Myofibroblasts and myoepithelial cells in the chicken Harderian gland

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Summary. An electron microscopic study of the myoepithelial cells in the chicken Harderian gland provides evidence that these cells can be transformed into myofibroblasts. After the application of a *Brucella ovis* suspension in sterile saline onto the eyeball, every 5 minutes for half an hour, myoepithelial cells gradually develop over a 90-minute period the characteristic features of myofibroblasts: bundles of intracytoplasmic microfilament; abundant rough endoplasmic reticulum; prominent Golgi complex; and surface membrane differentiations, that provide attachment to neighbouring epithelial cells. No typical desmosomes are observed. Besides, the intercellular space between epithelial cells and myofibroblasts increases and the basement membrane adjacent to myofibroblasts disappears. Hypoxia is hypothesized to be involved in the transformation of myoepithelial cells into myofibroblasts.

Key words: Myofibroblast, Myoepithelial cell, Ultrastructure, Harderian gland

Introduction

The myofibroblast is a cell sharing ultrastructural features of both fibroblasts — that is, rough endoplasmic reticulum and Golgi complex — and smooth muscle cells (namely, bundles of myofilaments with dense bodies) (Sánchez et al., 1978; Rüdolph et al., 1981).

Myofibroblasts were originally described in granulation tissue (Majno et al., 1971). Since then they have been observed, in normal conditions, in the synovial fluid (Ghadially and Imetha, 1971), capsule of the adrenal gland (Bressler, 1973), testes capsule (Boutet et al., 1974), testicular interstice (Bock et al.,

1972), duodenal villi (Guldner, 1972), pulmonary alveolar septa (Kapanci et al., 1990) and placenta (Feller et al., 1985). They are also present in a wide variety of pathological conditions, mainly chronic inflammation (Berlinger and Schachern, 1983) and neoplasia (Oda et al., 1988).

Myofibroblasts have contractile properties. They contain actin (Gabbiani et al., 1972) and non-muscle myosin suggesting that they utilize a non-muscle contractile system (Tomasek et al., 1987).

The origin of myofibroblasts is not a fully answered question. It has been clearly established that they derivate from fibroblasts (Díaz-Flores et al., 1979; Nakanishi et al., 1981; Oda et al., 1988; Bhawan and Majno, 1989), although it has also been proposed that they arise from endothelial cells and pericytes (Varela et al., 1978), myoepithelial cells (Díaz-Flores et al., 1979) smooth muscle cells (Abehsera, 1978), and macrophages (Bhawan and Majno, 1989).

The Harderian gland is the major paraocular gland of the domestic fowl. Its main functions are to clean and wet the cornea, and to lubricate the movement of the nictitanting membrane (Davis, 1929). The gland is longitudinally crossed by the main central duct, which is connected to the secreting portions by means of collector ducts (primary, secondary and tertiary ducts) (Maxwell et al., 1986). These ducts are lined by a columnar epithelium composed of glandular epithelial cells which contribute to the final secretion product (Wight et al., 1971). Myoepithelial cells are located immediately below the epithelium of the glandular ducts and at the base of the acini, inside the basal lamina (Rothwell et al., 1972).

The aim of our work is the ultrastructural study of the transformation of the myoepithelial cells of the ducts in the chicken Harderian gland into myofibroblasts.

Materials and methods

Seven-week-old White Leghorn chicken were used. They were killed by cervical dislocation.

In order to stimulate the secretory activity of the Harderian gland, a suspension of *Brucella ovis* reo-198 (non-CO_2 dependent) in sterile saline (SS) at a concentration of 10^9 microorganisms/ml, was droppped onto the eyeball, according to three experimental patterns.

The first test was carried out on 21 animals. The bacterial suspension (20 µl/eye) was administered only once to 15 chickens. The birds were killed at 30, 60 and 90 minutes after antigen administration. The remaining six animals were used as control and they received 20 µl SS/eye and were sacrificed at the above mentioned intervals.

The second test was performed on 12 animals. The antigen (20 µl/eye) was applied to 9 of them, three times to each animal at two-hour intervals. The 3 control birds received 20 µl SS/eye, again three times, and at the same intervals. The animals were killed at 30, 60 and 90 minutes after the last administration.

Twelve chickens were used for the third experiment. The bacterial suspension (10 µl/eye) was dropped onto the eyeball of 9 of the animals every 5 minutes for half an hour and they were killed at 30, 60 and 90 minutes after the last administration. SS was dropped onto the eyeball of the three control birds following the same pattern used for immunized animals, as described above.

Harderian glands were removed immediately after death and fixed in 2.5% glutaraldehyde for 90 minutes, washed in Millonig buffer and postfixed for 30 minutes in 1% osmium tetroxide. After final washing in buffer and dehydration in ethanol, fixed Harderian glands were cleared in propylene oxide and embedded in Epon-Araldite 1:1. Semithin (1 µm) and ultrathin (40-60 nm) sections were made and stained with toluidine blue, uranyl acetate and lead citrate respectively. Ultrathin sections were studied by transmission electron microscopy (Jeol T8).

Results

Both in the first and second experiments the results obtained show that the myoepithelial cells presented the common characteristics as described by Rothwell

et al. (1972) in the chicken Harderian gland.

However, the third experiment revealed that, although the cells of the animals used as control group did not show any modification, those of the immunized animals presented the series of alterations which are described below.

Animals killed at 30 minutes

A progressive loss of their usual location was detected in the myoepithelial cells. Furthermore, they
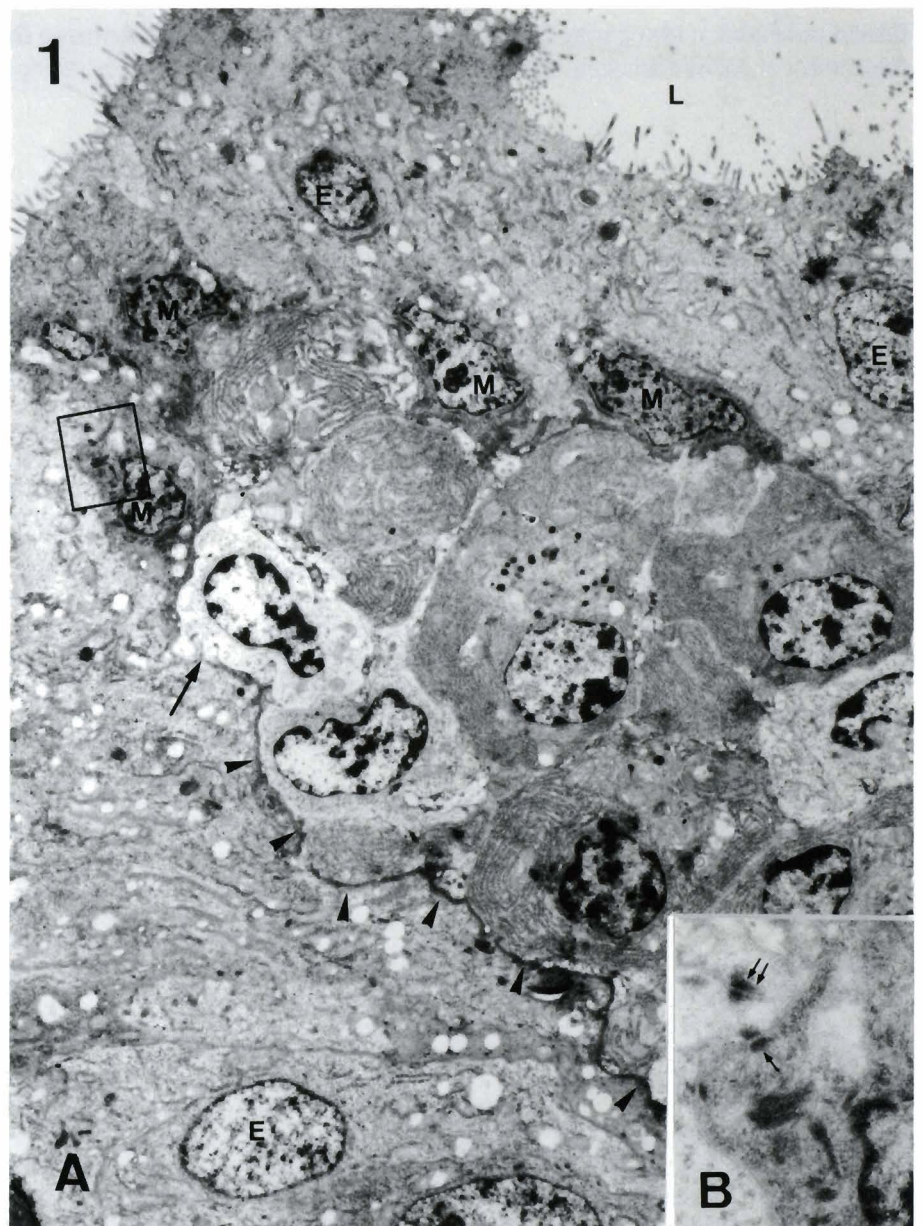

Fig. 1.A. Myoepithelial cells in transformation (M) with irregular nucleus. Note that the basal membrane of the epithelium (arrowheads) loses continuity (large arrow) in the neighbouring area. Epithelial cells (E). Lumen of the main duct of the gland (L). × 2,500. **B.** Detail of region in 1A. Desmosome attaching an epithelial and a myoepithelial cells (single arrow) and desmosome attaching two epithelial cells (double arrows). × 8,000. Animal killed 30 minutes after the last administration of *Brucella ovis.*
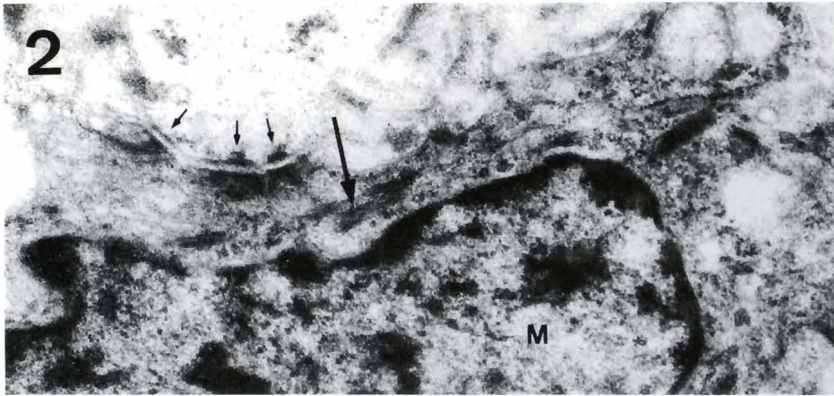
Fig. 2. Bundles of microfilaments (large arrow) in the cytoplasm of a myoepithelial cell in transformation (M). Desmosomes attach this cell to an epithelial cell (small arrow). Animal killed 30 minutes after last administration of *Brucella ovis.* × 8,000

lost their spindle shape and their long axis was no longer parallel to the basal membrane of the epithelium. At the same time the nucleus became irregular with numerous surface evaginations and invaginations (Fig. 1A).

In the areas close to the modified myoepithelial cells, the basal membrane became discontinuous (Fig. 1A). Modified myoepithelial cells and epithelial cells were attached to each other by desmosomes (Fig. 1B).

Microfilaments bundles of 5-7 nm were observed in the cytoplasm of the modified myoepithelial cells (Fig. 2).

Animals killed at 60 minutes

Ultrastructural modifications of myoepithelial cells became more apparent. The cellular body appeared located between the epithelial cells, its main axis being parallel to the long axis of these (Fig. 3A). The surface of the myoepithelial cells became irregular with small filiform cytoplasmic processes (Fig. 3A).

Modified myoepithelial cells showed few cytoplasmic organelles at this stage. The bundles of microfilaments, however, were more abundant than in the previous case (Fig. 3A).
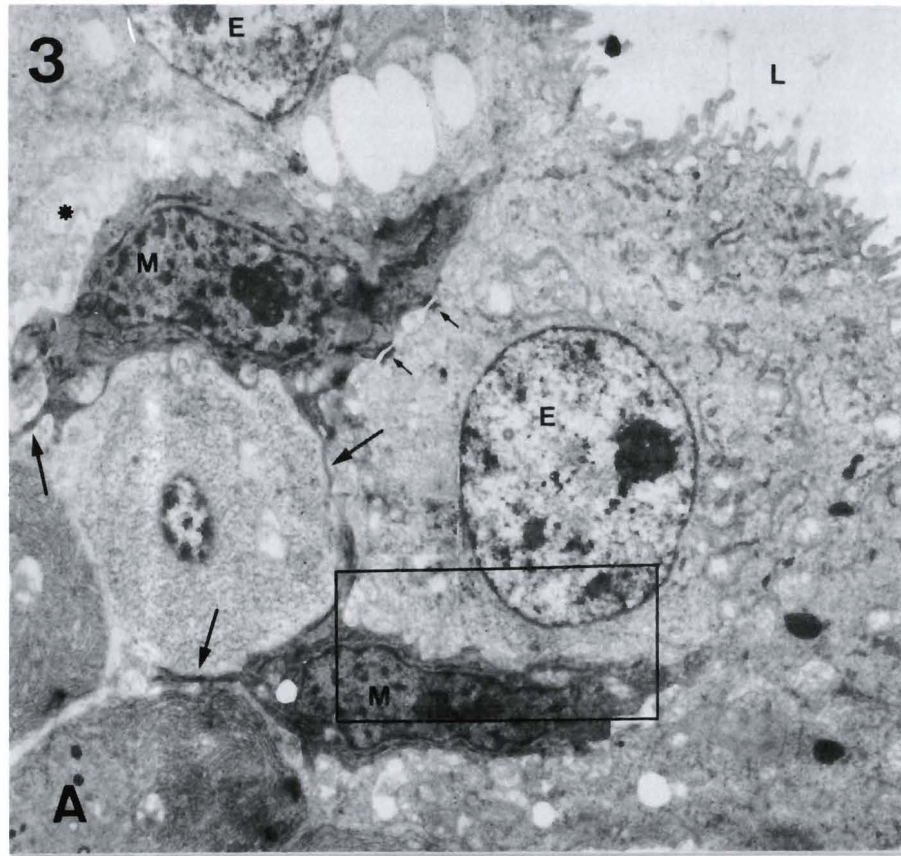
The basal membrane in the

Fig. 3.A. Myoepithelial cells in transformation (M) that present filiform cytoplasmic processes (large arrows) on their surface. Note the arrangement of these cells among epithelial cells (E) as well as the marked increase undergone by the intracellular space (asterisk) between both cell types. Desmosomes (small arrows). Lumen of the main duct of the gland (L) × 4,000.
B. Detail of region in 3A. Intercellular space between a myoepithelial cell in transformation (M) and an epithelial cell (E) occupied by microvilli that project from the surface of both cell types (arrowheads). Intercellular attachment showing a lower ultrastructural complexity than that of a desmosome (small arrow). × 11,500. Animal killed 60 minutes after last administration of *Brucella ovis.*
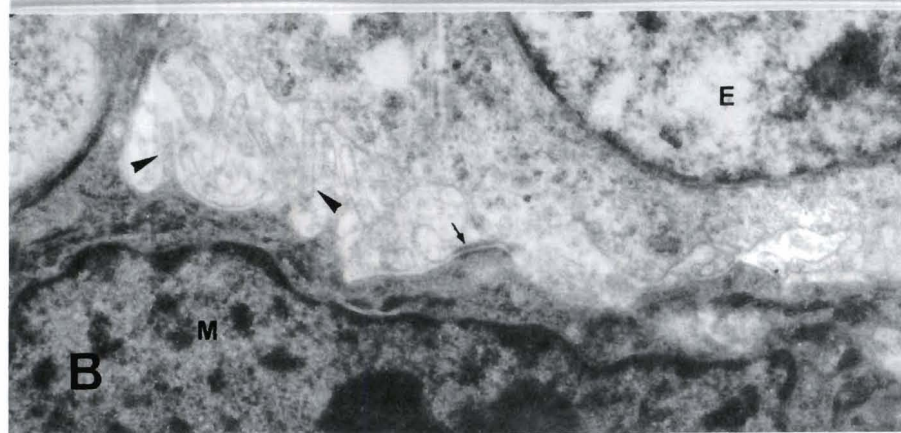
areas adjacent to the modified myoepithelial cells disappeared (Fig. 3A).

The intercellular space between modified myoepithelial cells and epithelial cells increased, presenting numerous microvilli extending from the surface of both epithelial and myoepithelial cells (Fig. 3B).

Desmosomes joining the epithelial and modified myoepithelial cells could be detected. However, the intercellular space was sometimes observed to be widened (Fig. 3A). This could indicate a modification in the desmosome. A new and less complex pattern of attachment was also observed (Fig. 3B).

Animals killed at 90 minutes

The myoepithelial cells, already transformed into myofibroblasts, showed the following ultrastructural cytoplasmic characteristics: prominent rough endoplasmic reticulum (RER) and Golgi complex, many mitochondria and abundance of microfilament bundles (Fig. 4). They occasionally presented vacuoles with lipids in their cytoplasm (Fig. 4).

The intercellular space between myofibroblasts and epithelial cells shared the characteristics described in the previous cases, except for a finely granulated material now present (Fig. 4A).

No desmosomes connecting myofibroblasts and epithelial cells were observed. Only junction structures with lower ultrastructure complexity than that of a desmosome could be detected (Figs. 4B, 4C).

Discussion

The findings support the myoepithelial origin of myofibroblasts. It has been observed that myoepithelial cells undergo a sequence of ultrastructural modifications in the course of their transformation into myofibroblasts which are basically the same as the ones describes by Ahmed (1978), Díaz Flores et al. (1979), Salamon and Hamori (1980) and Feller et al. (1985). Such modifications could be summarized as follows: loss of the basal membrane in areas adjacent to myofibroblasts, appearance of bundles of micro-filaments in the cytoplasm and marked development of RER and Golgi
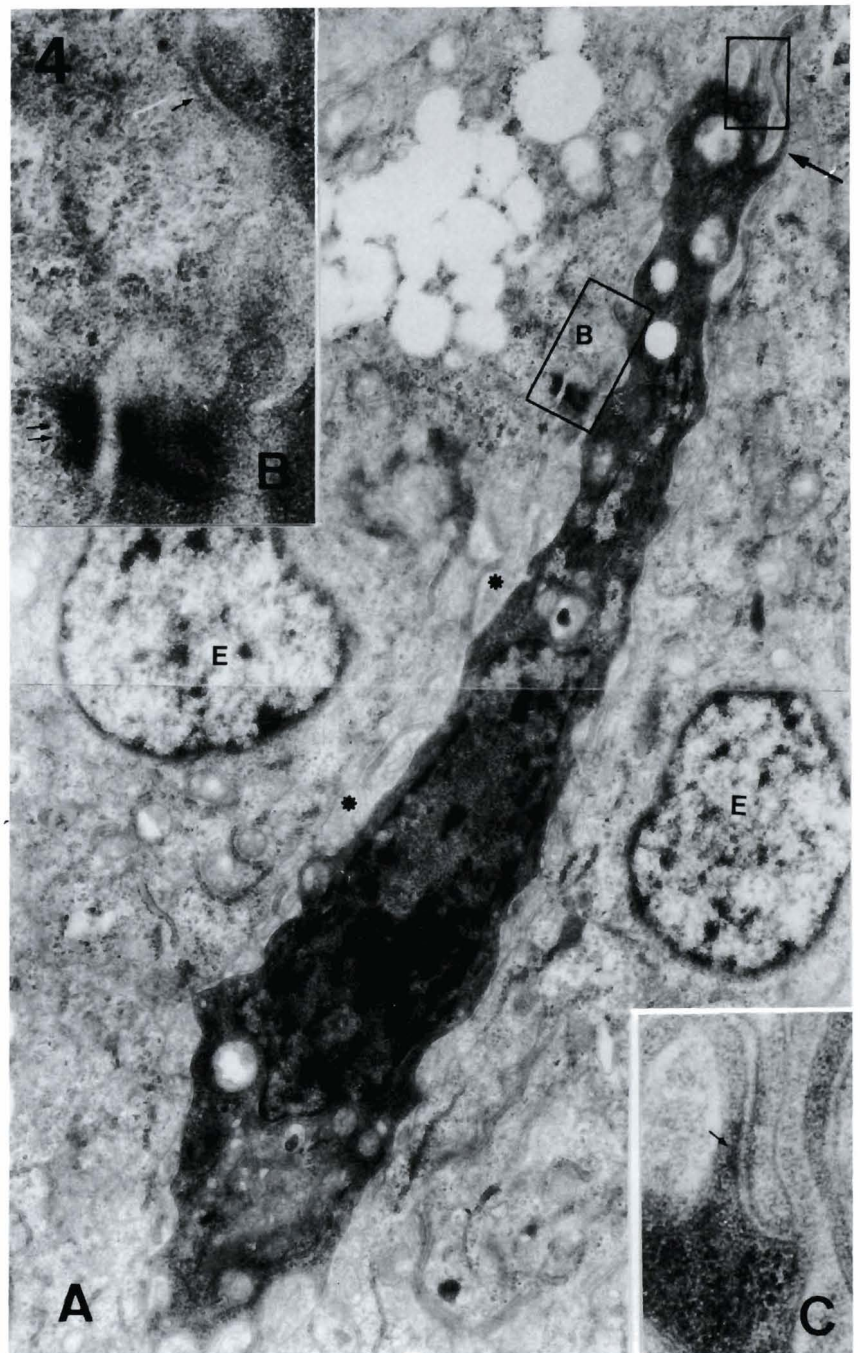

Fig. 4.A. Modified myoepithelial cell (myofibroblast) placed among epithelial cells (E), with a developed Golgi complex, numerous RER profiles and bundles of microfilaments. The intercellular space between both cell types is occupied by microvilli and a finely granulated material (asterisks). Filiform cytoplasmic projections (large arrow). × 8,000. **B.** Detail of region in 4A. Desmosome between epithelial cells (double arrow). Intercellular junction (single arrow) of lower ultrastructural complexity between a modified myoepithelial cell and an epithelial cell (E). × 30,000. **C.** Detail of region in 4A. Intercellular junction of lower ultrastructural complexity than that of a desmosome, joining the modified myoepithelial cell and the epithelial cell (small arrow). × 30,000. Animal killed 90 minutes after last administration of *Brucella ovis*.

complex; the intercellular space between myofibroblasts and epithelial cells was occupied by microvilli projecting from the surface of both cells and by a granular material that, according to Sánchez et al. (1978), could correspond to an intercellular material (characteristics of fibroblasts) synthesized by myofibroblasts.

The capacity of myofibroblasts to synthesize type I collagen (Oda et al., 1988) and type III collagen (Schuerch et al., 1981) has been evidenced. Nevertheless, we have not observed procollagen around the myofibroblasts, probably due to the fact that at 90 minutes, of the *Brucella ovis* suspension administration, the myoepithelial cell transformation into myofibroblasts has not finished. Thus, it can be assumed that they have not acquired collagen synthesizing property.

The transformation of myoepithelial cells into myofibroblasts occurred solely when *Brucella ovis* suspension was dropped onto the eyeball at intervals of 5 minutes for a half hour period, but not when the experimental conditions included applications at longer intervals. This suggests that the transformation may be a consequence of the functional hypoxia undergone by myoepithelial cells. The hypoxia could be due to the extended contraction which the cells were subjected to, in order to facilitate the excretion of the glandular secretion towards the conjunctiva. Therefore, the lubrication of the cornea would be increased as a response to the aggressive effect of the prolonged administration of *Brucella ovis*. Kapanci (1974) and Kischer et al. (1982) suggested that hypoxia was involved in the transformation of fibroblasts into myofibroblasts. We further suggest that this is also the factor that induced transformation of myoepithelial cell into myofibroblasts in the Harderian gland.

The fact that myoepithelial cells of ectodermic origin may transform into myofibroblasts suggests that myofibroblasts are not exclusively mesodermic in origin, as proposed by Ohtani and Sasano (1980). Since myofibroblasts may differentiate from myoepithelial cells and fibroblasts, their origin is ectodermic as well as mesodermic, that is to say, different embryonic layers might give origin to the same cellular type, as proposed by Díaz Flores et al. (1979). Mussini et al. (1977) have considered that myofibroblasts are bridge cells between different embryonic layers and therefore, embryonic layers should not be considered as separate non-related compartments (Díaz Flores et al., 1979).

Myofibroblasts are thought to be the cellular cause of wound and scar contraction (Squier, 1981; Rudolph et al., 1981) and of the retraction which characterizes many carcinomas (Schuerch et al., 1981) and are also responsible for the encapsulation of foreign bodies (Baker et al., 1981; Campbell and Ryan, 1983), and of the tissue distortion such as that which occurs in hepatic cirrhosis (Berlinger and Schachern, 1983). Under normal physiological conditions, myofibroblasts are found in organs or tissues that require certain structures that will provide temporal or isolated contractions such as in the case of the placenta when providing blood flow (Feller et al., 1985), in pulmonary alveolar septa as regulators of ventilation/perfusion ratio (Kapanci, 1974), in the human fetal spleen where they play a role in the excretion of lymphocytes from the follicles, and in pumping blood from the sinuses by their contraction (Fukuda, 1981).

All the above mentioned data together with the fact that myoepithelial cells are transformed into myofibroblasts in the Harderian gland under conditions of intense cell activity, suggest that the myofibroblast does not originate solely as a result of a pathological or degenerative process as proposed by Ohtani and Sasano (1980). On the contrary, it should be considered as a physiological condition of certain cellular types of ectodermic and mesodermic origin.

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