

X-Ray And Alizarin Studies On The Effect Of Bilateral Condylectomy In The Rat

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INTRODUCTION

In the last decade a number of authors have concluded that condylectomy in the rat causes remarkably little impairment in masticatory function and in the postnatal development of the mandible.^{1,2,3,4} Repair, including the development of a neoarthrosis and the growth of a new condylar process, has been described by Jarabak⁴ and analyzed by Jolly.⁵ In x-ray studies on growing animals near-normal dimensions of the condylectomized mandible have been observed. More recently, the seemingly close similarity between the condylectomized and the normal mandible has led some authors to repudiate the thesis that the condylar cartilage is the primary growth center of the mandible.⁶

No investigation directly comparing sites and rates of bone apposition in the intact and the condylectomized rat mandible seems to have been made. The present study was designed to fill this gap using Alizarin Red S as a bone marker. At the same time x-ray pictures were used to obtain a detailed comparison of dimensions at as many points as possible.

It was hoped that the combined use of two methods might help to clarify the role of the condyle in the postnatal

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development of the rat mandible.

MATERIAL AND METHODS

The present study is based on material obtained from forty-eight male albino rats of Charles River strain. One control group of ten animals was killed at 35 days of age. On nineteen animals a bilateral condylectomy was performed at 35 days of age. Ten of these were allowed to survive to 52 days and nine to 66 days of age. Ten days before sacrifice, they were given intraperitoneal injections of $\frac{1}{2}$ cc per 100 gm body weight of a 2 percent solution of Alizarin Red S in tap water. The same numbers of littermates, ten and nine animals, served as controls. They received alizarin 10 days before sacrifice and were sacrificed at the same ages as the experimental animals.

Operative Procedures: 1.2 cc per 100 grams of body weight of an intraperitoneal injection of 0.3 percent sodium pentobarbital in 10 percent ethyl alcohol was used to induce anesthesia. Anesthesia was obtained in 5-7 minutes and lasted for about half an hour. A few animals required reinforcement with ether. The skin over the condylar region was shaved and prepared with tincture of iodine and alcohol. Sterile instruments and drapes were used, but strict asepsis was found unnecessary. Only one animal had a superficial abscess following operation. This was incised and drained without further untoward effects. The surgical approach was similar in all essentials to that of

Jolly.⁵ Terramycin was added to the drinking water of all animals. They were given milk and powdered purina mouse food in addition to the standard purina rat pellets.

Preparation of Material: The animals were killed with ether. The heads were severed, skinned, and fixed in 10 percent buffered formalin solution. They were then halved, and the mandibles separated carefully from the cranium and cleaned.

Roentgenograms of the mandibles were taken on Kodak occlusal regular film and enlarged in order to improve the accuracy of measurement. To minimize distortion, the prints were made on Kodak commercial esterbase film.

Following roentgenography the mandibles were divided into antemolar, molar and postmolar segments. Antero-posterior ground sections of a thickness of 250-300 μ were made of antemolar and molar segments. The ground sections and the intact postmolar segment were dehydrated, cleared and mounted on troughed slides.

Measurements from Roentgenograms: In order to measure changes in size of mandibles without condyles, it was necessary to select landmarks that might give a representative picture. In a great number of trials those landmarks were chosen that yielded, in the control groups, measurements with reasonably small variability.

Length (Figure 1): A line through the two lowest points on the inferior border of the mandible was taken as the baseline. The measurements were made between projections of the landmarks on this line. The following points were used: (1) anterior edge of alveolar bone at the concavity of the incisor; (2) most anterior point of the crown of the first molar; (3) most posterior point of the crown of the third molar;

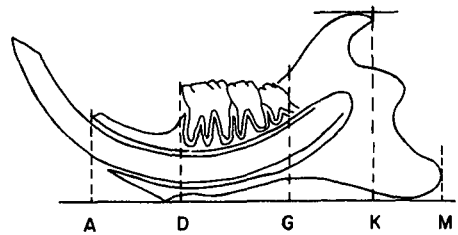


Fig. 1 Tracing of a magnified roentgenogram of a condylectomized mandible. Length measurements were taken between the projections of landmarks on the baseline.

- (4) tip of the coronoid process and
- (5) tip of the angular process.

Height (Figure 2): Height measurements were taken along lines at right angle to the baseline. The landmarks for placing these lines were the following: (1) a point midway of the length of the antemolar segment (B); (2) the lowest point on the inferior border of the mandible in the antemolar segment (C); (3) the highest point of the posterior interradicular septum of the first molar (E) and (4) the highest point of the interradicular septum of the second molar (F). The distance between the projections of the posterior end of the third molar and the most posterior point of the incisor socket was divided into three equal parts. The next three measurements were taken at (5) the border between anterior and

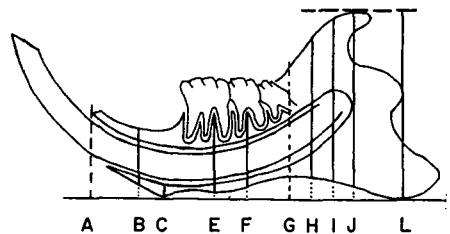


Fig. 2 Tracing of a magnified roentgenogram of a condylectomized rat mandible. Height measurements were taken on the solid lines.

middle one-third (H); (6) the border between middle and posterior one-third (I) and (7) at the posterior end of the incisor socket (J); (8) the measurement of maximal mandibular height was taken between the baseline and a parallel line touching the upper border of the coronoid process (L).

Growth between 35 and 52 days and between 52 and 66 days was determined as the difference in average size obtained from the magnified roentgenograms at the respective ages. After correction for the magnification, the differences were converted to microns of change per day in order to permit correlation with the alizarin measurements.

Alizarin Measurements: The distance between alizarin line and the border of the bone indicated the amount of bone laid down during ten days. The measurements that could be made were restricted by the diffuse deposition of alizarin in some areas. Except for measurements in the tooth sockets, which will be reported separately, bone apposition could be quantified at the anterior end of the incisor socket and at the four sites indicated in Figure 3. In the concavities of supracondylar and infracondylar notches the shortest distances from the alizarin line to the edge of the bone were measured at four to six neighboring points and an average determined. A similar procedure was followed at the tip and at the lower border of the angular process, as well as at the anterior edge of the incisor socket.

FINDINGS

Food Intake: All of the operated animals started taking soft food and milk within twenty-four hours after surgery, but it took most of them five or six days before they could eat the hard pellets. Some ate hard pellets for the first to second postoperative days, then switched to soft food. During the later

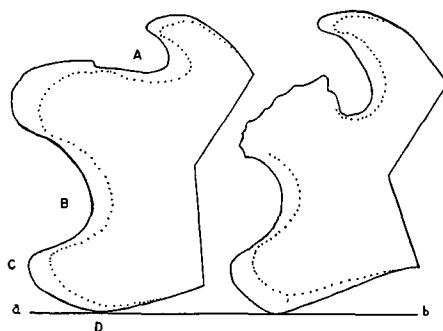


Fig. 3 Camera lucida drawings of the posterior parts of rat mandibles. Broken lines represent alizarin lines. 52 day old animals, 10 days after alizarin injection. Left: control. Right: 17 days after condylectomy. A. supracondylar notch; B. infracondylar notch; C. tip of angular process and D. lowest point of angular process. ab: line placed at the same angle as the base line shown in Figures 1 and 2. Magnification X 13.

days there was no difference in the number of hard pellets consumed by the control and experimental animals.

The injection of alizarin had a depressing effect on food intake in both the operated and control groups. For a day or two following alizarin the average number of pellets consumed was less than one, and gradually increased to three to four in the next seven days.

No abnormality was detected in the masticatory function of the operated animals. Although during some of the postoperative time their intake of hard food was smaller than that of the controls, all maintained their incisors adequately abraded. None showed restriction in extent and freedom of passive mandibular movements at autopsy.

Body Weight: The operation caused a mild drop in weight (2-3 grams) which was made up in a few days. The injection of alizarin caused a drop in weight of greater magnitude (7-16 grams), and it took the animals about five days to recover their previous weight. Control and experimental ani-

TABLE I
Average Changes in Length of Segments of the Mandible Between 35 and 52 Days of Age (From roentgenograms)

Group	Ante-molar Segment	Post-molar Segment
Control		
μ per day	36.5	160.6
Experimental		
μ per day	27.6	150.0
% of control value	76	93

mals had the same reaction to alizarin and the same average weights at autopsy.

Changes in Length

Since the anteroposterior extension of the mandible increased by apposition at the anterior and posterior margins, the effects of condylectomy on the length of both the ante- and the post-molar segment were considered.

35-52 Days; Roentgenograms — Controls: Between 35 and 52 days, the daily increase in length of the antemolar segment averaged 37 μ, and the increase of the posterior segment 161 μ (Table I). The molar segment remained unchanged within the limits of accuracy of the roentgenographic measurements.

Experimental: Both anterior and posterior segments of the mandible in-

creased in length in the condylectomized animals as well. The increase in size of the anterior segment was 76 percent of that in the controls, the increase of the posterior segment was 93 percent of the control increase. The molar-bearing segment remained unchanged.

These findings suggested continued increase in size of the condylectomized mandibles at rates that were moderately reduced anteriorly and near normal posteriorly.

42-52 Days; Alizarin: By means of alizarin injections bone apposition could be measured at the anterior end of the mandible and at three points along the posterior border (Table II). In addition, measurements could be made at the mesial walls of the tooth sockets. Since these do not relate to the growth of the mandible but to the antero-posterior position of the molars, they will not be reported here.

Controls: The measurements of bone apposition between 42 and 52 days of age confirmed the x-ray finding that there was growth in both segments of the mandible. Slowing of growth with age probably accounts for the smaller average increments given by the alizarin measurements.

Experimental: For the experimental animals also, the alizarin measurements confirmed the x-ray finding that at both

TABLE II
Average 10-Day Apposition of Bone at Anterior and Posterior Borders of Mandible Seen at 52 Days of Age (Alizarin)

Group	Anterior Border	Posterior Border		
		Tip of Angular Process	Supracondylar Notch	Infracondylar Notch
Control				
μ per day	23.1	78.9	79.0	79.2
Experimental				
μ per day	18.0	83.4	59.2	49.2
% of control value	78	118	75	62

TABLE III

Average Changes in Length of Segments of the Mandible Between 52 and 66 Days of Age (From roentgenograms)

Group	Ante- molar Segment	Post- molar Segment
Control		
μ per day	38.6	95.0
% of value in younger controls	106	59
Experimental		
μ per day	25.0	102.1
% of value in younger controls	65	107
% of value in younger experimental group	90	68

anterior and posterior ends of the condylectomized mandible growth continued between 7 and 17 days after condylectomy.

At the anterior end, the rate of apposition was 78 percent of the rate in the controls, a retardation comparable to that observed in the x-ray pictures.

At the posterior end, where the controls showed identical rates of bone formation at the three sites of measurement, the rates differed in the experimental animals. At the supracondylar notch there was a moderately reduced rate and at the infracondylar notch a more markedly reduced rate of apposition. But at the tip of the angular process, bone apposition was in excess of that in the controls.

More rapid apposition than in the controls probably did not commence before the 10 day period covered by alizarin. In the first postoperative week, bone formation may have been impeded by operative trauma. This may account for the x-ray finding of a slightly retarded growth of the postmolar segment.

52-66 Days; Roentgenograms — Controls: At the anterior segment the rate of increase was similar to that in the

younger control animals, but growth of the posterior segment was slower than at the younger age. The molar segment remained unchanged (Table III).

Experimental: In the experimental animals, increase in size between 17 and 31 days after condylectomy continued as in the earlier period, both in the ante- and postmolar segments.

Growth at the anterior segment was 65 percent of that in the controls which represents a slightly more severe retardation than before. Growth of the posterior segment was slightly in excess of that in the control animals.

56-66 Days; Alizarin — Controls: Alizarin lines could be seen in the same four locations as in the younger age group. The measurements in the controls showed apposition at all four sites to proceed at about two-thirds of the rates at the younger age (Table IV). As before, uniform rates were seen at the three points along the posterior margin of the mandible.

Experimental: Bone apposition in the condylectomized group also continued at all sites where it had been observed at the shorter postoperative interval. Except for a more severe reduction at the infracondylar notch, the rate changes from the first to the second period after condylectomy were similar to those in the controls, thus attributable to the increased age of the animals.

The rate of apposition at the anterior border was now almost the same as in the controls, the rate at the supracondylar notch remained moderately reduced, and the rate at the infracondylar notch was somewhat more reduced than before. Apposition at the tip of the angular process was still in excess of the control rate which explains the x-ray finding of greater length of the postmolar segment in the condylectomized animals.

TABLE IV
Average 10-Day Apposition of Bone at Anterior and Posterior Borders of Mandible Seen at 66 Days of Age (Alizarin)

Group	Anterior Border	Posterior Border		
		Tip of Angular Process	Supracondylar Notch	Infracondylar Notch
Control				
μ per day	16.6	52.4	48.4	51.8
% of values in younger controls	72	66	60	65
Experimental				
μ per day	15.3	55.6	38.2	22.8
% of value in controls	92	106	79	44
% of value in younger experimental group	85	67	65	46

The distortion of the growth pattern, manifested by different amounts of apposition at different levels on the posterior border, was slightly more marked than at 17 days after condylectomy.

Changes in Height

35-52 Days; Roentgenograms — *Controls*: Between 35 and 52 days of age the average height of the mandible in the control group had increased at all sites of measurement (Table V and Figure 2). The increase was slightest at a point just posterior to the third molar (H) and greater anteriorly and posteriorly, reaching its maximum between the upper border of the coronoid and the lower border of the angular process (L).

Experimental: Despite condylectomy 17 days previously, the experimental group also showed increases in height at all sites of measurement. In the antemolar segment and in the molar area the increases were of similar magnitude as in the controls and even tended to a slight excess. In the anterior part of the postmolar portion the condylectomized mandibles were slightly less high than the controls, but in the region of the angular process they were significantly higher.

42 and 52 Days; Alizarin — The distribution of alizarin in control and experimental animals seemed to be generally similar, but (excepting measurements at the tooth sockets) accurate measurements were feasible only at the

TABLE V
Average Changes in Height of the Mandible Between 35 and 52 Days of Age (Sites of Measurement Listed in Anteroposterior Direction; see Figure 2) (From roentgenograms)

Group	Site of Measurement						
	B	E	F	H	I	J	L
Control							
μ per day	29.4	62.9	57.6	43.5	74.7	86.5	100.0
Experimental							
μ per day	30.0	70.6	58.8	36.5	62.9	81.6	125.9
% of control value	102	112	102	84	84	95	126

TABLE VI

Average 10-Day Apposition of Bone as Measured at Lower and Estimated at Upper Border of the Mandible at 52 Days of Age (μ per day)

Group	Apposition at Lower Border (Alizarin)	Total Increase in Height (X-ray)	Apposition at Upper Border (Estimate)
Control	52	100	48
Experimental	80	126	46

lower border of the angular process.

Controls — At this site apposition in the control group averaged 523 μ in 10 days (Table VI).

Experimental: In the condylectomized group the apposition averaged 800 μ or an excess of 53% over the amount in the controls. This explained the greater increase in height in this region seen in the x-ray pictures.

Since bone apposition at the upper border of the coronoid process could not be measured accurately because here the alizarin lines were indistinct, measurements at the lower border in conjunction with the measurements of the increase in total height on x-ray pictures were used to estimate it.

In the control animals the average

daily increase at the upper border was estimated as 48 μ which was slightly less than half of the total gain in height. In the condylectomized group the estimate at the upper border was 46 μ . This was less than a third of their total increase in height and slightly less than the estimate in the controls. Thus the condylectomized mandible owed its advantage in total height seen on the x-ray pictures to the abnormally rapid apposition of bone at the lower border of the angular process.

52 and 66 Days; Roentgenograms — *Controls*: Between 52 and 66 days of age increases in height in the controls were less uniform in the different anteroposterior segments and were slower than during the younger age span (Table VII).

In the antemolar and molar segments the rates were about two thirds to three fourths of the rates at the younger age (Table I). In the anterior part of the postmolar segment, no changes, or a diminution of height, were noted. Posteriorly, the rates again rose to two thirds of the rates at the younger age.

Experimental: As in the immediate, so in the later postoperative period, increases in the height of the condylect-

TABLE VII

Average Changes in Height of the Mandible Between 52 and 66 Days of Age (Sites of Measurement Listed in Anteroposterior Direction; see Figure 2) (From roentgenograms)

Group	Site of Measurement						
	B	E	F	H	I	J	L
Control							
μ per day	22.9	42.9	37.9	-15.0	-0.7	36.4	70.7
% of value in younger controls	78	68	66	—	—	42	70
Experimental							
μ per day	10.0	17.1	18.6	-14.3	10.7	42.1	67.1
% of value in controls	44	40	49	—	—	116	95
% of value in younger experimental group	30	24	32	—	17	52	53

tomized mandible were noted at all points where increases were noted in the controls. Where the control animals showed stationary or declining dimensions, a similar lack of increase in height was observed in the condylectomized group.

There was, however, in the condylectomized group a sharp decline from the previous rates of growth at all points of measurement. Instead of increases in excess of those in the controls, as in the earlier period, rates less than half those in the controls were measured in the antemolar and molar segments, and rates like those in the control group in the segment posterior to the incisor socket. When the older animals are compared with the younger ones, the decline in growth rates in the later postoperative period appears even steeper. Whereas in the controls the rates in the older animals were two thirds to three fourths of the rates in the younger ones, a decrease to a half or less had occurred in the condylectomized group.

56 to 66 Days; Alizarin — Controls: In the controls bone apposition at the lower border of the angular process was reduced to 68% of the rate at the younger age, or 36 μ per day (Table VIII).

Experimental: In the condylectomized group apposition at this site was

reduced to 60% of the rate at the younger age and was 48 μ per day. Though it was still more rapid than in the controls, the excess was no longer marked.

The estimated amount of bone apposition at the upper border of the control mandible was 35 μ , or again about half of the total increase in height, 71 μ , seen on x-ray pictures. By contrast, the corresponding estimate for the operated group was 19 μ of a total increase of 67 μ . According to this estimate the increase at the upper border of the coronoid process was reduced to 53% of that in the controls. This was the same degree of retardation as that shown by x-ray in the antemolar and molar segments.

Additional Measurements — Two sets of roentgenographic measurements, one in anteroposterior direction and one of height, were not included in the tables because precise figures were difficult to obtain.

The anteroposterior measurement was that of the distance between the anterior end of the incisor socket (A, Fig. 1) and the tip of the coronoid process (K). It showed the average rate of increase in the experimental group to be by a third higher than the control rate between 35 and 52 days and slightly lower between 52 and 66 days. At both times the absolute dimensions were larger in the condylectomized group.

The vertical measurement, at C, measured the thickness of the convex alveolar bone of the incisor (Fig. 2). It showed the rate of increase in control and experimental animals to be similar between 35 and 52 days, and the experimental rate to be in excess of the control rate by about 50% between 52 and 66 days.

The alizarin measurements made at the fundus and at the interradicular crest of the molar alveoli, to be reported

TABLE VIII

Average 10-Day Apposition of Bone as Measured at Lower and Estimated at Upper Border of the Mandible at 66 Days of Age (μ per day)

Group	Apposition at Lower Border (Alizarin)	Total Increase in Height (X-ray)	Apposition at Upper Border (Estimate)
Control	36	71	35
Experimental	48	67	19

separately, showed apposition at these sites to continue in the condylectomized animals. The rate of apposition was only slightly retarded in the first and severely retarded in the second postoperative interval.

DISCUSSION

The present study of the effects of bilateral condylectomy in the rat confirmed previous investigations in showing that there was little impairment in masticatory function and that the condylectomized mandible continued to increase in size.

The anteroposterior dimensions as measured on x-ray pictures at 52 days and at 66 days were almost identical in the control and experimental animals. This might have been taken to indicate undisturbed increase in size of the condylectomized mandible. However, the combination of x-ray and alizarin measurements and consideration of growth at the anterior as well as the posterior border showed an altered pattern of growth in length in the experimental animals; the apposition of bone at the anterior border was retarded, growth in the upper half of the posterior border was retarded also, but excessive bone apposition occurred at the tip of the angular process. The deviations from normal growth were similar 17 and 31 days after removal of the condyles.

As to increase in height, growth at all points of the antemolar and molar segments was found to have been approximately maintained 17 days after condylectomy. Between 17 and 31 days, the rates declined sharply to half the control rates. In the posterior region the x-ray findings suggested growth in height in excess of normal up to 17 days and at normal rate between 17-31 days. The alizarin findings revealed at both time periods an excessive apposition of bone at the lower border of the angular

process. This simulated true growth in height and accounted for the larger than normal vertical dimensions seen on x-ray pictures at 17 days postoperative and for the normal dimensions seen at 31 days.

Thus growth in length did not, but growth in height did, suffer a sharp reduction of rate, largely masked on roentgenograms, in the later postoperative interval. With this qualification height as well as length of the condylectomized mandible may be said to have continued their increase, both following a distorted pattern.

The increase in length and height in the absence of the condyles might be taken as evidence against the leading role of the condylar cartilage in the growth of the rat mandible. We believe that such a conclusion would be in error.

To understand the difference in the growth of the normal and the condylectomized mandible it seems useful to distinguish between two modes of enlargement of a skeletal element. Normally, growth of bones is part of the development of the individual animal and follows an inherited pattern which determines not only their size but also their form. When pathology or experiment interfere with the normal harmony of growth and the functional balance of the skeletal elements, adjustments may occur, and these may include enlargement of bones. Such enlargement may be termed *adjustive growth* in contrast to the normal postnatal or *developmental growth*.

One of the characteristic signs by which adjustive differs from developmental growth is the distortion of the enlarged skeletal element.

This characteristic applies to the enlargement of the condylectomized mandible. The most significant result obtained by combining roentgenogram and alizarin measurements can be sum-

marized by stating: Though the condylectomized mandible may attain nearly normal size, its shape and proportions differ from those of the normal rat mandible (Fig. 3).

In the normal animal the positioning of the mandible in relation to maxilla and premaxilla is adjusted by condylar growth (which causes a forward and downward shift of the mandible) and is maintained by function of the craniomandibular articulation. In the condylectomized rat it is the musculature alone that keeps a functionally satisfactory position of the mandible. The functional balance of the mandibular musculature may well in part be established and maintained by proprioceptive reflexes from the periodontal ligaments, and it may be this mechanism that enables the condylectomized rat to maintain a fairly normal pattern of incisor wear and fairly normal masticatory movements, as described by Jolly.⁵

Jolly's study showed that the condylectomized mandible was in nearly normal position. There was a very slight degree of posterior displacement and of angular change of the occlusal plane. Jolly stressed the point that removal of the condyles, which entails removal of the lateral pterygoid muscles, disturbs the muscular balance in the rat to a much lesser degree than it does in man or in the monkey. In these, the protrusion of the mandible is exclusively the function of the lateral pterygoid muscles. In the rat these muscles are small and insignificant, and protrusion is the function of the superficial, almost horizontal, bundles of the masseter. This is the reason why, following condylectomy, the forward shift of the mandible remains almost normal in the rat. The downward shift, evidenced by increase of mandibular height in the molar area and eruption of the molars, does not give as clear a

picture. It is possible that under the new muscular balance the downward shift also is accomplished by the mandibular musculature, including the depressor of the mandible, the digastric muscle. The loss of the condyle and therefore the loss of the articulation as a center of rotation may lead to a compromise between pure translation and pure rotation by the balanced pull of elevators, protrusors and depressors. The decline of vertical growth and of eruption in the second postoperative period seems to indicate that the downward shift is less effectively maintained by the musculature than is the forward shift.

In the condylectomized mandible some areas were found to enlarge beyond normal, thereby masking the retardation in developmental growth: the alveolar bone of the incisor at the lower border of the mandible and the coronoid process to some degree and the angular process to a very marked degree. These are regions of attachment for the digastric, temporal, medial pterygoid, and masseter muscles, and the probable cause of their hypertrophy is the hypertrophy of the inserting muscles. Since the rat mandible that has lost its bracing at the craniomandibular articulation is nevertheless maintained in a satisfactory relation to the maxilla, it is not surprising to find evidence of hypertrophy resulting from the increased muscular load.

One could therefore say that the rat mandible adjusts to condylectomy by hypertrophic growth: by muscular hypertrophy primarily and under the stimulus of hypertrophic muscles by the enlargement of their bony attachments.

SUMMARY AND CONCLUSIONS

1. The enlargement of the condylectomized mandible was examined in nineteen male rats subjected to bilateral condylectomy at 35 days of age. The

animals were studied 17 and 31 days after condylectomy. They were injected with Alizarin Red S ten days before sacrifice. Two series of measurements were made, one on roentgenograms and one on suitably prepared alizarin-marked segments of the mandible.

2. In agreement with the work of others, the x-ray measurements of the main dimensions showed that at both time periods the condylectomized mandibles were as large or larger than those of the controls.

3. X-ray measurements of subsidiary dimensions and especially measurements on the alizarin preparations showed that in the condylectomized mandibles the pattern of bone apposition was altered and the shape distorted.

4. Bone apposition was in excess of normal in the areas that serve as attachment for mandibular muscles: alveolar bone of the incisor socket at the lower border of the mandible, coronoid process and especially angular process.

5. Since in the condylectomized rat mandible the musculature achieves and maintains the functional relation between mandible and maxilla, it appears likely that the muscles hypertrophy, thereby causing hypertrophy of their main bony attachment areas.

6. The combined use of roentgenograms and alizarin led to the conclusion that normal "developmental" growth does not occur in the absence of the condylar growth center. "Adjustive" growth does occur, and during the periods studied results in a mandible of approximately normal overall size but distorted shape.

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