

Differences in biologic response of the mandibular condyle to forward traction or opening of the mandible

An experimental study in the rat

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The effect of different mandibular postures on the growth of the mandible was studied. A total of 60 female Wistar rats were divided into 3 groups, and all the animals were anesthetized for 6 h daily from the age of 30 days onwards. The first group served as controls. The second had the mandible maintained in a protracted position, and the third had the mandible in an open position during the anesthesia. Macroscopic measurements showed the growth of the mandibular condyle to be increased in a posteroinferior direction and also in a superior direction in the animals with the mandible in an open position. The cartilage layer containing collagen type II was significantly thinned, whereas cell proliferation had significantly increased in the posterosuperior region. In association with mandibular protrusion the thickness of the cell layer containing collagen type II had increased, and cell proliferation in the posterosuperior region had significantly decreased. Significant differences in the growth of the condylar process were observed histologically and histochemically between the experimental animals, implying that the most rewarding aspect of the regulation of condylar growth seems to be the possibility to regulate the maturation rate of the cartilage cells. □ *Adaptability; condyle; forward traction; growth*

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The height of the vertical opening of a functional appliance used in association with the treatment of skeletal discrepancies affects the biologic response in terms of facial growth (1-4). High vertical opening has been reported to increase mandibular response (3, 4), and in association with hemifacial microsomia it is regarded as causing traction in the temporomandibular joint, inducing new bone growth (5, 6). Luder (3, 4) found high vertical opening to cause forward tilting of the maxilla as compared with the activators involving low vertical opening, and this has been verified in animal experiments (7).

Direct evidence of differences in the reactions to different heights of the construction bite at the temporomandibular joint is limited. McNamara (7) reported that the increased vertical dimension of the face directs condylar growth more posteriorly, but no increase in growth was found even though an end-on molar relationship developed. Woodside & Altuna (8) observed a two- to five-fold increase in condylar growth relative to control animals after lengthening of the vertical dimension of the face with bite blocks, and Buchner (9) reported induction of growth in the mandibular condyle after raising of the bite in the adult rat.

Variable reactions also seem to follow forward traction of the mandible with minimal opening. Longmire & Sanford (10) reported stimulated mandibular growth in congenital micrognathia after prolonged

protraction of the mandible at an early age, whereas Bakker & Johnston (11) reported decreased condylar growth in association with a class-II elastic force in an animal experiment. Several other contradictory reports exist with regard to the effect of forward traction of the mandible (12-16).

There is much truth in Bishara & Ziaja's statement (2), 'There is limited evidence that an activator with a large increase in the vertical dimension of the construction bite may be more effective in expressing mandibular growth in a relatively more horizontal direction. Further investigation into this potential is required.' The aim here was to pay special attention to the direction of the alteration of the mandibular posture, to differentiate reactions to protrusion of the mandible from those consequent on opening of the mandible.

Materials and methods

A total of 60 female Wistar rats (Fig. 1) were used, 30 of which were injected with alizarin red (200 mg/g body weight) at the age of 29 days. At the age of 30 days all the animals were anesthetized with an intraperitoneal injection of fentanylfluanisone (Hypnorm[®], 0.03 mg/kg body weight; Janssen, Beerse, Belgium). The rats were divided into 3 groups matched on the basis of their body weight, and 10 animals in each group were injected with alizarin red. The first group served as controls, the

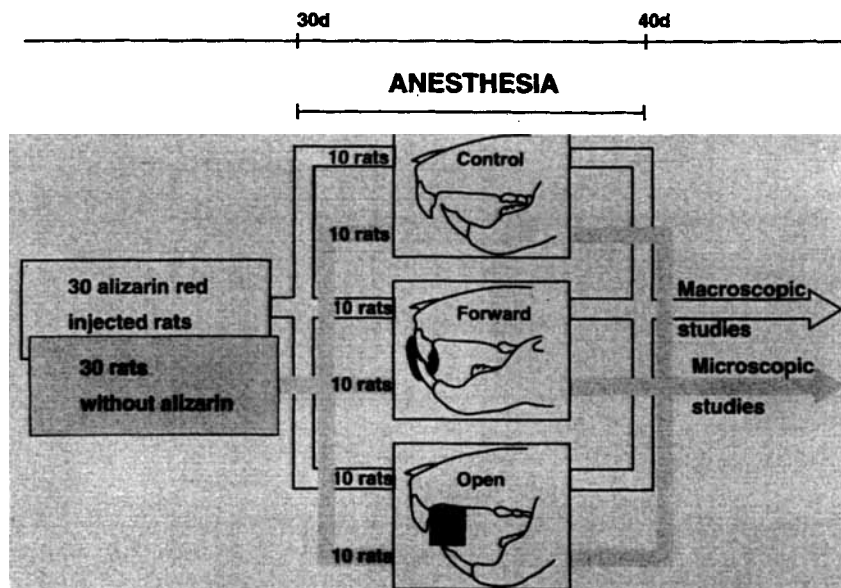


Fig. 1. A diagrammatic drawing of the use of rats for macroscopic and histologic studies.

second had the mandible maintained with phosphate cement (Lumicon®; Bayer, Leverkusen, Germany) in a protracted position so that the lower incisors reached in front of the upper ones, and the third group had a piece of plastic 7 mm thick inserted between the palate and the lower incisors to keep the mandible in an open position (Fig. 1). Control and experimental animals were kept anesthetized for 6 h daily with repeated injections, and after anesthesia the piece of plastic and phosphate cement were removed.

The animals were killed after 10 days, and those that had been injected with alizarin red were used for macroscopic examination. The heads were stripped of soft tissues, and the skulls and mandibular halves stored

in 95% ethanol. The following measurements were made under a microscope at $\times 10$ magnification with the aid of an ocular graticule (accuracy, 0.025 mm) (Fig. 2).

1. Growth in height perpendicular to the inferior border of the mandible, measured from the uppermost point of alizarin red staining to the superior surface of the condyle.

2. Growth in a posterosuperior direction measured parallel to the condylar process in the middle of the process from alizarin red staining to the posterosuperior surface of the condyle.

3. Growth in length parallel to the inferior border of the mandible measured from the extreme posterior point of alizarin red staining to the extreme posterior point of the condyle.

4. Growth in a posteroinferior direction, measured as the shortest distance from the alizarin red line to the posteroinferior border of the condylar process.

The differences in these values between the groups were tested with Student's *t* test.

The condylar processes of 10 animals in each group were transferred for 2 h to an organ culture in Dulbecco's modified Eagle's medium (Gibco, Paisley, Scotland) containing 10 $\mu\text{Ci}/\text{ml}$ radiolabeled thymidine (Amersham, Buckinghamshire, UK) and were then fixed in 10% neutral formalin. After 3 weeks of demineralization in 5% formic acid, they were embedded in paraffin, and sagittal sections 5 μm thick were cut from the central region for autoradiographic purposes and coated with Nuclear Emulsion (K5, Ilford, Maberly, UK). After 3 weeks' exposure in a freezer, the sections were developed and counterstained with hematoxylin. Thymidine-labeled cells were counted in

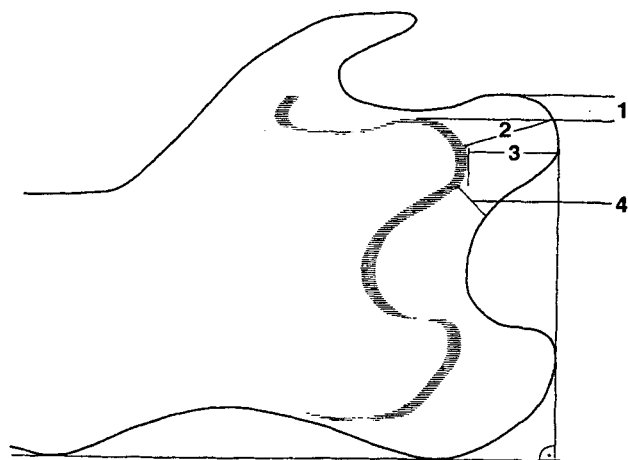


Fig. 2. Measurements made from the condylar processes of rats injected with alizarin red 11 days before being killed.

Table 1. Macroscopic (mm) measurements made on mandibular halves of 10 control rats, 10 rats anesthetized with the mandible in a protruded position, and 10 anesthetized with the mandible in an open position. The animals were injected with alizarin red at the age of 29 days and killed at the age of 40 days. Variable 1 is growth in height, 2 in a posterosuperior direction, 3 in a posterior direction, 4 in postero-inferior direction, and 5 the relation between variables 1 and 3

Variable	Control		Forward		Open	
	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD
1	0.36	0.026*	0.28	0.018*	0.33	0.013
2	0.63	0.078	0.54	0.023	0.44	0.013
3	0.56	0.032	0.53	0.028	0.48	0.020
4	0.11	0.014	0.10	0.012	0.14	0.016
5	0.65	0.028	0.53	0.035**	0.70	0.039

* $P \leq 0.05$; ** $P \leq 0.01$; Student's *t* test.

seven (1-7) adjacent sectors in the superior and posterosuperior region of the condyle. Each sector was 0.2 mm long when measured parallel to the surface of the condyle. The middle sector (4) was located in the region where the disc was thinnest, and three sectors (1-3) were taken anteriorly and three (5-7) posteriorly. Cells with at least 10 grains were regarded as labeled.

Five sections from the central region of each condyle were treated with 0.4% pepsin (P-7000, 1780 U/mg; Sigma, St. Louis, Mo., USA) for 1 h and then with a 1:100 dilution of monoclonal antibodies against collagen type II, a gift from Dr. Holmdahl, Uppsala (15). A Vectastain Elite kit was used for visualization of the sections. The sections and negative controls without primary antibodies were counterstained with hematoxylin. The superior and posterosuperior aspect of the condyle was divided into seven sectors as described above, and the distance of the cell layer secreting collagen type II from the surface of the condyle and the total thickness of this layer were measured in the middle of each sector.

The differences between the groups were tested with Student's *t* test. For evaluation of methodologic error, measurements in 10 condyles were repeated, and the error was calculated using the formula

$$s(i) \frac{\sqrt{\sum d^2}}{2n}$$

Results

The animals tolerated the operations well, and no untoward reactions were noticed except for a 6.5% lack in weight gain in the animals with the lower jaw maintained in an open position and a 3.7% lack in those with the lower jaw protruded as compared with the control animals.

The macroscopic measurements (Table 1) showed that the condylar process grew less in both experimental groups than in the control group, the only exception being growth in a postero-inferior direction, which was

greater in the animals anesthetized with the mandible in an open position (0.14 mm) than in control (0.11 mm) animals, but not statistically significantly so. The growth in height was greater ($P \leq 0.05$) in the animals anesthetized with the mandible in an open position (0.33 mm) than in the animals anesthetized with the mandible in a protruded position (0.28 mm) (Fig. 3).

The direction of mandibular growth (Table 1) had become significantly ($P \leq 0.01$) more vertical in the open-mandible animals than in the protruded-mandible group when measured as the relation between the values for growth in height and length. The result for the control group was between the two.

The numbers of proliferating cells had significantly increased in the posterosuperior regions of the condyle (sector 5, $P < 0.01$, and sector 6, $P < 0.001$) and decreased in the anterior regions in the open-mandible animals relative to the protruded-mandible ones (Fig. 4A), and the same was observed for the control animals, except that the differences were no longer statistically significant. Proliferation had significantly decreased anteriorly (sector 1, $P < 0.01$) with the mandible in an open position and posterosuperiorly (sector 6, $P < 0.001$) with the mandible in a protruded position as compared with the control animals.

The cartilage layer containing collagen type II was significantly thinner in all regions of the condyle (sectors 1-4, $P < 0.01$ and sectors 5 and 6, $P < 0.001$) in the open-mandible animals (Figs. 4B and 5) than in the controls or the protruded-mandible animals. The distance of this from the surface of the condyle did not differ significantly between the groups (Fig. 4C).

The methodologic errors were 1.1 for calculation of the numbers of thymidine-labeled cells and 0.4×10^{-2} mm for measuring the collagen layers and were considered negligible.

Discussion

Although statistically significant differences in the macroscopic measurements were observed between

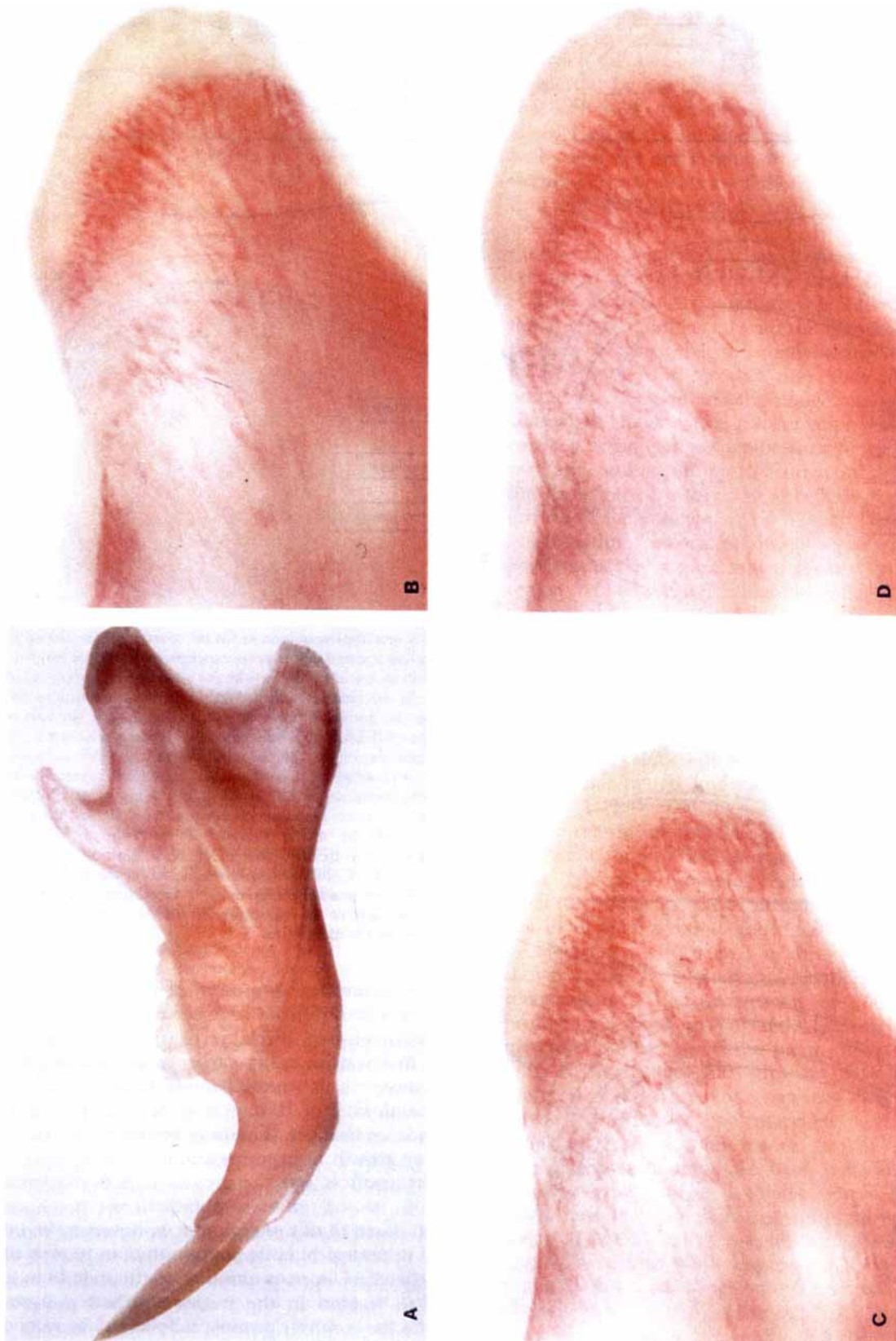


Fig. 3A. The mandibular half of a control rat injected with alizarin red 11 days before being killed. The animal was anesthetized for 6 h/day for 10 days. 3B. A higher magnification of the condylar process of the control rat in 3A. 3C. The condylar process of the mandibular half of an experimental rat anesthetized for 6 h/day for 10 days with the mandible in a protruded position. Alizarin red was injected 11 days before it was killed. The growth of the condylar process has decreased in the superior region relative to the control animal in 3B. 3D. The condylar process of the mandibular half of an experimental rat anesthetized for 6 h/day for 10 days with the mandible in an open position. Alizarin red was injected 11 days before it was killed. The growth of the condylar process has increased in the superior and posteroinferior regions relative to the control animal in 3B.

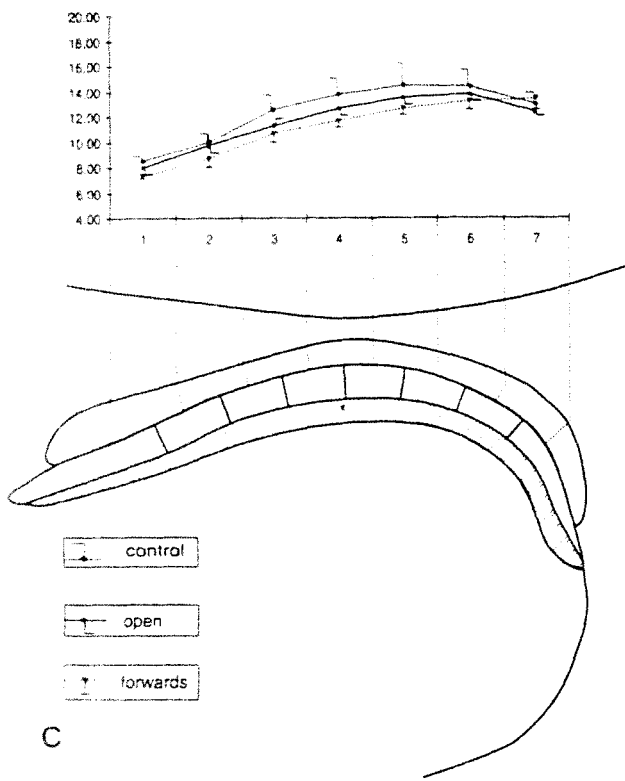
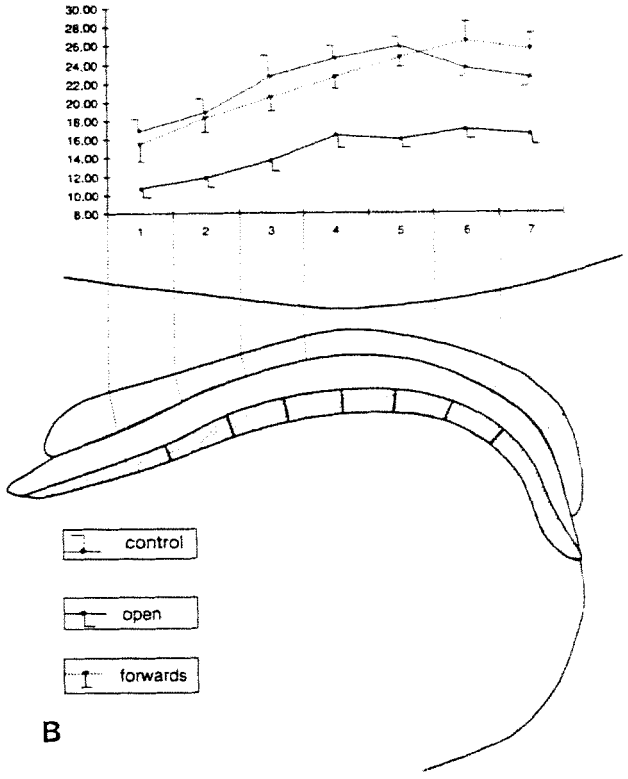
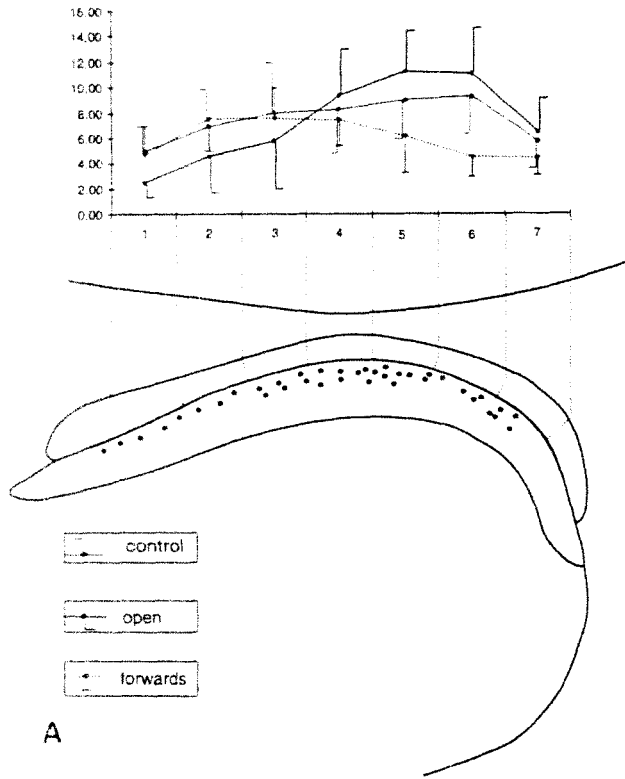


Fig. 4A. Schematic representation of the differences (mean and SEM) in thymidine incorporation between experimental and control rats. Cell proliferation has increased in the posterosuperior region of the condyles in the animals with the mandible in an open position and decreased in the anterior region as compared with the animals in other groups. Likewise, cell proliferation has decreased in the posterosuperior region of the condyle in the animals with the mandible in a protruded position. 4B. Schematic representation of the differences (mean and SEM) found in the thickness (10^{-3} mm) of the cartilage layer containing collagen type II. Its thickness is markedly reduced in all regions of the condyle in the rats anesthetized with the mandible in an open position as compared with the others. 4C. Schematic representation of the distances (mean and SEM 10^{-3} mm) of the cartilage layer containing collagen type II from the surface of the condyle. No marked differences are observed between the groups.

the two experimental groups, the investigation also showed that it is difficult to achieve any major increase in mandibular growth relative to controls, a conclusion that is in line with many earlier observations (14, 16, 18). However, it must be remembered that the observation time of 10 days may be too short to show macroscopic changes. The only tendency to increased condylar growth in experimental animals as compared with the controls was the growth in a posteroinferior direction in the animals anesthetized in an open position. Growth of this region may, however, be more related to periosteal bone apposition than to growth of the cartilage. The open position of the mandible may have led to tension in the tissues attached postero-inferiorly to the condylar process, followed by increased periosteal apposition. This tension concept is supported

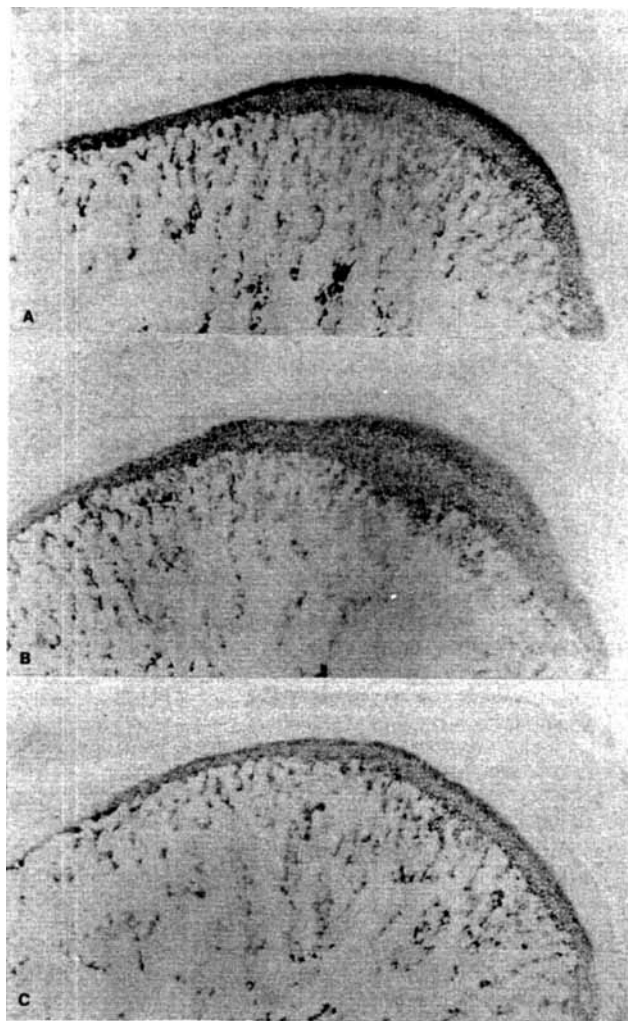


Fig. 5A. Sagittal histologic section from the condylar process of a control rat. The section was stained for the detection of collagen type II by using monoclonal antibodies. (Magnification, $\times 45$). 5B. Histologic section from the condylar process of a rat anesthetized with the mandible in a protruded position. The section was stained for the detection of collagen type II by using monoclonal antibodies. The cartilage layer containing collagen type II is thicker in the posterosuperior region of the condyle than in the control in 5A. (Magnification, $\times 45$.) 5C. Sagittal histologic section from the condylar process of an animal anesthetized with the mandible in an open position. The section was stained for the detection of collagen type II by using monoclonal antibodies. The cartilage layer containing collagen type II is thinner in all regions of the condyle than in the control in 5A. (Magnification, $\times 45$.)

by increased bone apposition on the postglenoid spine of primates in association with prolonged protrusion of the mandible (18).

The growth of the condyle was increased in the anterosuperior region and decreased in the posterior region in animals with the mandible in an open position as compared with those with the mandible in a protruded position. This is obviously related to alterations in pressure-tension conditions. The relief of

pressure in the anterior region of the condyle when the mandible is in an open position resulted in increased vertical growth, and posterosuperior growth was retarded for an analogous reason. This explanation is supported by the observation of Tuominen et al. (21) that increased condylar growth at the macroscopic level is observed in the rat after reducing functional pressure with a soft diet and cutting of the incisors. This increase occurs via acceleration of the maturation of prechondroblasts to chondroblasts and, finally, to fully hypertrophied chondrocytes (22, 23). Hypertrophy of chondroblasts is regarded as an important factor in the volume growth of the condylar cartilage (24).

The observed increased growth anterosuperiorly in the animals anesthetized with the mandible in an open position indicates that the condylar surface faces more to the back, and growth may thus also be more posteriorly focused in the long run. According to Isotupa & Koski (19), trabeculation underneath the cartilage is directed perpendicularly to the surface of the cartilage, and according to Stutzmann & Petrovic (20), the direction of trabeculation reflects the direction of growth of the condyle.

Thymidine incorporation was increased in the posterosuperior region and decreased in the anterior region with an open mandible relative to both the controls and the protruded-mandible animals. The fact that these changes point to an opposite direction from the macroscopic findings reflects the complicated nature of the relationship between individual factors in growth and macroscopic changes. It is common to measure growth by means of cell proliferation, but it can be seen that this does not come into effect until the cells secrete intercellular matrix and have hypertrophied, as stated above.

The reason for the changes in cell proliferation can be sought for in alterations in pressure-tension conditions. The posterosuperior region of the condyles was obviously under increased pressure when the mandible was in an open position, and significantly increased cell proliferation was observed in this region. It has been shown earlier (23) that increased pressure leads to a retardation in the progression of cells out of the proliferation pool, which could lead to the observed increased number of cells in the proliferative layer.

It is significant that growth in a posterior or posterosuperior direction was not increased by a forward traction of the mandible and that height growth decreased. This means that no such tension was generated that would have induced accelerated cartilage growth. The histologic observation that cell proliferation was not increased in the posterior region of the condyle and was even significantly decreased in one sector is in line with macroscopic findings. These findings are confusing when compared with the opposite findings of Charlier et al. (25), for instance. It must be remembered, however, that the appliance most often used for hyperpropulsion, as used by Charlier et

al. (25), causes a significant opening of the mandible as well, and thus it is reasonable to expect a combination of the results.

A significant thinning of the tissue layer containing collagen type II in all regions of the condyle was evident in the animals with the mandible in an open position as compared with the other groups. The occurrence of this thinning in the anterior region of the condyle could be explained by the findings in the rats on a soft diet, assuming that the loading had decreased in the anterior region in the present experiment. Decreased loading leads to accelerated maturation and to thinning of the cartilage layer as the mature cartilage is replaced by bone (21, 23). According to this hypothesis, a thickening would have been expected in the posterosuperior region due to increased loading. Reduction of the layer, however, was seen in this case. It could be speculated that the static pressure affected only the surface layers of the condyle and left the deeper layers unaffected. Thus, near the surface the progression of cells out of the proliferation pool to promote voluminous growth and collagen type-II production would have been retarded, but maturation in the deeper layers, and thereby replacement of cartilage by bone, would not. The net result would have been reduction of the cartilage.

In conclusion, we have shown that it is difficult to increase condylar growth at the macroscopic level. The most rewarding aspect seems to be via the maturation rate of the cartilage cells. Although the effect of functional appliances and situation here may not be directly comparable, it is obvious that several of our findings differ from those made earlier with regard to functional appliances. It is shown here that significant differences exist in the growth of the condylar process between animals with the mandible in an open position and those with it in a forward position.

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