ment. If the animals are treated with far red light first, further treatment of red light will not be able to bring the outer segment growth back to that equivalent to control. On the other hand, treatment of red light first followed by far red light will tend to lower the stimulating effect of red light to a very low level, so that the outer segment growth is about the same as that of control. Further additional treatment of red light (e.g. red - far red - red) will give no further promoting effect. The red and far red effect also reflect on the dopamine uptake of the retinas, and it is possible that they act through the neurotransmitter system.

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On the formation of the myotomes in avian embryos. An experimental and scanning electron microscope study[1]

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Summary. The formation of the myotomes of chick embryos was studied by using experimental and scanning electron microscope techniques. With the aid of the quail-chick marker system, it was demonstrated that the dermatome gives rise to the myotome plate. SEM studies support the conclusion that the myotome cells originate only from the edges of the dermatome.

Since the work of Remak[2], it is well known that during early development of the chick embryo each primary spherical somite divides into a ventral mesenchymal part (sclerotome) and an epithelial upper wall (dermatomyotome)[3]. The dorsal lamella then becomes a 2-layered plate consisting of an outer layer (dermatome) and an inner layer (myotome) which is considered to be the source of voluntary musculature[4,5]. The origin of the myotome layer has been a controversial issue. According to earlier investigations[4,6-8], the myotome is mainly formed by proliferation from the dorso-medial edge of the dorsal lamella and possibly completed by cells arising from the ventrolateral edge[6,9,10]. Langman and Nelson[11], however, concluded from their labelling experiments with tritiated thymidine that the cells of the myotome directly originate throughout the surface of the overlying dermatome. On the basis of ultrastructural studies, Mestres and Hinrichsen[12] believe that cells of the sclerotome reaggregate on the apical surface of the dermatome layer and constitute the myotome.

The aim of the present paper is to examine the validity of the various theories on myotome formation by using the quail-chick marker system and the scanning electron microscope. Since the interphase nuclei of Japanese quail cells exhibit a large mass of nucleolus-associated heterochromatic DNA which does not exist in chick cell nuclei, it is possible to identify individual cells in Feulgen-stained sections after experimental intermixture of quail and chick cells[13-15].

White Leghorn chick and Japanese quail *(Coturnix coturnix japonica)* embryos were used for the grafting experiments. From the somites 13-16 of chick embryos at stage 14, according to the criteria of Hamburger and Hamilton[16], the dorsal walls together with the overlying ectoderm were removed unilaterally and corresponding parts of somites and adjacent ectoderm isolated from quail embryos at the same stage were grafted into the defect (figure 1). After subsequent incubation (1 or 2 days), the host embryos were fixed in Serra's fluid, dehydrated with graded propanol solutions and embedded in paraplast. The 7 µm serial sections were treated according to the Feulgen and Rossenbeck technique[17] and post-stained with light-green. For the scanning electron microscope studies, chick embryos at stages 13-17 were fixed in a buffered (pH 7.4) paraformaldehyde and glutaraldehyde fixative[18], washed in Hank's solution and postfixed in osmium tetroxide[19]. After dehydrating with graded propanol solutions, specimens were dried by the critical-point method[20,21] and subsequently sputtered with gold. Pictures were produced utilizing a scanning electron microscope JSM 35.

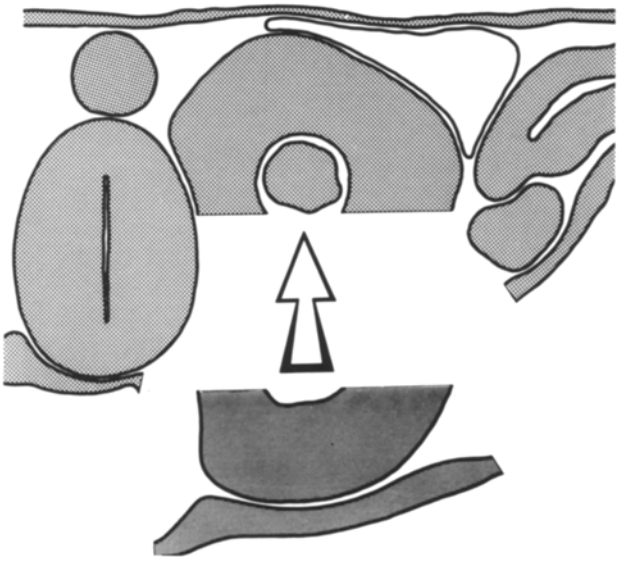

Fig. 1. Diagram showing the microsurgical procedure. After removal of the upper walls of somites 13-16 together with the overlying ectoderm of a chick embryo at stage 14 (H.H.) the corresponding parts of a quail embryo were implanted.

After 1 day of post-operative reincubation, the grafted somites are normally developed. On top of the mesenchymal sclerotome, which consists of cells containing nuclei of the chick type, the dermatome and myotome layer exhibit quail nuclei (figure 2, *A*). In the course of further development, the cells of the dermatome lose their epithelial arrangement and move towards the ectoderm, later to form the dermis. The most dorsal and ventral parts of the dermatome retain their epithelial structure. There seems to

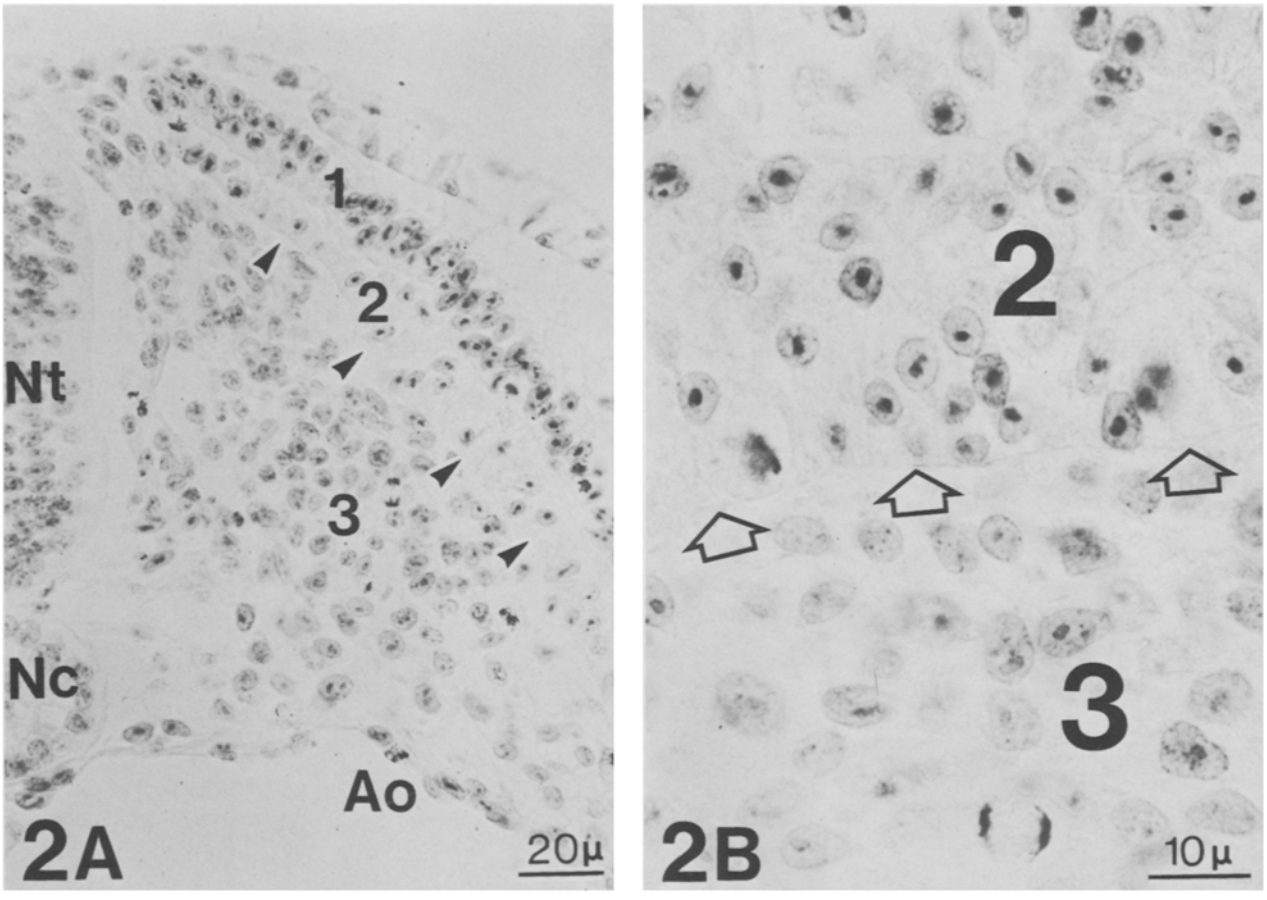

Fig. 2. *A* Transverse section through the operated region of a chick embryo after 1 day of reincubation. The dermatome (1) and the myotome (2) consist of cells with quail nuclei. 3 sclerotome, Nt, neural tube; Nc, notochord; Ao, Aorta. Arrows mark the border between myotome and sclerotome. Feulgen-Rossenbeck reaction and post-stained with light green. *B* Transverse section through the operated region of a chick embryo after 2 days of reincubation. Myotome (2) consisting of cells with quail nuclei; sclerotome cells (3) contain nuclei of the chick type. Arrows mark the basement lamella of the myotome. Feulgen-Rossenbeck reaction and post-stained with light green.

be a smooth and continuous transition between these parts of the dermatome and the myotome, which in the meantime has grown in length in the dorso-medial ventro-lateral direction as well as in thickness. All cells of the myotome are quail cells (figure 2, *B*).

As an attempt to further the understanding of how the myotome cells originate from the upper wall of the somites, scanning electron microscope studies were made. During formation of the sclerotome, only the dorsal somite wall and a small adjacent part of the medial wall maintain their pseudo-stratified epithelial structure. Along the dorso-medial edge of the somite, the myotome layer begins to appear directly in contact with the apical surface of the epithelial wall, which is now referred to as the dermatome (figure 3, *A*). Its dorso-lateral surface is strongly convex and formed like a cupping-glass (figure 3, *B*). Adjoining the dorso-medial 'lip' of the dermatome, the myotomic cells can be seen to be arranged in a relatively closed layer, whereas further laterally single and irregularly shaped cells are found. Since there is a continuous cellular transition between the growing myotome and the adjacent dermatome edges (figure 3, *B*), it can be concluded that, in addition to the dorso-medial 'lip', the cranial and caudal edges of the dermatome contribute to the myotome layer. Figure 3, *C* represents a sagittal fracture of the 17th somite of an embryo at stage 16 and shows the transitional zone between the caudal dermatome edge and the myotome layer. Here, too, no visible boundary between dermatome and myotome can be found. On the basis of our observations, it is very likely that during further development the ventro-lateral edge of the dermatome also contributes to the myotome, though the bulk of cells originating from this edge migrate into the somatic plate mesoderm, later to differentiate into myoblasts within the abdominal wall and limb buds[22-24].

Upto the time when the myotome layer reaches the lateral edge of the dermatome, the apical ends of the overlying dermatome cells have close contact with each other (figure 3, *D*). No cells were found to have originated directly from the surface of the overlying dermatome.

Our experimental results support the findings of Rabl[4] that only the dermatome gives rise to the myotome plate, while no experimental evidence was found that cells of the sclerotome participate in the formation of the myotome, as was postulated by Mestres and Hinrichsen[12] on the basis of electron microscope observations. On the other hand, we agree with these authors that a direct separation of myotome cells from the whole surface of the dermatome, as described by Langman and Nelson[11], does not occur. The SEM results described above are in accordance with the conception of Williams[6] that all dermatome edges contribute to the myotome. It is possible that the controversy

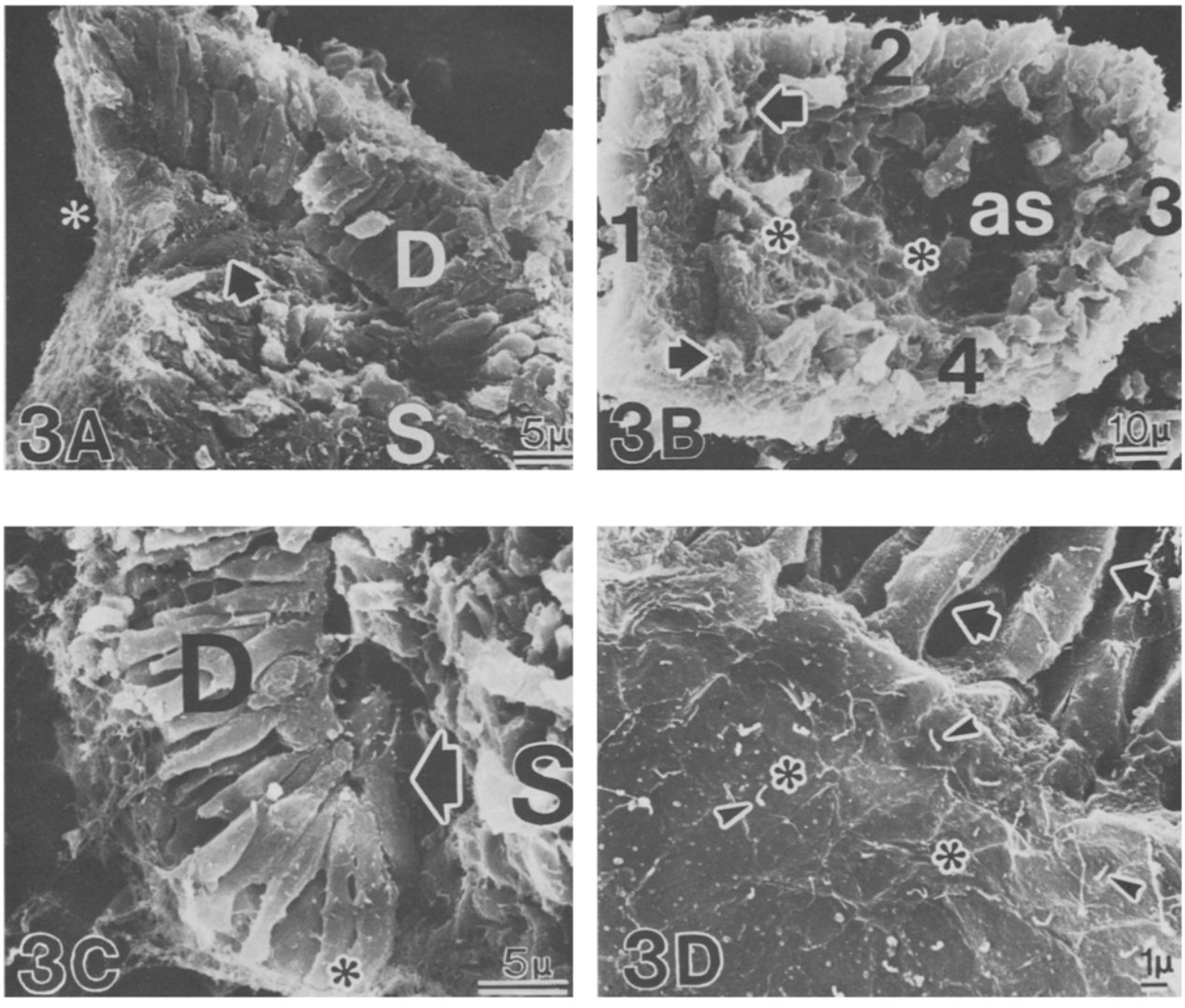

Fig. 3. *A* Cranial view of the fracture surface of the 13th somite of a chick embryo at stage 14. D, dermatome; S, sclerotome; asterisk: lower myotomic groove. The arrow marks the developing myotome layer. *B* Ventral view into the broken off upper wall of the 10th somite of an embryo at stage 14. Part of the medial edge (1) has been removed. 2 cranial edge, 3 lateral edge, 4 caudal edge. Asterisks: rudimentary myotome. Arrows mark the transitional zone between cranial edge, caudal edge and rudimentary myotome layer; as not yet covered apical surface of the dermatome. *C* Sagittal fracture showing the caudal edge of the dermatome (*D*). Somite 17, stage 16 (H.H.). S, sclerotome cells; asterisk: border of the dermatome edge. Arrow: cell in the transition zone between dermatome and rudimentary myotome. *D* View of the apical surface (asterisks) of the dermatome. Somite 10, stage 14 (H.H.). Small arrows: single cilia on the apical surface of dermatome cells. Large arrows mark dermatome cells.

concerning the myotome formation is derived from the fact that recent investigations were based only on transversal sections.

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