Calcification of Deciduous Teeth in Rhesus Monkeys

Abstract. *The rhesus monkey probably has the same sequence of calcification of deciduous teeth as man: central incisor, first molar, lateral incisor, cuspid, and second molar. Initial calcification of the deciduous molar cusps is somewhat similar to that of man.*

Rhesus monkeys have been widely used in dental research, but there still remain gaps in our knowledge concerning the processes of tooth development in this animal (*1*). Indeed, more is known about the mode of tooth formation in the rat, mouse, and rabbit than is known concerning odontogenesis in nonhuman primates. It seems appropriate to make detailed comparative studies of the growth and development of the hard dental tissues in nonhuman primates, for through such investigations ontogenetic patterns common to all primates and patterns representing peculiarities of differing primate groups may be revealed.

This report concerns observations on the initial calcification and maturation of the deciduous teeth of the rhesus monkey, and comparisons are made with the processes of early dental development in man. A series of 15 rhesus monkey fetuses ranging in age from about 2½ months to birth (168 ± 3 days) were cleared and stained with alizarin red S by the method of Noback and Noback (*2*). For evidence of calcifying teeth, the mandibular and maxillary crypts were dissected under a dissecting microscope (at ×40). The deciduous teeth were removed intact, photographed, and drawn from the occlusal view. Fetal ages were estimated from crown-rump length, using the method of Schultz (*3*).

Kraus has studied the different phases of deciduous tooth calcification found in human fetuses (*4*). For years it has been taught that calcification, proceeding in a regular sequence, begins in the central incisor and terminates with the second molar. Kraus's material, however, shows a different picture. He found that the central incisor begins to calcify first, the first molar appearing next, followed by the lateral incisor, cuspid, and second molar. Kraus believes this pattern of initial calcification to be extremely rigid for *Homo* and reports: "that the sequence itself is under strict genetic control can hardly be in doubt" (*5*).

We have compared this sequence with our data on the rhesus monkey. The two youngest fetuses had a gestation age of approximately 2½ months, and all deciduous teeth were in some stage of calcification.

At approximately 70 days of gestation, the central incisors have three small elevations (mamelons) along the incisal surfaces, the central elevation being the highest. At this same period, however, the lateral incisors have but a central projection which we interpret as a single center of calcification. The deciduous incisors of man develop from a single center of calcification (*4*).

Both the upper and lower central incisors had a greater mesiodistal diameter than the lateral incisors. Crown tips were present for the cuspids but were much smaller than the lateral incisors or the mesiobuccal cusps of the first molars. The first molars were represented by two or three cusps, while the second molars had but a single calcified cusp. Without specimens of an earlier gestation age it is impossible to state unequivocally the sequence of calcification.

If we assume, however, that the larger centers of tooth calcification appeared first, then the evidence, meager as it is, suggests the following sequence: central incisor (first molar, lateral incisor), cuspid, and second molar. If this sequence proves to be the correct one, then it would appear that the order of deciduous calcification is identical for man and rhesus monkey. Comparative data from many different primate species is required to answer the questions whether there is a common sequence of deciduous tooth calcification in the primates and to what extent the order varies among the primates. For example, Butler showed that the order of appearance of tooth buds in three primates, *Tupaia, Tarsius,* and *Homo* was similar and that this order differed from the sequence in the more primitive moles (*6*). In addition, he noted that the order of appearance of the germs does not necessarily coincide with the order of eruption. We have observed that the order of appearance of tooth buds in the three primates studied by Butler is not the same as the order of initial calcification in *Homo* or *Macaca*.

Among the developing deciduous molars in the rhesus monkey, the de-

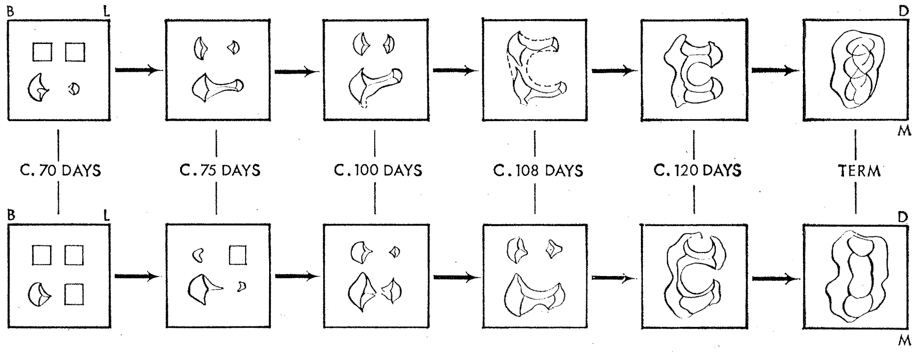

Fig. 1. (Top row) Sequence of calcification of the first lower deciduous molar. (Bottom row) Sequence of calcification of the second lower deciduous molar.

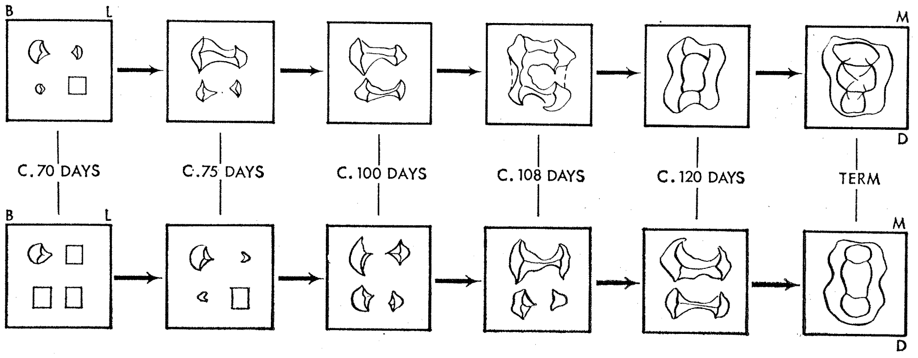

Fig. 2. (Top row) Sequence of calcification of the first upper deciduous molar. (Bottom row) Sequence of calcification of the second upper deciduous molar.

finitive deciduous molar, both maxillary and mandibular, has four major cusps. The two buccal cusps are connected with the two lingual cusps by transversely running enamel crests which give the tooth its characteristic bilophodont nature. Thus, the final morphology of these teeth is different from that of their counterparts in man. This difference, however, is not reflected during early molar odontogenesis. The first and second mandibular molars (Fig. 1) acquire the calcified cusps in the same sequence that Kraus noted for man—namely, protoconid, metaconid, hypoconid, and entoconid. The hypoconulid is absent in the lower deciduous molars of the rhesus monkey. In both upper molars calcification commences with the paracone, and calcification of the protocone, metacone, and hypocone (see Fig. 2) follows.

Variations from this cusp sequence probably can be expected when larger samples are examined. Indeed, Kraus found some variability in the order of cusp calcification for man and stated: "the lower first molar is the most variable while the upper second is the most constant, there being no exceptions to the proposed sequence in the latter" (*7*). If the proposed cusp sequence proves to be correct for the rhesus monkey, we may well be on the track of a generalized pattern of primate cusp formation, which represents an old, well-entrenched genetic complex within the order Primates. Or there may prove to be several intraprimate differences in the sequence which would indicate that changes have occurred in the timing of molar cusp formation. Such temporal alterations have undoubtedly played an important role in dental evolution and could be valuable taxonomic criteria.

At about 75 days' gestation, the molar cusps begin to coalesce with one another, eventually forming the bilophodont molar pattern characteristic of Old World Monkeys (Figs. 1 and 2). The mesiobuccal and mesiolingual cusps are the first to join, for an extension or spur of enamel develops toward the center of the tooth from the respective calcification centers. This is followed by the bridging of the two distal cusps. While the two mesial and the two distal cusps are being united transversely, enamel extensions develop along the buccal and lingual crown surfaces whereby the mesial and distal moieties of the tooth become connected. The buccal marginal ridge is formed before the union along the lingual surface. The bridging or joining of cusps does not begin until all the calcification centers of a molar have appeared.

DARIS R. SWINDLER
HARRIET ANN MCCOY
Department of Anatomy, Medical College of South Carolina, Charleston

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7. We thank Dr. L. H. Schmidt formerly of the Christ Hospital Institute of Medical Research, Cincinnati, and Dr. J. A. Gavan of the University of Florida for supplying the fetuses used in this investigation. Supported by grants C-2663, H-2417, and H-4176 from USPHS.

6 April 1964

Experimental Cardiac Hypertrophy: Concentrations of RNA in the Ventricles

Abstract. *Banding of the aorta or pulmonary artery in puppies produces a selectively increased concentration of RNA in the ventricle with the increased hemodynamic load as compared to the opposite side or to normal hearts. The increase in concentration of RNA following distortion of the myocardial cell may represent a fundamental response of growth and the system described may serve as a useful model for its study.*

Cardiac hypertrophy produced in rabbits by aortic banding was reported by Rossi and Mor (*1*) to be accompanied by an increased content of RNA in the myocardium in comparison with hearts of normal rabbits. They measured the total content of RNA in the myocardium but they did not compare right and left ventricles for differences in concentrations of RNA.

In dogs, the left ventricular hypertrophy produced by banding the aorta far exceeds the normal preponderance of the left side of the heart over the right. This response to an increased hemodynamic load by the left side of the heart suggested that there may be an associated increased concentration of RNA in the left ventricle as compared with the right. The studies reported here were initiated to evaluate whether an experimentally increased pressure load applied on either side of the heart is followed by a response of that side with increased concentration of RNA in the myocardium, and if so, how quickly this occurs and to what extent the opposite ventricle participates with an increased concentration of RNA.

A total of 23 5-week-old puppies from 5 litters were divided into an experimental group of 15 and a control group of 8. Each dog in the control group was a littermate of an operated dog. The 15 puppies chosen for operation were assigned at random into one group of nine for aortic banding and one group of six for pulmonary artery banding. Operations were done after intravenous injection of pentobarbital with respirations assisted by a constant volume respirator (*2*). Pressure studies showed systolic pressure gradients ranging from 42 to 55 mm-Hg across the banded ascending aortas and from 38 to 45 mm-Hg across the constricted pulmonary arteries.

The puppies from each operated group were killed under light ether anesthesia at various times after the operation as indicated in Figs. 1 and 2 and Table 1. Six of the control littermates were also killed at the same time since normal values for concentrations of RNA in the two ventricles were not previously known. Sham operations were carried out in two of the littermate puppies as additional controls, a band being placed around the aorta in one puppy and around the pulmonary artery in the other puppy, but neither band caused any constriction.

A standard dissection was done for each heart by removing the atria and separating the two ventricles longitudinally. The ventricular septum was included with the left ventricle because of its embryological origins.

Tissues were weighed, then homogenized and washed quickly in cold (2°C) 10 percent trichloracetic acid. They then were washed with a mixture of ether and ethanol (3 : 1, vol/vol) until the supernatant was clear and the lipids could be removed. The tissues were then divided for the various determinations among weighed tubes in which they were dried under high vac-