The Formation of the Ootheca by *Periplaneta americana*

II. The Structure and Function of the Left Colleterial Gland

By P. C. J. BRUNET

(From the Department of Zoology and Comparative Anatomy, University Museum, Oxford)

With two plates

**SUMMARY**

The anatomy of the left colleterial gland of the cockroach is described. It is convenient to subdivide the gland into four regions. At the posterior end, abutting on to the outlet of the gland, there is no secretory activity and the cells of this region are not unlike normal epidermal cells. Anterior to this region are the three secretory regions of the gland; of these, the anterior and posterior secrete the structural protein and the constituent cells are equipped with a complex end-apparatus, a thick-walled depression in the apex of the cell in which the final stages of the elaboration of the secretion occur. The body of the end-apparatus contains canalicules which lead to the lumen of the gland. Alkaline phosphatase is abundantly present in this organ. Protein is secreted continuously, and there is no cycle related to oviposition. The third region, between the protein-secreting regions, secretes an oxidase, whose function may well be to oxidize the phenolic tannin-precursor produced in the right colleterial gland when the products of the two glands come together at oviposition.

The gland becomes fully functional some 14 days after the final moult, the immature cells of the nymph developing directly into mature cells characteristic of the region in which they occur. Within each of the main regions the cells show some differences which suggest that there is a wave of change passing along the gland. It appears that the cells of the anterior end of the gland become defunct and the hitherto inactive cells of the posterior end take on a secretory function.

The structural protein has a high phenolic content, and contains no combined carbohydrate. The presence of lipoid in the secretory cells appears to be directly bound up with the secretory processes.

**INTRODUCTION**

The colleterial glands of the cockroach are the sexual accessory glands which produce most of the material used in the formation of the ootheca, the case which serves to protect each batch of eggs that is laid. The fabric of the ootheca and the substances which harden the arthropod cuticle are biochemically similar (Pryor, 1940b), and this gives to the results of the study of the colleterial glands a broad applicability and a more than parochial significance. The microanatomy and location of these glands has been described by Bordas (1909), Ito (1924), Voy (1949), and Brunet (1951). The glands are made up of a right- and left-hand component; each consists of a mass of branched tubules lying freely in the haemocoel, and each has its own opening into the genital vestibulum, an invagination of the posterior end of [Quarterly Journal of Microscopical Science, Vol. 93, part 1, pp. 47-69, Mar. 1952.]
the abdomen concerned with the bringing together of eggs, stored sperms, and other materials used to complete the formation of the ootheca and its contents.

In macroscopic appearance the left and right gland differ from one another. The left gland is composed of more tubules, and is therefore larger than the right, which it more or less surrounds. The colour of the left gland is mostly whitish on account of the presence of secretion product in the lumen, while the right gland stores little secretion in the lumen and is translucent and colourless. The function of the left gland is to secrete a protein which becomes shaped to form the ootheca (Pryor, 1940a) and the right gland secretes a diphenolic substance (Pryor, Russell, and Todd, 1946), which is converted by enzyme action to a quinonoid tanning agent that serves to cross-link protein molecules. The resulting tanned protein of the ootheca has a particularly resilient nature.

The aim of this paper is further to describe the anatomy of the secretory cells of the glands, and where possible to use histochemical methods to infer the part played by them in the production of the ootheca.

MATERIAL AND METHODS

*Periplaneta americana* has been used almost entirely for the investigation. This cockroach is easily cultured at a temperature of 28°C, which is favourable to the insect. The glands of *Blatta orientalis* have also been examined. There is a very close similarity between the colleterial glands of *Blatta* and *Periplaneta*.

Anatomical methods. It is possible to remove and examine each colleterial gland separately, in order to distinguish stages of the left gland from those of the right, and this method was used at first. The statement that the left gland appears whitish (since the lumen is filled with opalescent protein) while the right gland appears translucent (Ito, 1924; Pryor, 1940a) was used as a guide when disentangling the glands. However, it is only a general rule; for those cells which I have classified as type 3 cells of the left gland (Brunet, 1951) secrete little or no opalescent protein and the tubules in which they occur thus appear translucent, like the tubules of the right gland. Since also these same type 3 cells are taller than the remainder of the cells of the left gland, being approximately the same size as the cells of the right gland, unqualified acceptance of this method of distinction can be misleading. The tubules are entwined and bound together by tough and almost invisible tracheae; it is difficult to separate them without causing damage. It has been found preferable to deal with the glands while still attached at their exits to the dorsal wall of the vestibulum. Two longitudinal cuts along the edge of the full length of the abdomen allow the terga to be lifted up, and if these cuts are continued at right angles so as to meet below the anus, the terga and alimentary system are then made free and can be shifted anteriorly out of the way. The whole reproductive system and accessory glands are then clearly visible, and can be removed and examined or fixed and sectioned serially.
Histological and cytological methods. Fixation in Bouin’s fluid, followed by staining with Ehrlich’s haematoxylin and eosin proved to be an adequate general technique. Meves’s modification of Flemming’s chromic/acetic/osmium fixative (see Baker, 1945) followed by Heidenhain’s haematoxylin was used for general study of the cytoplasm and of the end-apparatus of the secretory cells. Altmann’s dichromate/osmium fluid was used as a non-precipitant fixative, and was likewise followed by Heidenhain’s haematoxylin. Many sections of tissue fixed in neutral formaldehyde/saline (Baker, 1949), and intended as controls for histochemical work, were used for the interpretation of general anatomy. As a basic dye 0.1 per cent. aqueous solution of toluidine blue G was often used in place of dye lakes; staining time is very short, and, as a brilliant blue counterstain, it is often useful in conjunction with histochemical techniques, for which purpose its transparency is an advantage.

For mitochondria, material was fixed in Helly's dichromate/mercuric chloride/formaldehyde and post-chromed for 48 hours at 37° C. in saturated potassium dichromate solution. Altmann’s acid fuchsin and other triphenyl-methane dyes dissolved in aniline water were used for colouring the mitochondria, and this treatment was variously followed by picric acid or aurantia (see Baker, 1933 and 1945), or methyl blue (Cain, 1948), or methylene blue derivatives (Volkonsky, 1928). Owing to its intensity, acid fuchsin used thus proved to be an excellent routine acid dye.

To determine whether fixing mixtures, known to be powerful evocators of myelin-forms (Palade and Claude, 1949), would show up the classical Golgi network, Flemming’s fluid was used, followed by post-osmication for several days at 37° C., and Aoyama’s formaldehyde/cadmium fixing mixture was used, followed by treatment with silver nitrate and subsequent reduction. This last fixative acted brutally upon the secretory cells, causing them, in many cases, to eject the secretory end-apparatus (an inpushing at the apex of the cell, larger than the nucleus).

Baker’s method (1949) using formaldehyde/dichromate for fixation followed by post-chroming and colouring with sudan black was used for demonstrating lipochondria, a term used by Ries (1935), Holtfreter (1946a), and Baker (1950), and used here in the last-named author’s sense to denote the lipoid granules and the lipoid sheaths customarily found to be present around secretory materials within a cell. The term lipochondria has recently been adopted by Baker (1950) to denote these organelles as a result of their dissimilarity to the original apparatus of Golgi. The term is equivalent to the Golgi bodies (of Baker, 1949); Golgi system (of Hirsch, 1948), and spheroids (of Thomas, 1948).

Except for this last technique, paraffin embedding was used. The presence of the cuticle does not seriously upset section-cutting. In the neighbourhood of the cockroach’s vestibulum there is not a great deal of heavily sclerotized cuticle, and the surfaces are sufficiently contorted to allow firm interlocking of wax and cuticle. Sections fixed for colouring with Baker’s sudan black were cut from frozen gelatine blocks. These and other sections for histochemical
purposes cut from gelatine blocks show that this is a most admirable method for cutting sections of cuticular tissues. There are limitations to the method: for example, the boundaries of cells or cuticle lying against supporting gelatine are often hard to define since the gelatine itself is coloured by dyes. Use was made of formaldehyde/alum solution (Baker, 1949) instead of neutral formaldehyde for hardening the gelatine blocks. Sections cut from blocks so hardened have the great advantage over formaldehyde-hardened blocks that they do not tend to stick to vessels and instruments.

Observations of living tissue. 0.01 per cent. solutions of various basic dyes in 0.7 per cent. saline (Baker, 1951) were used to demonstrate cell organelles. Since these so-called vital dyes are sometimes known to alter the nature of the constituents of the cell (Holtfreter, 1946b), unstained living cells were also examined by phase-contrast microscopy.

Histochemical methods. A number of qualitative colorimetric, mainly in situ, methods were used, which are discussed below in the text.

Observations

The Secretory Stages of the Left Gland

The four main types of constituent cell have been described (Brunet, 1951). The method of subdivision is arbitrary, and is such that any one of the four types of cell is quite unlike any other type (cf. figs. 1, 4, 12, and 18); and there occurs, between the region occupied by any one type of cell and its neighbour, a region of rapid change in cell character. The type 1 cell is found at the posterior end of the gland; it is not secretory and resembles an epidermal cell. The type 2 cell is a squat cell, much of the volume of which is occupied by the end-apparatus (fig. 4); a dense protein secretion is secreted in this region. The type 3 cell is a tall cell with an elongated end-apparatus, and with clearly fibrillated cytoplasm basally (fig. 12). The type 4 cell forms most of the gland and is a short cell with an elongated end-apparatus, and out of which protein passes into the lumen of the gland (fig. 18). There is no cyclical change occurring in the cells in relation to the formation of the oothecae: it is quite clear in types 2 and 4 cells—the two regions that secrete structural protein—that the lumen of the end-apparatus is always filled with protein, and there is no change in the cytoplasm indicative of such a secretory cycle. Protein is continually being produced and poured into the lumen of the gland.

The colleterial glands are present in the last instar nymph in an undifferentiated condition and the cells of both left and right glands are extremely similar, and are like cells of the epidermis. During the first day of the adult instar, the tubules of the left gland begin to show differentiation into the four definitive types of cell, but there is no secreted protein in the lumen of the tubules. On the second day of the adult instar, the four cell types are easily distinguishable and there is a trace of secretion in the lumen; and by the seventh day there has been a great increase in the size of the gland by growth on the
part of the cells, and in particular of their cytoplasm; by this time there is more protein in the lumen. By the fourteenth day the gland is mature and oviposition commences.

The disposition of the cells within the mature gland is not straightforward. As has been described, a tubule is composed of four types of cell arranged in linear sequence, and each type is separated by a transitional region, where there is a rapid but graded change in cell character. There is also, however, a slight change in cell character within the region occupied by any one cell type; for example, type 2 cells at the height of their activity are more or less equidimensional (fig. 1), but there is a slight progressive increase in height of the cells towards the anterior limit of the type 2 cell region until the transitional region between types 2 and 3 cells occurs and where the increase in height is rapid. Type 3 cells then show a further steady increase in height followed by a slight decrease, until the transitional region between types 3 and 4 cells occurs, where the decrease in height is rapid. Type 4 cells near the transitional region are relatively tall, but there is a steady decrease in height until the condition where all the cells are more or less equidimensional is reached.

The significance of this arrangement is not at once evident. It is clearly not what would be expected if the glandular cells were passing through a monophasic secretory cycle such that types 2, 3, and 4 cells represented early, middle, and late stages of secretion. In the first place, the well-defined transitional regions are not compatible with such an hypothesis, and also since in ontogeny all the secretory types differentiate out at the same time (i.e. on the fourteenth day of the adult instar). The presence of mitochondria and lipochondria in all three cell types suggests that they are all active secretory cells, and histochemical evidence shows that they are secreting not the same but three different products. This being so, the presence of three regions of secretory cells and the intermediate transitional regions is understandable, but the steady change within the regions occupied by the secretory cells still requires an explanation. It is tentatively suggested that there is a slow wave of change passing along the tubules of the gland such that as time passes each cell takes on the function previously carried out by a cell situated anteriorly to it. As a result of this, the most anterior type 1 cells become secretory (indeed, the anterior type 1 cells show first a dense apical region of the cytoplasm and later a rudimentary end-apparatus which is most clearly defined in the most anteriorly situated cells). In time these cells are thought to become fully secretory type 2 cells. These latter are destined to become type 3 cells, which themselves become type 4 cells; and as this process occurs the most anterior type become reduced in size and are evidently effete. The hypothesis is represented diagrammatically in Table 1. The reason as to why this process should occur is obscure. Table 1, being diagrammatic, of necessity suggests the presence of relatively more transitional cells than in fact exist. This being so, it can be seen that the number of cells which actually change from one cell type to another during the life-history is small, and what value such a slow wave of change can possess for the organism is not understood.
The Formation of the Ootheca by Periplaneta americana

**FIG. 1.** The secretory cells of the left colletorial gland as they appear after staining with iron haematoxylin. Their approximate position in the gland is indicated. All but one branch of the gland has been omitted for the sake of clarity.
The table illustrates the author's hypothesis as to the ontogeny of the four cell types in the left colletorial gland. The types of cell constituting the gland are indicated by numerals, and the table shows the development from the unspecialized nymphal condition to the adult condition, and the slow shift of function during the adult condition.

Arabic numerals indicate the types of cell present at any one period; two small numerals indicate transitional types; italic numerals indicate that the cells are pouring secretion out into the lumen.

The number of cells of any one type is only approximately indicated by the number of numerals, e.g. type 3 cells are in the minority, and type 4 cells in the majority.

**The Nature of the Structural Protein.**

Structural protein is secreted by two distinct regions of the left gland; cells of type 2 (fig. 4) and type 4 (fig. 18) carry out this function. Both types of cell have certain similarities in that they are short, squat cells with a somewhat rounded end-apparatus, and they contrast with the type 3 cells, which are tall cells with an elongated end-apparatus. It is not known why there should be two separate secretory regions; there are two also in the silk glands of *Cosus* and other silk-spinning larvae (Bradfield, 1951), and in this case it is believed that each secretes one of the two components of silk.

In *Periplaneta* large characteristic crystals of calcium oxalate (Kadyi, 1879) are mixed with the abundant protein secreted by the type 4 cells. These do not occur in the more scanty protein secreted by the type 2 cells. After formaldehyde fixation type 2 protein appears to be more coarsely granular than type 4. This difference is more evident in watery mounts than when the tissue is in balsam.

With in situ histochemical tests, whether on fresh material or paraffin sections, no distinction can be drawn between the two types of protein; but this does not necessarily indicate close similarity. Histochemical methods are not very suitable for protein analysis: many are exceedingly brutal in action, and many show simply an organic configuration (not that of an α-amino acid) from which one infers the presence of a protein. If an unknown substance reacts positively to several of these tests, there is considerable likelihood that it contains or consists of protein, and this likelihood is increased if a positive reaction is given with a reagent that demonstrates the presence of the peptic linkage or of amino-acid.

Ninhydrin in 50 per cent. glycerol, and alloxane 1 per cent. in 70 per cent. alcohol (Lison, 1936) both gave intensely positive reactions with the protein
The Formation of the Ootheca by Periplaneta americana in the lumen, thus indicating an amino-acid configuration. These reactions only occur with fresh material. Only a very weak reaction occurs after formaldehyde fixation, presumably as a result of the reaction of formaldehyde with the amino-group concerned in the test.

Baker's (1947a) modification of Sakaguchi's test showed the presence of arginine or a related guanidine derivative. The colour produced was not intense.

An intensely positive xanthoproteic reaction implied, and a similar reaction with Millon's reagent demonstrated, the presence of a phenolic nucleus. The result of coupling with several diazo-compounds in alkaline solution, in the manner specified by Lison, was to produce a coloured compound, and would thus further indicate the presence of a phenolic compound (Lison, 1931 and 1936). The specificity of this test is further considered below. Syrupy phosphoric acid (Romieu, 1925) caused the production of a pale pink colour attributable to the presence of tryptophane.

Oxidation with periodic acid (Hotchkiss, 1948; McManus, 1948) followed by the application of Schiff's reagent gave completely negative results on fixed and fresh material. Periodic acid oxidizes β-hydroxy-α-amino acids such as serine (Nicolet and Shinn, 1939) converting them to aldehydes, which can then be demonstrated with Schiff's reagent. The method has been used for the biochemical estimation of such acids, and Lillie (1950) has shown the possibility of demonstrating such hydroxyamino acids by this means.

With the same reagents, the presence of substances containing the α-glycol group would have been shown: periodic acid oxidizes the hydroxyl groups to aldehyde groups, which have been shown to be absent. Oxidation with 4 per cent. chromic acid (Bauer, 1933) again gave rise to no aldehyde groups. These two tests therefore demonstrate the absence of unsubstituted polysaccharides. A 0.1 per cent. aqueous solution of toluidine blue showed no metachromatic coloration of the protein, and demonstrated the absence of sulphate esters of high molecular weight (Lison and Fautrez, 1939), which are usually indicative of the presence of mucopolysaccharides. A negative reaction with Molisch's α-naphthol reagent confirmed the total absence of polysaccharides.

A characteristic of the protein secretion is its ability to reduce ammoniacal silver hydrosulphide solution (Lison, 1936), and Burtner and Lillie's (1949) modification of Gomori's (1946) methenamine (= hexamine or hexamethylene tetramine)/silver nitrate solution. Such reactivity indicates the presence of a reducing agent, e.g. sulphhydryl-, aminophenyl-, dihydroxyphenyl-, or aldehydic-group. Lison considers that only the latter three have the power to reduce silver solutions, and that the amino-groups are inactivated by formaldehyde fixation. Since aldehydes can be shown to be absent from the protein secretion by means of Schiff's reagent, the effect would seem to be due to the presence of a dihydroxyphenyl compound. In confirmation of this, the chromaffine test is positive: potassium dichromate, iodate, or iodic acid solutions colour the proteins yellow after prolonged treatment. This test is said to be highly specific for diphenols (Lison, 1936). Since all these reactions
occur even after fixation in absolute alcohol, the reducing agent is likely to be
dopa, which is insoluble in alcohol, and, as an amino-acid, would be bonded
to other amino-acids and thus fixed with the protein. The argentaffine
reaction is not intense, and the chromaffine reaction particularly weak, but
they indicate that there is probably some dopa present in the secretion.

It is noticeable that this reaction does not occur after fixation in Helly's
formaldehyde/dichromate/mercuric chloride solution, while it does occur after
fixation in a fluid of similar composition but with the mercury salt omitted.
This fact, and the observation, unqualified though it be, by Lison (1931) that
mercuric chloride should be left out of fixative mixtures if the tissue is later
to be analysed for phenols, suggests that the mercury salt may form com-
 pounds with phenols, as the result of which they lose their reducing power.
If this is so, then the results obtained by Chéremont and Frederic's (1943)
test for sulphhydryl groups should be treated with caution. The test involves
the location of reducing agents by means of ferric ferricyanide, which is
reduced by them to ferric ferrocyanide, both with and without previous
incubation in a mercuric chloride solution. Mercuric chloride is known to
block sulphhydryl-groups, and substances which react positively without incu-
bation and negatively with incubation in the mercuric chloride solution are
said to be sulphhydryl compounds. It would, however, be unreasonable to
persist with the criticism of this technique without further evidence, and also
because in the test for sulphhydryl-groups mercuric chloride solution is applied
for a brief period to fixed sections, while in the present case unfixed or partially
fixed tissues are allowed to react with the mercuric chloride for a prolonged
period.

Nitroferricyanide stabilized by means of zinc acetate solution (Giroud and
Bulliard, 1933) gave a result, when applied to the protein secretion, suggesting
that there was little if any sulphhydryl content.

It is not altogether impossible that the argentaffine reaction (and also the
diazo and chromaffine reaction) are positive as a result of pyrimidine deriv-
atives being present (Jacobson, 1939); and this point will be more fully dis-
cussed under the heading of the right colleterial gland.

The protein of both regions 2 and 4 colours intensely with acidic dyes, and
markedly so with triphenylmethene dyes in aqueous solution and also in
aniline water such as is used in techniques for demonstrating mitochondria.
It is coloured, but not intensely, by Heidenhain's haematoxylin and by acid
haematein after pyridine extraction (Baker, 1946).

In so far as comparison is possible, the structural protein of the left colleterial
gland resembles the proteins of the insect cuticle as described by Trim (1941),
who records a characteristically high aromatic amino-acid content, and a low
sulphur and carbohydrate content. In one respect the colleterial protein differs
from that of the cuticle: Trim records the presence of hydroxyamino-acids in
cuticular protein, while there appears to be none in colleterial protein. These
differences, however, may be reconcilable since evidence derived from a study
of the right colleterial gland would suggest that hydroxyamino-acids are
intermediate products in the formation of the oothecal tanning agent. This being so, it could be expected that such substances would occur in the cuticle, but not in the left colleterial gland, being kept separate (until oviposition) in the right gland.

The Oxidase Secreted by the Left Gland

Pryor (1940a) showed that the conversion of the diphenolic precursor to the quinone tanning agent was an enzymatic reaction. Polyphenol oxidases are known to exist (Keilin, 1936) and, as oxidases, to be demonstrable colorimetrically.

If the colleterial glands are immersed in a solution of Nadi reagent an overall blue colour appears in the cells of the glands, indicating either the presence of a powerful oxidizing agent or of an oxidative enzyme system. The ability to effect this reaction is abolished by prior treatment with 0.002 M cyanide solution at pH 7.3, which indicates that the reaction is the result of the action of an oxidative enzyme and not an oxidizing agent. 0.002 M sodium azide solution at pH 7.3 acts as an inhibitor of the cytochrome oxidase system, but leaves polyphenol oxidase unaffected, and it was found that treatment with azide solution before immersion in Nadi reagent greatly lessened the reactivity of most of the glands, and that a region of the left gland which, when dissected out and sectioned, proved to be composed of type 3 cells, retained their activity, and stood out a deep blue colour. From this it is concluded that an enzyme is present, that type 3 cells of the left gland produce it, and that it is most probably the polyphenol oxidase which oxidizes the diphenol produced by the right gland to a quinone when the contents of the left gland and right are mixed during the formation of the ootheca. This separation of enzyme from substrate until oviposition is what might well be expected.

Experiments similar to those carried out with Nadi reagent but with leucobase methylene blue (Roskin and Struve, 1947) gave comparable results. Dimethyl-paraphenylene diamine alone would give such a blue colour with quinones, if present, but the reaction was negative; nor was the blue colour derived from methylene blue the result of any ‘specific affinity’ of the tissue for methylene blue, since application of the unreduced dye showed no greater coloration of the type 3 cell region than of any other.

The Cytology of the Left Colleterial Gland

Type 1 cells. These cells occupy the most posterior end of the gland. They show no sign of secretory activity and have no end-apparatus. They form a single layer of basiphil cells with a relatively large nucleus, and lie beneath a thick layer of deeply folded chitin. The most posteriorly situated type 2 cells line the papilla on which the outlet of the gland into the vestibulum is situated, and here the chitinous lining of the duct is adorned with grouped bristles.

The cells change little during the differentiation of the glands which occurs in the first days of the adult instar; their change involves only a diminution
in height as is the case with the cuticular epidermal cells. Within the region of the gland occupied by type 1 cells the lumen becomes extremely narrow, and, as the elaborated protein secretion passes outwards, it must pass through this constricted region, around which there is an extremely thick layer of muscle. This constriction could act as a type of spinneret, and the shearing stresses caused to occur in the outflowing protein might bring about physical denaturation.

Type 2 cells. Between type 1 cells and definitive type 2 cells there is a transitional region in which the cytoplasm of the potential gland-cells stains more densely and the end-apparatus develops. Reorientation of the cells occurs: the single-layered epithelium, found where type 1 cells are present, becomes modified into a double-layered epithelium with chitinogenic cells lying around the lumen, and glandular epithelial cells with their bases upon the basement membrane. As a result of this, for a certain distance along the tubule, disorientation occurs and the cells are unevenly spaced. In addition, the tubule increases in diameter and there is a slight increase in the height of the glandular cells (fig. 2), which are at this stage taller than either type 1 or definitive type 2 cells. Since there is no increase in the volume of the cells corresponding with the increase in diameter of the tubule, spaces are left between neighbouring cells and these can be seen to persist even in the regions of definitive type 2 cells which are secreting protein into the lumen of the tubule.

The definitive type 2 cell is a most distinctive cell, but, despite this fact, it remains only briefly described (Brunet, 1951). It occupies the walls of the single, most posterior duct of the gland, the walls of the two tubules formed as the result of the first bifurcation, and the walls of the four ducts formed as the result of the second, though the extent of its location is variable. It is a cell which appears to be more or less cubical in longitudinal section, but with a broader base than apex; the end-apparatus is outstandingly large and complex (figs. 3, 4, 5). The nucleus is characteristically indented by the end-apparatus (fig. 4), a phenomenon which can be seen in living cells and is not caused by fixation.

A cell stained with Ehrlich’s haematoxylin and eosin after fixation in Bouin’s fluid preserves its general outline, and the end-apparatus is seen to have been stained with eosin and the cytoplasm weakly so. The acid dye, however, is not intense enough clearly to show up the details of the end-apparatus. Heidenhain’s haematoxylin after fixation in Meves’s fluid shows far more detail (fig. 4): the cytoplasm appears as a fine reticulum; the interstices seem to have been occupied by substances subsequently leached out during preparation. The end-apparatus colours a deep grey, which can be seen to be inhomogeneous: the grey is the result of the presence of deeply coloured radial striations lying within the body of the end-apparatus. There is evidence that such striations represent either the content of minute canalicules or the substance around such canalicules, which run from the cytoplasm to the cavity of the end-apparatus.

There is an outer wall to the end-apparatus, said, in this type of cell, to be
The Formation of the Ootheca by Periplaneta americana

a chitinous inpushing. It is clearly seen in fig. 5 as the uncoloured region between the body of the end-apparatus and the mass of secretion in the lumen of the end-apparatus. In these cells it is coloured when Burtner and Lillie's (1949) methenamine/silver is used after McManus's (1948) periodic acid oxidation, which should demonstrate polysaccharides (fig. 6); however, chitin generally and the chitinous intima of the tubules reacts extremely weakly with this test while the wall of the end-apparatus is deeply coloured, and this indicates that there is some difference between the intima and lining of the end-apparatus. It has been said that chitin is converted to a polyaldehyde as the result of oxidation with periodic acid (Hotchkiss, 1948), but I find that this is not so. The chitin of the cockroach gives at most a very weakly positive reaction with periodic acid/Schiff's reagent or periodic acid/silver complex. Nor should it be expected to give a positive reaction, for the acetylglucosamine residues in the chitin molecule possess no unsubstituted α-glycol group, according to the generally accepted formula for these residues (Stacey, 1946). Acyl-amino derivatives of glycols are stable in the presence of periodic acid (Jackson, 1944). Lillie (1947) specifies only fungal chitin as reacting positively to this test.

Histochemical tests for proteins show that while the cytoplasm reacts positively, it contains far less amino-acid than does the elaborated secretion in the lumen.

Tests for lipoids show how different are these cells from the silk-secreting cells of lepidopteran larvae (Bradfield, 1951, quoting Lesperon), where it is claimed that little or none is present. Sudan black after fixation in formaldehyde/dichromate and post-chroming (Baker, 1949) shows that the cytoplasm is packed with lipoidal granules, and that lipid is present in the end-apparatus, which shows a general diffuse coloration with more intense radial striations (fig. 2). Even without post-chroming, after formaldehyde/calcium fixation, these organelles are seen to be strongly sudanophil. Baker's (1946 and 1947) test for phospholipines shows the same cytoplasmic granules to be coloured intensely and picks out clearly the striae of the end-apparatus (fig. 5). Phospholipine preparations suggest that uncoloured granules surrounded by phospholipine occur between the nucleus and the end-apparatus, but analysis of the result is complicated by the presence of such large quantities of diffuse phospholipine.

Acid fuscin colours the elaborated secretion intensely, and, after fixation in formaldehyde/dichromate and post-chroming, the cytoplasmic granules are most intensely coloured.

With the 'classical' methods for impregnating the Golgi apparatus, no network system is found; indeed, the technique does not usually produce this result in invertebrate cells (Palade and Claude, 1949). Post-osmication following fixation in Flemming's fluid shows the cytoplasmic granules to be osmiophil; similarly, treatment with silver nitrate with its subsequent reduction by hydroquinone after fixation in Aoyama's fluid shows deposition of silver on the same granules, often incompletely so; the result being that the granules often
appear to have argentiphil crescents attached to them. Aoyama's fluid causes the end-apparatus to be extruded from the cell; this demonstrates that this fixative acts brutally until it has been separated into its components by differential diffusion through a tissue block; but the rigidity of the end-apparatus is implied as the result of this distortion.

The type 2 cell is thus a relatively small, highly active cell, synthesizing protein and secreting it. It is not a cell which first partly synthesizes its secretion, stores it, and later releases it (as is the case with some other cells in the coelenterial glands). Acid dyes and tests for protein suggest that there is at any one time no great concentration of protein within the cell, most of the cytoplasm of which is occupied by the secretory mechanism of the cell. The evidence suggests that the lipoid granules of the cytoplasm are probably both of mitochondrial and lipochondrial nature. The results of the acid haematein test for phospholipines is evidence for the presence of either or both: phospholipines constitute a considerable amount of mitochondrial substance, and they occur in the Golgi apparatus (Cain, 1947) which represents the lipochondria of Baker (1950), but the intense diffuse staining of the cytoplasm by acid haematein makes morphological differentiation impossible. Baker's (1949) sudan black technique for lipochondria involves post-chroming at 60° C. Mitochondria are usually not shown by this technique: less material is coloured by sudan black than is coloured by acid haematein in the previous test, and the shape of the objects coloured is often crescentic, suggesting their apposition to a chromophobe spherical granule. Acid fuchsin in aniline water after fixation of the tissue in formaldehyde/dichromate with post-chroming colours intensely what appear to be minute granules in the cytoplasm; their number prevents morphological study, but acid fuchsin used under these conditions electively colours mitochondria.

The end-apparatus is evidently the organ wherein the ultimate stages of protein synthesis occur. The staining reactions alter at this barrier, and alkaline phosphatase activity is centred within the end-apparatus; there is an over-all positive reaction to Gomori's technique (1939), used within the suitable limits prescribed by Danielli (1946), with a slight increase at the cytoplasm end-apparatus interface, and a strong increase at the outer wall (fig. 3), the canalicules appear more positive than the interstices. The role of alkaline phosphatase in fibrous protein secretion is not yet fully worked out, but the presence of the enzyme in regions where such substances are formed is characteristic (Bradfield, 1951). The nature of the phospholipine molecule indicates that it is a potentially highly active substance, and its presence in the radial canalicules is in harmony with the theory that these are the paths through which the secretion passes from the cytoplasm to the lumen of the gland. The fact that conglomerations of refringent material of sudanophil nature, and reacting positively in the acid haematein test are present on the walls of the lumen (fig. 5) and that they appear not to mix with the protein secretion favours the theory that the phospholipines in the end-apparatus are playing a role in the transport mechanism across the end-apparatus.
Type 3 cells. Rapid changes in the dimensions of the glandular cells occur over a distance of about 300 $\mu$, and the height of the cells increases from 15 $\mu$ to as much as 50 $\mu$. Accompanying this change, there is considerable modification of the rest of the cell. The end-apparatus of type 3 cells is altogether different from that of the previous type; fig. 6 shows the small cup-like wall of the end-apparatus of a type 2 cell; fig. 7 shows that there is a basal prolongation which extends (figs. 8, 9, 10) until the total length of the end-apparatus is more than three times its original length, and simultaneously the original cup disappears. The same figures show, but less clearly, that the main body of the apparatus is also modified; figs. 6 and 7 show the body as a pale striated region surrounding the outer wall, while fig. 10 shows no sign of it. Examination of the organelle stained with iron haematoxylin shows that the typical spherical end-apparatus of a type 2 cell, with its clearly demarcated boundaries, has changed to the elongate form, with its distinct outer wall but ill-defined inner limits, which merge into the general cytoplasm of the cell.

The remainder of the cytoplasm is clearly divisible into two regions: the basal region is markedly fibrillar, even after fixation in a non-precipitant fixative such as Altmann’s fluid. The fibres, which appear to be attached to the base of the cell, reach up to the level of the nucleus (fig. 12); they can be coloured with iron haematoxylin, eosin, and other dyes. In the middle region of the cell, between the nucleus and the end-apparatus, the cytoplasm is not fibrillar and is but weakly coloured by any dye. Iron haematoxylin and most basic dyes scarcely colour it, but toluidine blue does so weakly (fig. 10).

The orientation of the fibrils parallel to the main growth axis of the cell could be taken as an indication of a rapid intake of fluid by the cell from the haemolymph. The weak coloration by dyes suggests that the cytoplasm of the

---

**FIGS. 2–11**

*Fig. 2.* Early type 2 cells. Formaldehyde/dichromate: sudan black/carmalum. The lipoid contents of the end-apparatus and of the cytoplasm appear black. The disorientation of the cells at this level is evident.

*Fig. 3.* Type 2 cells. Alkaline phosphatase technique: eosin. Alkaline phosphatase in the end-apparatus appears black.

*Fig. 4.* Type 2 cells. Dichromate/osmium/acetic: iron haematoxylin. The characteristically large end-apparatus and indented nucleus are visible.

*Fig. 5.* Type 2 cells. Acid haematein technique. Phospholipine in the cytoplasm, in the canalicules of the end-apparatus, and lining the walls of the tubule, appears black. The outer wall of the end-apparatus appears colourless.

*Fig. 6.* Type 2 cell.

*Figs. 7 and 8.* Transitional type 2/3 cells. Methenamine/silver: toluidine blue. The outer wall of the end-apparatus appears black; around this the striated body of the end-apparatus is faintly visible.

*Fig. 9.* Transitional type 2/3 cells.

*Fig. 10.* Type 3 cells. Methenamine/silver: toluidine blue. The outer wall of the end-apparatus appears black. The body of the end-apparatus is no longer present. In figure 10 a region below the end-apparatus which is coloured by toluidine blue appears grey.

*Fig. 11.* Type 3 cells. Formaldehyde/dichromate: acid fuchsin. The secretion within the end-apparatus appears as a cast of the end-apparatus shown in figure 10. There are no radial canalicules.

The scale on fig. 3 also applies to figs. 4–11.
lumen
end-apparatus (canalicules more or less evident)
reticular cytoplasm
[indented]
nucleus
basement membrane
muscle layer

phospholipine on cytoplasmic walls of tubule
phospholipine
cytoplasmic
basement membrane
outer wall of end-apparatus

lumen
nucleus of chitinogenic cell
end-apparatus
radial structure
inner wall of end-apparatus
basal fibrillation
basement membrane
protein leaving end-apparatus
phospholipid in end-apparatus
protein in lumen
structural protein in lumen
calcium oxalate
end-apparatus
glandular cells
haemocoel
distorted end-apparatus
middle region is highly hydrated, certainly more so than the cytoplasm of
type 2 cells, and the proneness of the middle region of the cells to collapse
during fixation in (hypertonic) solutions is further evidence.

Phospholipines are present, but in far smaller quantities than in type 2
cells. There is a faint general positive reaction, showing that there is diffuse
phospholipine in the cytoplasm. Particulate bodies occur among the basal
fibres in the form of granules and roddles of diameter not exceeding 0.5 μ; in
the remainder of the cytoplasm they are present as spherical bodies ranging
from 0.5 μ in diameter at the tips of the fibres up to 3 μ in diameter around the
end-apparatus. The radially disposed phospholipine, so clearly seen in type
2 end-apparatus (fig. 5), is absent.

Baker's sudan black technique shows that lipochondria are disposed
especially at the poles of the cell, where they occur in the form of granules or
bipartite spheres of diameter ranging from 0.5 μ to 1.2 μ. In the middle region,
similar organelles are present but in smaller numbers. Mitochondria are
located among the basal fibres and around the end-apparatus.

There is an intense alkaline phosphatase reaction at the apical end of the
cell, and this alone shows that some radial system remains within the reduced
end-apparatus.

Type 3 cells clearly produce far less secretion product than either types 2
or 4, the regions that secrete structural protein. Such secretion as is produced
is sparse and of a more granular nature than the other secretions of the gland.
The cells themselves are twice as tall as the majority of the protein-secreting
cells, and, while it is evident that these latter cells store no unfinished secre-
tion within their walls, but rather store the finished secretion within the
lumen, which is always full, the fact that the lumen in the region occupied by
type 3 cells is more often than not empty supports the view that the increase
in height of these cells occurs so that their secretion product may be stored

---

**Figs. 12–19**

**Fig. 12.** Type 3 cells. *Alkaline phosphatase technique: eosin.* The alkaline phosphatase in
the end-apparatus is coloured black.

**Fig. 13.** Early type 4 cells. *Dichromate/osmium/acetic: iron haematoxylin.* Overexposed
negative to show radial structure of the end-apparatus and its well-defined inner wall.

**Fig. 14.** Early type 4 cells. *Acid haematein technique.* The ovoid end-apparatus, containing
phospholipine which appears black, is visible.

**Fig. 15.** Early type 4 cells. *Alkaline phosphatase technique: eosin.* Alkaline phosphatase in
the end-apparatus appears black.

**Fig. 16.** Later type 4 cells. *Acid haematein technique.* In this later stage the end-apparatus
is becoming rounder than in figure 14; the radial canalicules remain.

**Fig. 17.** Early (left) and later (right) type 4 cells. *Formaldehyde/dichromate: acid fuchsin.*
General view of tubules of the gland. Protein, visible as threads in the end-apparatus, can
be seen issuing into the lumen.

**Fig. 18.** Late type 4 cells. *Bouin: haematoxylin/eosin.* The dark appearance of the cells is
due to cytoplasmic basophilia. The end-apparatus is small. A crystal of calcium oxalate can
be seen among the protein.

**Fig. 19.** Late type 4 cells. *Alkaline phosphatase technique: eosin.* The weak alkaline phosphatase reaction of the end-apparatus contrasts strongly with that of earlier figures.

The scale on fig. 15 also applies to figs. 12–16 and 19; that on fig. 17 also applies to fig. 18.
within the cell and released when required. Type 3 cells resemble in shape the
cells of the right gland where storage of the secretion within the cell is easily
demonstrable; they too are tall cells and the lumen of the gland is but sparsely
filled. The end-apparatus is long and tubular and without the well-defined
radial disposition of canalicules.

The presence of mitochondria and lipochondria in the base of the cell on
the one hand, and around the end-apparatus on the other, is evidence that
some synthetic activity is occurring there. The middle region is far less well
equipped with these organelles, and it is there that the contents of the
cytoplasm possess the power to react with the Nadi reagent after azide
treatment.

The canalicules of the end-apparatus appear to be concerned with protein
synthesis. The fact that they are reduced in the type 3 cells is additive evidence
that this is the region responsible for secreting the oxidase. Where protein is
formed (in the regions of the type 2 and type 4 cells), the secretion passes out
of the cell through canalicules of the end-apparatus, but in this region alone
this is not the case; fig. 11 shows the elaborated secretion, coloured with acid
fuchsin, within the end-apparatus in the form of a cone such as would fit
into the end-apparatus shown in fig. 10, and the absence of any secretion
paths radially disposed about this cone is clear. The situation is quite unlike
that shown in figs. 5 or 14.

The region occupied by the type 3 cells is smaller than the regions occupied
by either type 2 or type 4 cells.

Type 4 cells. These cells constitute the bulk of the gland. Where they lie
adjacent to type 3 cells they are tall, but there is a gradual decrease in height
along the tubule. By far the majority are squat cells of dimensions similar to
type 2 cells.

The most noticeable change that occurs is the reappearance of the thick-
walled end-apparatus; this occurs while the cells are still tall. The difference
between thin-walled end-apparatus of type 3 cells (fig. 12) is markedly different
from the thick-walled end-apparatus of early type 4 cells (fig. 13). There is a
well-marked inner edge, separating end-apparatus from the rest of the cyto-
plasm, and striations in the wall of the apparatus indicate that canalicules are
present. This change in the end-apparatus occurs within the space occupied
by a few cells.

That the secretion product of the cells passes out through canalicules in
these cells, exactly as was the case in type 2 cells, can be seen in fig. 17, in
which the protein has been coloured with acid fuchsin, and in fig. 14 the pre-
sence of phospholipines in the regions of the canalicules is clearly evident.
Still retaining the radial striations, the end-apparatus changes from being
ovoid to being more or less spherical in shape (cf. figs. 14, 16). Eventually
the body of the end-apparatus decreases in volume and persists as a faintly
acidophil U-shaped depression in the cell (fig. 18).

As the cells lose height the basal fibrillation is lost. Basiphilia increases,
and the short cells are extremely basiphil. Experiments which would demon-
strate the presence of ribonucleic acid have not been made but that the basiphilia is due to its presence is inferred for a number of reasons. It is not insignificant that, while all histochemical tests for proteins show that the protein in the lumen is far richer in amino-acid residues than the cytoplasm of the cells, the test for arginine proves to be the exception. Arginine is known to constitute a considerable fraction of the protein moiety of nucleoproteins, and, indeed, Serra (1946) devised his modification of Sakaguchi’s in vitro test for arginine, with the view to using this test so as to be able to infer from the results of its use the presence of nucleoproteins. The fact that the cytoplasm of a cell secreting an acidophil protein is strongly basophil is, in itself, an indication that basiphilia is due to the presence of proteins other than precursors of the secreted protein, and suggests that the acidic nature of the protein (in the known absence of mucoproteins) is due to the presence of nucleoproteins. Anderson’s (1950) work on Popillia adds some weight to the theory, as does the fact that the cytoplasm of the cells is strongly coloured by pyronine.

Phospholipines steadily decrease in quantity as the cells decrease in height. The tall cells have phospholipines distributed in the cytoplasm in much the same way as in the type 3 cells, but the general diffuse reaction is weaker and there is far more in the end-apparatus; there are fewer large granules in the cytoplasm and it is evident that these are often of a composite nature, the phospholipine surrounding a chromophobe droplet. In the short definitive cell there is no diffuse phospholipine, and it is restricted to minute granules and rodlets in the cytoplasm which appear to represent incompletely coloured mitochondria. One or two large granules between 1 μ and 2.5 μ in diameter are present in each cell near the end-apparatus. The end-apparatus persists in staining with acid haematein in the short cells, but the coloration is no more intense than the coloration of the protein secretion itself in the control sections, the lipoids from which have been removed with hot pyridine. All that can be said is that there is either no, or only very little, phospholipine in the end-apparatus at this stage—there is definitely less than in earlier stages.

While phospholipines decrease in quantity, lipochondria and diffuse lipoid increase, and there is more lipoid in all type 4 cells than there is in type 3. Both in and around the end-apparatus diffuse lipoid is present, and in the tall cells there is diffuse lipoid in the basal region, below the nucleus. As the cell shortens, so these two sudanophil areas come together, leaving no uncoloured middle region; the whole cytoplasm then contains diffuse lipoid. Lipochondria are fairly generally distributed: in the tall cells the diameter varies from 0.5 μ to 1.5 μ, but in the short cells the maximum diameter becomes as large as 6 μ. Mitochondria are distributed in a similar pattern. At first they are concentrated apically and basally, and later at random throughout the cytoplasm.

Alkaline phosphatase activity is again confined to the end-apparatus. At first the reaction is intense (fig. 15), but it falls off as the cell ceases to be functional (fig. 19).
The present interpretation of the anatomy of the left gland is not in serious disagreement with that of any previous author; it rather tends to confirm what has been said, but also shows how very much more complex the organ is than has been previously supposed. No previous author, for instance, has done more than briefly describe early and late stages of type 4 protein secretion.

The type of cell comprising the gland is not unusual in insects, and such cells were already excellently figured by Leydig in 1859 and described as occurring in dermal, anal, venom, salivary, and accessory sexual glands. That the ductules of the female accessory sexual gland were lined with an ‘inner wall’ (chitinous intima) was known to von Siebold (1837) whose statement that the ‘vesicular mass’ (the cells) regularly showed a radiate texture extending to the inner wall shows that he was even dimly aware of the presence of the ductules (longer in many cases than those in the collereral glands) leading from the end-apparatus to the lumen of the gland.

The presence of the chitinous intima throughout the gland indicates its ectodermal origin, and this is confirmed by the developmental studies of Nel (1929) and Quadri (1940). The female sexual accessory glands generally possess such a structure and are of ectodermal origin: Metcalfe (1932) reports these conditions in Coleoptera, Cappe de Baillon (1920) in Orthoptera, and Bordas (1895) in Hymenoptera, but there is no homology with the larger of the male accessory sexual glands (the utriculi of the cockroach (Ito, 1924)) which, like that of Coleoptera (Anderson, 1950) and Hymenoptera (Bishop, 1920) is mesodermal in origin, has no chitinous intima, and in which secretion occurs as the result of imperceptible dissolution of the apex of the cell rather than through an end-apparatus. The conglobate gland of the male cockroach, however, is of ectodermal origin (Ito, 1924).

Dufour (1841) observed that the 'substance corneo-coriacee' of the blattid ootheca was derived from the collereral gland (glande sébifique), but believed that the spermatheca (glande sébifique) served to provide a 'varnish' for it. The cellular nature of the gland in *Blatta orientalis* was first figured by Duchamp (1878), but his diagram is rudimentary. He described the darkening process of the ootheca and likened it to the change in colour of the cuticle of a freshly moulted insect. He observed that the ootheca consisted of crystals bound together with a brown substance that he believed to be chitin. Kadyi (1879) described in detail the physical side of ootheca production, and noticed that the left and right halves of the collereral gland were unlike one another.

Bordas (1909) made the first detailed study of the collereral gland (glandes arborescentes). He noted the unequal proportions of the left and right glands, and described them and their secretion products. While noting that the left gland showed great differences in structure at different stages in the secretory process, he did not qualify this observation with clear descriptions of the stages. Evidently he did not discover the cells of the posterior part of the gland and, as his figures show, believed that cells resembling either type 3 or
early type 4 cells were presecretory and cells resembling type 4 cells active or postsecretory. Although he described the end-apparatus as occurring in the right gland, he does not mention its occurrence in the left gland, a remarkable fact since he was aware of Leydig’s work, and because his figures (especially fig. 17) clearly show a dense apical region of the cell which undoubtedly represents the end-apparatus. This omission may have been due to his opinion that the secretory process in the left gland was analogous with that occurring in the intestine, and that the chitinogenic cells broke up, forming the secretion product, and were replaced by the, albeit rare, ‘peritoneal’ cells lying beyond the basement membrane. His claim to have found nuclear debris in the lumen of the gland as a result of the breaking up of the chitinogenic cells can be shown to be erroneous by the use of Feulgen’s technique. Although he was aware that the ‘épithélium externe’ (the glandular epithelium) decreased in size as secretion proceeded, he does not pause to consider the significance of this. There can be little doubt but that his interpretation of the nature of the secretory process was erroneous. His observation that the crystals, which he described as being of calcium carbonate, found in the lumen of the left gland, were formed there and not within the cells is correct. He described how these crystals were passed out of the gland and served to give bulk to the ootheca; they were bound together by the mucilaginous secretion of the same gland.

Ito’s (1924) description of the left gland was brief and dogmatic. His analysis of the tubules into muscular coat, basement membrane, epithelium consisting of two types of cells—glandular epithelial and chitinogenic and chitinous intima is accurate, but his description of the component cells is brief: there is no figure of the left gland of Blatta, but he figures what are evidently type 4 cells as occurring in Loboptera. The statement that the glandular cells are cylindrical or cubical indicates that he had seen some of the various types of cell but did not localize them. Although he but briefly mentioned the end-apparatus of Blattids, his figure of the end-apparatus in the mantid accessory gland shows a clear understanding of their anatomy. He states that globules of secretion, passing through the vesicles (end-apparatus) take on the aspect of striae; and several of his figures representing their condition in mantids closely resemble my photomicrographs of Periplaneta.

Voy (1949) described the glands under the name ‘appareil sébifique’. His figures and descriptions show clearly that those which apply to the gland which he terms ‘glande droite’ (tubes du premier type) in fact apply to the left gland of all other authors, and vice versa. To prevent confusion I use the accepted term to denote the appropriate morphological entity. Voy’s histological details are sparse, but his figures of late and early type 4 cells are clear and correct. There is no mention of type 2 cells, and I find that the posterior region of the gland of Blatta orientalis which he describes differs from my observations on Periplaneta (Brunet, 1951), but this may be a generic difference. Pryor’s work on the function of the glands was unknown to him, and he does not discuss their function.

Crystals of calcium oxalate occur, mixed with the protein, in the lumen of
the left gland in such large quantities as would suggest that they play some active part in either the formation of, or the function of, the ootheca.

Kadyi (1879) first analysed these crystals, which had been recorded by Duchamp in 1878, and Hallez (1909) confirmed the fact that they were calcium oxalate. Neither postulated the reason for their presence. Bordas (1908 and 1909) made the error of believing them to be crystals of calcium carbonate, and attributed a structural function to them; as concrete consists of an inert filler, gravel, and a binding medium, cement, so the ootheca consisted of calcium carbonate crystals and a mucilaginous substance (the protein) respectively. There is probably some truth in this explanation; on the other hand, the structural function of the crystals may be entirely incidental, their presence being due in the first instance to more fundamental reasons. Crystals of calcium oxalate are indeed used protectively in the plant kingdom to deter predation by molluscs; the presence of these crystals interferes with the smooth running of the radula. But the ootheca is brittle and would almost certainly be shattered by a preying animal, in which case the crystals could have little deterrent effect against this occurrence; and the insolubility of the crystals and their being enclosed in an insoluble tanned protein matrix could not lead to their causing unpalatability.

A saturated solution of calcium oxalate would have a pH value on the alkaline side of neutrality, and herein lies the most likely explanation of the function of the oxalate. Ewins (1910) found that the Comessatti test for adrenalin (a diphenolic substance) worked best in alkaline solution; adrenalin can be oxidized to a red compound by mercuric chloride, and Commessatti when he discovered this had used tap-water as solvent. Ewins showed that this reaction also occurred in the presence of many salts of strong bases with weak acids. Ewins pointed out that this was in agreement with the work of Euler and Bolin (1909) who had shown that several so-called laccases, promoting oxidation of phenolic compounds (e.g. the laccase of Sedum) were not in fact enzymes at all but calcium salts of mesoxalic, citric, and malic acids; and from this he concluded that the rapid oxidation of phenolic compounds is dependent on the presence of free hydroxyl ions in solution. Calcium oxalate is probably present with the protein so that when the diphenolic substances from the right gland diffuse into it the pH is at an optimum for enzymatic and/or spontaneous oxidation of the phenol. Ito (1924) was of the same opinion, but his conclusion, in the absence of any reason for arriving at it, was evidently speculative.

Oxalates are known to occur within the Malpighian tubules of insects and it has not been disproved that the presence of oxalate in the left gland is due to its acting as an auxiliary excretory organ.

The glandular cells of the colletorial glands share in common with integumental dermal glands the possession of an end-apparatus. In the vestibulum, surrounding the outlets of the colletorial glands is an organ composed of massed dermal glands of the integumental type (Brunet, 1951) and at first it seemed likely that there might be a material connexion between these and
the secretory cells of the colleterial glands, but this is definitely not so: both
left and right glands have a region of non-secretory cells which separate the
dermal gland organ from the secretory cells. Integumental dermal glands
have, in general, a conspicuous duct leading from the end-apparatus, some-
times reaching 200 μ in length, and round the duct lie an unknown number
of cells supposed to have secreted the duct. In the colleterial glands, where
the end-apparatus is present, the duct is usually vestigial, as, for example,
in type 2 cells of the left gland (it is, however, 7 μ long in type 2 cells of the
right gland), but this marked difference is bridged by many examples of
intermediates in other similar glands of ectodermal origin (Beier, 1933). The
glandular epithelial cells of the colleterial gland are evidently cells related to
dermal gland cells, which have undergone similar morphogenetic processes,
and are accompanied by but one other type of cell (the chitinogenic) which
probably represents the unmodified epidermal (hypodermal) cell, and not
the cells found on the duct of integumental dermal gland cells.

Exactly what function the end-apparatus performs is not completely clear.
It is undoubtedly a complicated structure in the cells which secrete structural
protein; it is no mere filter, for the properties of substances which have
passed through it into the lumen differ from those present in the cytoplasm;
this fact and the fact that alkaline phosphatase is concentrated there show
that the last stages of metabolism occur in the end-apparatus. The anatomy
of the end-apparatus in structural protein-secreting regions suggests that it
may have yet another function: the minute radial canalicules in it, through
which the secretion has to pass, as well as providing a large surface for enzyme
action, could act as spinnerets, the shearing forces acting on the micelles
passing through them and causing partial physical denaturation, as is known
to occur in secreted silk (Meyer, 1950; Ramsden, 1938). The absence of
canalculi in regions which do not secrete structural protein adds some
support to this hypothesis. In these regions, the oxidase-secreting region of
the left gland, and in the cells of the right gland, histochemical tests and
staining reactions show little or no difference between the secretion when it
is in the cytoplasm and when it is in the lumen, which suggests that less active
metabolism occurs in the end-apparatus of these regions than occurs in the
region secreting structural protein.

The occurrence of alkaline phosphatase in the body of the end-apparatus
is consistent with previous reports of its presence at secretory surfaces
(Bradfield, 1951); and though the present results are not detailed enough to
confirm Bradfield’s statement that the presence of alkaline phosphatase and
ribonucleic acid tend to be mutually exclusive in the cytoplasm, they are not
in disagreement: the main protein-secreting region, type 4 cells, has a strongly
basophil cytoplasm, indicative of the presence of ribonucleic acid, while the
end-apparatus is acidophil and contains all the cytoplasmic phosphatase.
There is apparently no correlation between the presence of alkaline phos-
phatase and phospholipines.
REFERENCES

— 1947. Ibid., 88, 115.
— 1949. Ibid., 90, 293.
— 1951. Personal communication.
— 1948. Ibid., 89, 229.
Giboud, A., and Bulliard, H., 1933. Protoplasma, 19, 381.
— 1946b. Ibid., 102, 51.
— 1940b. Ibid., 128, 393.