The Spermatid Cell Membrane in Melanoplus differentialis.*

By L. E. Roth. (*From the Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Illinois.*)‡

A considerable interest in cell membranes has been shown in recent years, and approaches to the problem of molecular structure have been made from several different viewpoints. The physiological studies on different cells have been summarized by Danielli (2) who lists the fundamental properties of cell membranes as preferential permeability to lipoid soluble substances, existence of a low tension at the outer membrane surface, and high electrical resistance. Most studies of cell membranes, however, have been made on hemolyzed erythrocytes, since this is one of the few cells from which it has been possible to completely remove the cytoplasm. Parpart and Ballentine (6) have summarized numerous aspects of these studies and proposed a model of "molecular anatomy" of the red cell membrane.

The observations which are reported here were made during a study of the tail of the grasshopper sperm undertaken as one part of a general study of cilia and flagella. The results perhaps contribute to the picture of membrane structure proposed or implied in other studies, and also call attention to a variation in structure which has not been described previously.

Methods

Grasshoppers (*Melanoplus differentialis*) were decapitated and the single testis was excised. Individual follicles were freed of most of the surrounding connective tissue and immersed in a fixation fluid containing 1 per cent osmium tetroxide with 0.72 per cent sodium chloride and MacIlvaine's citric acid–phosphate buffer at 0.05 M and pH 7.4. After 3 hours fixation at room temperature, the follicles were washed in 4 per cent formalin wiith 0.72 per cent sodium chloride for 10 minutes. Dehydration was accomplished by 15 minute changes of 50 per cent, 75 per cent, 95 per cent, and absolute ethyl alcohol. After two 15 minute changes of methacrylate monomer, the polymerization was completed using a mixture of 60 per cent *n*-butyl methacrylate and 40 per cent ethyl methacrylate containing 1 per cent luperco CDB catalyst with ultraviolet light at 30°C. The sections were cut with a Porter-Blum microtome set at 0.025 μ and then mounted on carbon membranes from which the collodion

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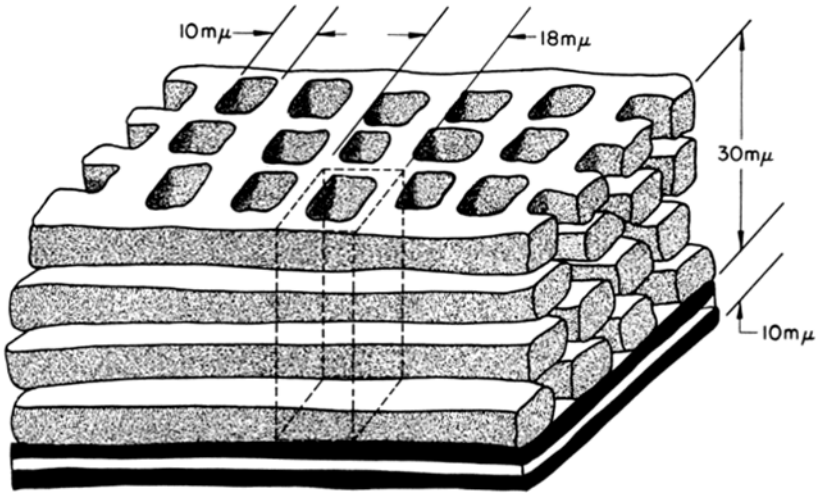
had been removed. They were examined in an RCA EMU-3A microscope with 100 kilovolt electrons.

OBSERVATIONS

The cell membrane of the spermatids in *Melanoplus* is unusually thick, 40 to 45 mμ. At higher magnifications (Fig. 1), the cell border is resolved into two principal regions. On the inner surface, there is a double-layered membrane 10 mμ thick, composed of two dark layers separated by a space of lesser density. Exterior to this membrane there is a thicker region measuring 25 to 35 mμ and exhibiting some regularity in its structural pattern (Figs. 1 and 2). In sections perpendicular to the membrane (Fig. 1, *L*), it is seen to consist of alternate dark and light bands radiating outward from the double-layered membrane. The center-to-center distance from dark band to dark band is 18 mμ. In tangential section, the pattern of the outer material seems usually to reflect square structural packing, but less regular arrangements are also found.

For clarity and the purposes of this report it is convenient to describe this outer material as composed of a repeating structural unit which is a rectangular solid, 18 mμ on each side and 25 to 35 mμ high. There is a thick peripheral region on each of the four long faces so that the inner, less dense portion measures about 10 to 12 mμ wide by 25 to 35 mμ long. This basic structure occasionally appears to be separated into four stacked plates, each about 5 mμ thick, arranged one on top of another parallel to the cell surface (Fig. 2, *LL*). This structural pattern is summarized in the drawing (Text-fig. 1).



TEXT-FIG. 1. A drawing of the spermatid surface showing an interpretation of the structura relationships. For purposes of description, the outer material is described as if composed of a basic geometric rectangular solid unit which is indicated by dotted lines.

This outer material on the cell membrane has not been observed in other species or in other stages of development in this species; it has been demonstrated to be present in the region of

the centriole (Fig. 1) and in the midpiece (Fig. 2) in the spermatid stage (the portion of the testicular follicle chosen for sectioning was in the proximal one-third where all the cysts contain spermatid stages.) While the structure may be present in other regions of the cell membrane, it has not yet been demonstrated.

DISCUSSION

The boundary between the cytoplasm and the extracellular environment varies in structure from one cell type to another. In metazoa, this boundary has been repeatedly observed as a single-layered membrane, but in other cells, more elaborate structures may be present. Piekarski and Giesbrecht (7) have demonstrated that the cell wall of *Bacillus megatherium* is composed of two dense layers separated by a slightly less dense layer, with an over-all thickness of 15 to 30 mμ. Chapman and Hillier (1) demonstrated portions of such a structure earlier in *Bacillus megatherium* and *B. cereus*. Examples are known also in the protozoa, *i.e.*, in *Euplotes patella* where two double-layered membranes are present except in the cilia, which have one double-layered membrane (8). In addition, Sedar and Porter (9) demonstrate more than one membrane in the pellicle of *Paramecium multimicronucleatum*. The most elaborate surface layers, of course, are found in the cuticular layers of insect eggs and eggs from certain parasites.

Grassé, Carasso, and Favard (3) have shown a structure somewhat similar to that described here in spermatids of *Helix pomatia*. However, the differences in intracellular position and geometric pattern would suggest differences in function. It is questionable whether the outer material observed here is a structural part of the cell membrane or a cuticle type covering. Cuticle layers are usually considerably thicker and not so intimately attached to the cell membrane. It has been observed in numerous tissues, *e.g.* see Palade (5), that when cell membranes are apposed in adjacent cells, a space is usually maintained between dense membranes. This space may in reality be the membrane with osmium precipitated on the surfaces, although the transition to a non-apposed, free surface membrane which is single-layered makes this seem unlikely. It is more probable that a thin layer of low density material also exists on the outer surface of each cell membrane.

The structure proposed in models of the cell membrane is based in a large part on studies of the membrane of the red blood cell. The concept described by Danielli (2) is that "of a continuous film of lipoid molecules, of which the two outermost layers are so oriented that the hydrated polar groups are in the oil/water interfaces, with a layer of protein molecules adsorbed on both of these surfaces." Hillier and Hoffman (4) have presented a theory of structure based on their observations of plaques averaging 20 mμ in diameter and fibrils 2 mμ in diameter, in the membranes of dried red cell ghosts. Since extraction with alcohol-ether-chloroform mixtures caused fragmentation of the membrane into free plaques and fibrils, they suggested a model that ". . . consists of plaques situated on the outside of a fibrous network joined together by ether extractable lipides." The model of "molecular anatomy" of the erythrocyte membrane proposed by Parpart and Ballentine (6) represents a concept which is based on considerable evidence and which allows explanations of many physiological events, *e.g.* hemolysis and

reversible hemolysis. In essence they propose, on both membrane surfaces, continuous protein layers except that aggregations of non-protein materials are present in areas about 5 mμ in diameter; the center-to-center distance of the circular areas is about 15 mμ (measured on their Fig. 2).

A correlation of the observations presented here with the above model systems has been attempted but seems to be satisfactory only to a limited extent. No structure has been demonstrated in the double-layered membrane; it is possible that the outer layer may represent a preferential adsorption onto a particular component of the double-layered membrane. If such adsorption took place on the continuous protein of the model of Parpart and Ballentine, an outer layer could conceivably be formed which would be similar in both pattern and dimensions to that described here. However, such conjecture does not argue for or against the proposed models, neither does it allow an understanding of the significance of the structure of this spermatid cell membrane.

SUMMARY

1. The cell membrane of the spermatid of the grasshopper *Melanoplus differentialis* is shown to be composed of a double-layered membrane 10 mμ thick. Closely applied to this is an additional outer covering 25 to 35 mμ thick which has a regular geometrical pattern.

2. Models of cell membrane structure are discussed briefly. It is suggested that the pattern results from preferential adsorption of material onto a particular component of the double-layered membrane.

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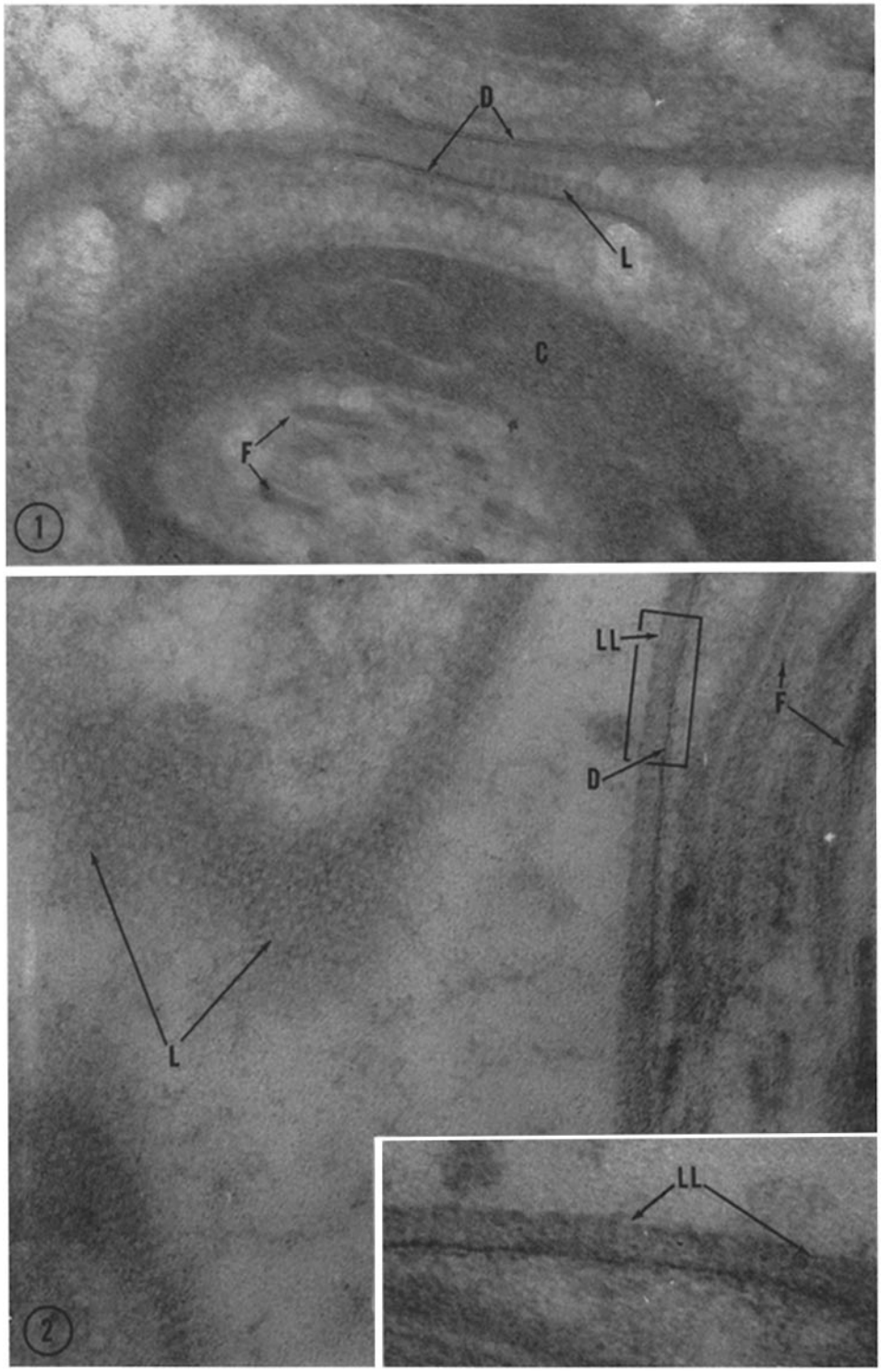
EXPLANATION OF PLATE 258

FIG. 1. Cross-sections through the centriole (*C*) in the spermatids of the grasshopper, *Melanoplus differentialis*. The cell membrane is composed of an inner, double-layered membrane (*D*) with additional outer material (*L*) which exhibits a banded appearance when sectioned in this plane. The cluster of tail filaments (*F*) is also shown. × 83,000.

FIG. 2. Sections through spermatids in the region of the mid-piece. In tangential section, the outer, thick material shows a regular pattern (*L*) which may be described as if composed of rectangular solids measuring 18 mμ on two sides and 25 to 35 mμ high. In perpendicular section, the double-layered membrane is seen (*D*) below the outer material which is composed of four layers (*LL*) parallel to the membrane. Tail filaments are also shown (F). × 116,000. Inset × 193,000.



(Roth: Spermatid cell membrane)