Radioautographic Study of Chondrocytic Proliferation in Nasal Septal Cartilage of the 5-Day-Old Rat

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The precise factors controlling maxillofacial growth are unknown. Researchers (*1*, *2*, *4*, *6–9*, *15*, *16*, *21*) in this field have formulated many hypotheses attempting to account for the mechanisms involved in facial growth and a subject of especially active and prolonged investigation has been the role played by nasal septal cartilage in midfacial development. Many studies (*3*, *5*, *10–14*, *17*, *19*, *20*) involving a variety of experimental animals have been conducted in which parts of the septal cartilage have been extirpated. Results have varied.

In 1968 Moss et al (*10*) removed the nasal septa in 12 and 20-day old rats with electric cautery applied to the full length of the internasal sutural region and found only collapse of the dorsal surface of the nasal cavity, with relatively little retardation of growth of the overall maxillofacial complex. They concluded that "the nasal septal cartilage grows as a secondary, compensatory response to the primary growth of related oro-facial matrices and that mid-facial skeletal growth is *not* dependent upon a primary growth impetus of the nasal septal cartilage." Stenstrom and Thilander (*19*) using a different experimental animal (4 to 7-day-old guinea pigs) and various surgical ablations (removal of the perpendicular plate of the ethmoid removal of the entire septal cartilage etc.) also found only slight growth changes. They concluded that significant skeletal changes could only be established after removal of at least the anterior two-thirds of the nasal septum and that these changes were not due to retarded growth but to decreased snout stability because of loss of septal support. Bernstein (*3*) reported similar results in his study of 4–6 week-old mongrel pups

However in 1973, Kvinnsland and Breistein (*5*) surgically excised the septa in a series of rats ranging in age from day of birth to 28 days and reported dramatic skeletal changes but only when the midportion of the cartilaginous nasal septum was removed at birth or at 14 days. Considerably less change was found when animals were operated on at later ages (21 and 28 days).

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Such diverse findings would appear to indicate that there are several contributing factors to the varied results being reported. Among these are: 1) The several species of animal on which surgery has been performed, 2) the surgical techniques employed, 3) the precise portion of septum removed and 4) the age at which surgery takes place.

In an attempt to provide meaningful data on two of these variables (precise portion of septum to be removed and optimum age of animal at time of surgery), a series of experiments is being undertaken in our laboratory utilizing radioactive tracers. Patterned after a study by Long, Gruelich and Sarnat (*8*) (in which tritiated thymidine was used to label the growing septal cartilage in a young rabbit) similar techniques are being used to determine the location of sites of high and low chondrocytic proliferative activity in the nasal septum of the growing rat. It is assumed that once such growth sites are identified, selective extirpation of these areas should cause facial deformity *if* the cartilaginous nasal septum is a primary growth center and *if* these centers are maximally active at the time of surgery.

Data have been reported previously (*17*) concerning such sites in the 10-day-old albino rat and similar information is being collected currently from in-utero and newborn rats. The present study reports data collected from the 5-day-old rat.

Materials and Methods

The laboratory animal used in this study was the 5-day-old albino rat.[1] Seven rats from the same litter were selected on the basis of uniformity in size and weight. Males and females were chosen indiscriminantly. Each animal was weighed and a dose of tritiated thymidine[2] in the concentration of one microcurie (specific activity 6.7 Ci/mM) per gram of body weight was injected intraperitoneally.

One hour after injection, each rat was killed by ether inhalation and the nasal septum (Figure 1) was removed, using the following surgical approach: The head was divested of its integument by making a circular incision at the neck line and peeling this tissue anteriorly over the snout. The anatomic midline between the nasal bones was identified and a posteroanterior incision was made through the skull; the incision began approximately 5 mm. posterior to the nasofrontal suture, immediately lateral to the midline. The cut was extended completely through the head and mandible, moving anteriorly through the end of the snout. A second cut was made from the posterior end of the first incision laterally through the temporal bone and body of the mandible. The nasal bones were dissected free, the septum was disengaged posteriorly at the spheno-occipital synchrondrosis and was placed in a vial of 10% buffered formalin[3] for 48 hours. Each septum was washed in tapwater for 4 hours and demineralized (chelated) in EDTA for 36 hours. Subsequently, each septum was washed in running tapwater,

[1] Sprague-Dawley Co., Madison, Wisconsin.
[2] New England Nuclear Corpn., Boston, Massachusetts.
[3] Calcium acetate, 20 gms/liter.

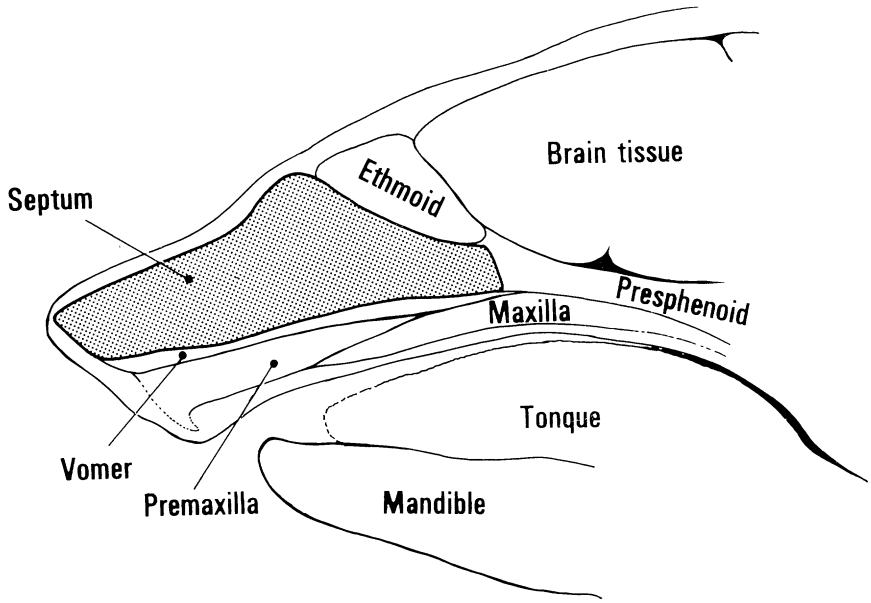

FIGURE 1. Midsagittal section of 5-day-old rat head shows relationship of cartilaginous nasal septum to contiguous skeletal structures.

rinsed in distilled water, run through a series of graded alcohols (50% to absolute), cleared in xylene and embedded in Paraplast.[4]

The resultant blocks were oriented so that each septum could be sectioned anteroposteriorly (i.e., coronally) on a rotary microtome set at 6 μm. Every tenth section was mounted, four sections per slide, so that each slide represented a 240 μm section of septum. After the mounting of sections on slides, each was dip-coated with photographic emulsion[5] in a darkroom illuminated by a Wratten series no 2[6] safelight at a distance of 3 feet. After two weeks' exposure time, the slides were developed, fixed, allowed to dry and stained metachromatically with toluidine blue. An arbitrary decision was made to perform counting procedures on the first and third sections of each slide. Thus, for each rat, the sections on which counting was performed were approximately 120 μm apart.

Results

Technical difficulties in sectioning the seven labeled septa led to deletion of two from the portion of the study reported here. Data in this manuscript, therefore, are from grain counts on sections from five rats.

The counting of labeled cells was done on a Leitz[7] binocular microscope using 10× oculars and a 43× objective with a 1.25× tube increment.

[4] Fisher Scientific, Chicago, Illinois.
[5] NTB-3, Eastman Kodak Co., Rochester, New York.
[6] Eastman Kodak Co., Rochester, New York.
[7] Ernst Leitz, GMBH, Wetzlar, West Germany.

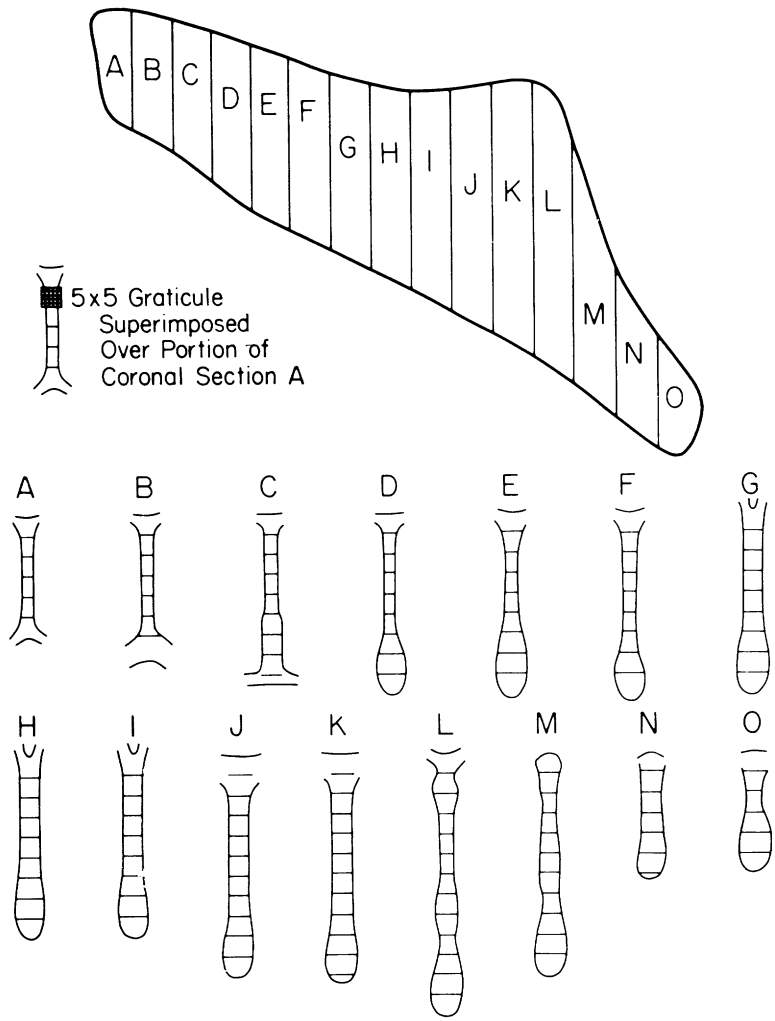

FIGURE 2. The nasal septum was divided arbitrarily into 15 separate parts for purposes of this study. Coronal sections in lower half of figure represent average size and shape of sections taken from corresponding portions of midsagittal septum seen above.

Within the right ocular was placed a 5 × 5, 25-square graticule[8] measuring 10 mm. on each side, with each subdivisional square being 2 mm. × 2 mm. The glass slides were positioned on the microscope stage so that the top edge of the septal tissue coincided with the top edge of the graticule. An outline (Figure 2) of that portion of the septum was drawn to scale on a piece of 20 × 20 graph paper[9] whose squares were also divided into 25 smaller subunits. Grains appearing in a particular subunit of the graticule were recorded in the appropriate square on the graph paper, resulting in precise

[8] Ernst Leitz, GMBH, Wetzlar, West Germany.
[9] Eugene Dietzgen Co., Chicago, Illinois.

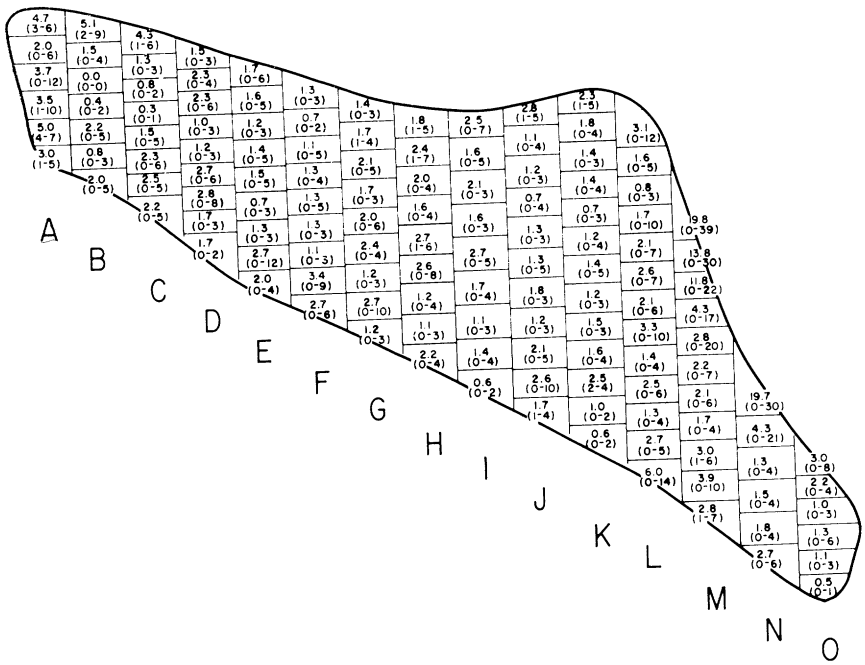

FIGURE 3. Mean counts of labeled cells in representative fields of the cartilaginous septa of 5-day-old rats; range is shown in parentheses. (Because of the relatively small area examined in one graticular field and because of the random and haphazard distribution of grains, it was not unusual to find one field completely devoid of label while a contiguous field showed a relatively high grain count. This caused the range to be unrealistically exaggerated.)

spatial orientation within the lateral outlines of the septum. After all grains within the boundaries of the graticule (and septum) were recorded, the slide was moved upward so that the top boundary of the new section to be counted coincided with the bottom boundary of the preceding section. This process was repeated until the entire length of coronal section was reconstructed on graph paper and all metallic grains were properly recorded.

Figure 3 represents a midsagittal reconstruction of the 5-day septum from the coronal sections seen in Figure 2. Mean data and ranges derived from the labeled cell counts of the five septa are tabulated. Figure 4 is a graphic representation of the same data. The following generalizations can be made from these data: 1) The region of highest chondrocytic proliferative activity is found at the septoethmoidal junction. Counts in this area (some in excess of 19 grains/field) are considerably higher than in any other region. 2) The area of second highest proliferative activity is in the anterosuperior portion of the septum. 3) Other areas vary in intensity of cellular proliferation but none establishes a definite pattern of increase or decrease in activity.

Discussion

The proliferation of chondrocytes within the nasal septum obviously contributes to the mass of this structure. Cells, however, are not the only

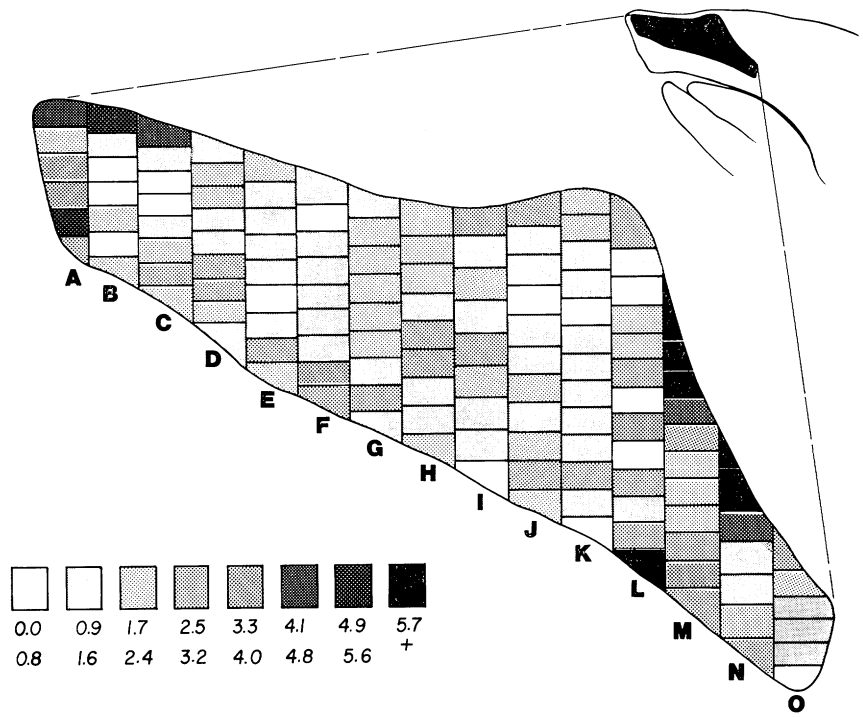

FIGURE 4. Graphic midsagittal reconstruction of rat nasal septum depicts relative area differences in chondrocytic proliferation. Numerical figures below septum represent average numbers of labeled cells per microscopic (graticular) field counted.

component of the cartilaginous septum. In fact, they are less conspicuous than the intercellular matrix in which they lie. Therefore, a pilot study (*18*) has been done in this laboratory to determine the approximate contribution of the intercellular matrix to the overall mass. Radioactive sulfur (S-35), in various concentrations, was injected into 5-day rats and the labeling of septal cartilaginous matrix was observed microscopically. Labeling was so intense, even in moderate dosages, that grain counts were deemed impractical to carry out.

However, upon observing the tissues at low microscopic magnification, it became obvious that the radioisotope was distributed pretty much uniformly, with no one area showing appreciably higher concentrations than any other. Armed with this information, it seemed reasonable to assume that all cells are synthesizing matrix at a more or less constant rate. If this is true, it would also seem reasonable to identify those regions with a higher concentration of cells as areas of greatest growth, since the total amount of intercellular matrix produced would be in direct proportion to the number of cells in a given portion of septum.

Assuming this to be correct, growth sites can be identified just anterior to the septoethmoidal junction (presumably an area of endochondral bone

formation) and in the anterosuperior portion of the septum. This is somewhat at variance with earlier results of Searls and Kinser (*17*), in which they reported, in the 10-day rat, the area of greatest activity to be the presphenoidal tail, although the septoethmoidal junction also showed a high rate of proliferation. The presphenoidal tail in the 5-day rat is relatively inactive (Figure 3). The 10-day rat also showed high proliferation in the center of the septum, an area that is spotty in the 5-day rat. In the latter, very high grain counts were observed in the anterosuperior portion of the septum, a region which showed virtually no activity in the older rat. Thus, it would seem that growth sites, in the rat septal cartilage at least, change as the animal matures. Those areas (with the exception of the septoethmoidal junction) which show high cellular proliferation in the 5-day rat are relatively quiescent in the 10-day rat. This would indicate that some growth sites do shift, thus making selective extirpation of portions of the nasal septum a haphazard manipulation unless proliferative activity has been charted beforehand.

Summary

Relative rates of chondrocytic proliferation in the 5-day-old rat nasal septum are determined radioautographically, using tritiated thymidine as the labeling agent. The results are tabulated and charted graphically; they show that the zone of greatest activity is the portion just anterior to the septoethmoidal junction. The area of second highest activity is the anterosuperior tip of the septum. All other regions are either relatively inactive or show only sporadic areas of proliferative activity.

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