duced may be obtained by subcortical than by cortical stimulation. Especially suggestive are the data showing behavioral differentiation of stimulation sites in mesencephalic auditory system structures, particularly as there is some electrophysiological evidence for tonotopic organization within the inferior colliculus (*4*). It is hoped that the combined behavioral and stimulation techniques of this preliminary study will prove to be powerful tools for the investigation of theories of the central code of the auditory system (*5*) and perhaps of the visual system as well (*6*, *7*).

PHILIP C. NIEDER*
WILLIAM D. NEFF
Laboratory of Physiological Psychology, University of Chicago, Chicago, Illinois

References and Notes

1. See W. Penfield and T. Rasmussen [*The Cerebral Cortex of Man* (Macmillan, New York, 1950)] for presentation of data on sensory effects from cortical stimulation in a long series of human subjects.
2. R. B. Loucks [*J. Comp. Psychol.* **25**, 315 (1938)] reported conditioning leg flexion or salivation in dogs with direct stimulation of "visual" cortex as the conditioned stimulus; R. W. Doty and L. T. Rutledge [*J. Neurophysiol.* **22**, 428 (1959)] found that cats trained in leg flexion with cortical direct stimulation as the cue were more easily trained to respond to photic or sound stimuli than naive animals, and vice versa; R. W. Doty and C. Giurgea [*The Physiologist* **1**, 17 (1958)] conditioned leg flexion in dogs by using direct stimulation of cortical points as the conditioned and unconditioned stimuli.
3. A preliminary report of this work was presented by W. D. Neff, P. C. Nieder, and R. E. Oesterreich [*Federation Proc.* **18**, 112 (1959)].
4. W. R. Thurlow, N. B. Gross, E. H. Kemp, K. Lowy, *J. Neurophysiol.* **14**, 289 (1951).
5. For a presentation of theoretical work on this subject see J. C. R. Licklider [in *Information Theory*, C. Cherry, Ed. (Butterworth, London, 1956)].
6. A suggestion for central coding of visual pattern information has been outlined by P. Nieder [*Science* **131**, 934 (1960)].
7. This research was supported in whole or in part by the U.S. Air Force under contracts No. AF 49(638)-925 and No. AF 19(604)-5526 monitored by the Air Force Office of Scientific Research and the Operational Applications Office of the Air Force Command and Control Development Division.
* Postdoctoral fellow, National Institute of Mental Health, U.S. Public Health Service.

7 December 1960

Water and Myotonia in Goats

Our work on myotonia, in goats, was frustrated by the great variation in the gravity of the symptoms in one and the same animal. Our efforts to correlate these variations with meteorological changes, food, exercise, or other factors failed. Lately, we have found indication that the gravity of the symptoms depended on the water intake. To test this point, three myotonic goats were kept on mixed dry food, only very limited grazing being allowed. Water

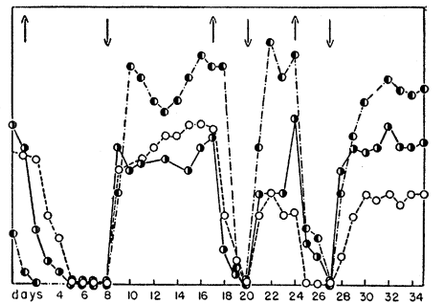

Fig. 1. Effect of water on myotonic symptoms in goats.

was withheld for periods of 3 to 7 days, these periods being followed by similar periods in which water was offered ad libitum. We found that on withholding water, the myotonic symptoms disappeared altogether within 3 days to return with full gravity within 2 to 3 days when water was given. These results are illustrated in Fig. 1. The abscissa shows days and the ordinate, the gravity of symptoms on an arbitrary scale (the intensity of stiffness was marked by one to five crosses, and the number of crosses was multiplied by the duration of stiffness in seconds). Upward arrows mean withholding water, downward arrows mean giving water (*1*).

ANDREW HEGYELI
ALBERT SZENT-GYÖRGYI
Institute for Muscle Research at the Marine Biological Laboratory, Woods Hole, Massachusetts

Note

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6 December 1960

Odontoblasts: Vacuoles and Inclusions

Abstract. Lipid granules have been found in the cytoplasm of odontoblasts and in the odontoblastic extensions within the dentinal tubules. It is suggested that these granules represent specific activity on the part of the normal cell as well as an increase in number after injury. While they are not limited to occurrence within the vacuoles in the cytoplasm of the odontoblasts, the relationship of the granules to the vacuoles suggests that the vacuoles are also a part of the physiologic activity of the adult odontoblasts.

This report is a preliminary account of the observation of lipid inclusions in the cell body of odontoblasts and in the cytoplasmic extension of the odontoblasts into the dentinal tubules. The granules were first observed during attempts to determine the contents of vacuoles which previously had been reported as occurring in odontoblasts. While other investigators have considered the vacuoles to be indicative of degenerative change (*1*) or inadequate fixation (*2*), or have mentioned them without attaching apparent significance to them (*3*), I have assumed them to be of physiologic significance.

Clinically healthy human teeth, both deciduous and permanent, were sectioned longitudinally within minutes after extraction. They were fixed in either neutral formalin or osmium tetraoxide as Flemming's strong fluid. Decalcification was achieved by using 0.5*M* sodium triethylenediamine tetraacetate buffered to *p*H 8.5 at 60°C. The specimens were embedded in paraffin after dehydration by graded ethyl alcohol or were washed and embedded immediately in polyethylene glycol. Paraffin sections were cut at 5 μ while the water wax sections were cut at 3 μ for observation by phase-contrast microscopy. Sections from each specimen were stained with hematoxylin and eosin, silver stains (*4*) and Sudan B. The material for phase microscopy was unstained. In addition to the sectioned material, fresh suspensions of odontoblasts scraped from the pulp chamber were studied and utilized as heat-fixed smears and as smears made from pulps prepared in the manner described above.

Vacuolization of the odontoblasts in the coronal portion of the pulps was found in each specimen; approximately every third to fifth cell was affected. The vacuoles varied from several microns in diameter to a proportion which appeared to engulf the nucleus. Cells in all levels of the palisade of odontoblasts were vacuolated. The shrinkage which was evident in the paraffin-embedded material distorted the vacuoles and produced spaces between the cell bodies of the odontoblasts. The vacuoles were observed in approximately the same frequency in fresh smears, heat-fixed smears, and smears made from fixed material.

Spherical bodies 1 to 2 μ in diameter were found in the cytoplasm of the odontoblasts fixed with osmic acid and in the formalin-fixed material, if this latter tissue was sectioned in water wax or smeared and if washing was minimized. In the material fixed with osmic acid, the granules could be preserved through alcohol dehydration and paraffin embedding and then could be demonstrated as silver-positive bodies. Bulk staining of the formalin-fixed material with Sudan B, with subsequent preparation of smears, also revealed the black to dark-green granules. Phase-contrast microscopy of fresh material, wet smears fixed with formalin or osmic acid, or water wax sectioned material

mounted in water consistently demonstrated the granules, although they were less numerous in the formalin-fixed material.

The granules appear to form in the cytoplasm among the mitochondria in the "Golgi zone" and then to be engulfed by the forming vacuoles in many instances or to pass peripherally into the odontoblastic process within the dentinal tubule. When the granules are seen in the vacuoles, they exhibit Brownian movement which is apparently limited by filamentous partitions within the vacuoles. The granules also appear to be 'discharged' into the predentin among the Korff's fibers when the odontoblast disintegrates as a part of its cycle. In addition, the granules appear in greater numbers in the odontoblastic processes subadjacent to areas of experimental injury to the tooth surface.

Inasmuch as the fixation of the odontoblasts can be assumed to have been complete within minutes after the teeth were removed from the mouth, the occurrence of vacuoles in the cytoplasm of these cells can be considered to be indicative of physiologic activity. This is further supported by the appearance of the vacuoles in the heat-fixed smears and in the fresh material.

The origin of the granules may be explained on the basis of degeneration or specific secretory activity of the mitochrondria (*5*). An unknown spherical body was described by Nylen and Scott as occurring in the Golgi zone in young odontoblasts (*6*). The granules described in the present report may well represent similar bodies in the more mature odontoblasts.

It would appear that the vacuoles and granules described in this report represent specific physiologic activity in odontoblasts and that the migration of the granules into the peripheral dentin through the odontoblastic processes is a function of the maturation of dentin. Also, since the granules are increased in number in the injured cells, they may represent a specific response to stimulation by injury. It may further be concluded that the granules are basically lipid in character because of the solubility factors and the response to osmification (*7*).

J. M. STEWART
Ruth M. Stewart Memorial Research Laboratory, Good Samaritan Hospital, West Palm Beach, Florida

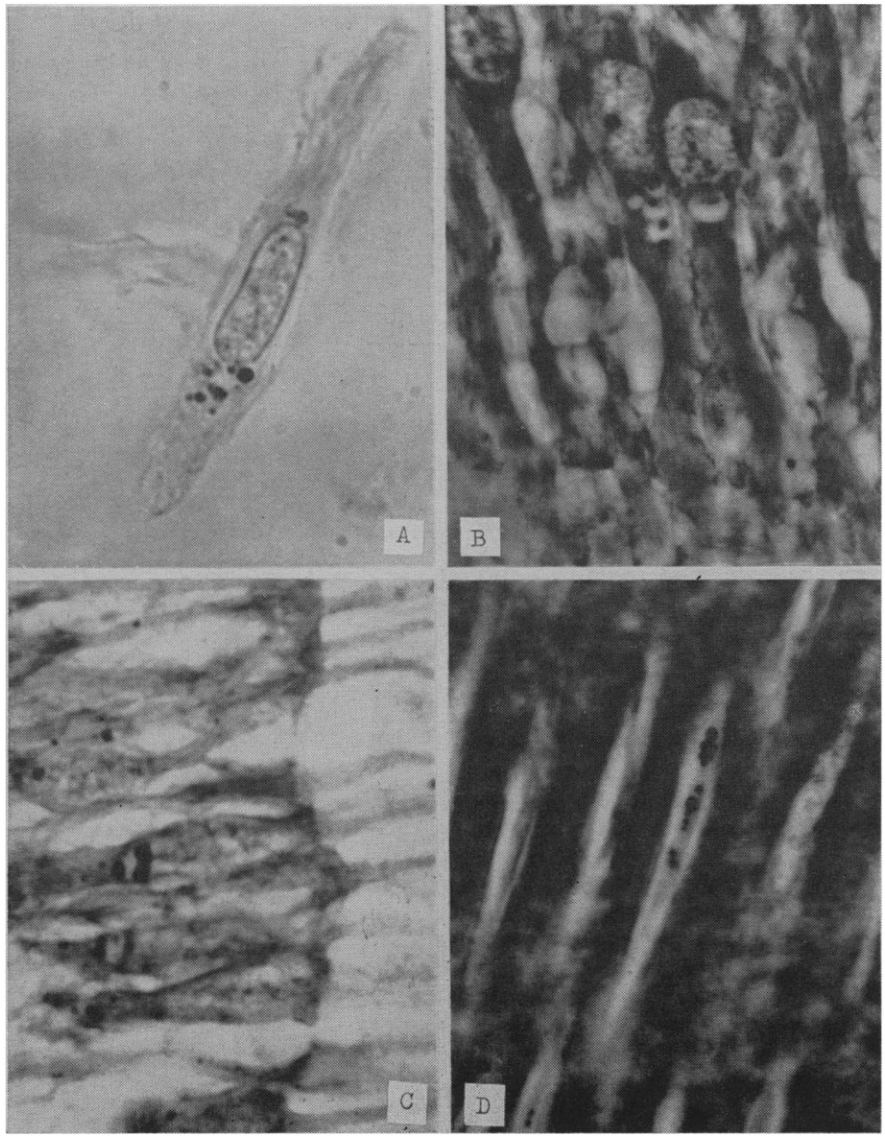

Fig. 1. Human odontoblasts, original × 2200, osmic acid fixation. (*A*) Unstained odontoblast in wet preparation showing granules at each end of nucleus. (*B*) Paraffin section with silver stain showing many granules in the cytoplasm and inside the vacuoles. (*C*) Carbowax section viewed with phase contrast showing vacuoles and phase-positive granules. (*D*) Section of mature dentin showing granules inside the cytoplasmic extension of the odontoblasts within the dentinal tubule.

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7. This study was aided in part by a grant (D928) from the U.S. Public Health Service.

9 November 1960

Quantitative Analysis of Evolution of the Brain in Mammals

Abstract. Empirical equations derived from brain size (E) and body size (P) of archaic-Eocene, Oligocene, and Recent mammals were all of the form, $E = kP^{2/3}$; $k = 0.03$ for the Eocene, 0.06 for the Oligocene, and 0.12 for the Recent groups. It is suggested that k, which has been used as an index of cephalization in contemporary mammals, may be an appropriate measure of brain evolution in the mammals as a class.

The purpose of this report is to determine general expressions for brain development in mammals at various stages of their evolution. At the turn of the century, Dubois (*1*) proposed a quantitative measure of cephalization in contemporary mammals based on an equation relating brain weight, E, to body weight, P:

$$E = k P^{\beta} \quad (1)$$

where k and β are constants; Dubois' index of cephalization was E/P^{β} or k. Despite criticisms of this index (*2*) it