

## Brief Notes

### The Fine Structure of the Desmosomes in Frog Mesothelium.\* BY KIYOSHI HAMA.† (From the Department of Anatomy, School of Medicine, University of Washington, Seattle.)§

#### INTRODUCTION

Several investigators (Kolossow, 1892; Linzbach, 1952; Muscatello, 1895) have described "intercellular bridges" between mesothelial cells. Others have denied the existence of a protoplasmic continuity between mesothelial cells but have postulated the individuality of each cell (Baron, 1941; Cunningham, 1926; Lewis, 1923). However, all agree that mesothelial cells are very closely associated with each other. Recent electron microscopic observations (Felix and Dalton, 1956; Odor, 1954, 1956) have provided little information about the relationship among adjacent mesothelial cells. In the present study the fine structure of mesothelial cells of the frog, *Rana pipiens*, was examined by means of electron microscopy, with special attention to intercellular relationships, including areas of attachment, here called "desmosomes." The term desmosome is used in a general sense according to that proposed by Wood (1959) and much as the term "desmochondria" is used by Dahlgren and Kepner (1908). It refers to any specialized attachment body of whatever shape which binds together apposing plasma membranes of cells. By this definition, the nodes of Bizzozero, terminal bars, and intercalated discs would all be special types of desmosomes.

#### Materials and Methods

The adult frog, *Rana pipiens*, was examined. Specimens were fixed *in situ* by injecting into the peritoneal cavity cold fixative consisting of equal parts of 5 per cent OsO<sub>4</sub> and *s*-collidine buffer, pH 7.4-7.5 (Bennett and Luft, 1959). After about 3 minutes, portions of the mesentery and the urinary bladder were removed, cut

into bits, and placed in fresh fixative for 2 hours at 0°C. The specimens were dehydrated, treated with 0.1 per cent phosphotungstic acid for 5 minutes in absolute alcohol, embedded in methacrylate, cut as usual, and examined in an RCA EMU-2C electron microscope equipped with a special stabilized lens power supply.

#### OBSERVATIONS

The cytoplasm of the mesothelial cells contains various structures such as mitochondria, endoplasmic reticulum, vesicles, and caveolae of diverse size, filaments about 7 m $\mu$  in diameter (Figs. 1 and 3).

Each mesothelial cell overlaps and makes intimate contact with adjacent cells (Fig. 1). Over most of this surface of contact a narrow interval about 7 to 10 m $\mu$  in width separates the apposed plasma membranes. This interval contains a homogeneous material similar to that found in basement membranes. Deeper in the mesothelium, the apposing cell membranes run tortuous courses, sometimes of such complexity that it is difficult to trace the membranes. No intercellular gap between adjacent mesothelial cells wider than that described above has been detected.

Specialized attachment structures or desmosomes similar to those described in epidermal tissue (Horstmann and Knoop, 1958; Odland, 1958; Porter, 1956; Selby, 1955) can be observed occupying a portion of the mutual contact surfaces of mesothelial cells (Fig. 1). These desmosomes consist of local thickenings of the apposing plasma membranes (attachment plaques of Odland), accumulations of dense cytoplasmic material on the inner aspect of the thickened area of the cell membranes, and tufts of fine filaments radiating from the dense material into the cytoplasm of each of the adjacent cells. The thickness of the cell membranes in the attachment plaques is about 12 m $\mu$ . Intervening between the thickened plasma membranes is material of lesser density, about 15 to 20 m $\mu$  in thickness. Within this material, half-way between apposed plasma membranes, a

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thin dense line about 5  $m\mu$  thick can be observed (Fig. 2). This structure seems to correspond to the "intercellular contact layer" described by Odland (1958). The intercellular material in the desmosome is much denser than that found elsewhere in regions of mesothelial cell contact.

The intracytoplasmic portion of the desmosome seems to include dense granules of about 7  $m\mu$  in diameter. Whether these represent a true granular material or cross-sections of filamentous structures is not known at present (Figs. 1, 2).

Numerous filaments are seen running up to the attachment plaques of both cells. These filaments seem to end in the dense plaque itself. They are about 7  $m\mu$  in diameter. Scattered filaments of the same sort, running serpentine courses, can be observed elsewhere in the cytoplasm of the mesothelial cells (Fig. 3).

A basement membrane about 30  $m\mu$  thick forms a continuous layer at the basal surface of the mesothelial cells. Material of the same density seems to extend into the intercellular 7 to 10  $m\mu$  space between adjoining mesothelial cells up to the region of the desmosome.

Large intercellular openings, stomata or stigmata of von Recklinghausen (Allen, 1936; Allen and Vogt, 1937; von Recklinghausen, 1865), which have been questioned by many authors (Baron, 1941; Hertzler, 1901; MacCallum, 1903; Muscatello, 1895; Notkin, 1924) were not detected in the present study.

#### DISCUSSION

Since the desmosomes observed in this material closely resemble, in fine structure, those of epidermis described by many authors (Horstmann and Knoop, 1958; Odland, 1958; Porter, 1956; Selby, 1955), they are considered to be corresponding structures, and to form the basis for the "intercellular bridges" (seen in the light microscope) (Kolossow, 1892; Linzbach, 1952; Muscatello, 1895). In fact, no protoplasmic continuity between adjoining cells has been seen anywhere. In the present study, a desmosome binding apposing plasma membranes was seen in every instance in which the section encountered an intercellular contact—although in some cases the structures were not as well developed as the one shown in Fig. 1. Though the evidence is limited, it may be that the desmosomes of frog mesothelial cells are arranged in belts completely encircling each cell. In that event they would correspond to the

terminal bars of columnar epithelium (Yamada, 1955).

The filaments which fray out from the "plaque" into the cytoplasm of each of the cells are like tonofilaments. These make up the fine structural equivalent of the tonofibrillae commonly encountered in the attachment areas of epidermal cells. They are probably keratinous in nature and serve to anchor the cytoplasm of the cell to the desmosome.

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## EXPLANATION OF PLATE 316

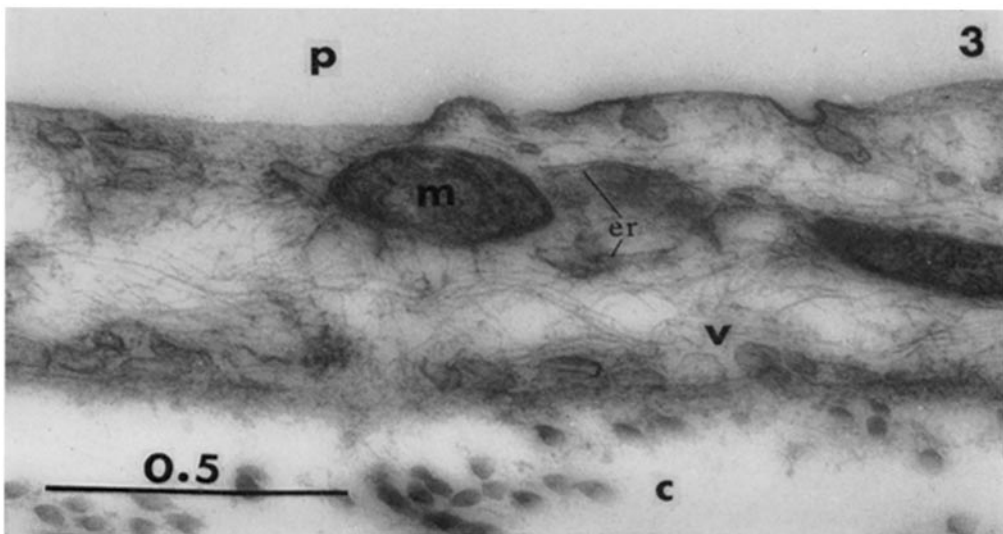
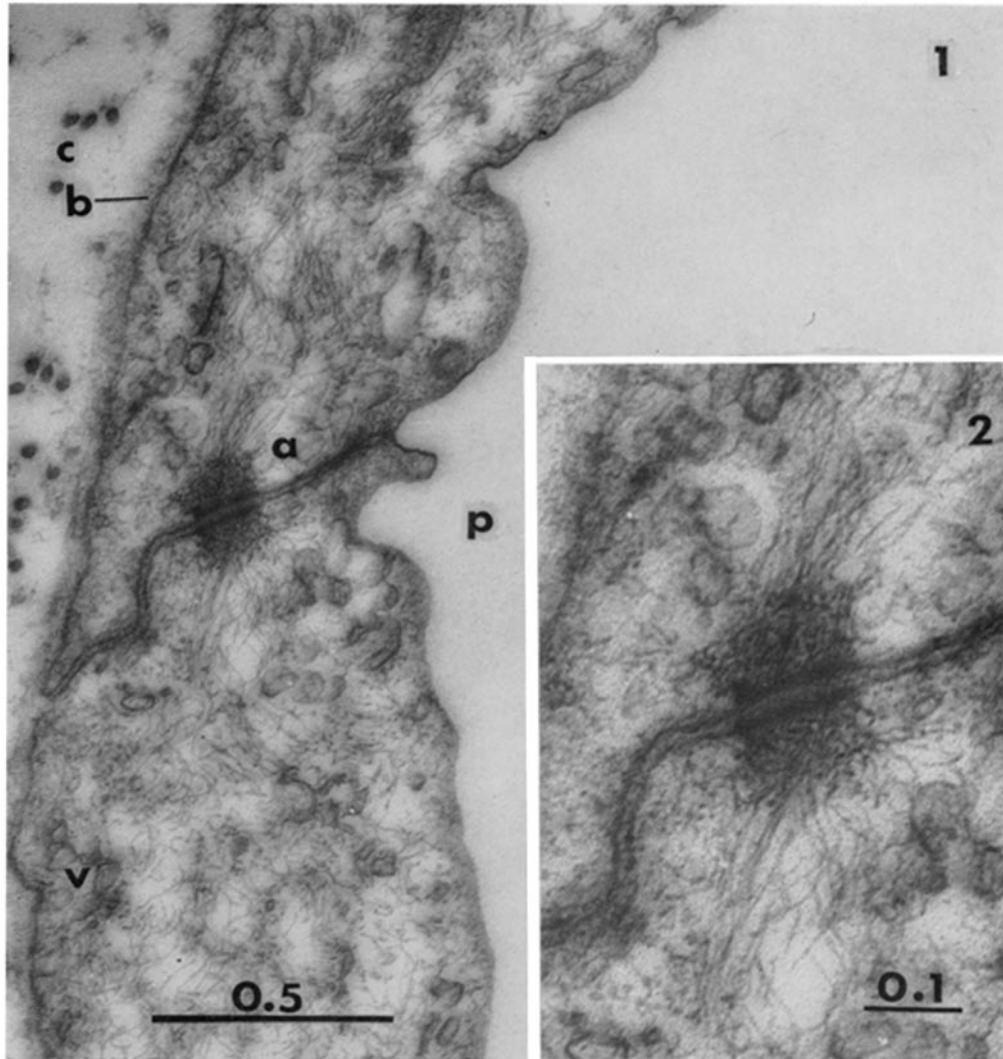
*Legend for all Figures*

<i>a</i>	desmosome	<i>m</i>	mitochondria
<i>b</i>	basement membrane	<i>p</i>	peritoneal cavity
<i>c</i>	subserous connective tissue	<i>v</i>	vesicle
<i>er</i>	endoplasmic reticulum		

FIG. 1. A high power electron micrograph showing two adjoining mesothelial cells from the serosa covering the urinary bladder of the frog. Near the middle portion of the cell contact surfaces, one can see a typical desmosome consisting of local thickenings of apposed plasma membranes, accumulations of dense cytoplasmic material on the inner aspect of the thickened areas of the cell membranes, and fine filaments radiating from the dense material into the cytoplasm.  $\times 66,000$ .

FIG. 2. Higher magnification of the desmosome in Fig. 1 showing details of desmosome fine structure. The intercellular material of the desmosome is very dense and displays a central lamina of accentuated density.  $\times 131,000$ .

FIG. 3. A high power electron micrograph of a part of a mesothelial cell showing fine tonofilaments running tortuous courses in the cytoplasm.  $\times 82,000$ .



(Hama: Mesothelial desmosomes)