Development of the Cuticle in the Rotifer \textit{Asplanchna brightwelli}

Ann E. Brodie*
Department of Genetics, University of California, Berkeley
California 94720, U.S.A.

Received December 22, 1969

Summary. Ultrastructural studies of developing \textit{Asplanchna brightwelli} embryos support the following hypothetical scheme of cuticle formation. First the external hypodermal membrane invaginates, and deposition of a dense intracellular layer commences next to this membrane. Then the rough endoplasmic reticulum synthesizes fibrous protein which is transferred to the Golgi complex. Here polysaccharide is synthesized and added to the protein, and the resulting filamentous complex is enclosed in large irregularly shaped vesicles which bud off from the Golgi elements. Maturation of the filamentous material to condensed cuticle material occurs as the vesicles move to the invaginations. Each vesicle fuses with an invagination, thus forming a hypodermal bulb; then the cuticle material is discharged through the neck of the bulb to its extracellular location. After the bulbs are formed, new, smaller, spherical vesicles begin to bud off from the Golgi elements. They too contain the filamentous complex which is refined to condensed cuticle material as the vesicles near the bulb. These vesicles fuse with the hypodermal bulbs contributing the cuticle and membrane necessary for the growth of the hypodermis of the embryo and newborn animal. Ruthenium red staining has confirmed that the cuticle consists of glycoprotein.

Key-Words: Cuticle --- Rotifer --- Development --- Electron microscopy.

The integument of the rotifer \textit{Asplanchna brightwelli} is composed of a syncytial layer, the hypodermis, covered by an extracellular layer, the cuticle (Nachtwey, 1925). The integument covers the entire surface of the animal except the anterior head region, where the ciliated corona is located. The hypodermis and the corona differentiate from ectoderm, after all of the other organ primordia have moved into the interior (Tannreuther, 1919). The hypodermis develops from large ectodermal cells by forming a syncytium and stretching into a very thin layer. The cuticle is secreted by the hypodermis shortly before the embryos hatch.

The ultrastructure of the integument of the adult \textit{Asplanchna sieboldi} was described by Koehler (1965). According to J. Gilbert, the stock used for this study was actually \textit{Asplanchna brightwelli} (personal communication). This description of the integument refers to several unusual components, such as bulb-like invaginations, a dense lamellar band, extracellular acid polysaccharide-protein material (cuticle), and a five-layered external plasma membrane. Two questions were raised by Koehler’s investigation. The five-layered external hypo-

* I would like to express many thanks to Dr. C. W. Birky, who provided encouragement, suggestions, and laboratory facilities for this project. I would also like to thank Dr. D. Pitelka, Dr. J. Koehler, Dr. C. Hermanns, Dr. M. Bentfeld, and Mrs. E. Reid for their assistance in the preparation of this manuscript. This work was supported by National Institute of Health Predoctoral Traineeship No. GM-367.
dermal membrane (cuticular side) appeared to be continuous with the three-layered hypodermal bulb membrane and the three-layered internal (pseudocoel side) hypodermal membrane. The precise manner in which the transition was made from a five- to a three-layered membrane was unclear. Secondly, the function of the bulblike invaginations was uncertain. Koehler’s tentative hypothesis of the developmental aspects of cuticle formation suggested that the bulbs were involved in cuticle distribution.

The present study of the embryonic development of the cuticle will show that the bulblike invaginations are involved in the secretion of the cuticular material, as hypothesized by Koehler (1965); that the bulbs may be important for the addition of external membrane during the growth of the newborn animals; that the five-layered external membrane results from an intracellular layer becoming closely apposed to the plasma membrane; and, by means of ruthenium red staining, that the cuticle is a mucoprotein with a polysaccharide component and numerous acid residues.

Materials and Methods

All experiments were done with amictic Asplanchna brightwelli females, which reproduce by diploid parthenogenesis. An amictic clone (inbred clone 5B4S3 from Bloomington, Indiana) was maintained on Paramecium aurelia in infusions of Scottish grass (M43) as described by Birky (1964, 1968). To select females with the desired embryonic stages, bright field and phase contrast observations were made in vivo on animals immobilized in a micro-compression chamber (Biological Inst., Philadelphia, Penn.).

For proper fixation it was necessary that the animals be in M43 immediately prior to being placed into the fixative. Fixation was usually carried out in 1% osmium tetroxide in Palade’s (1952) veronal acetate buffer with 0.05% calcium chloride (pH 7.5) for 45 minutes at 4°C. Some animals were fixed in a mixture of 1% osmium tetroxide and 1.4% glutaraldehyde in 0.069 M Sorenson’s (Gomeri, 1955) phosphate buffer for 45 minutes (pH 7.4) at room temperature. Dehydration was carried out by a graded series of ethyl alcohols from 50% to absolute. In order to prevent shrinkage in Epon, the animals were punctured with a sharpened needle. Embedding was carried out in Epon 812 according to the procedure of Luft (1961), but without the use of propylene oxide. Silver or silver-grey sections were cut with a diamond knife on an MT-1 Porter-Blum ultramicrotome and “stained” at room temperature with Reynolds’ (1963) lead citrate and aqueous uranyl acetate. The grids were carbon coated before being observed in the RCA EMU 3E or 3F electron microscopes.

For ruthenium red staining (Luft, unpublished and 1964), animals were placed into a mixture of 1% osmium tetroxide and 645 ppm ruthenium red in Palade’s (1952) veronal acetate buffer (pH 7.5) for 45 minutes at 4°C. The normal procedure was followed for dehydrating and embedding. The sections were not stained with lead citrate or uranyl acetate.

Observations

The first evidence of change on the external hypodermal membrane occurs in mid to late stage 18 (Birky, 1967, unpublished stage series). At this stage, all of the organs have begun to differentiate and most of them can be recognized easily. The muscle cells have formed desmosome attachments to the hypodermis, but muscle fibers have not yet differentiated. The coronal cilia beat strongly and septate junctions between the hypodermis and corona are well developed. The hypodermis becomes syncytial at the end of stage 17 or beginning of stage 18 and starts to stretch out at mid to late stage 18.