

DEMONSTRATION OF "GAP JUNCTIONS" BETWEEN SMOOTH MUSCLE CELLS

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There has been considerable interest recently in the precise structure of the membranes of closely related smooth muscle cells in various systems. Most workers have claimed that there is true fusion of the outer lamellae of apposing unit membranes (1, 2), but one note (without illustration) published in this journal has reported that the areas of close apposition of membranes of smooth muscle cells do not represent fusion but rather "gap junctions" with a separation of the outer lamellae of apposing cell membranes of about 20 Å (5).

The electron micrograph presented in Fig. 1 clearly demonstrates the gap type of junction between the smooth muscle cells of the sheep ureter, although the intermembrane gap varies between 25 and 30 Å in this preparation. Gap junctions were also found between heart muscle cells in the guinea pig (Fig. 2), as has also been shown for the mouse heart (4).

The fixation procedure used in this work was as follows. Tissue blocks were fixed initially with phosphate-buffered 1% OsO₄ (pH 7.3) for 1 hr, immersed in 3% glutaraldehyde (in water) for 1 hr, and then postfixed with 2% OsO₄ (in water). The tissue was stained in block with uranyl acetate solution (T. Kanazeki, Y. Uehara, and M. Imaizumi, data in preparation). Specimens were dehydrated through graded concentrations of ethanol and embedded in Epon 812 according to the method of Luft (3).

With the same fixation procedure, it was possible to demonstrate gap junctions in only very short episodes of the order of 100 Å between muscle cells in the chicken gizzard (Fig. 3), in which Cobb and Bennett (1) have claimed that there is true fusion of adjacent muscle cell membranes with a wide variety of fixation procedures. Fig. 3 also demon-

strates variation in thickness of the fused layer, which suggests intermediate gradation between close fusion and near separation into "gap junctions".

Using improved fixation techniques and higher resolution electron microscopy, it may be possible to demonstrate gap junctions in most smooth muscles. However, it is still not known to what extent preparative procedures alter the appearance of smooth muscle cell membrane relationships or how much variation in structure there is between different systems of the same animal, or in the same system between different animals.

Received for publication 1 July 1969, and in revised form 15 September 1969.

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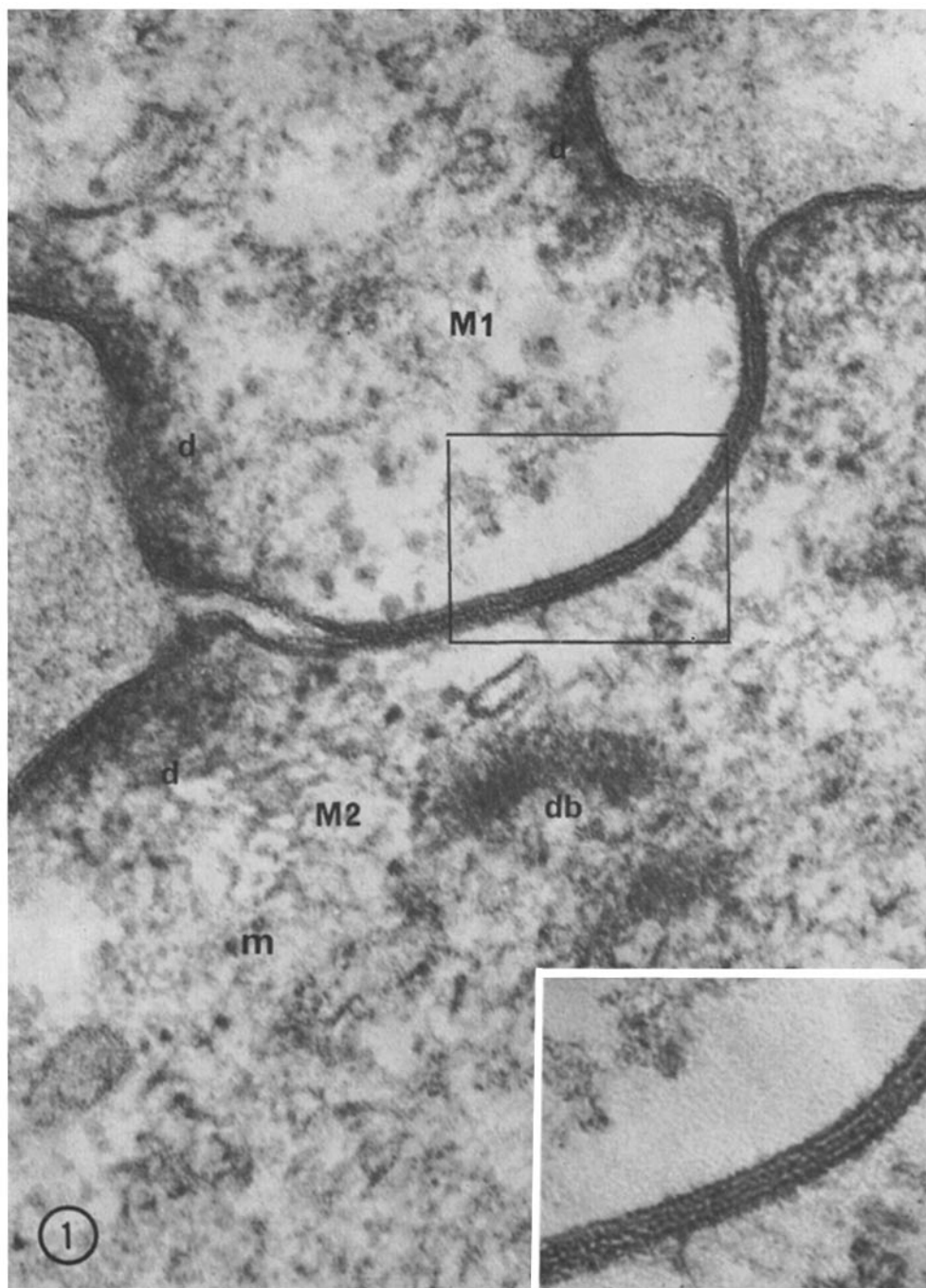


FIGURE 1 Electron micrograph of the sheep ureter showing close apposition between two muscle cells (*M1* and *M2*). $\times 150,000$. *d*, dense area; *db*, dense body; *m*, myofilament. The *insert*, an enlargement of the region outlined, clearly demonstrates the presence of a gap junction with separation of less than 30 Å between the outer lamellae of apposing cell membranes. $\times 250,000$.

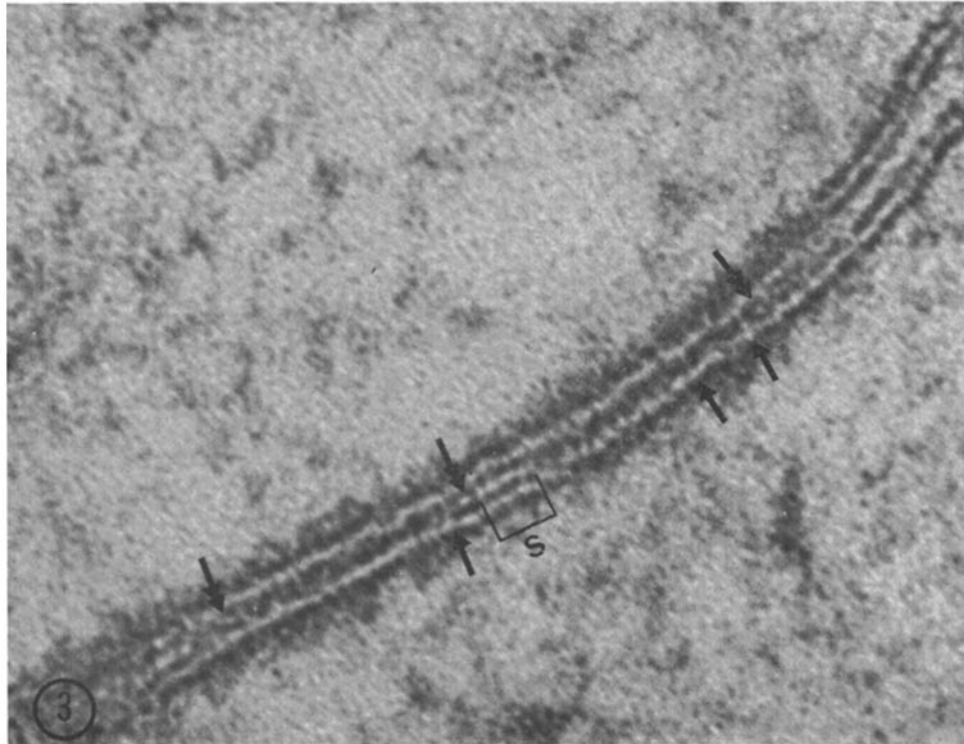
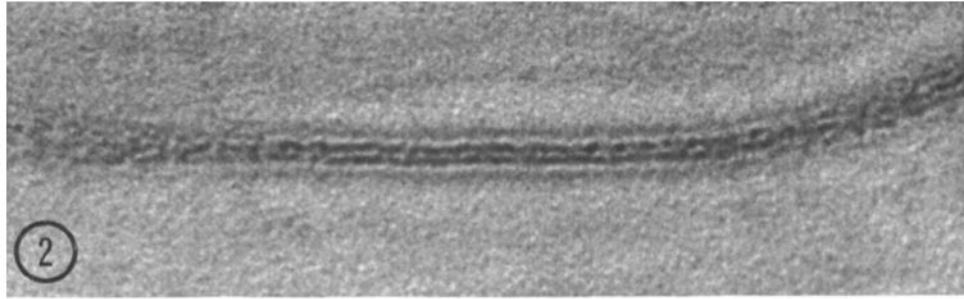


FIGURE 2 Electron micrograph of ventricular heart muscle of the guinea pig, showing that a region of membrane apposition at the intercalated disc is also represented by a gap junction. $\times 300,000$.

FIGURE 3 High power electron micrograph of a junction between two muscle cells in the chick gizzard. Double arrow shows a gap of 15–20 Å in width between the outer lamellae of apposing membranes. In the area indicated by *S*, the junction appears as a seven-layered gap junction with a middle layer about 25 Å wide. Note the septal structures (single arrow) across the middle layer, suggesting the occurrence of punctate fusion of the outer leaflets. $\times 600,000$



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Title:

Demonstration of "gap junctions" between smooth muscle cells.

Date:

1970-01

Citation:

Uehara, Y. & Burnstock, G. (1970). Demonstration of "gap junctions" between smooth muscle cells.. J Cell Biol, 44 (1), pp.215-217. <https://doi.org/10.1083/jcb.44.1.215>.

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