AN INSECT DESMOSOME

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INTRODUCTION

During the pupal stage of moths, a mass of connective tissue develops on the dorsal side of the abdominal nerve cord. The cells, which secrete the fibrous matrix, are originally close together, but as they secrete the matrix, they are pushed apart, with a corresponding decrease in their size. As the process proceeds, it was noticed that there is a gradual development of desmosomes where the membranes of two cells remain adjacent, and of hemidesmosomes where cell membranes adjoin the fibrous tissue. The desmosomes and hemidesmosomes are similar in structure and are of a type which, at present, appears to be confined to insects and other arthropods. Desmosomes are found between many different cells, for example, between the blood cells forming a capsule around foreign bodies in *Galleria mellonella*,[1] between glial cells in the nervous system of *G. mellonella*,[2] and between cells of the prothoracic gland of *Antheraea pernyi* (3), while hemidesmosomes are reported between epidermal cells and cuticle (1, 2, 6, 9) and epidermal or rectal cells and connective tissue (7, 9–13). The most elaborate desmosomes are found in the muscle attachments of insects, crustaceans, and arachnids, in which a muscle cell is attached by desmosomes to an epidermal cell, which, in turn, is attached by hemidesmosomes to the cuticle (1, 4, 6, 9, 14, 15).

The desmosomes consist of a plaque of dense material on the inner surface of the cell membrane with an associated group of microtubules. It has been suggested that the microtubules insert into

[1] A. R. Fisher. 1969. Personal communication.

[2] D. E. Ashhurst. 1969. Unpublished observation.

the dense material in a manner analogous to that of the tonofilaments in vertebrate desmosomes suggested by Kelly (8). It has been shown by tilting these desmosomes in the electron microscope that this is not their true relationship to the dense material.

METHODS

The abdominal nerve cords of pupae and adults of the wax-moth *Galleria mellonella* were fixed in 2.5% glutaraldehyde in 0.05 M cacodylate buffer pH 7.2 at 4°C for 1½–2 hr. They were then washed overnight in several changes of buffer at 4°C and post-fixed in 1% aqueous osmium tetroxide at room temperature. They were taken straight to 70% ethanol, then dehydrated in graded ethanols, passed through propylene oxide, and embedded in Araldite. The thin sections were stained in saturated uranyl acetate in 50% ethanol and lead citrate. The micrographs of tilted desmosomes were taken in an A.E.I. EM 801 electron microscope, using the tilting stage which allows tilt angles of ±20° in two axes at right angles.

RESULTS

The micrograph in Fig. 3 shows two desmosomes where the cells are adjacent and a small hemidesmosome where one cell adjoins fibrous tissue; the section has not been tilted. The dense material, which forms plaques beneath the cell membranes, has no apparent structure. A large number of microtubules form a halo around the desmosomes in both cells. The microtubules appear to have differing orientations, and some seem to be approaching the dense material. None is seen in true transverse section, but they appear as short tubules since they are seen throughout the thickness of the section. In Fig. 1, this group of desmosomes has been tilted through 10° in the direction shown by the arrow; no further information about the relationship of the microtubules and dense material is gained. When this same section is tilted through 20° in the opposite direction (Fig. 4), the microtubules associated with the hemidesmosome are now seen simply as dense rings, that is, in true transverse section. It is immediately apparent that a distinct gap is present between the tubules and the dense material. In Fig. 2, the section has been tilted by 20° on the other tilt axis. All the structures are now seen as in a very oblique section, and the microtubules clearly appear to penetrate the dense material. In the last micrograph of the series (Fig. 5), the section has been tilted by 20° in the opposite direction, that is, there is a difference in tilt angle of 40° between Figs. 2 and 5. Here, most of the microtubules around the two desmosomes are seen in true transverse section and, clearly, they are separated from the dense material by an appreciable gap. In other words, it appears that the microtubules might be parallel, rather than perpendicular, to the plaque of dense material. This is further substantiated by the longitudinal section of a desmosome (Fig. 6), in which the tubules lie parallel to the plaque.

It may be argued that thin strands of material can be seen in Fig. 5 which appear to link some of the microtubules to the dense plaque. These strands are at best irregular, but they might represent a modification of the cytoplasm near the plaques which could serve to maintain the relationship between the microtubules and dense material. The number of microtubules around desmosomes is variable, ranging from a few to many; hemidesmosomes always have fewer associated microtubules.

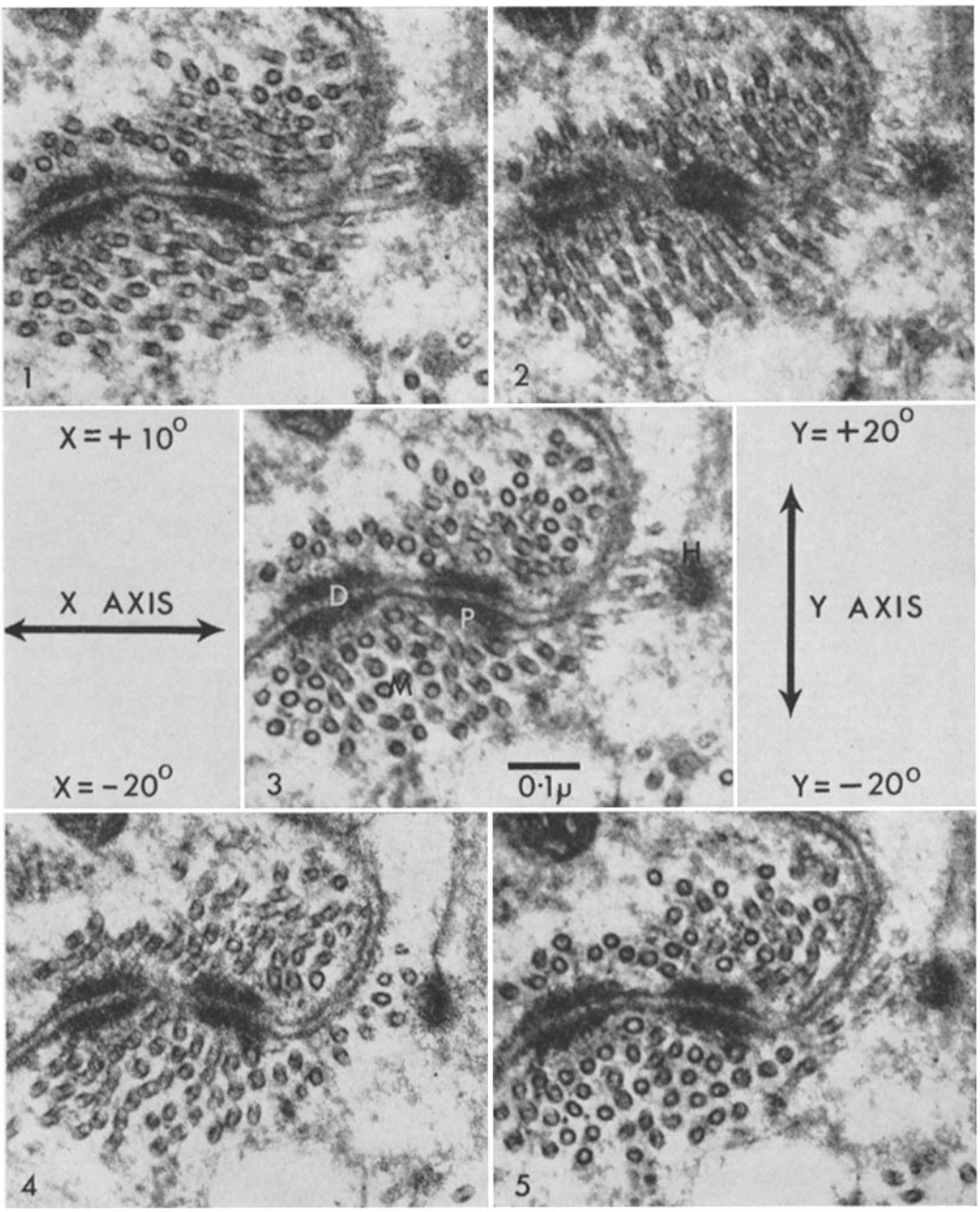
In longitudinal sections (Fig. 6), the dense material covers a greater length than in transverse sections. This suggests that the dense material forms an oval, rather than a circular, plaque on the inner surface of the cell membrane. There is no evidence to suggest the nature of the dense material or its intimate relationship with the membrane. In the longitudinal section (Fig. 6), there are indications of a dense strand forming loops or circular profiles within the plaque; each profile seems to originate at the cell membrane. No unequivocal *en face* views of the desmosome have so far been observed.

The two adjacent cell membranes in the desmosome are held about 170 A apart. There is no specialized structure in this space; a few tenuous strands of material may be seen joining the two membranes, but these also occur where there are no desmosomes.

It is, therefore, suggested that the structure of this desmosome is as shown in the drawing (Fig. 7). The bundles of microtubules overlie the dense plaque and protrude over the ends. At no time are the tubules in contact with the dense plaque, but the possibility that the cytoplasm in the whole area is specially modified cannot be excluded; indeed, this would seem highly likely.

DISCUSSION

This description of these insect desmosomes, or hemidesmosomes, differs from that of other authors



FIGURES 1–5 These five micrographs show two desmosomes (*D*) and one hemidesmosome (*H*) at different angles of tilt. Each consists of a plaque of dense material (*P*) and an associated group of microtubules (*M*). Fig. 3 shows the desmosomes in the untilted position. Fig. 1 is tilted by +10° on the X axis, and there is no tilt on the Y axis. Most of the microtubules appear to run towards the dense plaques. Fig. 4 is tilted by −20° on the X axis and, while the microtubules still appear to approach the dense material of the desmosomes, those near the hemidesmosome are seen in transverse section and there is a clear gap between the tubules and the plaque. A tilt of +20° on the Y axis, the X axis being untilted, produces the appearance seen in Fig. 2 where all the tubules appear to run towards the dense material and some to penetrate it. A reversal of this tilt on the Y-axis to −20° gives the situation in Fig. 5, where the majority of the microtubules around the desmosome are seen in transverse section and there is again a clear gap between the tubules and the plaque. × 100,000.

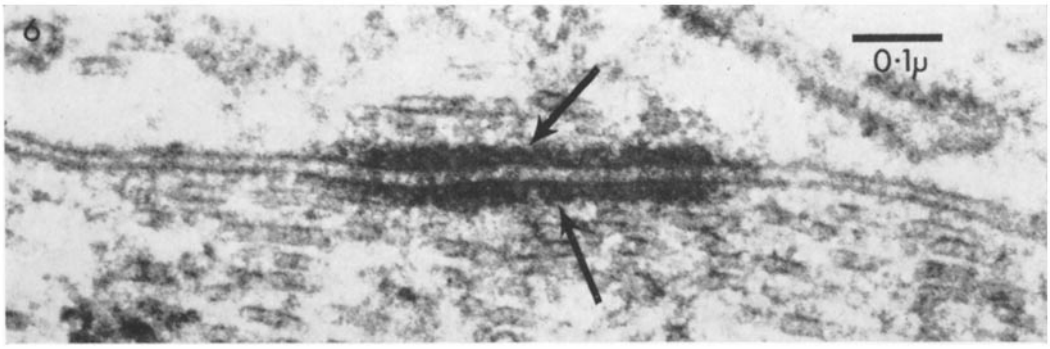


FIGURE 6 A desmosome seen in longitudinal section. There appear to be denser strands within the plaque which form loops or circular profiles originating at the cell membrane (arrows). The microtubules run parallel to the dense material. × 120,000.

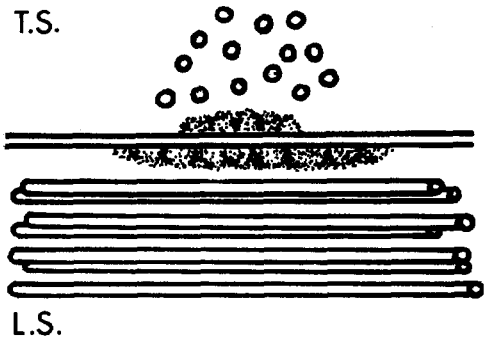


FIGURE 7 This is a diagrammatic representation of a desmosome in both transverse (*T.S.*) and longitudinal (*L.S.*) section.

in that it is suggested here that the microtubules lie parallel to the dense plaque on the inner surface of the cell membrane, whereas it has previously been considered that the microtubules are perpendicular to the dense material and insert into it, thus achieving an attachment to the cell membrane (2, 7, 9–12). None of the previous descriptions of these desmosomes has been based on micrographs of tilted specimens. The value of this facility is clearly illustrated here, since, as can be seen from the figures in this paper, it is extremely difficult to assess the true relationships from sections which are not truly transverse or longitudinal. The views of the desmosome in Figs. 5 and 6, however, are transverse and longitudinal, respectively, and these indicate the relationships described here. In areas of previously published micrographs in which the microtubules are cut in transverse section, it is interesting to note that a distinct gap occurs between the tubules and the layer of dense material (2, 6, 7, 9, 10, 12). It is, admittedly, more difficult to determine the precise relationship of the microtubules and dense plaque in some of the junctions where the cuticle forms "pegs" which insert into the epidermal cells (2, 6), and it would be inadvisable to extrapolate the observations of this study to include those junctions. It is possible, however, that more precise information might be obtained by tilting sections of these structures through the angles used in this study.

It may be argued that the results presented here could have been obtained by examining many desmosomes in differing orientations. This is, in part, correct, but a desmosome with the microtubules in exactly transverse section is a rare occurrence. In most instances, the desmosomes are cut obliquely, and in a series of such micrographs the relationship of the differing views is not precisely known. The advantage of tilting the specimen in the microscope is that one obtains different views of the same desmosome. Thus, one can observe the microtubules inserting into the dense material in one micrograph, while at a different angle of tilt the same microtubules are clearly separated from it; the difference in angle between the two views is accurately known. Such evidence is readily obtained and is more convincing than that from a series of micrographs of different desmosomes. In this instance, the information about the orientation of the microtubules obtained by tilting has enabled a more feasible assessment of their function to be made; previous authors (7) have suggested that they might be concerned in the transport of substances to the cell membrane, a view which the evidence here cannot support.

The bundle of microtubules has been considered analogous to the tonofilaments of vertebrate desmosomes and hemidesmosomes which run perpendicularly to the dense plaque and loop through or near it (8, 9, 10). More recent evidence suggests that, in many instances, the tonofilaments are parallel, or at shallow angles, to the dense plaque and run past without coming into direct contact with it.[3] Tonofilaments have not been reported in association with an insect desmosome; their role has presumably been taken over by the microtubules.

The next question concerns the function of the microtubules; it is suggested that their function is skeletal. The purpose of desmosomes and hemidesmosomes is to hold two cells, or a cell and matrix, together so that their spatial relationship is undisturbed by movements of the tissue. In order to achieve this, it is necessary to maintain the proximity of the two opposing cell membranes or one membrane and the matrix. A bundle of stiff microtubules lying parallel to a cell membrane above the desmosome would help to prevent any distortion of the adjacent cytoplasm which could lead to the breakdown of the desmosomal, or hemidesmosomal, contacts. A parallel bundle would, in this context, be more efficient than a bundle at right angles to the cell membrane.

The connective tissue, in which the desmosomes described here are found, is in constant motion since it serves for the insertion of muscles which produce a constant sideways movement of the abdominal nerve cord. Thus, a supporting role, such as has been suggested for microtubules in many other cells (5, 16, 17), would seem to be the most appropriate role for them in this insect desmosome.

[3] D. E. Kelly. 1969. Personal communication.

SUMMARY

A desmosome which at present appears to be peculiar to insects has been examined by tilting sections in the electron microscope. It has been shown in this way that the desmosome consists of a plaque of dense material on the inner surface of the cell membrane with a bundle of microtubules lying parallel to it in the adjacent cytoplasm.

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