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Differentiating abilities of avian somatopleural mesoderm[1]

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Summary. Quail-to-chick grafting experiments were performed on 2-day embryos in order to test the differentiating abilities of the somatopleure. After orthotopic and heterotopic transplantations of different parts of quail somatopleural mesoderm into chick embryos it is demonstrated that avian somatopleural cells differentiate into skeletal elements, smooth muscles, tendons and connective tissues. However, skeletal muscle fibres do not originate from somatopleural cells.

In previous studies using the quail-chick marker technique according to Le Douarin and Barq[2] it has been shown that the limb, thoracic and abdominal muscle cells are of somitic origin whereas the connective tissues and the tendons originate from the somatopleural mesoderm[3-11].

In contrast to our findings that the muscle cells solely differentiate from somitic cells, Chevallier et al.[9,10] have the idea that the somatopleure can also give rise to muscle fibres. These authors believe that under special conditions, the somatopleural mesoderm seems able to compensate for somitic deficiency. In view of this concept a new series of experiments was undertaken in order to test the differentiating abilities of the somatopleural mesoderm.

Material and methods. The experiments were carried out on 2-day chick and quail embryos (White Leghorn, *Coturnix coturnix japonica*). Parts of somatopleural mesoderm and adjacent ectoderm previously isolated from different levels of quail donors were orthotopically or heterotopically grafted on chick embryos. The affected somatopleural fragments had at that time not been invaded by myogenic cells from the somitic mesoderm.

The orthotopically implanted somatopleural fragments participate in the normal development and differentiation of limb and ventral body wall structures. Analysing their cellular composition, it can be stated that with the exception of the ribs the remaining cartilage elements and the complete connective tissue are made up of quail cells. The blood vessels can be identified as composite structures in which the endothelia mainly consist of chick cells whereas the surrounding connective tissue cells of the tunica media exhibit nuclei of the quail type (figure 1). From this it may be concluded that somatopleural cells can differentiate into smooth muscle fibres.

The skeletal muscular bulks within the operated regions are generally found to be of bispecific composition. While the tendons as well as the intra- and peri-muscular connective tissue are of quail (somatopleural) origin, the muscle fibres are of chick (somitic) origin. After histological examination of 50 embryos operated in this way no indication was found that somatopleural cells can form muscle fibres, since the myotubes exclusively contain nuclei of the chick type (figure 2). Moreover, hybrid myotubes cannot be observed.

From these results regarding the normal differentiating abilities of somatopleural cells, the question arises whether the somatopleural mesoderm in the absence of somites can undergo muscular differentiation. In order to test this possibility, in the 2nd experimental series, parts of somatopleural mesoderm and adjacent ectoderm of quail embryos were grafted into the coelomic cavity of chick embryos.

If the grafts consist of prospective limb mesoderm, well developed wings and legs with quite reasonable distal elements can be found. Examination of the pattern in such

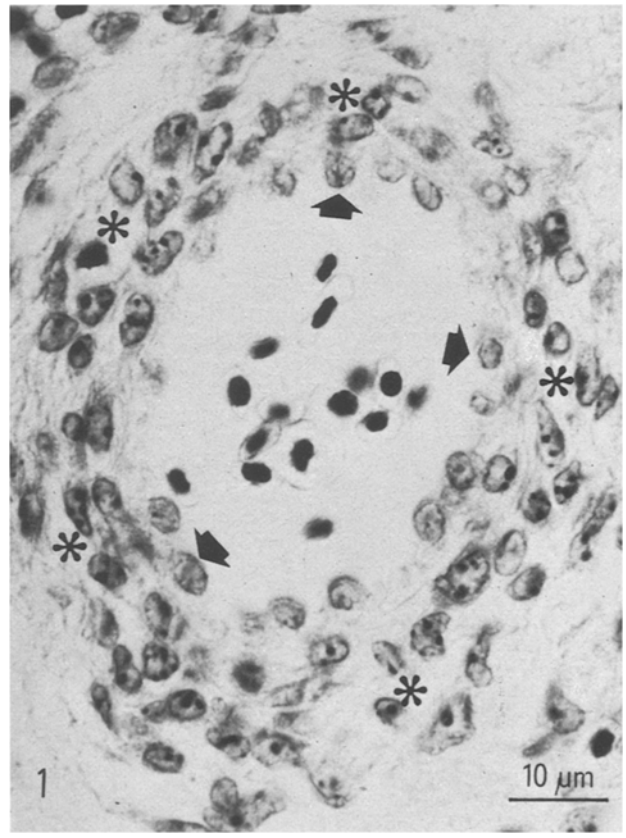

Fig. 1. Rudimentary blood vessel within a leg of a chick embryo 9 days after orthotopical replacement of somatopleural mesoderm by quail somatopleure. Endothelial cells (arrows) exhibit chick nuclei. The tunica media (asterisks) made up by quail cells. Feulgen-Rossenbeck reaction, post-stained with light green.

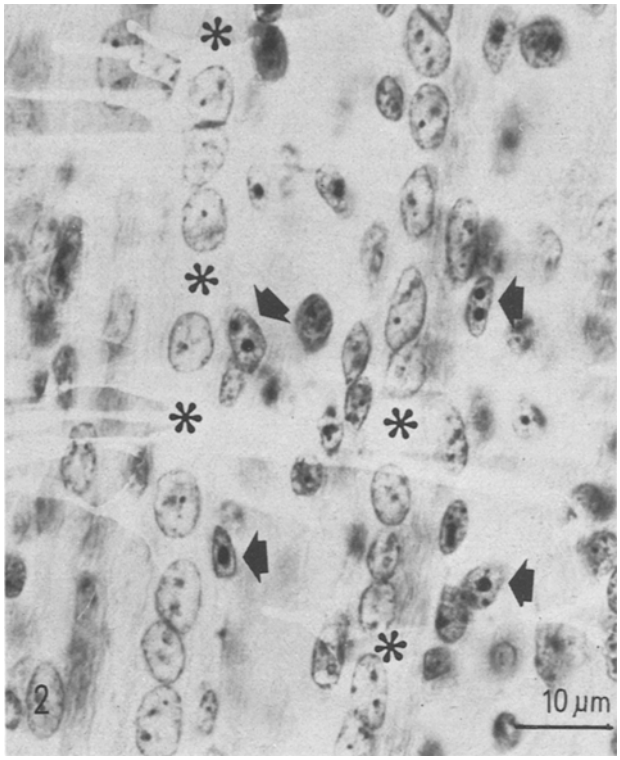

Fig. 2. Detail of a leg muscle 7 days after orthotopical replacement of somatopleural mesoderm of the chick by that of a quail. Myotubes (asterisks) contain chick nuclei. Intramuscular fibroblasts (arrows) are characterized by quail nuclei. Feulgen-Rossenbeck reaction, post-stained with light green.

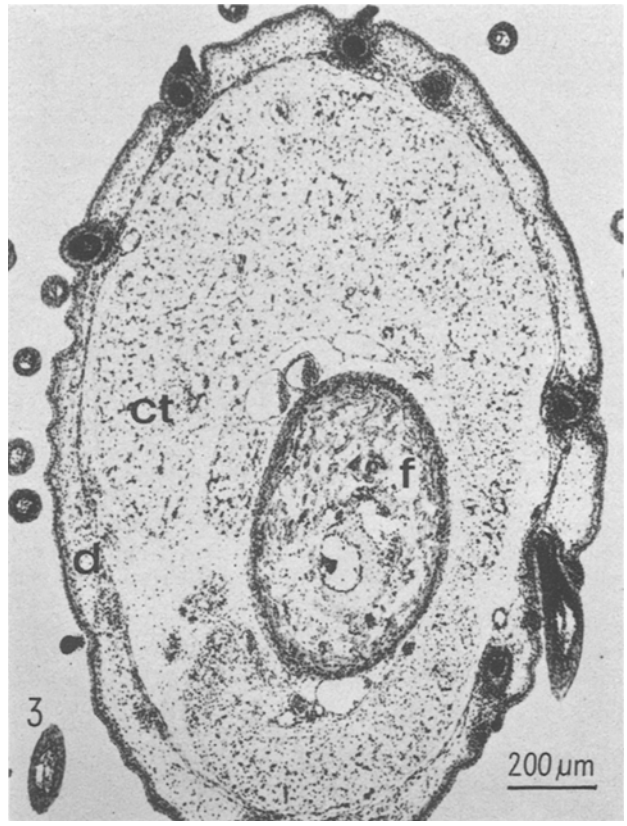

Fig. 3. General view of the upper part of a leg developed for 11 days in the coelomic cavity of a chick. The grafted quail somatopleural mesoderm has given rise to following structures: dermis (d), areas of differentiated connective tissue (ct), femur (f). Feulgen-Rossenbeck reaction, post-stained with light green.

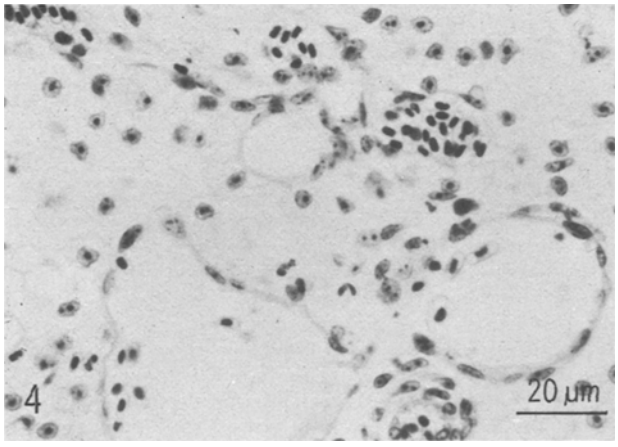

Fig. 4. Detail of the connective tissue within the myogenic zone. Numerous blood vessels are present. Muscular elements failed to differentiate. Feulgen-Rossenbeck reaction, post-stained with light green.

limbs grown in the absence of the myogenic cell source shows that the skeletal elements are well developed and are in their normal position. However, these grafts are always characterized by a total absence of the muscle bulks. Between cartilage elements and subectodermal mesenchyme loosely arranged mesenchymal cells can be seen. In other cases the mesenchyme of the muscle-forming zone undergoes organisation and appears to be arranged in a lobular manner (figure 3). It is remarkable that the demar-

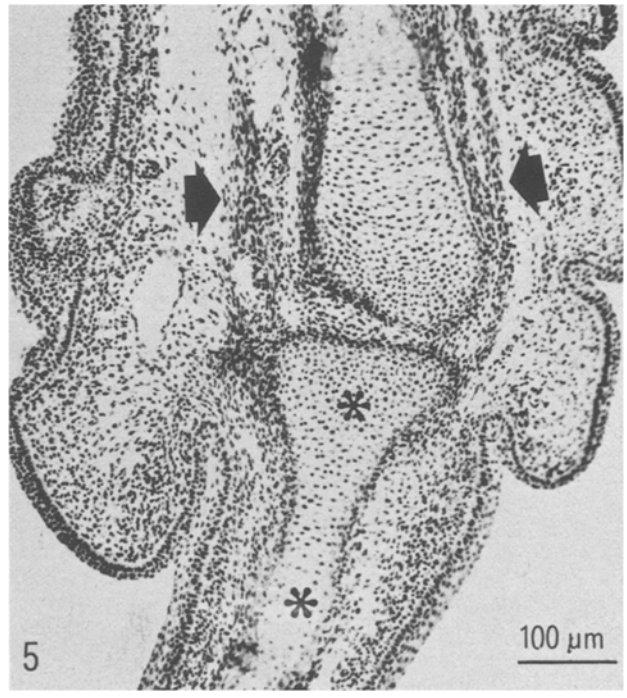

Fig. 5. Distal part of a leg developed out of quail somatopleural mesoderm and adjacent ectoderm, 10 days after transplantation into the coelomic cavity of a chick. Tendons (arrows) attach to the distal phalange (asterisks). Feulgen-Rossenbeck reaction, post-stained with light green.

cated areas show numerous blood vessels (figure 4). During further development these lobules differentiate into adipose tissue.

Despite the complete absence of musculature in the distal parts of the limbs, tendons have differentiated. Their distal ending attaches to the perichondrium of the phalanges, while the proximal part passes progressively into the loose mesenchyme of the muscle-forming zone (figure 5). These observations are in conformity with previous findings on the autonomy of tendon development[12-14].

The results of the 2 experimental series show that the avian somatopleural mesoderm gives rise to skeletal elements, smooth muscles, tendons and connective tissues. However, skeletal muscle fibres do not differentiate from somatopleural cells. The fact that hybrid myotubes cannot be found within the operated regions supports our point of view that the myoblastic component of the muscles solely originates from the somites. Since the somatopleural fragments after heterotopical cultivation exhibit no musculature, it may be excluded that the somatopleural cells undergo a change in their programme of development in order to compensate for somitic deficiency. This is in line with the findings of Dienstman et al.[15] which indicate that the mesoderm cells of the early limb bud are already determined as cartilage or muscle precursor cells.

Our results are in partial contradiction to those reported by Chevallier et al.[9,10]. After removal of the brachial somitic mesoderm these authors observed muscles within the wing. Moreover, after the replacement of quail somites by chick somites they found muscle bulks of mixed constitution. In our opinion these observations offer no proof that the somatopleure gives rise to muscle fibres, because it was expressly conceded by the authors that the somites of the host embryos have not been completely removed. With regard to the remarkable capacity of the somitic mesoderm to regulate deficiencies[16], it is worth considering that from the remaining somitic parts the migration of myogenic cells can still occur.

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Correlation of immunogenicity with suppression of lymphocyte adenosine 3′,5′-monophosphate-dependent protein kinase

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Summary. Cyclic-AMP-dependent protein kinase activity was depressed in whole spleen as well as in isolated splenic lymphocytes from 3-methylcholanthrene (MCA), R3230 AdCa mammary adenocarcinoma, N-hydroxy-2-acetylaminofluorene, and 4-dimethylaminoazobenzene (DMAAB) tumor-bearing Fischer rats as compared to control animals. The magnitude of depression increased with the immunogenicity of the tumor. The depressed enzyme activity was the result of a reduced V_{max} for adenosine 3′,5′-monophosphate (cAMP)-stimulated histone phosphorylation.

Adenosine 3′,5′-monophosphate (cAMP) plays an important role in regulation of the immune responsiveness[2,3]. There is extensive evidence to indicate that cAMP inhibits immune responsiveness which leads to enhanced growth[4-7]. cAMP mediates its physiological effects by cAMP-dependent protein kinase[8]. The present studies report on cAMP-dependent protein kinase in whole spleens and spleen lymphocytes of normal and tumor-bearing rats. The tumors examined possessed various degree of antigenicity so that the cAMP-dependent protein kinase and immunogenicity of the neoplasm could be correlated.

Materials and methods. Tumors. 4 tumor systems, sarcoma MCA, mammary R3230 adenocarcinoma (R3230 AdCa), mammary carcinoma AAF and hepatoma DMAAB (table 1) were induced and carried on in inbred Fischer rats (80–90 g) which initially were obtained from Charles River Breeding Laboratory (Wilmington, Mass.).

Rat splenic lymphocytes were obtained from a Ficoll-Hypaque gradient (8 ml of 8% Ficoll plus 2 ml of 50% Hypaque) as described by Simon et al.[9]. The cells were next washed 2 times in hypotonic Tris buffer (2.00 g Tris plus 7.47 g ammonium chloride/l, pH 7.2) to remove contaminating erythrocytes[10]. The final cell preparations had more than 98% small lymphocytes (as indicated by Wright's Stain) and 95% were viable (as determined by trypan blue dye exclusion).

Preliminary experiments showed that the supernatant fraction resulting from a 50,000 × g centrifugation (20 min) of spleen lymphocytes homogenized in 20 mM 2 N-morpholineethanol sulfonic acid (MES) buffer, pH 7.0, contained over 85% of the cAMP-dependent protein kinase in whole homogenate. In brief, the protein kinase reaction[11] was initiated by addition of the enzyme preparation to a reaction mixture containing: 0.5 mM EGTA, 10 µg calf thymus type II-A histone, 2.0 mM theophylline, 5.0 mM sodium fluoride, 0.01% bovine serum albumin (BSA), 30.0 mM $MgCl_2$, 20.0 mM MES, 0.1 mM ATP containing 5×10^6 cpm of gamma labeled ^{32}P-ATP, and with or