another in the brood sac and produce offspring. The results are summarized in table 1. There is no significant difference in fertility (i.e., females which carried embryos to the emergence of nymphs) between the females from which fat body or colleterial glands were removed. Removal of either or both colleterial glands from *Diploptera punctata* does not prevent the development of young.

β -glucosidase in the Right Glands

When the right gland of *Diploptera punctata* is incubated overnight at 37° C. with the left gland of *P. americana* the latter darkens. Paper chromatographic analyses of the solution in which *P. americana* left glands and *D. punctata* right glands have been incubated indicate the presence of glucose and protocatechuic acid. Neither glucose nor protocatechuic acid were detected when the glands were incubated separately. Right glands of *D. punctata* were also incubated overnight at 37° C. in a 0.5% solution of salicin (a β -glucoside of saligenin). Glucose and saligenin were demonstrated by paper chromatography. These products were absent in solutions of salicin incubated under the same conditions in the absence of right glands or with right glands which were heated three minutes at 99° C. at the start of incubation. These facts indicate that the right gland of *Diploptera punctata* contains a β -glucosidase similar to that of *Periplaneta americana*.

β -glucosidase was similarly demonstrated in the right colleterial glands of *Blattella germanica*, *Leucophaea maderae* and *Nauphoeta cinerea*. It appears that the secretion of β -glucosidase is a function characteristic of the right colleterial glands of cockroaches representing different egg-laying behavior.

Ethanol Extracts of Left Colleterial Glands

Ethanol extracts of left colleterial glands were run on paper chromatograms, using butanol: acetic acid: water as solvent and ferric chloride/potassium ferricyanide as developer. In the absence of authentic 3-hydroxy-4-O-(β -D-glucopyranosido)-benzoic acid, each chromatogram was run with extract of left colleterial glands of *Blatta orientalis*, *Periplaneta americana* or *Eurycotis floridana* as a control. The chromatogram in figure 9 shows left glands of four species of oviparous cockroaches in subfamily Blattinae compared with *Blatta orientalis*. All four species show the same two spots. In *B. orientalis* the upper one is stronger than the lower. The upper spot (Rf 0.42 to 0.48) is the 3-hydroxy-4-O-(β -D-glucopyranosido)-benzoic acid identified by Brunet and Kent (1955); the lower (Rf 0.29 to 0.32) is an unidentified substance, Gl-A. Extracts of left colleterial glands of 19 species of cockroaches contained substance Gl-A. In addition to Gl-A, another unidentified substance, Gl-B (Rf 0.35 to 0.40), was demonstrated in *Supella supellectilium* and *Parcoblatta pennsylvanica*. Paper chromatograms (fig. 10) indicate that Gl-A is more abundant than Gl-B in *S. supellectilium* and vice versa in *P. pennsylvanica*. Table 2 summarizes the occurrence of substances Gl-A and Gl-B and the β -glucoside of protocatechuic acid in extracts of colleterial glands from different species of cockroaches in relation to the taxonomic classification, the egg-laying behavior and oöthecal color of the species.

By extraction of left glands of *Periplaneta americana* of different ages it was indicated that the earliest secretion of the gland (i.e., in a 2-day-old adult) contains mostly Gl-A; the β -glucoside of protocatechuic acid appears later.

Comparison of Oöthecal Fluids

Oöthecal fluids from five species of oviparous cockroaches were run on paper chromatograms along with protocatechuic acid. Clear fluid was gently squeezed from pin holes in the ends of the oöthecae and applied directly to the paper, producing a spot about 3 mm. in diameter. The spot was allowed to dry and another drop superimposed. Two drops were generally sufficient to be detected with ferric chloride/potassium ferricyanide or diazotized *para*-nitroaniline. In all five species the oöthecal fluid exhibited a spot with the same Rf value (0.76 to 0.80) as protocatechuic acid. In addition, an unidentified substance A with a slightly smaller Rf value (0.67 to 0.70) than protocatechuic acid occurred in all species, more conspicuously in *Eurycotis floridana*, *Periplaneta brunnea*, and *Periplaneta australasiae* than in *Periplaneta*

americana and *Blatta orientalis* (fig. 12), those species previously studied (Pryor et al. 1946, Brunet and Kent 1955). Brunet and Kent (1955) noted that an unknown spot close to protocatechuic acid sometimes occurred on chromatograms of oöthecal fluid of *Periplaneta americana*.

Change in the composition of the oöthecal fluid as tanning of the oötheca proceeds is illustrated in *Eurycotis floridana* (fig. 13). Fluid from an oötheca during formation plainly shows protocatechuic acid and the substance A as well as two spots corresponding to those obtained with extracts of left colleterial glands (fig. 9). Presumably, the two lower spots are the precursors of the two higher ones. Fluid from a completed oötheca not yet abandoned by the female shows none of the precursor substances and little of the substance A (fig. 13).

Oöthecae of other available species of oviparous and ovoviviparous cockroaches did not readily yield sufficient oöthecal fluid for paper chromatographic analysis.

Interaction of Left and Right Glands and Hydrolysis of Left Gland Extract

Left and right glands were partially homogenized in a little acetate buffer or distilled water and incubated at 37° C. for 2 to 4 hours. Alternately, ethanol extracts of left glands were dried and in-

cubated in buffer solutions either with a right gland from *Eurycotis floridana* or glucosidase prepared from *Aspergillus luchuensis*. Dried left gland extracts were also hydrolyzed with 1 N sulfuric acid at 120° C., 15 pounds pressure, for 15 minutes. The resulting solutions were tested paper chromatographically. Before spotting on paper the solutions of homogenized left and right glands were mixed with one volume of 95% ethanol; the acid solution was neutralized. As controls, right glands and left glands or left gland extracts were incubated separately.

In those species in which the left gland showed the β -glucoside of protocatechuic acid and the unknown Gl-A, the interacted glands showed the unknown A. The interaction of left and right glands of *Supella supellectilium* exhibited A and B (Rf 0.73 to 0.76). Sometimes a faint spot with an Rf value slightly greater than those of protocatechuic acid occurred.

The same results were obtained when extracts of left glands were incubated with right gland of *Eurycotis floridana* or glucosidase of *Aspergillus luchuensis*. The right gland of *Eurycotis floridana* incubated alone showed either no spot or a faint one with a lower Rf value than the spots resulting from interacted gland and extract. The enzyme solution showed no spot.

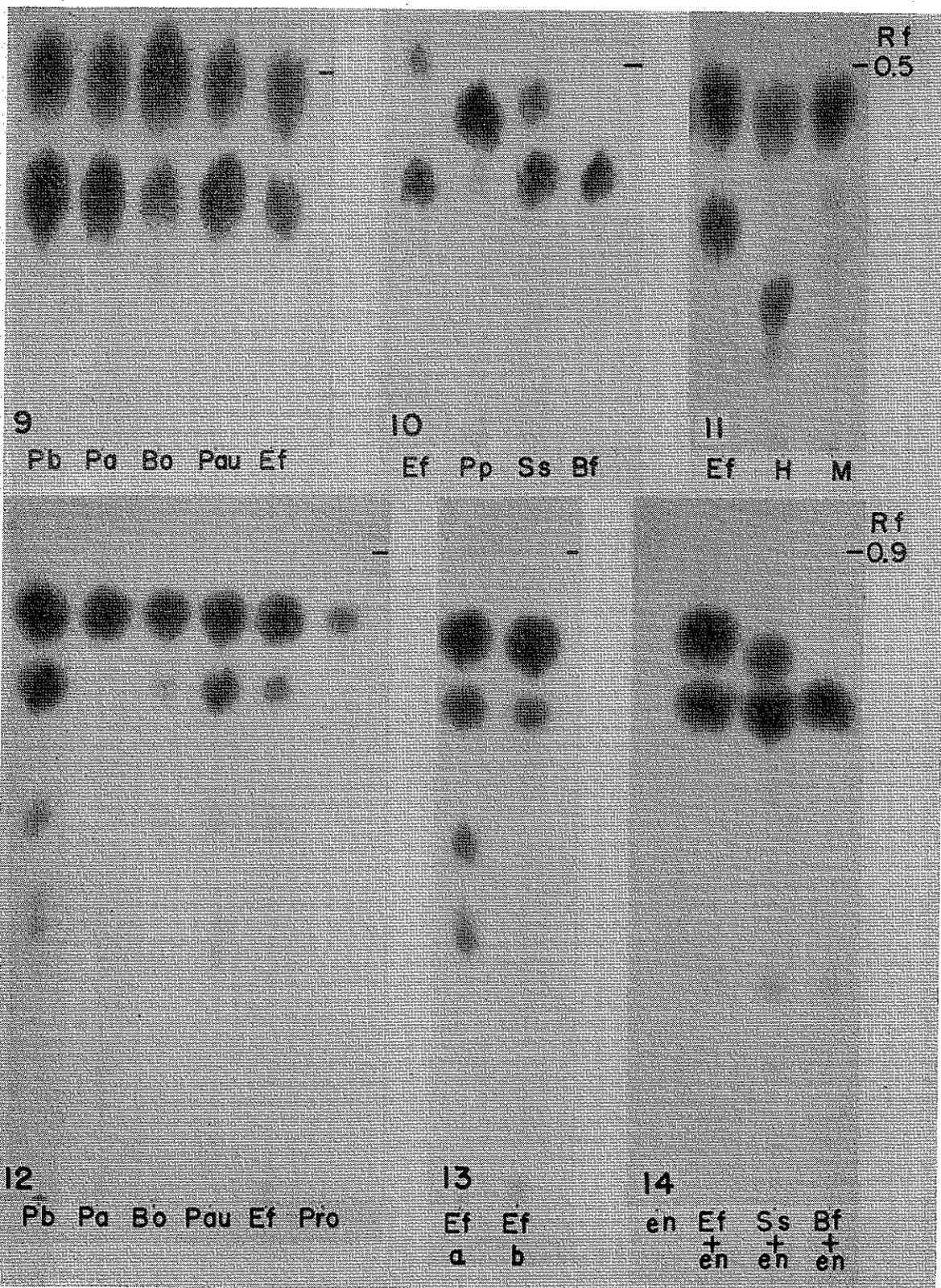
Glucose was demonstrated paper chromatographically in the interaction products of left gland extract

Table 2.—Characteristics of colleterial glands and oöthecae of various cockroaches.

Family Subfamily ^a Species	Disposition of oötheca during embryogenesis ^b	Source of water during embryogenesis ^b	Color of oötheca	Left colleterial gland contains glucoside of		
				Un- known Gl-A	Un- known Gl-B	Proto- catechuic acid
BLATTIDAE						
Blattinae						
<i>Blatta orientalis</i> L.	Abandoned	Oötheca	Dark brown	+	—	+
<i>Eurycotis floridana</i> (Walker)	"	"	"	+	—	+
<i>Neostylopyga rhombifolia</i> (Stoll)	"	"	"	+	—	+
<i>Periplaneta americana</i> (L.)	"	"	"	+	—	+
<i>Periplaneta australasiae</i> (Fabr.)	"	"	"	+	—	+
<i>Periplaneta brunnea</i> Burm.	"	"	"	+	—	+
Ectobiinae						
<i>Ectobius pallidus</i> (Olivier)	"	Substrate	"	+	—	—
Pseudomopinae						
<i>Parcoblatta pennsylvanica</i> (DeG.)	"	Undetermined	"	+	+	—
<i>Parcoblatta virginica</i> (Brunner)	"	Substrate	"	+	—	—
<i>Supella supellectilium</i> (Serv.)	"	Oötheca	Light Brown	+	+	—
<i>Blattella germanica</i> (L.)	Borne externally	Female	"	+	—	—
<i>Blattella vaga</i> Hebard	"	"	"	+	—	—
Blaberinae						
<i>Blaberus craniifer</i> Burm.	Borne internally	"	"	+	—	—
<i>Blaberus giganteus</i> (L.)	"	"	"	+	—	—
<i>Byrsotria fumigata</i> Guérin	"	"	"	+	—	—
Epilamprinae						
<i>Leucophaea maderae</i> (Fabr.)	"	"	Yellow	+	—	—
<i>Nauphoeta cinerea</i> (Olivier)	"	"	Light yellow	+	—	—
<i>Pycnoscelus surinamensis</i> (L.)	"	"	Yellow-tan	+	—	—
DIPLOPTERIDAE						
Diplopterinae						
<i>Diploptera punctata</i> (Esch.)	"	"	Light yellow	+	—	—

^a Classification after Rehn (1951), but not according to his linear arrangement.

^b Roth and Willis (1958).



of *Blaberus craniifer* and right gland of *Eurycotis floridana*. No glucose could be detected in either when incubated singly. Extract of 20 left colleterial glands of *Blattella germanica* incubated with glucosidase of *Aspergillus luchuensis* gave a dinitrosalicylic acid test for reducing sugars (Sumner 1924) corresponding to 0.35 mg. glucose per milliliter; whereas, the enzyme and left gland alone showed 0.14 mg./ml. and 0.07 mg./ml., respectively. Paper chromatograms of the reaction products run in butanol: acetic acid: water or isopropyl alcohol: acetic acid; water and sprayed with benzidine showed the presence of glucose. The unknown substance from extract of left glands of *Eurycotis floridana* was isolated by paper column chromatography. The extract showed no glucose, whereas acid hydrolysate of the extract showed a spot corresponding to glucose.

The absence of glucose in the presence of substance Gl-A in left gland extracts and the presence of both glucose and substance A in hydrolysate of left gland extract suggest that Gl-A is a glucoside and A is its aglucone.

Characteristics of the Unknowns

The unknown A and B and their glucosides formed blue spots on paper chromatograms sprayed with ferric chloride/potassium ferricyanide. When sprayed with diazotized *para*-nitroaniline the glucosides Gl-A and Gl-B appear rose-colored whereas A and B are more orange.

Gl-A and A were located on paper chromatograms under ultraviolet light (wave length peak at 2537 Å) as dark blue spots. The glucoside of A for absorption spectra study was obtained from extracts of left glands of *Eurycotis floridana* or *Byrsotria fumigata*. The glucoside of B was obtained from *Supella supellectilium*. Extracts were run on paper using butanol: acetic acid: water as solvent. The unknown glucoside was located with ultraviolet light or by spraying a small strip of each paper with ferric chloride/potassium ferricyanide. The strips of unknown were cut out and eluted with water or phosphate buffer. Alternately, but less satisfactorily, the unknown glucoside was separated by running extracts on columns of cellulose. The aglucone was prepared by hydrolyzing the separated glucoside with glucosidase for 4 hours at 47° C. and extracting the aglucone with ether. The ether was removed at low temperature under vacuum, resulting in a light yellow solid which darkened on exposure to air. The dark brown

solid gave a bright red color with concentrated sulfuric acid. Solutions of the unknown in water or buffer solution were scanned from 3300 to 2200 Å in a 10-mm. cell of an automatic recording Cary Model 11 MS spectrophotometer. The peaks of maximum absorption of the glucosides Gl-A and Gl-B are similar. In 0.1 M phosphate buffer pH 7.0 the maximum peaks of absorption of the glucosides Gl-A and Gl-B is 2770 Å. In ether the aglucones A and B both have maximum absorption peaks at 2810 Å.

Relative Concentrations of Phenolic Secretions of Left Gland

Since darkly colored oöthecae are produced by species which do and do not elaborate the β -glucoside of protocatechuic acid, the difference in color cannot be ascribed to the different nature of the presumed precursors of the tanning agent. One possible explanation in color difference might be a difference in amount of phenolic material produced relative to the protein.

Left colleterial glands full of secretion were quickly rinsed in distilled water and spread on a glass slide. After drying at 40° C. for several days the glands were put in a small weighed test tube, crushed with a glass rod, weighed and extracted once with 1 ml. 95% ethanol per 1.1 mg. of dry weight. Paper chromatograms showed little else than the unknown glucosides and the glucoside of protocatechuic acid. The Folin-Ciocalteu colorimetric test (Lowry et al. 1951) was run with 0.5-cc. samples from five different species. The relative concentrations of phenolic glucosides were *Periplaneta americana* 100, *Supella supellectilium* 97, *Blaberus craniifer* 21, *Byrsotria fumigata* 19, and *Leucophaea maderae* 5.5. These measurements would indicate that with the exception of *S. supellectilium* the coloration of the oötheca (see table 2) varies with the quantity of phenolic glucoside.

That factors other than the concentration of phenolic material are involved in the coloration of the oötheca is suggested by the reactions of isolated left colleterial glands of different species to a solution of authentic protocatechuic acid or oöthecal fluid of *Blatta orientalis*. Left colleterial glands were incubated overnight at 40° C., one-half of each gland in 0.5% protocatechuic acid in 0.05M phosphate buffer pH 7.4, the other half in buffer alone. Alternately, portions of left colleterial glands were kept at room temperature for 2 to 3 days in a drop of

EXPLANATION OF PLATE 2

FIG. 9.—Ethanol extract of left colleterial glands of several oviparous species of cockroaches belonging to the subfamily Blattinae. FIG. 10.—Ethanol extract of left colleterial glands. FIG. 11.—Ethanol extract of left colleterial gland of a mantid (H), and extract of the accessory gland of a grasshopper (M). FIG. 12.—Oöthecal fluid from oöthecae of several species of Blattinae. FIG. 13.—Oöthecal fluid of *E. floridana*; (a) from an oötheca in the process of formation; (b) a finished oötheca still carried by the female. FIG. 14.—Ethanol extracts of colleterial glands reacted with β -glucosidase.

The chromatograms were run in butanol: acetic acid: water and sprayed with ferric chloride/potassium ferricyanide. Pb, *Periplaneta brunnea*; Pa, *Periplaneta americana*; Bo, *Blatta orientalis*; Pau, *Periplaneta australasiae*; Ef, *Eurycotis floridana*; Pp, *Parcoblatta pennsylvanica*; Ss, *Supella supellectilium*; Bf, *Byrsotria fumigata*; H, *Hierodula patalifera*; M, *Melanoplus femoratus*; Pro, protocatechuic acid; en, glucosidase from *Aspergillus luchuensis*.

fluid from oöthecae of *B. orientalis* diluted with a drop of phosphate buffer pH 8.0, and in buffer alone. The glands in buffer alone remained white. In protocatechuic acid or oöthecal fluid, the left glands of *Periplaneta americana* and *Eurycotis floridana* became dark brown; those of *Leucophaea maderae*, *Diploptera punctata* and *Pycnoscelus surinamensis* attained no more coloration than pink or dark pink.

COLLETERIAL GLANDS OF OTHER ORTHOPTERA

The colleterial glands of the praying mantids appear similar to those of the cockroach in the relatively large size of the left gland and the small right gland. Chromatograms of ethanol extracts of the left colleterial glands of *Hierodula patalifera* and *Paratenodera sinensis* both showed similar spots when sprayed with ferric chloride/potassium ferricyanide (fig. 11). One spot corresponds to the β -glucoside of protocatechuic acid in extracts of left gland of *Eurycotis floridana*, the other spots have a smaller Rf value than the unknown GI-A of *E. floridana* (fig. 11). The right glands from these mantids hydrolyzed the glucoside in extract of left gland of *E. floridana*, indicating the presence of glucosidase in the right glands of mantids. Extracts of the left gland of the mantids *H. patalifera* and *P. sinensis* were hydrolyzed with enzyme from *Aspergillus luchuensis*. Paper chromatograms showed, in addition to the spots of the left gland extract, two spots with higher Rf values, one of which corresponds to protocatechuic acid.

Ethanol extracts of the accessory gland and the calyx of the ovary of the two-lined grasshopper, *Melanoplus femoratus*, were also compared with left gland extract of *E. floridana*. A spot corresponding to the β -glucoside of protocatechuic acid was present, and, in addition, two faintly visible unknown substances apparently different from the unknowns of both mantids and cockroaches. Enzyme hydrolysis of the grasshopper extract produced a spot corresponding to protocatechuic acid.

These preliminary observations would indicate that the colleterial glands of the mantids and probably the calyx of the grasshopper produce compounds similar to those formed by the colleterial glands of cockroaches.

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Paratenodera sinensis and *Parcoblatta pensylvanica*; and Mr. John Sousa and Dr. R. B. Clayton for much help in characterizing the unknown phenols.

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