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An Electron Microscopic Study on the Interstitial Cells of the Gizzard in the Love-Bird (*Uroloncha domestica*)*

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Summary. A type of cells morphologically resembling fibroblasts or Schwann cells and identical with the interstitial cells as firstly described by CAJAL was studied electronmicroscopically.

Examinations of serial sections reveal that cell membranes of these cells make close appositions (nexus) to those of all surrounding smooth muscle cells. The surfaces of these cells are also provided with nerve endings of certain axons derived from plexus myentericus.

On the basis of these findings, the possible nature and function of interstitial cells are discussed.

A type of cells closely associated with muscle cells and nerve fiber bundles in the muscular layer in the avian gizzard was firstly described by CAJAL as interstitial cells. Many histologists using light microscopes regarded these cells as either neurons (CAJAL, 1893; ESVELD, 1928; BOEKE, 1949; MEYLING, 1953; HONJIN, 1956; DUPONT and SPRINZ, 1964) or modified connective tissue cells (DOGIEL, 1898; KUNTZ, 1923; JOHNSON, 1925; KNOCHE, 1952) or Schwann cells (lemnoblasten) (LAWRENTJEW, 1926; SCHABADASCH, 1934; STÖHR, jr. 1935; BOEKE, 1933). Also, in electron microscopic studies, RICHARDSON (1958) and ROGERS and BURNSTOCK (1966) regarded them as connective tissue cells and BRETTSCHNEIDER (1962) as Schwann cells.

SUZUKI (1963) compared their function to that of an electric transformer, calling them "transmittal cells". However, studies up to date, whether light or electron microscopic, have failed to describe in detail how an interstitial cell connects with muscular cells or nerve fibers. The present work, placing a special emphasis on this point, was designed to detect any structural characteristics suggestive of the nature and function of the interstitial cells of the gizzard in the love-bird by the use of an electron microscope.

Materials and Methods

Tissues from the gizzard of the love-bird (*Uroloncha domestica*) were fixed in one of the following:

1. 2% osmium tetroxide for 2 hours.
2. 3% glutaraldehyde for 2 hours followed by 2% osmium tetroxide for 1 hour.
3. 2% osmium tetroxide and 3% glutaraldehyde for 1 hour each followed by 1% osmium tetroxide for 30 minutes (KANASEKI *et al.*, unpublished paper).

All fixatives used were buffered at pH 7.2—7.4 with s-collidine (BENNETT and LUFT, 1959) and contained 4% sucrose. Tissue blocks were stained with 2% uranyl acetate for 15 minutes,

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then dehydrated by a graded series of alcohol and embedded in epoxy resin (LUFT, 1961). Thin sections were cut in series on a Porter-Blum microtome with glass knives, stained with lead hydroxide (MILLONIG, 1961), and examined by the use of a Hitachi, HU-11A, electron microscope.

As comparisons, tissues from gizzards of other birds such as chicken, zebra finch, sparrow, quail and canary and tissues from the intestine of the mouse were observed similarly.

Observations

The muscular coat of the gizzard of the love-bird was very thick, consisting of three layers of smooth muscle cells, inner oblique, middle circular and outer longitudinal. Electron-microscopically, there are no remarkable differences among smooth muscle cells comprising these three layers of muscle.

A type of cells closely associated with smooth muscle cells and nerve fiber bundles derived from plexus myentericus is frequently observed. These cells are considered to be interstitial cells as firstly described by CAJAL. They show angular shapes in cross sections, filling up the space among smooth muscle cells and having many slender processes (Fig. 1).

There is a basement membrane around an interstitial cell. The nucleus is centrally placed and generally rounded in shape, without any indentation. The cytoplasm surrounding nucleus is very scanty, containing profiles of granular endoplasmic reticulum, free ribosomes, vacuoles, mitochondria, glycogen granules, pinocytotic vesicles and small amount of filaments.

Certain axons in nerve fiber bundles make nerve endings on the surface of the interstitial cells. In such ending, the axon contains many clear vesicles measuring 300—600 Å in diameter.

The gap existing between an interstitial cell and a nerve ending measures 800 to 1,000 Å, containing fused basement membranes of both components. There are no special modifications in either density or thickness of axon membrane and interstitial cell membrane (Fig. 2). Thus, the fine structure of the nerve ending on the surface of the interstitial cell is similar to that on the smooth muscle cell heretofore reported.

A special emphasis should be laid on a striking feature of the interstitial cell in that its cell membrane has a close apposition (nexus) to smooth muscle cell membrane (Figs. 2, 3). This status is apparently similar to that of so called "nexus" firstly described by DEWEY and BARR (1962) in smooth muscle cells. The overall thickness of this close apposition is 120—150 Å, when measured in the materials fixed with osmium tetroxide alone. The thickness of dark line on either cytoplasmic side in this apposition is 25—30 Å. The center dark line measures about 20—30 Å (Fig. 4). Also, processes of an interstitial cell often make similar close appositions to those of another interstitial cell. Moreover, serial sections demonstrate that the cell membranes of the interstitial cells always make nexus to those of all surrounding smooth muscle cells (Figs. 5, 6).

Similar interstitial cells are also found in the gizzards of the chicken, zebra finch, sparrow, quail and canary and in the intestine of the mouse. Interstitial cells in these animals are were provided with close appositions to surrounding smooth muscle cells and with nerve endings on their surface.

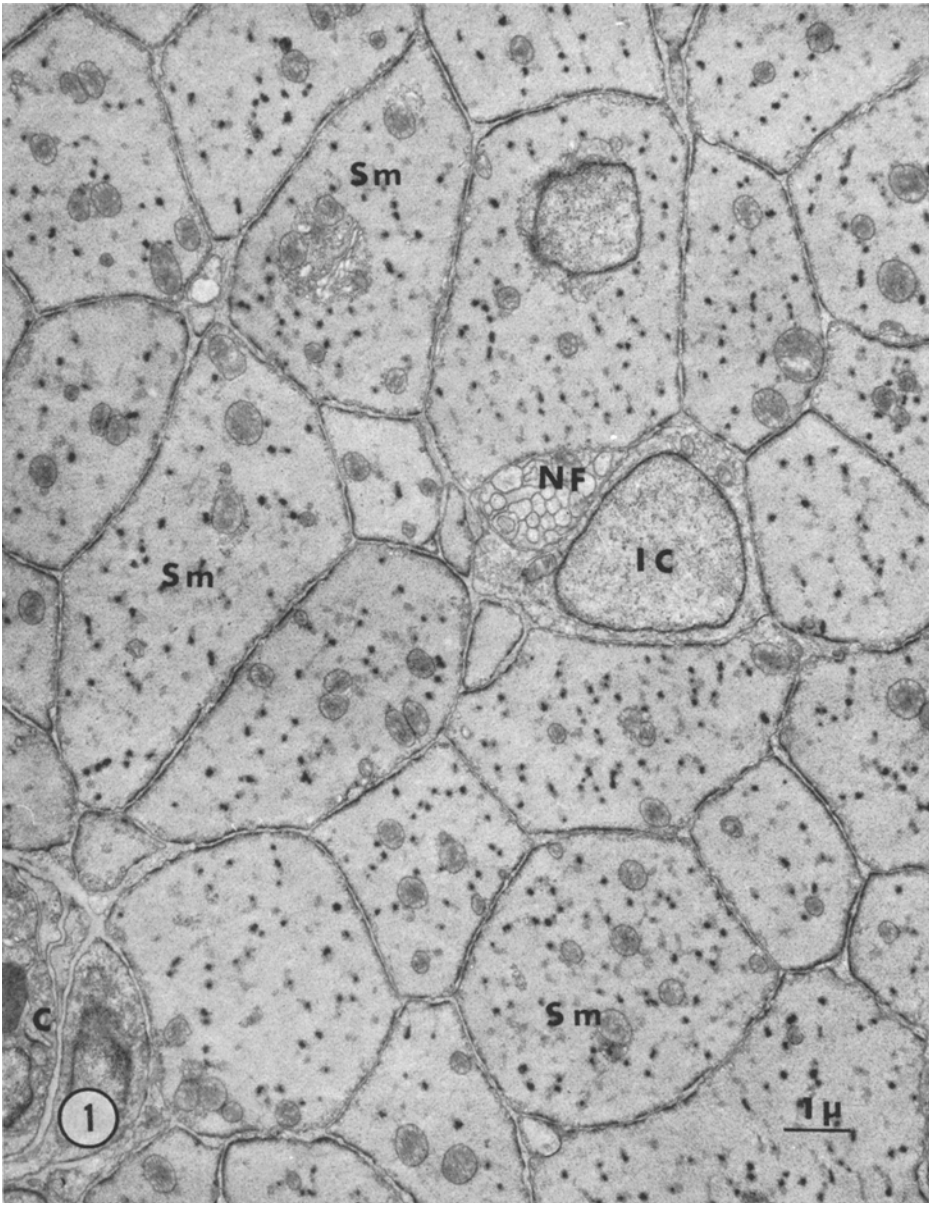


Fig. 1. An electron micrograph showing the interstitial cell (*IC*) closely associated with smooth muscle cells (*Sm*) and nerve fiber bundle (*NF*). The interstitial cell shows angular shape in cross sections, filling up the space among smooth muscle cells. *C* capillary. × 9,000

Discussion

Our observed interstitial cells are invested by a basement membrane and do not cover the outer margin of the axon as did Schwann cells. Moreover, the most

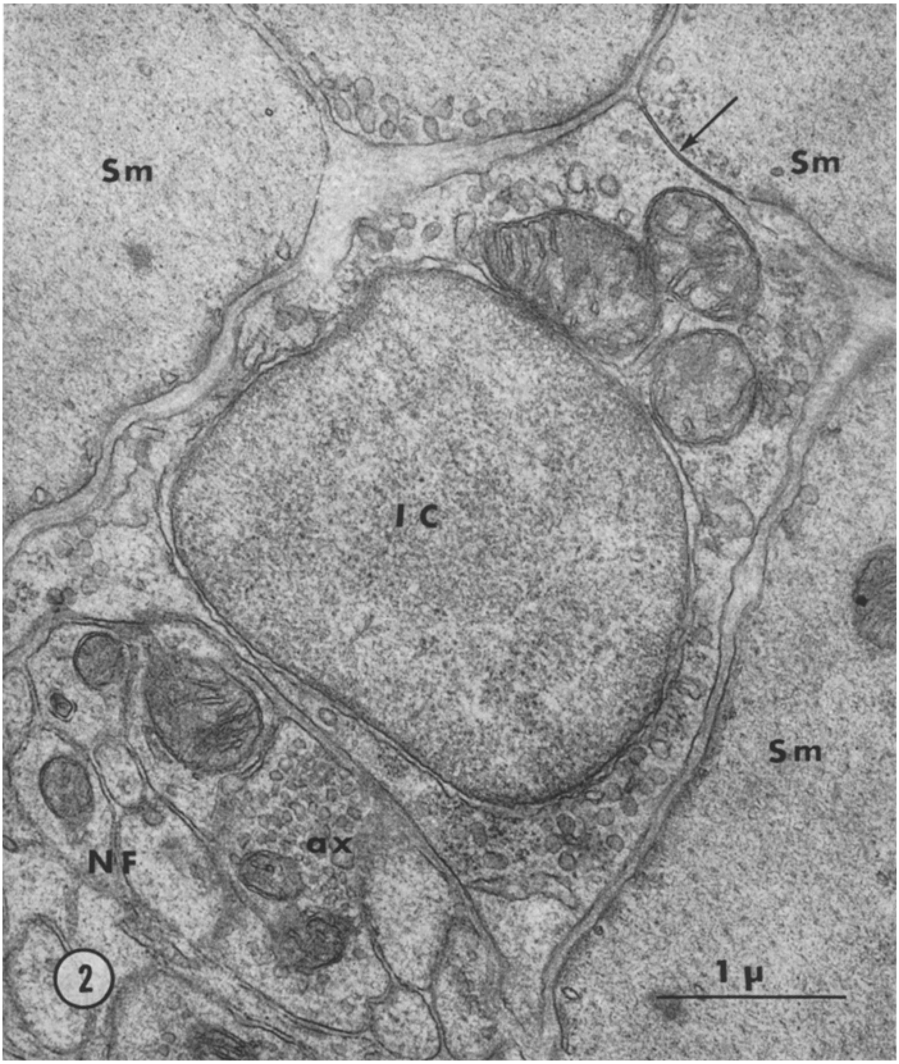
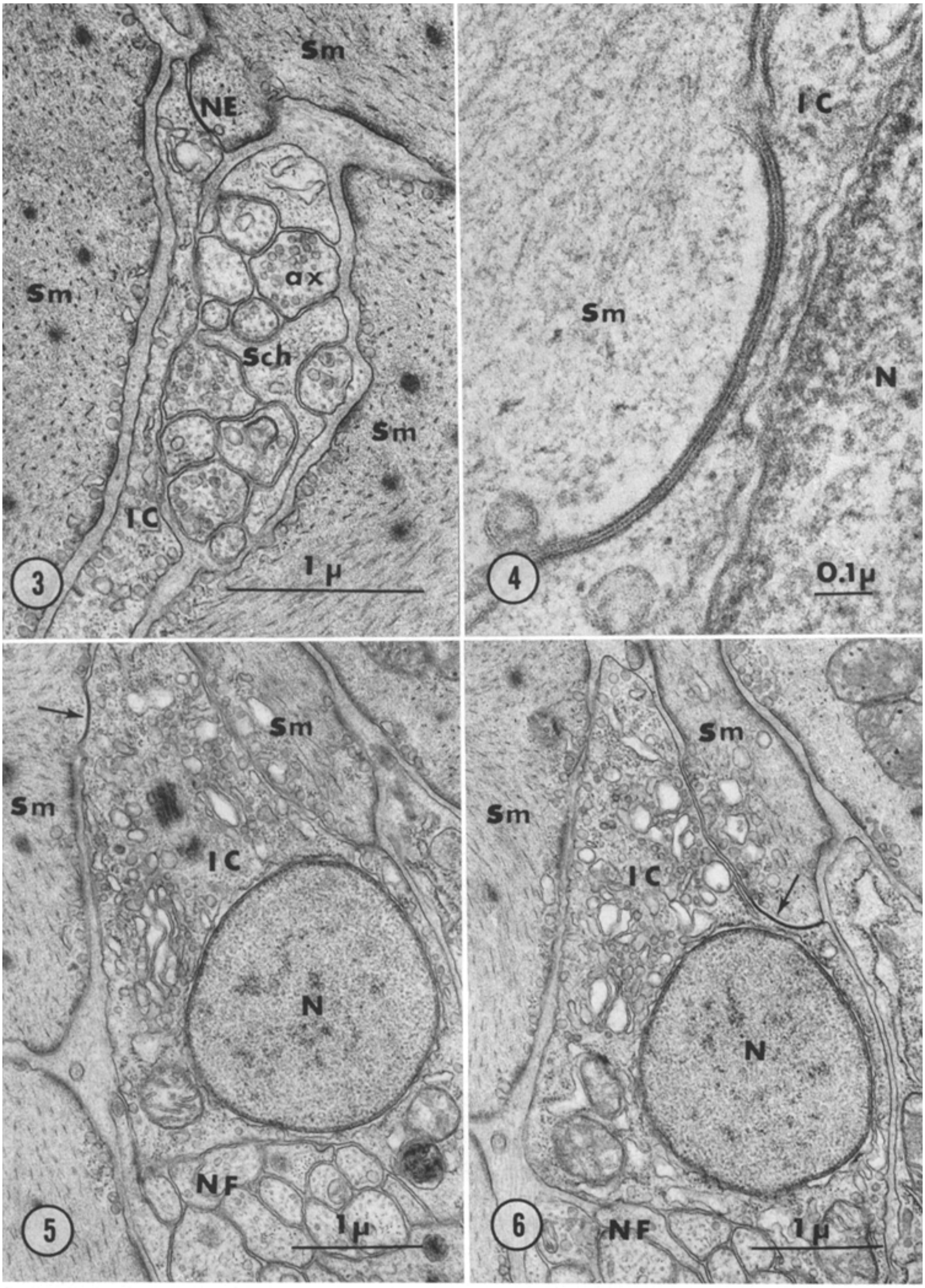


Fig. 2. An axon (*ax*) in nerve fiber bundle makes nerve ending on the surface of the interstitial cell (*IC*). Many clear vesicles measuring 300—600 Å in diameter are observed in the axon. The interstitial cell membrane has a nexus to smooth muslce cell (*Sm*) membrane. (see arrow). *NF* nerve fiber bundle. × 25,000

Fig. 3. An electron micrograph showing the nexus (*Ne*) between the process of interstitial cell (*IC*) and smooth muscle cell (*sm*). The process is close relation to nerve fiber bundle. *ax* axon, *Sch* Schwann cell. × 30,000

Fig. 4. An electron micrograph at high magnification showing the nexus between interstitial cell (*IC*) and smooth muscle cell (*Sm*). The nexus, as demonstrated here with OsO_4 fixation, appears as three dark lines separated by two light lines. *N* nucleus of interstitial cell. ×88,000



Figs. 3—6

Figs. 5 and 6. An electron micrograph showing the interstitial cell (*IC*) in serial sections. The interstitial cell membrane has nexus to smooth muscle cell (*Sm*) membranes in different parts (see arrows). *N* nucleus of an interstitial cell, *NF* nerve fiber bundle. × 20,000 characteristic feature of interstitial cells is the existence of parts of close apposition (nexus) of their cell membranes and surrounding smooth muscle cell membranes. Therefore, interstitial cells are neither Schwann cells nor fibroblasts, but rather possibly either nerve cells retaining their primitive character or cells derived from smooth muscle cells, the latter possibility being more likely because of their intimate relation to muscular cells. Such interpretation is in line with the finding that interstitial cells are stained violet selectively with methylene blue in contradistinction to Schwann cells or fibroblasts (MEYLING, 1953; HONJIN, 1956).

Further, the surface of the interstitial cell is provided with nerve endings formed by certain axons of nerve fiber bundles. These observations suggest that the interstitial cell may play a role in transmitting stimuli received from the axon to surrounding smooth muscle cells by an electrotonic response. Such functioning of the interstitial cell would be comparable to that of a connector or a socket in an electric circuit.

Incidentally, RICHARDSON (1962) described two types of nerve endings in the rat vas deferens; one type contains clear vesicles and the other cored vesicles. The former is generally believed to be cholinergic and the latter adrenergic. Therefore, our observed interstitial cells of love-bird gizzard seem to be supplied mainly with cholinergic nerves in the light of the abundance of clear vesicles in supplying axons.

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