from data for laboratory animals to justify further study of a vaccine prepared from virus propagated in chick embryo cell cultures, for use in the immunization of humans against eastern equine encephalomyelitis.

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References and Notes

- 1. R. Randall, Vet. Bull. 34, 7 (1940)., J. W. Mills, L. L. Engel, J. Immunol. 55, 41 (1947).
- 3. The excellent technical assistance of Miss Mette Strand and Mrs. June Cole is acknowledged.

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Calcification of the Permanent First Mandibular Molar in Rhesus Monkeys

Abstract. Evidence suggests that in rhesus monkeys calcification in the mandibular permanent first molar commences much earlier than has been previously reported. This tooth is the first secondary dental element to calcify, as it is in man.

To my knowledge there are no studies relating directly to the calcification of either the deciduous or permanent teeth in the rhesus monkey. Perhaps the only exception to this is in the widely used Anatomy of the Rhesus Monkey (1933), which has recently been reprinted (1). In the chapter on the teeth, Marshall discusses rather briefly the complex problem of tooth calcification. Because of the obvious importance this subject has for the dental researcher, and since there are several inconsistencies in Marshall's presentation, it seems appropriate to present some new observations on the calcification of the permanent first mandibular molar.

In his study of the development of the deciduous and permanent teeth, Marshall made the following comments:

1) In still-born animals of a gestation period ranging from 153 to 169 days, x-rays indicated, "The calcification of the deciduous second molars and of the permanent first molars is just beginning" (1, p. 85).

2) In 14 animals between 1 and 2 months old, "There was no evidence at

this age of even the beginning of calcification of the permanent teeth" (1, p. 85)

3) "The calcification of the second set of teeth was not demonstrated roentgenographically until after the sixth month. At this time the permanent upper central incisors are just beginning to be formed" (1, p. 86).

It seemed advisable, therefore, to reexamine these statements in the light of our observations in the developing deciduous and permanent teeth of rhesus monkeys.

To date, 13 animals have been born in our colony at Charleston. Of these 13 animals, four had x-rays taken on the day of birth, whereas the initial xrays of the other animals were taken at various ages (see Table 1). One male rhesus fetus of approximately 129 days gestation (170 days average gestation period) (2) was dissected, cleared, and stained with alizarin red S, the method of Noback and Noback (3) being used.

Table 1 presents the findings on the live animals via roentgenography. For two animals the lower molar is listed under "questionable," because the identification was not positive. I believe that the crown tips are present; however, I prefer to record the animals in the "not sure" category. Animal number 109 did not have jaw films taken again until he was 1 year old, at which time the permanent first mandibular molar was well formed. Number 119 was 1 day old at the time this report was written. It should be mentioned that No. 113 has a large, well-formed crown which is clearly visible on the x-ray film. It is evident, therefore, that the present x-ray evidence does not substantiate statements 2 and 3 of Marshall; on the contrary, it suggests a much earlier calcification time for the permanent first mandibular molar, more in line with Marshall's first pronouncement. Incidentally, I have never observed calcification occurring in the permanent maxillary central incisors earlier than in the permanent first mandibular molars, as noted by Marshall in his statement 3. The present findings indicate that the permanent first mandibular molar is the first permanent tooth to calcify, and in this respect the rhesus monkey is similar to man.

The fetal monkey that was dissected would seem to confirm this. While all the deciduous teeth were present and well developed morphogenetically, the Table 1. Calcification of permanent first mandibular molar. In column three, zero indicates that the first x-ray was made at birth.

| Animal No. | Sex | Postnatal age at first x-ray (days) | Lower molar |
|---------------|--------------|---|--------------|
| 108 | F | 14 | Present |
| 110 | F | 21 | Present |
| 112 | F | 0 | Present |
| 117 | M | 14 | Present |
| 104 | F | 32 | Present |
| 106 | \mathbf{F} | 44 | Present |
| 109 | Μ | 3 | Questionable |
| 111 | Μ | 113 | Present |
| 113 | Μ | 170 | Present |
| 115 | м | 56 | Present |
| 119 | М | 0 | Questionable |
| 121 | Μ | 0 | Present |
| 114 | F | 0 | Present |

crypts for the mandibular and maxillary permanent first molar were discovered. The crypts for the upper molars contained only a membranous dental organ at a stage preceding the formation of hard structures, whereas the mandibular crypts had the membranous dental organ with two of the four crown tips beginning to calcify. X-rays were unable to detect this early formation of calcified material but alizarin red S was absorbed by these minute cusp tips. (For a more elaborate comment relating to this question, see 4.) These primordial dental organs were characterized by their bilophodont appearance, two mesial and two distal cusps connected by a transverse ridge (5). The two calcified cusps would correspond to the mesial buccal (protoconid) and distal buccal (hypoconid) cusps of dental terminology. It is interesting to note that Kraus found the mesial buccal cusp the first to calcify in his investigations of human teeth (6, 7).

DARIS R. SWINDLER

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References and Notes

- 1. C. G. Hartman and W. L. Straus, Jr., Eds., The Anatomy of the Rhesus Monkey (Macaca mulatta) (Hafner, New York, 1933, reprinted
- minata) (Hainer, New Tork, 1953, Teprinted 1961).
 2. J. A. Gavan and G. van Wagenen, Am. J. Phys. Anthropol. 13, 399 (1955).
 3. C. R. Noback and E. Noback, Stain Technol. 19, 51 (1944).
- . Kraus, J. Am. Dental Assoc. 59, 1128 (1959). W. E. Le Gros Clark, The Antecedents of Discourse of Chicago (1960). **W**. Е 5.
- Man (Quadrangle Books, Chicago, 1960).
- B. S. Kraus, personal communication. This research was supported by grants (C-2663 and H-2417) from the U.S. Public Health

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