Influence of beta-aminoproprionitrile (BAPN) on cell growth and elastic fiber formation in cultures of auricular chondrocytes

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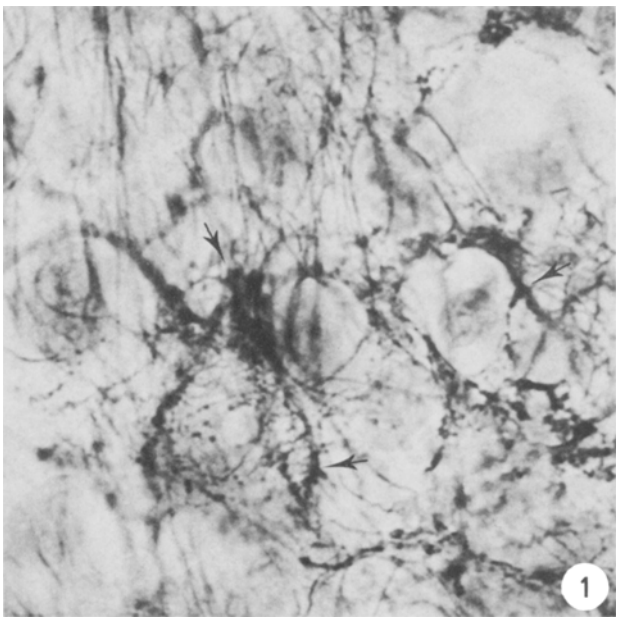
Summary. Auricular chondrocytes isolated from 4-day-old rabbits and grown in vitro for 14 days, proliferated rapidly and produced a conspicuous network of elastic fibers. Beta-aminoproprionitrile (BAPN), which in vivo inhibits cross-linking of elastin, decreased the formation of elastic fibers at a concentration of 10–20 µg/ml and prevented formation at 40 µg/ml. At a concentration of 5 µg/ml only the so-called patches of elastin appeared to be absent. The inhibitory effect of BAPN on cell growth did not exceed 10%, which indicates that BAPN is only slightly harmful to auricular chondrocytes and can safely be used in studies on elastin deposition by these cells in vitro.

Beta-aminoproprionitrile (BAPN) administered in vivo prevents cross-linking of elastin by inhibition of the enzyme lysyl oxidase[3]. Reports concerning its effectiveness in vitro are, however, conflicting. In one study BAPN was found to inhibit elastic fiber formation[4], whereas another showed no effect on cross-linking of elastin[5]. Furthermore,



Influence of BAPN on the increase in DNA content in cultures of rabbit auricular chondrocytes

Concentration of BAPN, µg/ml	DNA content, µg/dish ± SD* After 1 day of culture	After 14 days of culture
0	12.7 ± 0.3	53.2 ± 0.7
10	–	51.4 ± 0.9**
20	–	48.1 ± 0.6***
40	–	49.4 ± 0.8***

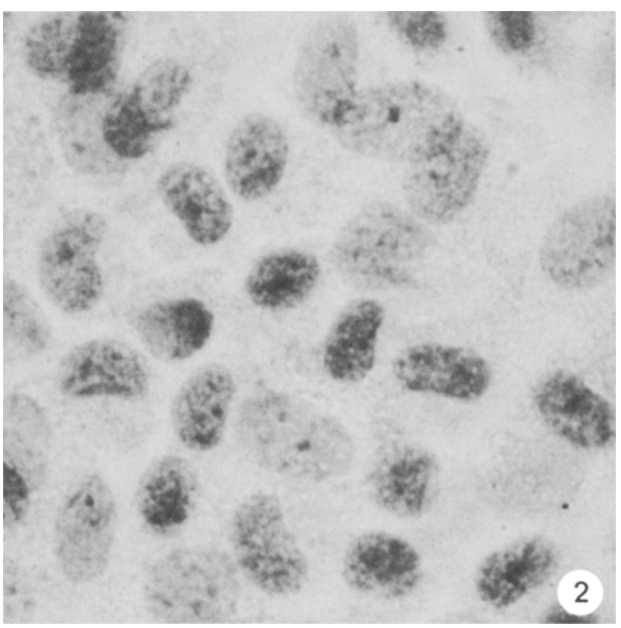
*In each group cells from 5 dishes were analyzed. **Different from control with $p < 0.01$, according to Student's t-test. ***Different from control with $p < 0.001$.

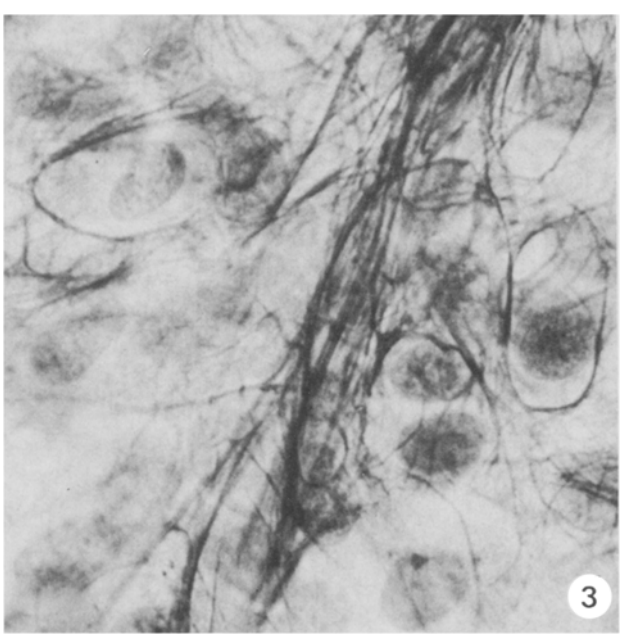
Figures 1–3. 14-day-old cultures of auricular chondrocytes stained with orcein-haematoxylin. × 980.

Figure 1. Control culture with numerous elastic fibers running straight or forming loops around the chondrocytes. The latter show patches of elastin (arrows).

Figure 2. Culture exposed to 40 µg/ml BAPN during 13 days. No elastic fibers were formed.

Figure 3. Culture exposed to 5 µg/ml BAPN during 13 days. Patches of elastin are absent, but elastic fibers are still abundant.





BAPN has been found to exert a toxic effect on cultured smooth muscle cells[6]. Since BAPN represents a potentially useful agent in experiments on elastin formation in vitro, its influence on cell growth and elastic fiber formation in cultures of auricular chondrocytes was examined in more detail.

Materials and methods. Chondrocytes were isolated from auricular cartilage of 4-day-old rabbits, as described elsewhere[7,8]. The chondrocytes were cultured in Medium 199 containing Earle's salts and supplemented with 10% newborn calf serum as well as 10 mg penicillin, 10 mg streptomycin, and 1 mg mycostatin per 100 ml medium. Cultures were started at a density of 1×10^6 cells per 35 mm plastic dish (Falcon) and kept in an atmosphere of 5% CO_2 in air at 37 °C, usually for 14 days. The medium was changed every 2–3 days. Before the addition of BAPN (BAPN-fumarate, Sigma) the cells were cultured overnight to permit adherence to the plastic surface of the dishes. The DNA content of the cultures was determined according to Karsten and Wollenberger[9]. After fixation of the cultures in 70% ethanol, elastic fibers were stained with orcein.

Results and discussion. As can be seen in the table, the DNA content of the cultures increased considerably during the 14-day period, thus indicating intensive proliferation of chondrocytes. BAPN exerted only a slight, although statistically significant inhibitory effect. Control cultures contained numerous elastic fibers (fig. 1). As in previous studies[4,7,8], some fibers had a straight course, whereas others formed loops around the chondrocytes. These loops were usually provided with patches of elastin. The number of fibers was higher than in previously-studied cultures of chondrocytes from 1-week-old rabbits[4], which is consistent with recent data[10] suggesting that the amount of elastin produced by auricular chondrocytes declines sharply during the first few weeks of post-natal life. BAPN used at a concentration of 40 µg/ml completely prevented the formation of elastic fibers (fig. 2). This indicates that the drug effectively inhibited cross-linking of elastin and thus conversion of the soluble into the insoluble form[11]. At a concentration of 10–20 µg/ml the formation of elastic fibers was depressed and only thin fibers were formed. At a still lower concentration (5 µg/ml), BAPN only prevented the deposition of the patches of elastin, whereas the fiber formation appeared unaffected (fig. 3). This suggests that the patches constitute an addition to the fibers and not a stage in their formation.

The concentration of BAPN required to inhibit the formation of elastic fibers completely, was higher than that reported previously[4], but this discrepancy is probably attributable to the more vigorous formation of elastic fibers by the younger chondrocytes in the present study.

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The effect of exposure to gaseous ammonia on the duration of diapause II in the embryos of the annual fish, *Nothobranchius guentheri*

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Summary. The exposure of the embryos of *N. guentheri* to gaseous ammonia at stages prior to the onset of diapause II caused a reduction in the frequency of diapausing embryos and the shortening of the duration of diapause. The optimum exposure time was approximately 30 min. There was a prolongation in the duration of diapause II when the ammonia treatments were conducted using embryos which were undergoing diapause II.

Instances of diapause or developmental arrests have been described in the embryos of the killifish, *Nothobranchius guentheri* (Pisces: Cyprinodontidae)[1,2]. This species belongs to a unique group of freshwater teleosts known as annual fishes[3]. *N. guentheri* is native to the seasonal ponds and stagnant pools along the coastal lowlands of East Africa[4]. The evaporative waterloss during periods of drought results in the death of the adult and juvenile fishes. The survival of the species during the dry season becomes dependent entirely upon the embryonic population deposited in the muddy substrate. It has been postulated that annual fishes escape extinction by undergoing diapause at specific stages of their normal embryonic development[1,5–7].

Many of the factors that may induce the onset of diapause in *N. guentheri* are known[2,8,9]. However, the conditions which regulate the termination of diapause remain unclear. Since the physiology of the annual fish diapause bears a striking resemblance to the well studied diapause in insects, the phenomena which influence diapause termination in insects might provide valuable insights on the mechanism that may control diapause termination in fishes. This report demonstrates that, like in insects[10,11], the arrest periods in this fish may be influenced by the exposure of the embryos to ammonia.

Materials and methods. All embryos were collected from a randomly bred laboratory population of the annual fish, *N. guentheri.* The source of fishes, husbandry conditions, and method of embryo collection have been reported previously[2]. The fishes were kept at a photoperiod of 9 h light and 15 h darkness. This photoperiod has been report-