Aspects of Growth in the Cranium, Mandible, and Teeth of the Rabbit as Revealed Through the Use of Alizarin and Metallic Implants

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Our knowledge of osseous growth in the cranium has come from many sources. Important studies have been carried out in man, using cephalometric roentgenograms, and in living animals through the use of cephalometric procedures, metallic implants and vital dyes. Broadbent, in 1930, commenced a long-range growth study on humans in the course of which he developed a reliable instrument for the taking of cephalometric head films.[6] Through the use of this instrument, the Broadbent-Bolton cephalometer, Broadbent has been able to record craniometric points accurately and repeatedly over many years.

Cephalometric studies have since been carried out on a variety of animals. To facilitate this type of work, Jarabak designed an animal-head fixation attachment which could be placed on the Broadbent-Bolton cephalometer.[12] Recently (1953) Selman and Sarnat succeeded in further adapting the cephalometer to the anatomy of the rabbit head by a change in the head post angulation of the apparatus.[27] An attachment similar to that of Jarabak's was adapted for the taking of head plates during the present study.

Metallic implants have been used on numerous occasions in the study of animal growth. Selman and Sarnat employed cranial and facial implants in rabbits, while Gans and Sarnat used them in the Macaca Rhesus monkey.[25 26 11] Robinson and Sarnat, on the other hand, confined their implant study to the pig mandible.[24] Levine, working with rabbits, employed cranial metal implants, but also used alizarin concurrently to demarcate growth.[16]

More recently an introduction to the use of metallic implants in the study of facial growth in man has been made by Bjork. Cephalometrics and metallic implants were used concurrently and preliminary data on the vertical development of the face and the mode of eruption of the teeth were obtained.[4]

The vital dye, alizarin red S, has been used many times to demonstrate growth in animals.[21 22 29 31] In an early and extensive work, Brash fed alizarin to pigs in its natural form (within madder) and studied the bone growth lines which were formed in due course.[5] Most investigators, however, have used the synthetic form of the dye (alizarin red S) because of its broader range of usefulness.[2 8 9 13 15]

When administered in vivo, alizarin red S becomes linked to metallic hydroxides present at calcification sites

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within the body and insoluble dye "lakes" are formed. So rapid is this union of dye with hydroxide that, within fifteen minutes of an alizarin injection, a permanent marking line is formed in all dentin and bone calcifying at that time. This tinctorial activity of alizarin red S has been studied by Hoffman through the use of an artificial bone matrix. It is evident that the dye has a selective staining effect upon the calcifying or calcio-receptive zone of the collagenous matrix within which the calcium salts are being deposited.[30]

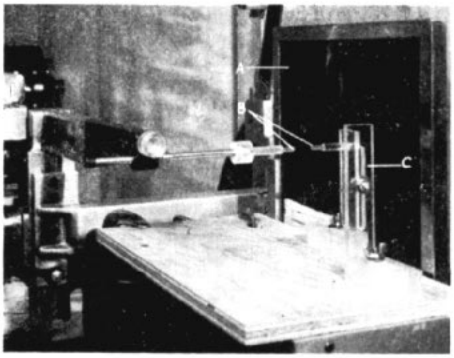


Fig. 1 Broadbent Bolton cephalometer head holder attachment for rabbits. A. film cassette; B. earposts; C. anterior post for centering and holding the upper incisors.

Methods and Materials

The young New Zealand rabbit was selected as an animal well suited to a study of dental and osseous growth. Preparatory to actual experimentation with the animal, it was found necessary to devise a technique for the taking of serial roentgenograms of the rabbit on the cephalometer. A special cephalometric attachment was designed, consisting of a plywood base with plastic up-rights upon which were mounted: 1) an ear post and 2) an anterior post for centering and holding the upper anterior teeth of the rabbit. Another plastic ear insert was machined to fit one of the ear posts on the cephalometer. In this way, the entire attachment was oriented exactly in the midsagittal plane and the transmeatal axis of the cephalometer (Fig. 1). Thus, a fixed target distance and fixed film placement were obtained. Exposure time was subsequently evolved through trials with a rabbit head on this apparatus.

Before detailed experimentation on the rabbit could be undertaken, it was necessary to determine the commensurate dosage which would give recognizable markings in the cranial bone of the rabbit, and which would at the same time be within the tolerance range of the animal. For these reasons, introductory experiments were conducted upon two eight-week old New Zealand rabbits. One rabbit served as the control, the other as the experimental. Two injections of alizarin red S were given this latter animal within the dosage range 50-100 mg./kg. of body weight as established by Schour et al.[30] The first injection was made at the level of 50 mg./kg. of body weight and the second at the level of 75 mg./kg. of body weight. When the animal was sacrificed, it proved extremely difficult to locate the first alizarin red S line. The line resulting from the second injection was visible upon the bone surface, but did not demonstrate the areas of growth as strongly as might have been desired. No toxic effect was noted following the injection of either quantity of alizarin red S. For this reason, the maximum dosage (100 mg./kg. body weight), presumably well within the tolerance range, was considered preferable in terms of the bone marking which it would produce.

The experiment proper was built around a group of six New Zealand rabbits, 8 weeks of age. Five rabbits served as experimentals, the sixth as a control. At the outset, metal implants were placed surgically on each side of the fronto-parietal and fronto-nasal sutures of the cranium in all experimental animals. Each rabbit was then given an intraperitoneal injection of a 2% solution of alizarin red S in 0.45% sodium chloride (100 mg./kg. of body weight).

A second injection was given 32 days later. The animals were sacrificed 14 days after this second injection. Just seven days before the animals were sacrificed, a mark was placed on the upper and lower anterior teeth at the free gingival margin. Two animals died prior to the termination of the experiment; one at the time of surgery, because of an overdose of nembutal, and the other after the second injection of alizarin for unknown reasons.

The rabbits were fed twice daily a ration of cereal grains and mash concentrates. Greens were given twice weekly. They were weighed every day for the first four days and then every other day.

Roentgenograms were taken under nembutal narcosis at the time when the metal implants were placed, at the time of the second injection, and at the time of sacrifice. At the time of sacrifice, records were also made of the distances, (a) between the amalgam inserts on the cranial surface, and (b) between the horizontal marks upon the anterior teeth and the free gingival margin. The heads were then placed in a 10% formalin solution and, several days following this, the soft tissue was removed from about the skulls.

Experimental Results

A. Roentgenographs

Tracings of the three successive films showed that the inserts moved apart an average of 3.86 mm. at the fronto-nasal sutures and an average of 0.766 mm. at the fronto-parietal sutures during the entire period studied. There was, in other words, five times more growth at the fronto-nasal sutures than at the fronto-parietal sutures. The two

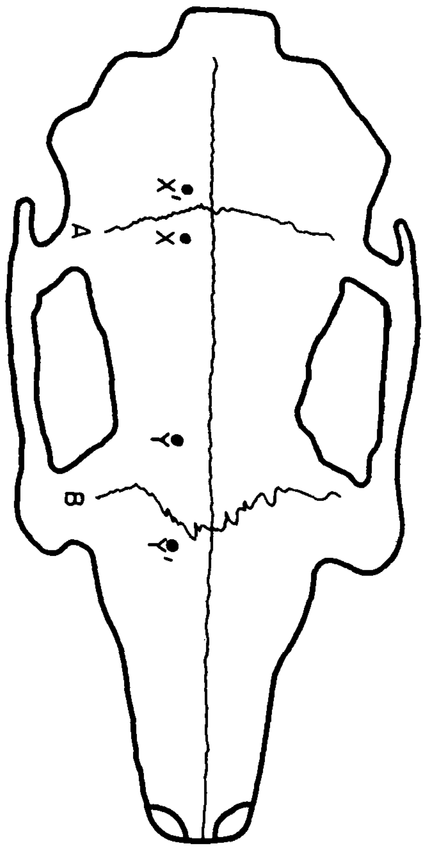


Fig. 2 Tracing of superior view of skull showing location of metal inserts. A. fronto-parietal suture; B. fronto-nasal suture; X', X, Y, Y', metal inserts.

inserts (X and Y) in the frontal bone proper showed no movement apart (Fig. 2), demonstrating further that longitudinal growth of this bone is concentrated in the area of the suture line. Using these two inserts as a guide in superimposing the roentgenogram tracings, a definite growth pattern of the head in a forward and downward direction could be observed (Fig. 3). Furthermore, under dissecting microscope inspection, there could be observed overlying each metal insert a film of dense tissue resembling new osteoid bone.

B. Alizarin red S

Since a decalcification procedure

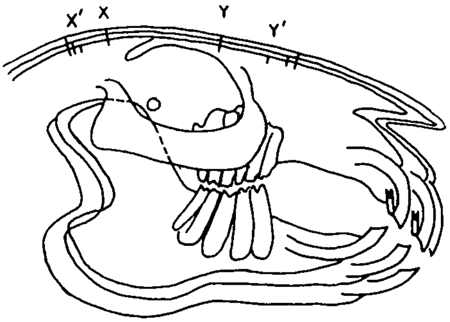
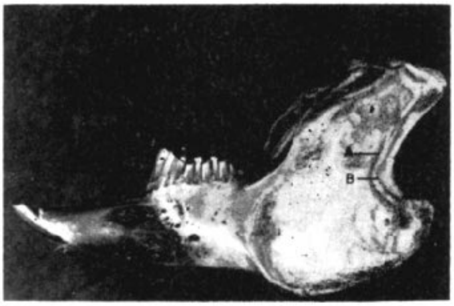
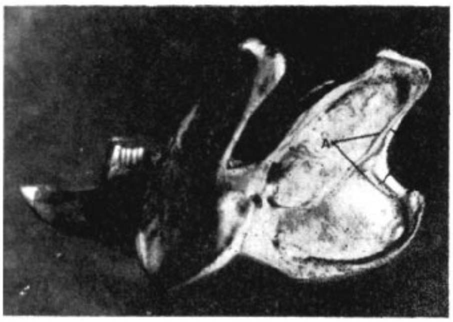


Fig. 3 Roentgenographic tracings superimposed on X and Y to show forward and downward growth pattern. X', metal insert in parietal bone. Y' metal insert in nasal bone. X and Y metal inserts within the frontal bone.

would have removed alizarin red S, it was necessary to base the experimental analysis upon visual observation of gross specimens and microscopic observation of ground sections of the bones and teeth.

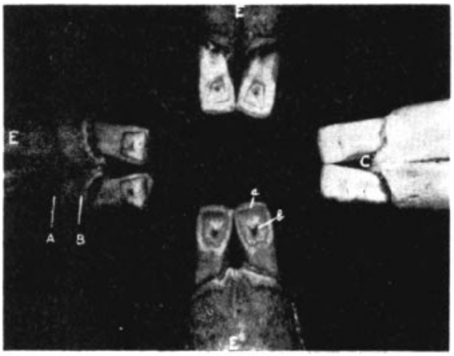


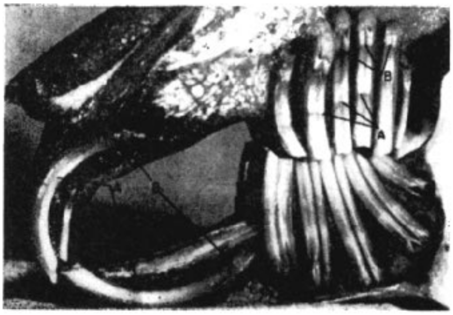


Figs. 4 & 5 Lateral and lingual views of mandible. A. line produced by first injection of alizarin red S; B. line produced by second injection of alizarin red S.

The surfaces of the skull and mandible were completely covered by the alizarin red S, showing that appositional growth was generalized. The posterior border of the mandible and the condylar region showed two definite alizarin lines from the two injections (Figs. 4 and 5). Similar lines were visible in the alveolar process surrounding the mandibular central incisor (Fig. 6). The alveolar processes generally showed heavy staining.

The teeth in animals subjected to





Figs. 6 & 7 Superior views of lower incisors. E - experimental animals; C - control animal. a. line produced in dentin by first injection of alizarin red S; b. line produced in dentin by second injection of alizarin red S; A. line produced in alveolar process by first injection of alizarin red S; B. line produced in alveolar process by second injection of alizarin red S.

Lateral view of skull and mandible with alveolar bone removed. A. apical termination of line produced by first injection of alizarin red S; B. apical termination of line produced by second injection of alizarin red S.

alizarin red S also showed distinct red lines in the many ground sections prepared. In ground cross sections there were dual lines one within the other. Distance measured between these lines, divided by the number of days between injections, gave the daily appositional growth rate of the dentin. On their external facial surfaces the teeth were marked by two red bands which actually formed the apical terminations of two internal cones (Fig. 7). A measurement made between the apical terminations of these two successive bands or cones on the external surfaces, divided by the number of days between injections, gave the daily eruption rate of the teeth. For the upper and lower incisors, measurements of both labial and lingual surfaces were obtained and the mean eruption rate was determined. The total measured length of the teeth divided by this calculated eruption rate provided the estimate of the time required for complete replacement of these teeth.

As a further check on the eruption rate of the anterior teeth, measurements were made between the prepared mark cut in the enamel and the free gingival margin. This latter procedure lacked complete accuracy in that the free gingival margin was quite movable. The average distance measured between tooth mark and free gingival margin was 2.2 mm. for the upper incisors, and 2.3 mm. for the lower incisors. A range of plus or minus 0.3 mm. was noted.

When the distance between alizarin lines on the facial surfaces of the teeth was measured, the average weekly eruption rate calculated for the upper incisors was 2.0 mm. and for the lower incisors, 2.36 mm. As noted in Table I, the lower teeth were erupting a little faster than the upper. The calculated replacement time for the upper incisors was 12 weeks, for the lower incisors, 10 weeks and for the upper and lower posterior teeth, 10 weeks. The third molars showed extreme variation in their growth rates from one animal to another. In one animal, for instance, the estimated third molar replacement time was 7 weeks, while in another, it was 32 weeks.

Cross sections were made of certain teeth in the upper and lower dental arch of each animal. Teeth of the upper arch which were studied included the central incisors, the first, second and third premolars, and the first and second molars. The lower teeth studied

TABLE I

WEEKLY ERUPTION RATE OF TEETH

Teeth	*Number of Measurements*	* *Mean*	*Standard Error of Mean*	*Standard Deviation*
Upper Central Incisors	6	2.00	± .18 mm	.44
Lower Central Incisors	6	2.36	± .11 mm	.289
Upper Posterior Teeth	18	1.19	± .09 mm	.40
Lower Posterior Teeth	15	1.30	± .07 mm	.30
All Teeth	45	1.71	± .05 mm	.34

*Millimeters per week.

TABLE II

Apposition of Dentin Per Day				
Teeth	*Number of Measurements*	* *Mean*	*Standard Error of Mean*	*Standard Deviation*
Upper Central Incisors	24	12.00	± .58	2.88
Lower Central Incisors	24	12.76	± .53	2.61
Upper Posterior Teeth	103	10.99	± .40	4.14
Lower Posterior Teeth	105	10.10	± .49	5.05
All Teeth	256	11.46	± .26	4.31

*Microns per day.

were the central incisors, the first and second premolars, and first and second molars. Ground sections were made of each tooth (Figs. 8 and 9). In each section the distance between the lines produced by the alizarin was recorded. Eight or more measurements were made on each section. The measured distance divided by the number of days between the injections gave the daily appositional growth rate of the dentin (Table II).

The average daily apposition of dentin was calculated to be 11.46 microns. The observed rate of apposition varied considerably from a minimum of 1.87 microns a day to a maximum of 20.93 microns per day.

C. Weight variations

The experimental animals demonstrated a weight loss following each injection of alizarin red S. This weight loss continued on each occasion for three to four days.

The control animal was kept in the same environment and given care identical to that received by the experimental animals. By contrast, no surgery was performed upon this control animal and no injections were administered to it. The first weight loss registered by the control, almost paralleling in time and severity the weight loss of the experimentals at the time of first injection, may be attributed

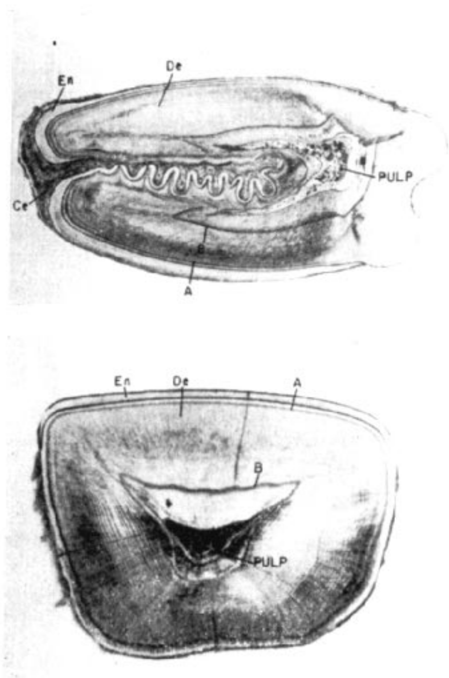


Fig. 8 & 9 Cross section of lower incisor. A. line produced in dentin by first injection of alizarin red S; B. line produced in dentin by second injection of alizarin red S; En - enamel; De - dentin.

Cross section of upper first molar. A. line produced in dentin by first injection of alizarin red S; B. line produced by second injection of alizarin red S; En - enamel; De - dentin; Ce - cementum.

to an accidental water deprivation for five to eight hours. The weight drop seen in the control on the 42nd day was in all likelihood produced by a similar, short-term, accidental dehydration.

Discussion

A. Appositional growth and calcification

Appositional growth and calcification are two different processes, characteristic of bone, enamel, cementum and dentin. Even though these processes are separate and distinct, with calcification following the appositional growth, the two are closely associated with one another. In this connection Schour and Hoffman point out that "the rate of incremental calcification and the rate of apposition in enamel and dentin are the same, and the calcification pattern under normal conditions acts as a reliable indicator of the growth pattern."[29] When the alizarin red S injection is employed, it is the incremental pattern of calcification that is being measured, rather than the appositional growth.

Such a sequence is well illustrated in dentin. As one layer of organic matrix material or predentin is being produced under odontoblast cell influence, the predentin formed the previous day is undergoing calcification.[28] Thus, when the predentin reaches a width in the range of 10-20 microns, that portion of it farthest from the odontoblasts becomes a newly calcified zone known as the intermediate dentin.[28] This is the zone that is stained by the alizarin red S in the initial stages of calcification.

B. Dentition of the rabbit

The teeth of the rabbit grow and erupt continuously throughout the life of the animal. These continuously erupting teeth are referred to as teeth of persistent growth.[28] No structural distinction is made between crown and root in this situation. Instead, a single crown exists, the constant growth of which is due to the continuous apposition of tissue at the basal portion.

Because of varied growth rates on the labial, buccal, and lingual surfaces, brought about by differential cellular activity of the epithelial base, all the teeth exhibit a distinct curvature (Fig. 7). This is seen most readily in the upper and lower incisors, although the upper premolars and molars do have a slight buccal curvature and the lower premolars and molars a slight mesiodistal one.

C. Eruption

Because of the pronounced curvature of the upper and lower incisors, a single mark placed only on the labial surface of the tooth does not give the average eruption rate for the entire tooth. Preferably a mark should be placed on both the labial and lingual surfaces. An even more accurate determination can be made by using the basal termination of each alizarin band. Such a procedure was followed here. Measurements were made on each side of the tooth and the mean eruption rate was determined from these figures.

D. Growth of dentin

The daily appositional rate of dentin varies within a single tooth and between different teeth. This variation is dependent upon the anatomy of the tooth, the amount of tooth structure already formed, the position of the tooth, the method of sectioning and measuring, and the age and species of the animal. In the rabbit upper incisors, for example, growth of dentin was found to vary from 4.68 microns in one area to 15.62 microns in another, while in lower incisors the range was 7.81 to 18.43 microns. Schour and Hoffman point out that, even though there is a marked growth gradient within a single tooth, it follows a definite and orderly pattern of distribution.[29]

The posterior teeth demonstrated the most variation in growth. Their complex geometrical configuration greatly influenced the measurements taken and it was with difficulty that an accurate mean of their growth was obtained.

E. Cranial growth

Both this study and that of Levine reveal that there is approximately five times as much growth at the fronto-nasal suture as at the fronto-parietal suture, and that this growth differential produces an elongation of the face in an anterior direction.[16] Levine attributes the relative lack of activity at the fronto-parietal suture to an early stabilization of the cranium. Selman and Sarnat, while investigating growth at the fronto-nasal suture of the rabbit, measured the distance between metal implant and suture line as well as the distance between metal implants. They found that approximately 65% of the total growth at the suture could be attributed to the nasal bone and approximately 35% to the frontal bone.[25]

The mechanisms of cranial growth are still being investigated and debated. Majority opinion credits the primary growth of the cranium to sutural activity. Brash, on the contrary, did not attribute the primary increase in size of the cranium to suture line activity, but rather explained such growth on the basis of surface accretion and surface absorption.[5] Where growth sites in maxilla and mandible are concerned, however, the findings of Brash are not at variance. Likewise, Robinson and Sarnat, through the use of metal implants in the pig mandible, demonstrated the same growth sites that have been brought out here by the use of alizarin red S.[24] In the present study, growth at the suture lines was demonstrated both by metal implants and by alizarin red S injection. Moore and Craven, using the latter technique, and Jarabak, et al., using radioactive calcium, have also demonstrated growth at the suture lines and have suggested this mechanism to be fundamental to cranial enlargement.[22, 9, 14]

Conclusions

1. The major portion of cranial growth took place at the suture lines.
2. Measurements made of the distance between metal inserts at two cranial suture lines showed an average separation of 0.766 mm. at the fronto-parietal suture and 3.86 mm. at the fronto-nasal suture. These observations demonstrated that there was five times as much growth at the fronto-nasal suture as at the fronto-parietal. Such growth differentiation between the cranial and facial portions may be characteristic of long snouted animals.
3. The cranium showed marked appositional growth on all internal and external surfaces.
4. In the mandible the location of alizarin lines showed the growth sites to be at the posterior border of the ascending ramus, in the condylar region and in the alveolar process.
5. In the incisal region of the mandible, the growth appeared localized to the superior one-third of the alveolar process.
6. Both maxilla and mandible showed marked appositional growth over the entire alveolar process in the molar area.
7. The average daily increment of dentin in the upper incisors was found to be 12.00 microns; in the lower incisors, 12.76 microns; in the upper posterior teeth, 10.99 microns; in the lower posterior teeth, 10.10 microns. An overall average rate of dentin formation of 11.46 microns per day was noted.
8. The eruption rate for the upper incisors was calculated to be 2.00 mm. per week; for the lower incisors, 2.36 mm. per week; for the

upper posteriors, 1.19 mm. per week; and for the lower posteriors, 1.30 mm. per week. The overall average rate of tooth eruption was 1.71 mm. per week.

9. The use of metal implants and the vital dye alizarin red S has been shown to be of great value in the study of normal growth.

Summary

A study was conducted on New Zealand rabbits to determine the rate of growth at the fronto-parietal and fronto-nasal sutures, to follow the pattern of cranial growth, to determine the appositional rate of growth in the dentin and to ascertain the eruption rate of the anterior teeth. The use of metallic implants and the employment of serial roentgenograms made it possible to trace the rate of growth at the suture lines as well as the general pattern of cranial growth. Appositional growth rate in the dentin and the eruption rate of the teeth were determined by means of peritoneal injections of alizarin red S.

Two groups of rabbits were studied. The first group was used to determine the dosage levels of alizarin necessary to reveal growth in bones and dentition. On the basis of results with these first animals, it was found that dosages of 50 and 75 mg. alizarin per kilogram body weight, although well tolerated, did not demonstrate the areas of growth as strongly as might have been desired.

The second group consisted of five experimentals and one control animal. The experimentals were given two injections of 100 milligrams of alizarin red S per kilogram of body weight 32 days apart. This alizarin dosage revealed satisfactorily the growth sites in the skull and mandible and the incremental growth pattern in the teeth.

Cranial expansion was found to occur primarily as a result of bone growth at the suture lines, although pronounced appositional growth was evident on the internal and external surfaces of all cranial bones. Appositional bone growth was also very noticeable upon the posterior border of the ramus and upon the alveolar process of both maxilla and mandible in the molar area.

The growth differentiation between the cranial and facial portions of the head may be characteristic of long-snouted animals. Five times as much growth occurred in a given time interval at the fronto-nasal suture as at the fronto-parietal suture.

Apposition of dentin in the teeth of the rabbit occurs at the mean rate of 11.46 microns per day while eruption of the teeth preceedes at the mean rate of 1·71 mm. per week.

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