The effect of increased oxygen tension on the growth of the mandibular condyle

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Forty-eight Long Evans/Turku rats were exposed to increased oxygen tension at the age of 11 or 13 days. Three control and three experimental rats were killed after 3, 7, 11 and 14 days of exposure and 1, 5, 10, and 15 days after the animals had been returned to normal laboratory conditions. Glycosaminoglycan synthesis decreased when the oxygen tension increased, as indicated by reduced metachromasia of the cartilage. After the animals had been returned to normal laboratory conditions the glycosaminoglycan synthesis of chondroblasts and chondrocytes seemed to recover. Disturbances were seen in the intermediate cell layer of the condyle and later in the condylar cartilage. The results seem to indicate that there are differences in the metabolic state of the cells in different regions of the condyle. Variances in the metachromasia of the condylar cartilage appear to be affected by different oxygen tensions, which seem to be lowest in the superior region. The mesenchymal cells in particular seem to be sensitive to a drop in oxygen tension. □ *Condyle; histochemistry; rats; structure*

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Pressure has been found to affect the amount of cAMP in the cartilage tissue, which is obviously one of the growth-regulating factors (1, 2). Pressure also affects enzyme activities (3), and the role of oxygen in cartilage growth is well known (4). Because of the articulating function, oxygen can only be supplied by diffusion from the blood vessels of the peripheral joint regions (5, 6). It is thus possible that there are differences in the oxygen tension between the various regions of the condyle.

The purpose of this investigation was to shed further light on the role of oxygen in the function of mesenchymal and cartilage cells in different regions of the mandibular condyle.

Materials and methods

Forty-eight Long Evans/Turku rats were exposed to increased oxygen tension in a chamber at the age of 11 or 13 days and onward. An air flow (1 l/min and containing 28% oxygen) was passed through the chamber at 0.5 bar over the atmospheric pressure. Water and food were available ad libitum, and the chamber was opened every other day so that the water and food could be changed and the cages cleaned. The 40 animals comprising the control group were kept under the usual laboratory conditions.

Three experimental rats were killed after 3, 7, 11, and 14 days of exposure to hyperbaric oxygen, together with three control animals, for microscopic studies.

After 14 days of exposure 12 rats were returned to normal laboratory conditions. Three control and three experimental rats were killed at the age of 26, 30, 35, and 40 days for microscopic study. The skinned heads were fixed in neutral formalin and demineralized in formic acid. Sagittal paraffin sections, 5 μm thick, of the temporomandibular joint were stained with hematoxylin and eosin or with toluidine blue.

Results

The animals tolerated the experimental con-

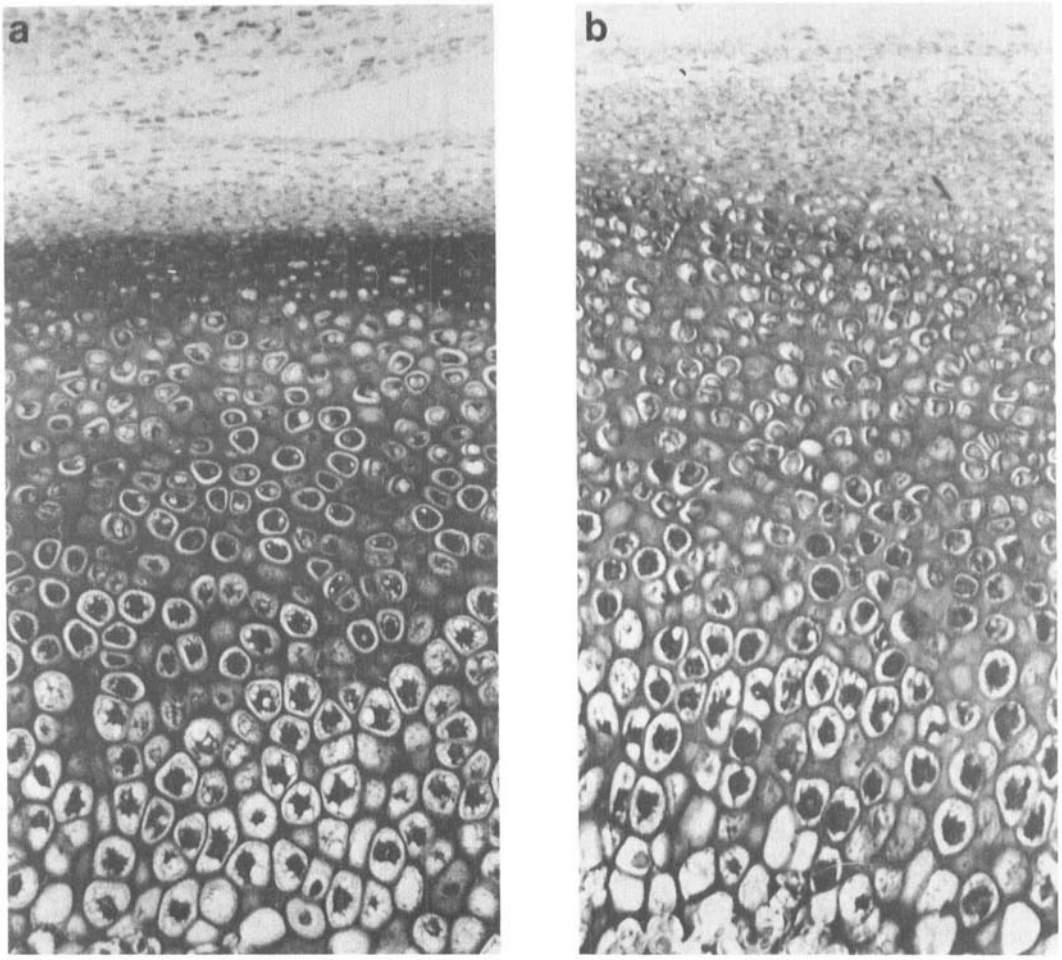

Fig. 1a. Histological section of the temporomandibular joint of a 20-day-old control rat. (Magnification, ×240.) 1b. Histological section of the temporomandibular joint of a 20-day-old experimental rat exposed to oxygen for 7 days. The mesenchymal cell layer is slightly thickened, and the strongly metachromatic deeper layer is deficient in cells, which have increased in size. (Magnification, ×240.)

ditions well. No untoward signs or differences in weight gain between control and experimental animals were observed.

Microscopic examination of the mandibular condyle showed that the structural changes were generally most marked in the anterior and superior regions and least marked in the posterior region. Slight thickening of mesenchymal and chondroblast cell layers was observed after 3 to 7 days of exposure (Fig. 1) but was no longer noticeable after 14 days (Fig. 2). The thickness of the cartilage seemed to have decreased by the 11th to 14th day of the experiment (Fig. 2).

A general reduction in metachromasia of the intercellular matrix was observed in the course of the oxygen treatment. The strongly metachromatic layer under the mesenchymal cells was observed to be located at greater depth, and the cells in this layer were increased in size after 3 days of exposure (Fig. 1). The thickness and metachromasia of this layer gradually decreased. Erosion

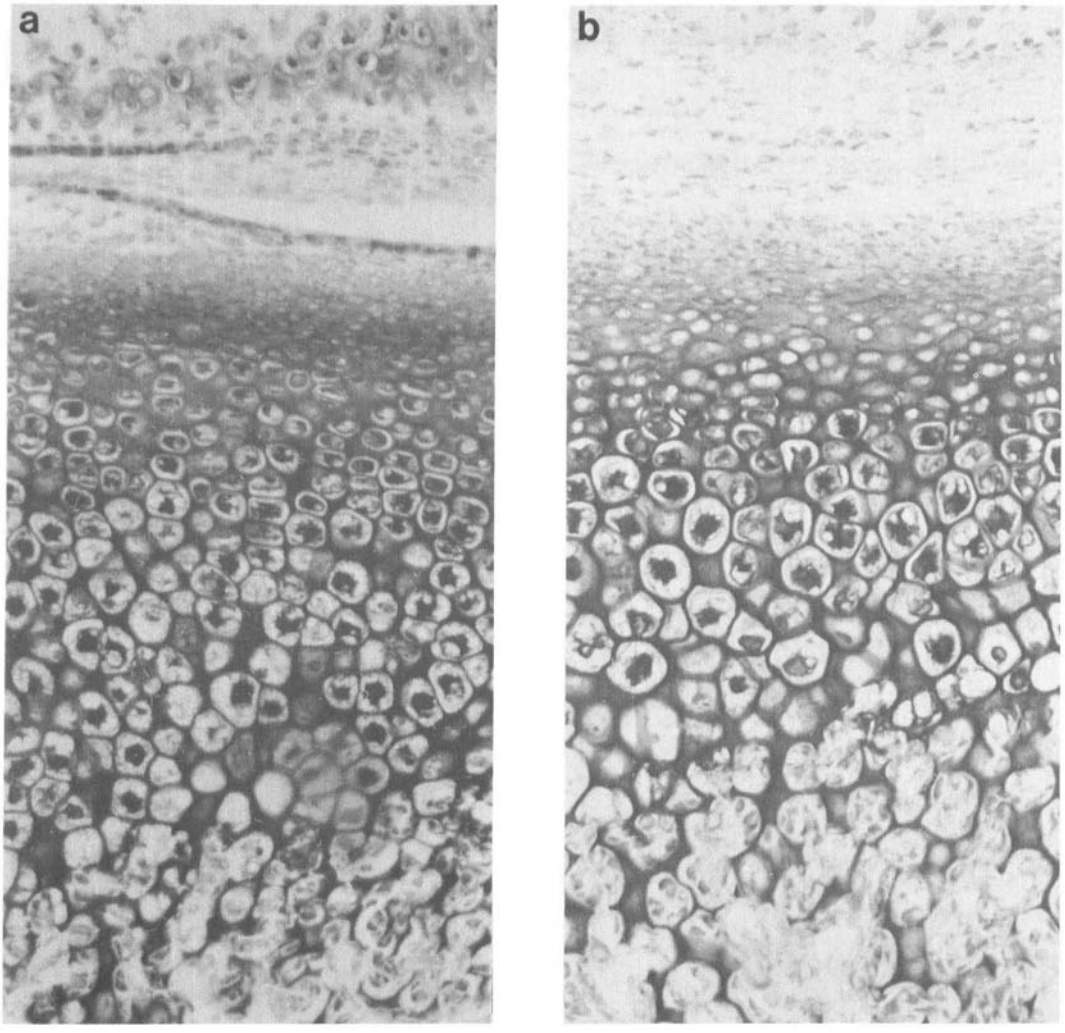

Fig. 2a. Histological section of the temporomandibular joint of a 25-day-old control rat. (Magnification, ×240.) 2b. Histological section of the temporomandibular joint of a 25-day-old experimental rat exposed to increased oxygen saturation for 14 days. The thickness of the cartilage has decreased, and the erosion front seems to be thicker. The metachromasia of the whole cartilage has decreased, and the strongly metachromatic layer under the mesenchymal cells has almost disappeared. (Magnification, ×240.)

of the cartilage seemed to be accelerated (Fig. 2).

Five days after the animals had been returned to normal laboratory conditions strong metachromasia appeared inside the mesenchymal cell layer, whereas the deeper part of the same layer remained weakly stained.

In the superior region, the metachromasia of the mesenchymal layer seemed to be uneven. On later observation days the hypertrophying of the mesenchymal cells into chondroblasts and further into mature hypertrophied chondrocytes was apparently disturbed (Fig. 3).

Acellular regions reaching the erosion front were observed in the cartilage tissue at 35 days (Fig. 3).

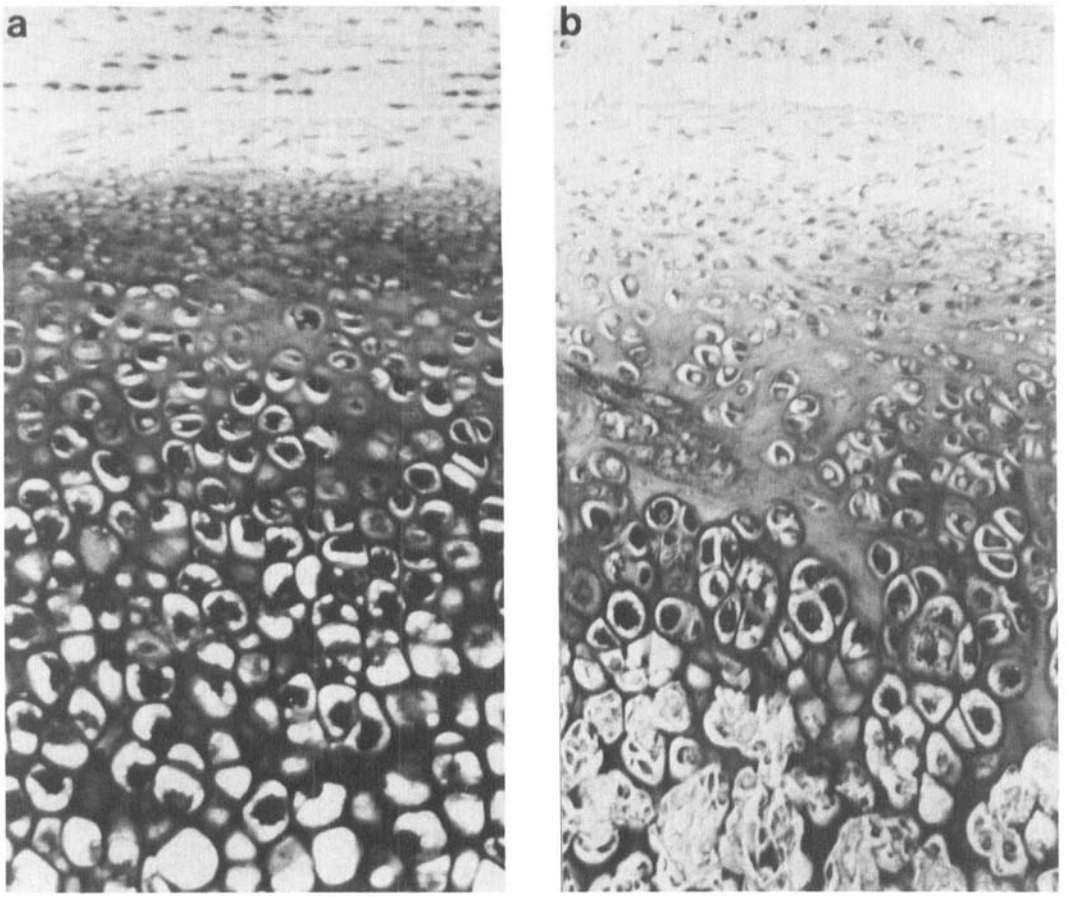

Fig. 3a. Histological section of the temporomandibular joint of a 35-day-old control rat. (Magnification, ×240.) 3b. Histological section of the temporomandibular joint of a 35-day-old experimental rat. The metachromasia in the intermediate cell layer seems to be uneven, and there is structural derangement, including disproportionately small cells and acellular regions, superiorly in the condylar cartilage. (Magnification, ×240.)

Discussion

High oxygen tension

Increased oxygen tension did not seem to have any untoward effects on the animals, possibly because the oxygen percentage and overpressure used in this experiment were far lower than those of earlier experiments (7).

The metachromasia of the intermediate cell layer of the condylar cartilage disappeared from the anterior region and weakened in the superior and posterior regions. These findings seem to imply that differences in metachromasia between different regions and cell layers of the condyle (8) are dependent on oxygen tension. The assumption is in accordance with the findings of Stockwell (9), indicating that synthesis in cartilage cells is increased if oxygen tension is decreased. Thus under normal conditions oxygen tension would be lowest superiorly in the pressure-bearing area, where strong metachromasia under mesenchymal cells is observed, second lowest posterosuperiorly, and highest anteriorly, where the strong metachromatic reaction suddenly disappeared (8).

The influence of oxygen on the cells may depend on their distance from the articu-

lating surface of the condyle, as the strongly metachromatic layer was at a greater depth in the experimental animals after 3 days of exposure. The assumption is consistent with events in the limb bud during embryogenesis, where the distance from the blood vessels seems to direct the differentiation (10). It is possible that the slight thickening of the mesenchymal cell layer resulted from the direct increasing effect of the oxygen on the mesenchymal cell proliferation, as cell density has been found to vary linearly with oxygen tension (9).

The increased cell size in the strongly metachromatic layer under the mesenchymal cells could have resulted from the increased distance of these cells from the surface. Thus the cells in the state of hyperoxia had more time in which to increase in size before intensive glycosaminoglycan synthesis started. A direct effect of the oxygen on the growth of the cells in this layer cannot be ruled out either.

Low oxygen availability has been found to reduce the production of thyroxin and growth hormone, both of which affect the growth of cartilage (11, 12). The accelerated erosion of the cartilage in this reverse situation could perhaps be attributed to enhanced thyroxin production. This, however, should also increase the chondrogenesis, which did not occur in the present experiment. The growth hormone increases the growth of the cartilage, but, although the mesenchymal cell layer was slightly thickened, the thickness of the whole cartilage was reduced. The reactions found therefore cannot be explained exclusively by enhanced hormone production but should rather be attributed to the direct effect of the oxygen on the condylar tissues.

Return from high to normal oxygen tension

The reinforcement of the metachromasia in the hypertrophied cartilage cell layer soon after the animals had been returned to normal laboratory conditions implies broad oxygen tolerance with regard to the glycosaminoglycan synthesis of these cells, which is consistent with findings in other studies (13–15). Disturbances were, however, found in the mesenchymal and chondroblast cell layers in the superior region after the animals had been returned to normal laboratory conditions. The strong metachromasia normally present in the chondroblast cell layer under the mesenchymal cells now appeared inside the mesenchymal cell layer. This may further support the assumption that the effect of oxygen on glycosaminoglycan synthesis depends on the distance from the surface from which the oxygen is diffused. The deeper part of the mesenchymal cell layer had obviously already adapted to higher oxygen tension, and then a sudden drop in the tension led to disturbances in chondrogenesis.

Although low oxygen tension has been regarded as necessary for chondrogenesis, the features of the mesenchymal cell layer in the condyle, the peripheral location, and the high cellularity (9) seem indicative of a higher oxygen consumption than that of cartilage tissue. This would explain why a sudden drop in oxygen tension induced pathological reactions that were carried over into the cartilage tissue during growth. It is noteworthy that increased articulating function in association with weaning, which induced somewhat similar disturbances (T. Kantomaa. Weaning and the histology of the mandibular condyle in the rat. Unpublished observations), might also decrease the oxygen tension.

The posterosuperior region of the condyle appeared structurally quite normal throughout this experiment. This might be because under normal conditions oxygen tension is higher in this region than elsewhere, as the main blood supply comes from the posterosuperior region (5, 6) and as this area is not under continuous pressure. Thus, after treatment the oxygen tension did not drop to as low levels as in other regions. This assumption supports the earlier suggestion (8) that the cells in the posterior region are in a metabolic state different from those in the superior region; it is also in agreement with findings that there are differences in the reactions to the compressive forces between various regions of the condyle (3).

In conclusion, this experiment clearly demonstrates the effect of oxygen on glycosaminoglycan synthesis in the condylar car-

tilage. Regional differences were found in reactions to an increase and to a drop in the oxygen tension, implying differences in the metabolic state of the cells. Furthermore, the mesenchymal cells in particular appeared sensitive to a drop in oxygen tension.

The effect of oxygen on the growth of the condylar process was studied histologically in this experiment. It seems important to conduct experiments of longer duration to establish what, if any, macroscopic changes occurred and to test the conclusions drawn on the basis of the histological findings.

References

1. Hall BK. Developmental and cellular skeletal biology. New York: Academic Press, 1978.
2. De Witt MT, Handley CJ, Oakes BW, Lowther DA. In *vitro* response of chondrocytes to mechanical loading, the effect of short term mechanical tensions. Connect Tiss Res 1984;12:97–109.
3. Copray S. Growth regulation of the mandibular condylar cartilage *in vitro* [Thesis]. Groningen, The Netherlands: University of Groningen, 1984.
4. Hall BK. Cellular differentiation in skeletal tissues. Biol Rev 1970;45:455–84.
5. Boyer CC, Williams TW, Stevens FH. Blood supply of the temporomandibular joint. J Dent Res 1964;43:224–8.
6. Wright DM, Moffett B. Postnatal development of the human temporomandibular joint. Am J Anat 1974;141:235–50.
7. Clark JM, Lambertsen CJ. Pulmonary oxygen toxicity: a review. Pharmacol Rev 1971;23:37–133.
8. Kantomaa T. New aspects of the histology of the mandibular condyle. Acta Anat 1986;126:218–22.
9. Stockwell RA. Biology of cartilage cells. Cambridge: Cambridge University Press, 1979.
10. Capalan AI, Koutroupas S. The control of muscle and cartilage development in the chick limb: the role of differential vascularization. J Embryol Exp Morphol 1973;29:571–83.
11. Hunter C, Glegg EJ. The effects of hypoxia on caudal vertebrae of growing mice and rats. J Anat 1973;116:227–44.
12. Hoskins WE, Asling CV. Influence of growth hormone and thyroxine on endochondral osteogenesis in the mandibular condyle and proximal tibial epiphysis. J Dent Res 1977;56:509–17.
13. Pawalek JM. Effects of thyroxine and low oxygen tension on chondrogenic expression in cell culture. Dev Biol 1969;19:52–72
14. Nevo Z, Horowitz AL, Dorfman A. Synthesis of chondromucoprotein by chondrocytes in suspension culture. Dev Biol 1972;28:219–28.
15. Brighton CT, Lane JM, Koh JK. *In vitro* rabbit articular organ culture model. II. Incorporation in various oxygen tensions. Arthritis Rheum 1974;17: 245–52.

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