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*).

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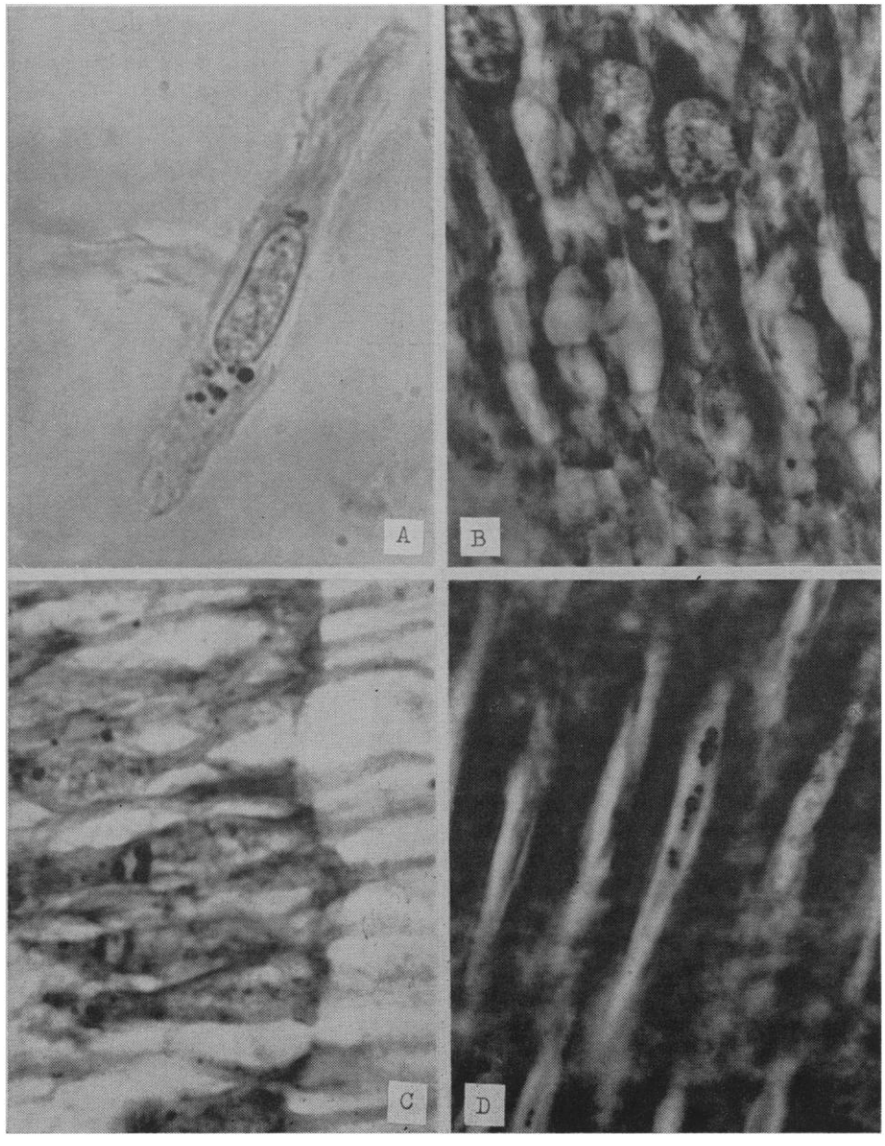

Fig. 1. Human odontoblasts, original × 2200, osmic acid fixation. (*A*) Unstained odontoblast in wet preparation showing granules at each end of nucleus. (*B*) Paraffin section with silver stain showing many granules in the cytoplasm and inside the vacuoles. (*C*) Carbowax section viewed with phase contrast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

References and Notes

1. A. F. Gardner, *Oral Surg. Oral Med. Oral Pathol.* **12**, 1102 (1959).
2. V. Kalnins, *J. Dental Research* **36**, 437 (1957); T. J. Hill, *J. Am. Dental Assoc.* **21**, 820 (1934).
3. K. Langeland, *Oral Surg. Oral Med. Oral Pathol.* **12**, 1235 (1959); *Odontol. Tidsskr.* **65**, 314 (1957).
4. L. H. Ungewitter, *Stain Technol.* **18**, 183 (1943); T. K. Nassar, M. Issidorides, W. M. Shanklin, *ibid.* **35**, 15 (1960).
5. S. L. Palay, Ed., *Frontiers in Cytology* (Yale Univ. Press, New Haven, 1958).
6. M. U. Nylen and D. B. Scott, "An electron microscopic study of the early stages of dentinogenesis," *U.S. Public Health Serv. Public Health Serv. Publ. No. 613* (1958).
7. This study was aided in part by a grant (D928) from the U.S. Public Health Service.

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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

remains "the only neurological character for which a correlation with behavioral capacity in different animals is supported by significant evidence" (*3*).

In an earlier paper (*4*) I assumed Eq. 1 with $\beta = 2/3$ to hold for archaic as well as for Recent mammals. I also suggested that the primitive value for k could be determined by using the contemporary opossum to represent the primitive condition, because this animal is similar to didelphids from early periods in the adaptive radiation of mammals. The research reported now (*5*) presents direct tests of these hypotheses, using endocranial volumes and body volumes for fossil mammals at early and intermediate evolutionary stages.

Although I was able to measure the endocranial volumes of about 50 specimens, I could find illustrations of mounted skeletons of only 16 of these, eight Oligocene and eight Eocene or earlier, from which to estimate body volumes. The Oligocene sample is from the White River area of South Dakota and Nebraska. It is a relatively homogeneous group representing the fauna of that area over the short time, geologically speaking, of about 5 million years, and dates back about 30 million years. The more diverse "Eocene" sample (which includes one Paleocene form) covers a 20-million-year period, from about 60 to 40 million years ago, and is based on specimens from various European and North American fossil deposits.

Body volumes for extinct mammals were determined either from accurate scale models of restorations based on mounted skeletons, from other published estimates (*6*), or, in those cases where dimensions were very similar to contemporary forms, from body-weight data on the contemporary forms. Volume and weight were assumed equal. The error introduced by this assumption will not exceed 10 percent of the absolute weight, and because the logarithms of the weights or volumes were used, the error was no more than 2 percent for the smallest specimen and less than 1 percent for the largest.

Data on the opossum, *Didelphis marsupialis*, which are particularly important for this analysis, had to be revised from those used earlier (*7*). The earlier data, taken from von Bonin (see *4*), were $E = 6.3$ g and $P = 1700$ g, but a road-kill from the Ohio Fish and Wildlife Service with linear dimensions in the middle of the range given by Hall and Kelson (*8*) weighed about 4500 g. I therefore sought larger published weights and found these in Weber (*9*): $E = 6.5$ g and $P = 3480$ g. With $\beta = 2/3$ these result in $k = 0.028$. By using the opossum as our type, the hypothetical relationship for primitive mammals, that is, mammals at an early stage in their adaptive radiation, could now be written:

$$E = 0.028\, P^{2/3} \qquad (2)$$

For comparative purposes the brain and body weight relationship in Recent mammals was reanalyzed by selecting a sample from published data on specimens similar in adaptive niche and body configuration to the fossil sample. Only specimens within the range of body sizes reported in standard works on mammalogy (for example, *8*) were included.

The results are presented in Fig. 1. It should be noted, first, that the logarithmic form of Eq. 1 is:

$$\log E = \log k + \beta \log P \qquad (1a)$$

If Eq. 1 accounts for the data, the points in Fig. 1, which uses logarithmic scales for the coordinates, will be distributed about straight lines with slopes, β, and intercepts, k. (The intercept will be the value of E at $P = 0.001$ kg.) If β is a mammalian constant equal to 2/3, the slopes should all be about 2/3, and if Eq. 2 is correct the lowest k should be 0.028.

The lines drawn on Fig. 1 are least-squares fits (*10*) of Eq. 1a for each evolutionary stage. The empirical equation for the Recent sample, excluding the opossum, is:

$$E = 0.115\, P^{0.664} \qquad (3)$$

For the Oligocene sample, the numbered points in circles, it is:

$$E = 0.055\, P^{0.655} \qquad (4)$$

The Eocene sample, the numbered points in squares, can be divided into two groups on evolutionary grounds. Points 1, 4, 5, and 6 in Fig. 1, which are near the Oligocene line, are for a primate and three perissodactyls from the Bridger basin middle Eocene, orders that were to continue their adaptive radiation into later geological periods. The other points, 2, 3, 7, and 8, in Fig. 1 are for a creodont from the upper Paleocene, a condylarth and a pantodont from the lower Eocene, and a uintathere from the middle Eocene. These latter specimens are from groups that had achieved their maximum radiation

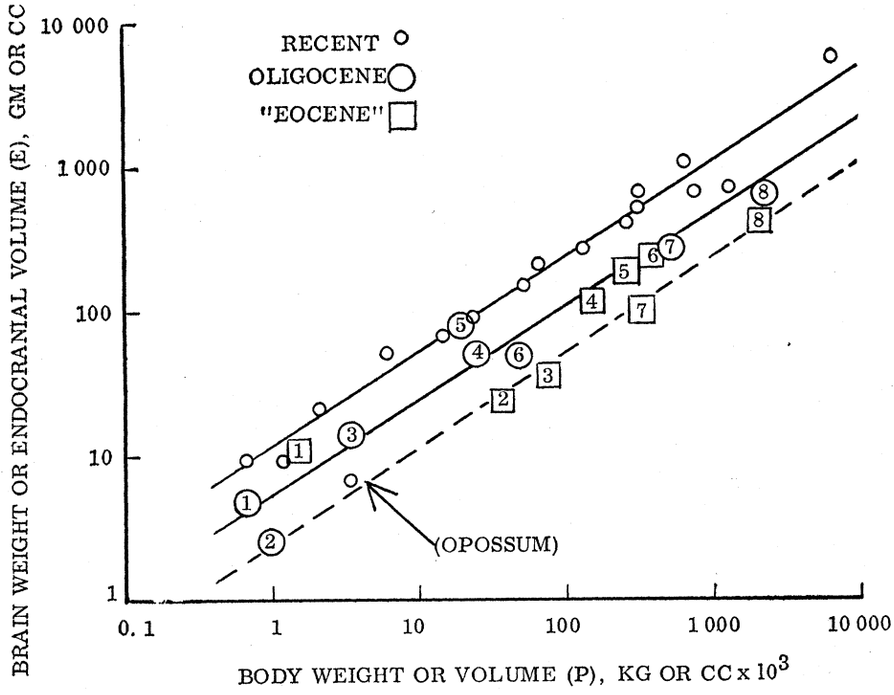

Fig. 1. Relation between brain and body measures for mammals at three evolutionary levels. Lines are least-squares fits to Eq. 1a; upper line, Recent; middle line, Oligocene; lower dashed line, archaic "Eocene" and opossum. Fossils numbered in the graph and museum catalogue numbers (see *5* for museum initials) of the endocranial casts used are: Oligocene: 1, *Ischyromys typ.* USNM 15934; 2, *Palaeolagus haydeni* AMNH 5674; 3, *Pseudocynodictis* AMNH 9766; 4, *Hoplophoneus primaevus* CNHM-UM 2 and USNM-22538; 5, *Mesohippus bairdi* AMNH 3940 and USNM-22539; 6, *Merycoidodon culbertsoni* CNHM-UC 256; 7, *Subhyracodon* USNM-22540; 8, *Menodus giganteus* AMNH 15599. Eocene: 1, *Smilodectes gracilis* USNM 17997; 2, *Arctocyon primaevus* AMNH 10431; 3, *Phenacodus primaevus* AMNH 4369; 4, *Hyrachyus modestus* AMNH 11651; 5, *Mesatirhinus petersoni* AMNH-P39558 (Princeton-10041a); 6, *Palaeosyops leidyi* AMNH 1544; 7, *Coryphodon hamatus* AMNH 15598; 8, *Unitatherium mirabile* USNM-11770 (AMNH-1036).

by early or middle Eocene times and were approaching extinction. They can, therefore, be considered to be better representatives of archaic mammals at an early stage of evolution than the entire Eocene group. The dashed line in Fig. 1 is a least-squares fit to the "Eocene" points, 2, 3, 7, and 8, and the contemporary opossum, which was assumed to be representative of forms of that type. The equation of the dashed line is:

$$E = 0.026\,P^{0.657} \qquad (5)$$

It should be emphasized that the only restriction imposed by this analysis was that the lines in Fig. 1 be straight. They have slopes that are essentially 2/3 and are displaced as they are in the vertical dimension because these slopes and displacements fit the data best.

Despite the small number of points, the equations are relatively well determined. The exponents of Eqs. 3, 4, and 5, that is, the slopes of the lines in Fig. 1, stated with their standard errors, are: for the Recent sample, $\beta = 0.664 \pm 0.012$; for the Oligocene sample, $\beta = 0.655 \pm 0.020$; and for the archaic "Eocene" and the opossum, $\beta = 0.657 \pm 0.028$. The linear regression accounts for 97 percent of the variance of the Recent sample, 94 percent of the Oligocene, and 99 percent of the archaic "Eocene" and the opossum. We can conclude that for these specimens the likelihood of a true β much different from 2/3 is small, and that the linearity implied by assuming Eq. 1a is supported by the data.

In addition to confirming my speculations about the primitive relationship between brain and body weight in mammals (compare Eqs. 2 and 5), perhaps the most important aspect of the results is that they give simple, yet mathematically precise, statements about the evolution of the brain. The same general rule that describes brain-to-body relationships in contemporary mammals was found in the earlier evolutionary stages sampled here, and the parameter, k, differentiated those stages. This parameter is not as successful in differentiating subgroups of contemporary mammals (see *7*), but the present results support its consideration as an index of cephalization for the mammals as a class at different stages in evolution.

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References and Notes

1. E. Dubois, *Bull. Soc. Anthropol. (Paris)* **8**, 337 (1897).
2. The major criticism has been of Dubois' choice of $\beta = 5/9$, which is certainly incorrect [see D. Sholl, *Proc. Roy. Soc. London B* **135**, 243 (1948); K. Wirz, *Acta Anat.* **9**, 133 (1950)].
3. K. S. Lashley, *Quart. Rev. Biol.* **24**, 28 (1949), p. 33.
4. H. J. Jerison, *Science* **121**, 447 (1955). Equation 1 with $\beta = 2/3$ was proposed O. Snell [*Arch. Psychiat. Nervenkrankh.* **23**, 436 (1891)] and was first given empirical support for contemporary mammals by G. von Bonin [*J. Gen. Psychol.* **16**, 379 (1937)].
5. Most of this research was performed under grant M-3145(a) from the National Institutes of Health and was supported in part by the U.S. Air Force under contract No. AF 49(638)-536 monitored by the Air Force Office of Scientific Research of the Air Research and Development Command. The results were included in a paper presented to the Psychonomic Society, 2 September 1960. Fossil specimens used in this work are from the departments of vertebrate paleontology at the American Museum of Natural History (AMNH), Harvard's Museum of Comparative Zoology (MCZ), the U.S. National Museum (USNM), and the Chicago Natural History Museum (CNHM). I wish to thank E. H. Colbert, G. G. Simpson, A. S. Romer, C. L. Gazin, and W. D. Turnbull of those museums for allowing me access to these materials. Special thanks are due Dr. Gazin for identifying *Subhyracodon* and *Hoplophoneus* at the U.S. National Museum on the basis of associated teeth, and for copies of endocranial casts of *Subhyracodon* and *Uintatherium*. My visit to Harvard's Museum of Comparative Zoology was made especially memorable by the gracious hospitality of Dr. Tilly Edinger. Her monograph *Evolution of the Horse Brain* [*Geol. Soc. Am., Mem. 25* (1948)] suggests important departures from the general evolutionary trend described here and includes an invaluable bibliography.
6. E. Dubois, *Biol. Generalis* **6**, 247 (1930).
7. H. J. Jerison, *Science* **121**, 447 (1955).
8. E. R. Hall and K. R. Kelson, *The Mammals of North America* (Ronald, New York, 1959).
9. M. Weber, *Festschr. f. Carl Gegenbaur, 3* (Engelmann, Leipzig, 1897).
10. R. S. Burrington and D. C. May, *Handbook of Probability and Statistics with Tables* (Handbook Pubs., Sandusky, Ohio, 1953), sec. 12.5.

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Alterations in Sialic Acid Content of Human Transferrin

Abstract. Starch gel electrophoresis of human transferrin treated with neuraminidase revealed a pattern of five bands whose intensities varied with neuraminidase concentration. Sialic acid analysis after starch block electrophoresis suggested that the bands represented the stepwise removal of sialic acid from the transferrin molecule. Evidence was also obtained for the purification of a particular genetic transferrin variant.

Genetically determined polymorphism has been described in several proteins in man, and in certain instances the variation has been localized to a single aminoacid substitution (*1*). The experiments reported in the present study suggest that observed variations in electrophoretic mobility may also represent differences in carbohydrate content.

Poulik (*2*) has described alterations in the electrophoretic mobility of serum protein components after treatment with diphtheria toxin. Using the technique of immunoelectrophoresis, Schultze and Schwick (*3*) have shown a decrease in mobility of the "β_1-iron-combining globulin" upon incubation with neuraminidase, an enzyme which cleaves the glycosidic bond joining sialic acid to a protein molecule. After the introduction of starch gel electrophoresis by Smithies (*4*), several genetically controlled variations in human serum β-globulins were described and subsequently identified as transferrin, the iron-binding component of serum. Neuraminidase-treated transferrin has recently been reported to separate into two sub-units in the starch gel (*5*).

In the present experiments transferrin was detected by its characteristic position in vertical starch gel electrophoresis in borate buffer, and its iron-binding property was confirmed by autoradiography (*6*). Transferrin C was prepared from the beta fraction of 20 ml of normal serum isolated by starch block electrophoresis in *p*H 8.6 barbital buffer (*7*). The beta fraction was chromatographed on TEAE cellulose and eluted with 0.025*M* Na_2HPO_4. The purified transferrin migrated as a single component in starch gel electrophoresis. Transferrin concentration was determined by the Folin-Ciocalteau procedure, and a standard curve was calculated from a transferrin sample dried to constant weight. Neuraminidase experiments on normal human serum and on the purified transferrin preparations were carried out by incubating samples at 37°C at various enzyme concentrations for 12 hours. The neuraminidase preparation was a ten times concentrated filtrate of *Vibrio cholerae*. The unconcentrated filtrate inhibited the agglutination of chicken erythrocytes at a 1:128 dilution when titrated against the Lee strain of influenza B virus (*8*). Sialic acid was determined by a modification of Bial's orcinol reaction (*9*), and optical density was measured in a Beckman Model DU spectrophotometer at 570 and 670 mμ, as suggested by Svennerholm (*10*). A standard curve was calculated from sialic acid purified from human ovarian cyst fluid.

Treatment of normal transferrin with neuraminidase split the single transferrin C (Fig. 1, band 4) into four additional slower-moving, iron-binding components (bands 3 to 0) whose relative intensities depended upon concentration of neuraminidase. A similar pattern was obtained by varying incubation time. No further bands appeared with an additional tenfold increase in neuraminidase concentration.

Starch block electrophoresis of purified transferrin treated with neuraminidase (Fig. 2) showed that the protein peak of the treated material migrated slower than an untreated control. Ultracentrifugal analysis could detect no difference in *S*-rate between the treated and untreated material. Sialic acid analysis revealed that the treated material contained approximately one to two sialic acid residues per molecule of