nergic receptor is coupled with the adenylate cyclase catalytic unit[14]. Myometrial cells in vitro share some other properties with uterine smooth muscle cells in vivo. Results presented here suggest but do not prove that E_2-sensitivity of β-adrenergic receptor-mediated cAMP synthesis may be one of these.

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Differentiation of L6 myoblastic cells into chondrocytes

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Summary. Under the influence of demineralized bone pieces L6 cells differentiate into chondrocytes. The cartilage formed is identifiable histologically. The results demonstrate that these myoblastic cells, which are committed to produce muscle, may still be influenced to express another potentiality of their genome.
Key words. Demineralized bone matrix; cell culture; L6 myoblastic cells; chondrocytes; chondrogenesis; cellular differentiation.

The implantation in animals of demineralized bone matrix (DBM), generally prepared in pulverized form, induces in a first step the formation of cartilage, even in ectopic sites, i.e. under the skin or intramuscularly. The test animal is usually the rat, and optimal results are mainly obtained with allogenic implants. After invasion of the implant by blood capillaries, bone marrow forms, and around it, osteoblasts begin to secrete osteoid, which subsequently mineralizes. A regular cancellous ossicle is formed[1].

It is generally assumed that the cells from which chondrocytes originate under the influence of a cytokine contained in DBM, are undifferentiated mesenchymal cells.

In vitro, upon incubation of embryonic rat muscle with rat DBM pieces, prepared by cutting demineralized bones longitudinally, chondrocytes secreting cartilage matrix appear[2,3]. As angiogenesis cannot take place under these conditions, no bone is formed.

Thompson, Piez and Seyedin[4] did not detect any proteoglycan synthesis in 14-day cultures of DBM extracts with L6 cells, assayed for cartilage proteoglycan by an ELISA, although mesenchymal cells prepared from rat embryonic muscle and incubated under the same conditions consistently gave a positive result. On the contrary, Glowacki and Mulliken[5] observed incorporation of ^{35}S-sulfate into chondroitin sulfate by cells of mesenchymal origin, i.e., 3T3, L6 and swine aorta smooth muscle cells. A discrepancy exists between the above mentioned results which could be due to differences in sensitivity between the methods used.

On the other hand, as the implantation of pulverized DBM into the rat abdominal rectus muscles consistently elicits the formation of cartilage and bone[6], the possibility of a differentiation of chondrocytes from myoblastic cells seems worth testing. Therefore, we have tried in a series of experiments to find out whether L6 cells, which behave characteristically as myoblasts, would express a chondrocytic phenotype as a result of incubation with rat DBM pieces.

Experimental procedures. L6 cells were purchased from ATCC (number CRL 1458). The culture medium was essentially the same as that utilized by Nathanson and Hay[3]: CMRL 1066, with 15% heat inactivated fetal calf serum, containing penicillin (100 units/ml) and streptomycin (100µg/ml). Incubation was at 37 °C in an atmosphere containing 5% CO_2.

Demineralized bone pieces were prepared from the humerus, femur and tibia of adult male Wistar rats killed with Nembutal i.p. (50 mg/100 g b.wt). The bones were defatted and dehydrated by 2 washes of chloroform: methanol (1 vol.: 1 vol.). They were then broken into 3–4 pieces each. From then on they were handled under aseptic conditions, since any

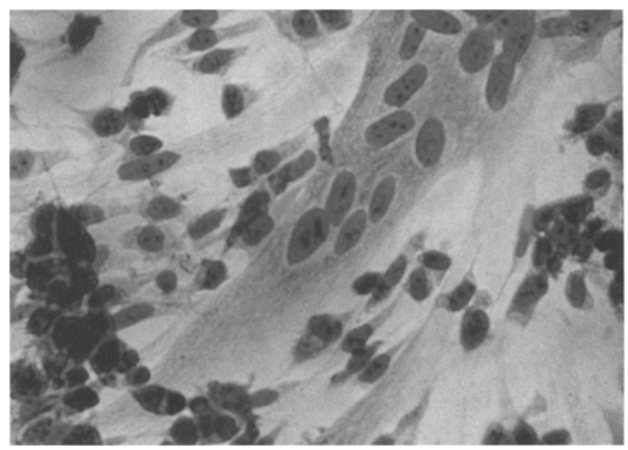

Figure 1. L6 myoblastic cells, aspect after short trypsinization as described in the text: partial disruption of the upper mononucleated cell sheet leaves the polynucleated myotubes intact. Fixation in methanol, staining by May-Grunwald-Giemsa[10].

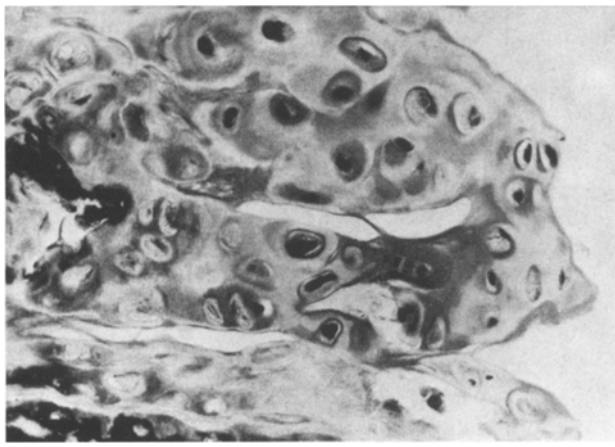

Figure 2. Cartilage bulging out of bone matrix. L6 cells cultured for 30 days on rat DBM pieces. The differentiated chondrocytes display a more or less spherical form; they are pink. The cartilage matrix, which is secreted by these cells in large amounts and separates them from one another, shows with variable intensity the bright blue which is characteristic for this substance after staining with Movat's pentachrome I (modified by Olah et al.[8]).

sterilization procedure causes a loss of osteogenic activity[6]. They were demineralized with two changes of HCl 0.6 N (70 vol./g) at 0 °C for 20 h. The bone pieces were then treated with phosphate buffer 0.2 M, pH 5.5, washed twice with water, and freeze-dried.

For each series of experiments, 12 petri dishes (diameter 5 cm) each containing 4–5 bone pieces were seeded with L6 cells suspended in the medium. Two thirds of the medium was changed twice a week. At first the bone pieces moved freely in the medium, but after 1 week they were held together by a multilayered cell sheet.

Half of the cultures were fixed at 20 days, the other half at 30 days; they were washed with Hank's solution and fixed with phosphate buffered formol 4%.

The bone pieces supporting the cells were dehydrated and embedded in methylmethacrylate. They were sectioned at 3µm with a rotation microtome type Biocut Jung No. 1130, and stained by a modification of Movat's pentachrom I stain[8].

Results. L6 cells, when incubated alone, behave essentially like myoblasts as described by Richler and Yaffe[7]; upon incubation some of the cells fuse into multinucleated myotubes. To observe the latter phenomenon in culture dishes, a disruption of the upper myoblastic sheet, leaving the myotubes intact, can be provoked by a 2-min treatment with Hank's solution containing 0.25% trypsin, followed by inactivation of the proteolytic enzyme with the normal incubation medium (fig. 1).

After 20 and 30 days incubation of the L6 cells with demineralized bone pieces, the cells show a high level of mitotic activity; they are piled up in many layers and invade the DBM pieces, lodging even in the lacunae formerly occupied by osteocytes. In one out of three culture vessels, formation of chondrocytes can be observed. These form rows of cells which are recognizable by their secretion of cartilage matrix brightly stained by alcian blue. The larger islets are bulging out of the bone pieces and the cells are separated by mucopolysaccharide in quantities as large as the diameter of the cells (fig. 2). The cells are pink and the bone pieces pinkish, in contrast to the blue stain of the cartilage matrix.

Discussion. L6 cells are thought to be cells of myoblastic origin, that can form muscular syncytia or myotubes[7]. After cellular differentiation the cells stop dividing. However mononucleated cells continue to proliferate. These cells, when cloned, again differentiate into myotubes or remain proliferating mononucleated cells. Thus, L6 cells appear to be myoblasts or committed muscle precursor cells. Nevertheless, it seems that the expression of the genome of the L6 cells can be modified by the presence of an active principle contained in the bone matrix in such a way that they differentiate into chondrocytes. This is relevant to the discussion on the origin of chondroblasts in ectopic bone formation[9]. L6 cells may be common precursor cells for both myoblasts and chondroblasts; the direction of the differentiation can be determined by exogenous factors and by the culture conditions.

In vivo, the cells that differentiate into chondrocytes in implants of bone matrix into muscle may also be of myoblastic origin by de-differentiation, or originate from a common precursor cell, rather than arising from non-differentiated mesenchymal cells.

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Membrane damage by coelomic fluid from *Holothuria polii* (Echinodermata)

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Summary. Rabbit erythrocyte membranes lyzed by *Holothuria polii* coelomic fluid, observed under the electron microscope, present lesions consisting of irregular holes which are heterogeneous in size (ranging from 50 Å to 250 Å) and ultrastructurally different from the ring-like structure produced by human complement. The protein pattern associated with the lyzed membrane was also examined.
Key words. Holothuria polii coelomic fluid; hemolysin; rabbit erythrocyte membranes; complement lesions.

The lysis of cells is one of the most important functions of the complement system. Observed using the electron microscope, this phenomenon is observed as the formation of holes in the damaged cells[2]. Typical ultrastructural complement lesions have