pensions was done in a Balzers BA 360 M apparatus, as described by Moor (*8*). Examination of the carbon replicas of the surfaces was by an AEI-6B and a Siemens 101 electron microscope.

Replicas of surfaces of lamellae of never-dried, freeze-fractured pellicles from both species of bacteria which produce cellulose show little evidence of the characteristic microfibrillar morphology (Fig. 1A). Instead, a lamella is composed of broad strands (frequently 30 to 40 nm in diameter, as opposed to the 10- to 12-nm microfibrils revealed by other techniques) of an amorphous material within which there is sometimes a core. In some parts of the pellicle there is no perceptible evidence for cores, only a mass of strands, but both core and sheath are readily perceived when the strands are pulled apart (Fig. 1A). In other areas, the cores may extend hundreds of nanometers. When strands are broken by fracturing, the replicas show that the ends of the cores often extend above the sheath and that there is a visible separation between the material of the sheath and the surrounding ice (Fig. 1B). A sheath and its core may be seen attached to or apparently extending from a bacterial cell incubated for 10 or 20 minutes (Fig. 1C). Sometimes the proximal tip can be seen within a fracture plane of the bacterial cell envelope, and sometimes this tip extends into the external milieu from a protrusion on the surface of the cell wall (Fig. 1D). Some strands intimately associated with freshly incubated bacterial cells have a diameter of almost 100 nm. The discreteness of the sheath (and its core) is made particularly visible when extended etching of the fracture surface leaves the sheath partially elevated above the surface of its ice matrix (Fig. 1C).

Replicas of the fracture surfaces of pellicles dried before freezing show microfibrils one-fourth to one-third as wide as the strands in the never-dried pellicles (Fig. 1E). There is little evidence for the strands found in never-dried pellicles. Instead, these replicas suggest an interlacing, felt-like structure of discrete threads similar to those recognized previously (*4*). In brief, the process of drying (which was applied to all previously examined specimens) converted the strands from a broader, unconsolidated form to a smaller, more condensed thread.

Because there is no evidence of extracellular, fibrillar material other than cellulose in cultures of either bacterium, it is reasonable to assume that the observed strands in the lamellae of the pellicles or in the suspensions of cells are a form of nascent cellulose microfibril, hitherto unrecognized. With this assumption, a strand consisting of a sheath of amorphous gel about a central core is an initial stage in the formation of the microfibril: this stage is physically altered by drying or chemical digestion of the film. Details of the process are unknown but it is plausible to suppose that water molecules are progressively removed from between the polyglucan chains, allowing the chains to associate irreversibly and to form the entity that has been recognized as the cellulose microfibril for at least 25 years (*4*). Even when drying or digestion does not occur, this association may take place slowly, leading to formation of a consolidated fibril which becomes the standard microfibril. The presence of such an early, unconsolidated sheath is consistent with the notion of intermediate, transient polymers in the biosynthesis of cellulose (*5, 6*), with the suggestion of multiple, glucan-synthetase activities (*9*), and with earlier, indirect inferences about hydration of cellulose (*4*, p. 61).

The presence of the sheath does pose additional problems of microfibril genesis because if the sheath were simply a cellulose-water gel, past experience suggests that the dried material would adopt the cellulose II lattice. If small cellulose I aggregates were already present these might assemble to give a cellulose I microfibril. However, there is as yet no evidence in the core or sheath for the crystalline, elementary fibrils which have been postulated from x-ray diffraction or electron microscope investigations (*10*).

Nonetheless, irrespective of molecular interpretations in detail, the presence of a transient gel sheath about a core represents a new, additional stage in the physical formation of the cellulose microfibril.

GARY G. LEPPARD
Inland Waters Directorate,
Environment Canada, Ottawa K1A 0E7
L. C. SOWDEN, J. ROSS COLVIN
Division of Biological Sciences,
National Research Council of Canada,
Ottawa K1A 0R6

References and Notes

1. K. Hess, *Die Chemie der Zellulose und Ihrer Begleiter* (Akademische Verlagsgesellschaft, Leipzig, 1928); M. V. Tracey, *Rev. Pure Appl. Chem.* 7, 1 (1957).
2. K. H. Gardner and J. Blackwell, *Biopolymers* **13**, 1975 (1974).
3. W. Z. Hassid, *Science* **165**, 137 (1969); H. M. Flowers, K. K. Batra, J. Kemp, W. Z. Hassid, *J. Biol. Chem.* **244**, 4969 (1969); F. Barnoud, D. Gagnaire, L. Odier, M. Vincendon, *Biopolymers* **10**, 2269 (1971); M. Dankert, R. García, E. Recondo, in *Biochemistry of the Glycosidic Linkage; An Integrated View*, R. Piras and H. G. Pontis, Eds. (Academic Press, New York, 1972), p. 199; J. Kjosbakken and J. R. Colvin, in *Biogenesis of Plant Cell Wall Polysaccharides*, F. Loewus, Ed. (Academic Press, New York, 1973), p. 361; M. Marx-Figini and B. G. Pion, *Biochim. Biophys. Acta* **338**, 382 (1974).
4. J. R. Colvin, *Crit. Rev. Macromol. Sci.* **1**, 47 (1972).
5. I. Ohad, D. Danon, S. Hestrin, *J. Cell Biol.* **12**, 31 (1962); E. Macchi, M. Marx-Figini, E. W. Fischer, *Makromolek. Chem.* **120**, 235 (1968); E. Macchi and A. Palma, *ibid.* **123**, 286 (1969).
6. J. Kjosbakken and J. R. Colvin, *Can. J. Microbiol.* **21**, 111 (1975).
7. S. Hestrin and M. Schramm, *Biochem. J.* **58**, 345 (1954).
8. H. Moor, *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **261**, 121 (1971).
9. G. Shore and G. A. Maclachlan, *Biochim. Biophys. Acta* **329**, 271 (1973).
10. A. Frey-Wyssling and K. Mühlethaler, *Makromolek. Chem.* **62**, 25 (1963).
11. We thank L. Ng and R. Whitehead for their technical assistance and J. Rak for preliminary investigations which led to the observations reported here. Issued as NRCC No. 14904.

31 March 1975

Incisor Size and Diet in Anthropoids with Special Reference to Cercopithecidae

Abstract. *In 57 species of anthropoids relative size of incisors is highly correlated with diet. Anthropoids that feed primarily on large food objects (large fruits) have larger incisors than those that feed on smaller food objects (berries or leaves). This difference reflects a need for more extensive incisal preparation of larger food objects before mastication. Extensive incisal preparation causes increased tooth wear; therefore, enlarged incisors are probably an adaptive response to increase their wear potential.*

The relationship between incisor morphology and diet has been investigated in both African apes and baboons (*1*). Within these two groups of primates, the more frugivorous forms (*Pan* and *Papio*) tend to have larger incisors than the more folivorous or graminivorous forms (*Gorilla* and *Theropithecus*). The adaptive significance of this finding is thought to be related to differential tooth use. Extensive incisal preparation prior to mastication (cutting, tearing, or pulping of food objects before chewing them with the postcanine teeth) ordinarily is not necessary for leaves, stems, berries, grasses, seeds, buds, or flowers; however, it is necessary when ingesting large, tough-skinned fruits. The increased frequency and duration of incisal preparation in both *Pan* and *Papio* causes increased amounts of attrition and abrasion of the anterior dentition, and therefore their enlarged incisors represent an adaptive response to delay dental obsolescence under these conditions. Accordingly, anthropoids whose diets consist primarily of large, tough fruits should have larger incisors than anthropoids which eat leaves, grass, or berries. The purpose of this study is to test the expected relationship between diet and size of the anterior dentition among anthropoid primates.

I analyzed 553 anthropoid skulls from the British Museum, the Musée Royal de l'Afrique Centrale, the Field Museum of Natural History, the American Museum of Natural History, and the United States National Museum. This sample comprises 57 species representing all catarrhine genera and subgenera (*2*). Only two species of platyrrhines, representing two genera (*Alouatta* and *Saimiri*), were examined. When possible, each taxon sampled was represented by at least five males and five females. Individuals with extensive incisor tooth wear were not included in this study. Two measurements were taken on each skull: the maximum linear distance between the distal cemento-enamel junction of the left and right lateral upper incisors, and a similarly measured distance between the lateral lower incisors. These measurements are estimates of the length of the incisal cutting edge and the wear potential (size) of the maxillary and mandibular incisal tooth rows. For each species studied, the mean values of these two measurements were analyzed relative to mean body mass (*3*). The natural logs of both the incisal width mean values and the body weight mean values were plotted against one another. Regression lines, with body weight acting as the independent variable, were fitted by the method of least squares (*4*). Males and females were analyzed separately.

As is shown in Fig. 1, there is a near separation of colobines from cercopithecines; most colobines fall below the regression line while most cercopithecines fall above it. Thus, relative to body size, the leaf-eating colobines have smaller incisors than the more frugivorous cercopithecines. (This generalization holds even when colobine mean body weights are reduced 25 percent to compensate for possible stomach contents.) There are, however, several cercopithecine data points that fall within or near the colobine range. The gelada baboon (*Theropithecus*), a terrestrial primate that feeds primarily on grasses (*5*), falls among the leaf-eating colobines. As noted by Jolly, small incisor size in this species may be related to its habit of feeding on small objects, and to the rather infrequent use of its incisors for food preparation (*1*). The patas monkey (*Erythrocebus*), another predominantly grass-eating terrestrial primate, also has relatively small incisors. Although this primate does eat small fruits and berries it ignores the large, tough, sausage-shaped fruit of *Kigelia aethiopica*, a fruit that is commonly eaten by sympatric populations of *Papio* (*6*). Like *Theropithecus*, *Erythrocebus* normally eats objects so small as to require relatively little incisal preparation.

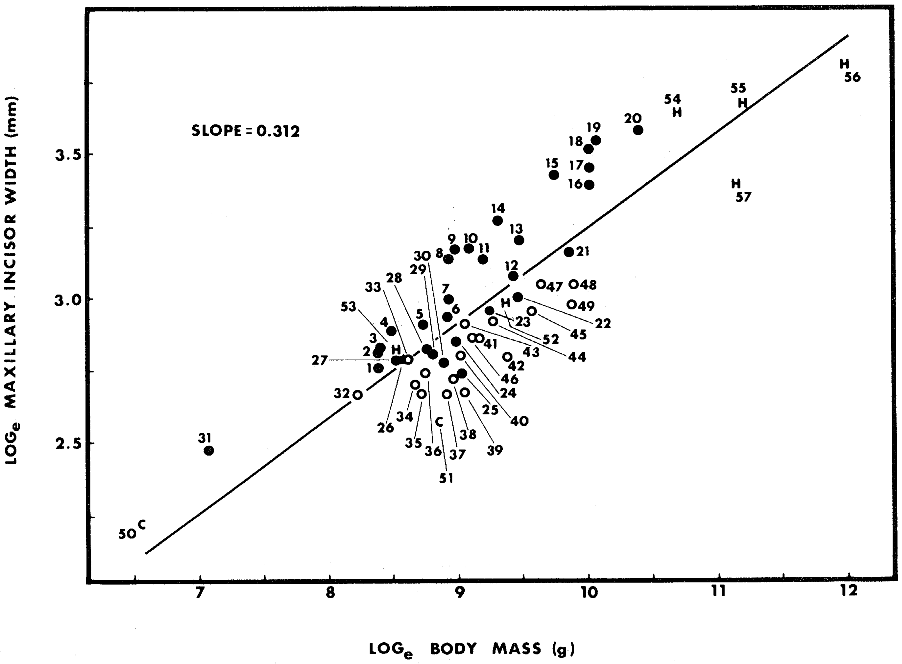


Fig. 1. A plot of mean body mass versus width of the maxillary incisor tooth row for all male anthropoids examined. Numbers in parentheses indicate the number of animals. Numbers preceding each species are keyed to numbers in the plot. Cercopithecines (closed circles): 1, *Cercopithecus pogonias* (4); 2, *Cercopithecus denti* (5); 3, *Cercopithecus cephus* (5); 4, *Macaca fascicularis* (5); 5, *Cercopithecus nictitans* (5); 6, *Macaca cyclopis* (4); 7, *Macaca mulatta* (5); 8, *Macaca nigra* (5); 9, *Cercocebus albigena* (7); 10, *Macaca maura hecki* (4); 11, *Cercocebus torquatus* (5); 12, *Macaca sylvana* (4); 13, *Macaca speciosa* (4); 14, *Macaca nemestrina* (5); 15, *Papio hamadryas* (6); 16, *Mandrillus sphinx* (6); 17, *Mandrillus leucophaeus* (4); 18, *Papio anubis* (4); 19, *Papio cynocephalus* (1); 20, *Papio ursinus* (10); 21, *Theropithecus gelada* (5); 22, *Macaca fuscata* (4); 23, *Erythrocebus patas* (7); 24, *Cercopithecus mitis* (5); 25, *Cercopithecus lhoesti* (4); 26, *Cercopithecus ascanius* (5); 27, *Cercopithecus aethiops* (6); 28, *Allenopithecus nigroviridis* (7); 29, *Cercopithecus neglectus* (5); 30, *Cercopithecus hamlyni* (5); 31, *Miopithecus talapoin* (6). Colobines (open circles): 32, *Procolobus verus* (5); 33, *Presbytis frontatus* (4); 34, *Presbytis aygula* (5); 35, *Presbytis rubicundus* (5); 36, *Presbytis potenziani* (5); 37, *Presbytis cristatus* (5); 38, *Simias concolor* (5); 39, *Presbytis senex* (3); 40, *Presbytis obscurus* (4); 41, *Colobus angolensis* (5); 42, *Presbytis johni* (5); 43, *Colobus badius* (7); 44, *Pygathrix nemaeus* (5); 45, *Presbytis entellus entellus* (5); 46, *Colobus guereza* (5); 47, *Rhinopithecus roxellanae* (4); 48, *Presbytis entellus achilles* (4); 49, *Nasalis larvatus* (5). Ceboids (C): 50, *Saimiri sciureus* (5); 51, *Alouatta villosa* (5). Hominoids (H): 52, *Symphalangus syndactylus* (5); 53, *Hylobates moloch* (5); 54, *Pan troglydytes* (5); 55, *Pongo pygmaeus* (5); 56, *Gorilla gorilla* (5); 57, *Homo sapiens* (5).

The only Asiatic cercopithecine to fall below the regression line is the Japanese macaque, *Macaca fuscata*. For many months of the year it subsists almost exclusively on bark, leaves, buds, twigs, roots, berries, and even lichen (*7*). Again, although this animal eats some fruit, the bulk of its diet consists of food objects that probably require little incisal preparation. The remaining cercopithecines that fall below the regression line are *Allenopithecus* and several species of *Cercopithecus*. The *Cercopithecus* species are *C. neglectus*, *C. mitis*, *C. hamlyni*, and *C. lhoesti*. As seen in Fig. 1, the last two species have very small incisors. Unfortunately, very little is known about their ecology. *Cercopithecus lhoesti* is said to include fruit, berries, and leaves in its diet (*8*, *9*). There is no dietary information about the owl monkey, *C. hamlyni*. The blue monkey, *C. mitis*, in addition to eating fruits and bamboo shoots, is said to include a large amount of leaves in its diet (*10*). Very little is known about the ecology of either *Allenopithecus* or *C. neglectus*. Stomach contents and observations on a small number of animals suggest that the diet of *C. neglectus* consists of both leaves and fruit (*11*). Pournelle states that *Allenopithecus* eats snails, shrimp, fish, insect larvae, nuts, and fruit (*12*).

Relative to body size, *Miopithecus*, *Cercopithecus cephus*, *Macaca fascicularis*, *Cercocebus albigena*, *Macaca nigra*, *Macaca nemestrina*, and *Papio* have very large incisors (they fall well above the regression line in Fig. 1). Although not shown in Fig. 1, *Macaca silenus* females have extremely large incisors. (No males were sampled.) *Miopithecus*, *Cercopithecus cephus*, *Cercocebus albigena*, *Macaca fascicularis*, and *Macaca nemestrina* apparently eat large quantities of fruit (*8*, *13*). Very little is known about the diet of *Macaca nigra*. *Macaca silenus* is said to eat fruits, flowers, nuts, and buds, but its favorite food is the unripe nut of a chestnut-like fruit (species unknown) (*14*). Using both teeth and hands the animal apparently takes about 10 minutes to crack open these unripe nuts. Many of the above primates with large incisors are known to be highly frugivorous. For many (possibly all) of them, the fruits eaten require extensive amounts of incisal preparation.

As seen in Fig. 1, the various species (or subspecies) of *Papio* also have relatively large incisors. Although a few populations of *Papio* are said to be highly folivorous or graminivorous (*15*), when ecological conditions permit, *Papio* includes a large amount of fruit in its diet (*16*). Jolly notes that these fruits often require extensive incisal preparation (*1*). In addition to food object size, another factor that probably affects the amount of incisor tooth wear is the inclusion of dietary grit. Presumably, a terrestrial animal that selects food objects from the ground ingests more grit than an animal that selects the same food object directly from a tree or shrub. An increase in the amount of dietary grit would have the effect of increasing the amount of tooth wear. The inclusion of large amounts of grit (from both roots and fallen fruit) might in part explain why various *Papio* populations have such large incisors. For example, *Papio anubis* often pulls grass rhizomes from the ground with its incisors, unlike sympatric populations of *Theropithecus* that pull these same rhizomes out with their hands (*5*).

Drills and mandrills also have relatively large incisors, that is, they fall well above the regression line in Fig. 1. Unfortunately very little is known about their dietary habits. It has been suggested that the frugivorous *Mandrillus* has larger incisors than *Papio*, and also that incisor size in *Papio hamadryas* is intermediate between *Theropithecus* and other populations of *Papio* (*17*). Neither of these observations is supported by these data.

Unfortunately the diets of many colobines are not well known. However, it has recently become apparent that the diets of certain sympatric colobines differ in the percentage of leaves and fruits ingested. Populations of *Colobus badius* include a larger percentage of fruit in their diet than do sympatric populations of *Colobus guereza* (*18*). Similarly, *Presbytis entellus* is more frugivorous than *Presbytis senex* (or *Presbytis johni*) (*19*). In both of these groups, the more frugivorous of the two has the larger incisors (see Fig. 1).

On the basis of the relation between incisor size and diet, certain dietary predictions can be made. It is hypothesized that, compared to those *Cercopithecus* species that fall above the regression line, the diet of *C. hamlyni, C. mitis*, and *C. lhoesti* (and possibly also *Allenopithecus* and *C. neglectus*) consists of food objects that require relatively little incisal preparation. The fact that *Allenopithecus* has incipient sacculation of the stomach (*20*) takes on additional interest here, because this type of visceral specialization is usually present only in folivorous primates. Of the sympatric colobines found on the Mentawi Islands, *Presbytis potenziani* is probably more frugivorous than *Simias concolor*. There are several colobine species on Borneo that are also sympatric in portions of their range. Unfortunately, except for *Nasalis larvatus*, little is known of their diets. Judging from incisor dimensions, *Presbytis frontatus* is probably much more frugivorous than the highly folivorous *Nasalis larvatus* (*21*), and *Presbytis rubicunda* and *Presbytis aygula* are probably dietetically intermediate between these two forms. The olive colobus, *Procolobus verus*, falls slightly above the regression line. On the basis of incisor size, one would predict that this colobine takes into its diet at least as much fruit as *Colobus badius*. Stomach contents of 33 specimens, however, revealed only leaves and a few flowers (*22*). It is possible that these animals were collected at a time of the year when few if any fruits were available.

In addition to presenting data for cercopithecids, Fig. 1 also includes data for hominoid and the two ceboid genera. Among the ceboids and hylobatids, *Alouatta* and *Symphalangus* have a more folivorous diet than *Saimiri* and *Hylobates*, respectively (*23*). These dietary preferences are reflected in the size of the anterior dentition, as seen in Fig. 1. The frugivorous forms have larger incisors than the more folivorous forms.

Of the remaining primates, the chimpanzee (*Pan*) is highly frugivorous, the orang (*Pongo*) is both frugivorous and folivorous (depending on the season), and the gorilla (*Gorilla*) is highly folivorous (*24*). The diet of *Homo*, of course, varies considerably from one population to the next. As expected, *Pan* falls well above the regression line, *Gorilla* falls below it, and *Pongo*, although located above the line, is positioned intermediate to *Pan* and *Gorilla*. *Homo*, like *Gorilla*, falls below the line; but, unlike that of *Gorilla*, the diet of *Homo* is not highly folivorous. The relatively small incisors in *Homo* are probably related to food preparation techniques that result in less incisal preparation of foods. This particular population of *Homo sapiens* is apparently from India. If the incisor tooth row of Australian aboriginals or Europeans had been measured in constructing Fig. 1, these populations would fall closer to and farther from the regression line, respectively. However, it is doubtful whether any human population would closely approach the line.

An identical analysis of female anthropoids yielded essentially the same results; that is, the scattering of data points around the regression lines exhibited the male pattern. The analysis of the mandibular dentition also exhibited a similar pattern in both males and females. An analysis of both incisor and molar morphology among primates has been described (*25*). Independent studies on molar morphology and diet in primates by Kay have yielded remarkably similar results in both the correlation between morphology and known diet, and in dietary predictions for various cercopithecoids (*26*). In conclusion, Jolly's original observation on the relation between diet and incisor morphology in African apes and baboons appears to hold for all catarrhines and the two sampled platyrrhines.

WILLIAM L. HYLANDER

Departments of Anatomy and
Anthropology, Duke University,
Durham, North Carolina 27710

References and Notes

1. C. J. Jolly, *Man* **5**, 1 (1970).
2. J. R. Napier and P. H. Napier, *A Handbook of Living Primates* (Academic Press, New York, 1967); R. W. Thorington and C. P. Groves, in *Old World Monkeys* (Academic Press, New York, 1970), p. 620.
3. Mean body weight estimates were determined in various ways. The majority of the primate skulls analyzed in this study are housed in the U.S. National Museum, and body weight data are available for many of them. Body weight data for various species and subspecies were also taken directly from the literature. These sources provided enough information to estimate male and female mean body weight values for all the ceboids and hominoids, and the great majority of cercopithecids included in this study. Mean values were estimated for the remaining cercopithecids (approximately 10 percent) in the following manner: Male and female cercopithecids with known body weights were treated separately. Each sex was then divided into the following natural groups: (i) a *Macaca-Cercocebus-Papio* group, (ii) a colobine group (excluding *Simias* and *Nasalis*), and (iii) a *Cercopithecus* group (including *Miopithecus, Allenopithecus*, and *Erythrocebus*). Then, with the use of multiple stepwise regression procedures, mean body weight values were predicted on the basis of (i) foramen magnum area, (ii) interorbital width, and (iii) mandibular bicondylar width mean values (the explained variance ranged between .93 and .99). Using these multiple regression formulas for the three natural groups, body weights were estimated for taxa where body weight data are unavailable.
4. G. G. Simpson and A. Roe, *Quantitative Zoology* (Harcourt, Brace, rev. ed., New York, 1960).
5. R. I. M. Dunbar and E. P. Dunbar, *Folia Primatol.* **21**, 36 (1974).
6. K. R. L. Hall, in *The Baboon in Medical Research* (University of Texas Press, Austin, 1965), vol. 1, p. 43.
7. A. Suzuki, *Primates* **9**, 31 (1965); K. Izawa, *ibid.* **12**, 191 (1971); *ibid.* **13**, 201 (1972).
8. A. J. Haddow, K. C. Smithburn, A. F. Mahafey, J. C. Bugher, *Trans. R. Soc. Trop. Med. Hyg.* **40**, 677 (1947).
9. J. S. Gartlan and T. Struhsaker, *J. Zool. Proc. Zool. Soc. Lond.* **168**, 221 (1972); I. T. Sanderson, *The Monkey Kingdom* (Doubleday, New York, 1957).
10. A. J. Haddow, *Uganda Wildlife and Sport* **1**, 22 (1956); J. S. Gartlan and C. K. Brain, in *Primates: Studies in Adaptation and Variability* (Holt, Rinehart & Winston, New York, 1968), p. 253.
11. J. P. Gautier and A. Gantier-Hion, *La Terre et La Vie* **23**, 164 (1969).
12. G. H. Pournelle, *Zoonooz* **32**, 10 (1959).
13. A. Gantier-Hion, *La Terre et La Vie* **25**, 427 (1971); J. A. Kurland, *Primates* **14**, 245 (1973); J. Fooden, *Fieldiana Zool.* **59**, 1 (1971); M. Malbrant and A. Maclatchy, *Faune de l'Equateur African Francais*, tome 2, *Mammiferes* (Lechevalier, Paris, 1949).

14. Y. Sugiyama, *J. Bombay Nat. Hist. Soc.* **65**, 283 (1968).
15. S. L. Washburn and I. Devore, *Sci. Am.* **204**, 62 (1961); V. Nagel, *Folia Primatol.* **19**, 104 (1973).
16. F. P. G. Aldrich-Blake, T. K. Bunn, R. I. M. Dunbar, P. M. Headley, *Folia Primatol.* **15**, 1 (1971).
17. C. J. Jolly, in *Old World Monkeys* (Academic Press, New York, 1970).
18. T. H. Clutton-Brock, *Nature (Lond.)* **250**, 539 (1974).
19. C. M. Hladik and A. Hladik, *La Terre et La Vie* **2**, 149 (1972); R. H. Horwick, *J. Bombay, Nat. Hist. Soc.* **69**, 255 (1972); N. Muckenhirn, thesis, University of Maryland (1972).
20. W. C. O. Hill, *Primates, VI, Cercopithecoidea* (Wiley-Interscience, New York, 1966).
21. J. A. Kern, *Zoologica (N.Y.)* **49**, 183 (1964).
22. A. H. Booth, *Proc. Zool. Soc. Lond.* **129**, 421 (1957).
23. C. M. Hladik, A. Hladik, J. Bousset, P. Valdebouze, G. Viroben, J. Delort Laval, *Folia Primatol.* **16**, 85 (1971); D. J. Chivers, in *The Gibbon and Siamang*. (Karger, Basel, 1972), vol. 1; J. Fooden, *Am. J. Phys. Anthropol.* **22**, 227 (1964); R. R. Tenaza and W. J. Hamilton, *Folia Primatol.* **15**, 201 (1971).
24. A. Suzuki, *Primates* **1**, 481 (1966); R. K. Davenport, *Folia Primatol.* **5**, 247 (1967); G. B. Shaller, *The Mountain Gorilla* (Univ. Chicago Press, Chicago, 1963).
25. R. F. Kay and W. L. Hylander, *Arboreal Folivores* (Smithsonian Press, Washington, D.C., in press).
26. R. F. Kay, *Am. J. Phys. Anthropol.*, in press; *Proceedings Fourth International Symposium Dental Morphology*, in press.
27. I thank the curators of the primate collections at the British Museum of Natural History, the Musée Royal de l'Afrique Centrale, the Field Museum of Natural History, the American Museum of Natural History, and the United States National Museum for allowing access to the materials upon which this study is based. I am also grateful to K. Brown, R. F. Kay, and M. Cartmill for help throughout this investigation.

28 April 1975

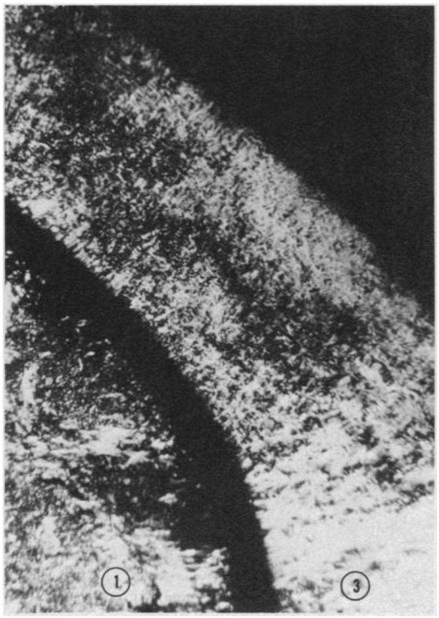
Crustacean Intestinal Detergent Promotes Sterol Solubilization

Abstract. *Although crustacean tissue cholesterol content is high, Crustacea, like other arthropods, are incapable of cholesterol synthesis, and presumably are dependent for maintaining tissue cholesterol stores on the intestinal absorption of ingested sterol. A detergent,* N-(N-*dodecanoylsarcosyl)taurine, representative of a set of detergents synthesized by the crustacean hepatopancreas and secreted into the intestine, is capable of efficient cholesterol solubilization, and thus of promoting sterol absorption.*

Micellar solubilization is an essential step in the biliary secretion and efficient intestinal absorption of endogenous and dietary lipids (*1*). In vertebrates aqueous solubility of lipids is enhanced by mixed micelle formation with bile salts, a family of steroidal hepatobiliary detergents derived from cholesterol (*2*). The formation in bile of mixed micelles of bile salts with phospholipids and cholesterol promotes the excretion of these otherwise insoluble lipids. Similarly, the formation in the intestine of mixed micelles of bile salts with fatty acids, monoglycerides, and cholesterol augments the solution concentration of these luminal lipids and thus promotes their absorption from the gut (*3*).

Less is known about the intestinal absorption of essential lipid in subvertebrate species (*4*). While the cholesterol concentrations of crustacean tissues are among the highest recorded for animals (*5*), Crustacea, like many other arthropods, are incapable of cholesterol synthesis from nonsteroidal precursors, such as acetate and mevalonate (*6*). Also, some marine invertebrates contain a wide variety of sterols in addition to cholesterol (*7*). We therefore propose that the sterols of crustacean tissue are derived from the diet. Since dietary sterols are largely insoluble in water, it seemed reasonable, by analogy to vertebrate physiology, to seek a mechanism for sterol solubilization in the crustacean intestine. The crustacean hepatopancreas, unlike the vertebrate liver, is incapable of bile salt synthesis (*8*) although enzyme systems capable of catalyzing the conversion of phytosterols to cholesterol and of cholesterol to a variety of steroid hormones are present (*9*). However, a mixture of straight-chain fatty acid detergents containing predominantly *cis*-dodec-5-enoic acid conjugated with the dipeptide sarcosyltaurine has been isolated from crustacean (*Cancer* spp.) gut contents (*10*). These detergents have been shown to be synthesized endogenously from acetate (*11*) and have been assumed to aid lipolysis by playing a role in the intestinal emulsification of dietary fat (*12*).

Fig. 1. Photomicrograph of phases observed during flooding of DST with water (crossed nicols; (× 55) 23°C. Phases: (1) Neat phase; (2) viscous isotropic phase (cubic); (3) middle phase texture (cloudy); (4) isotropic solution. This photograph demonstrates the microscopic textural appearances of DST as progressive hydration occurs. A small quantity of crystalline DST was placed between slide and cover slip, and a drop of water at *p*H 6.8 was added. The changes in the preparation were observed within a few seconds of hydration. As the water comes in contact with the sample, a water gradient is created with the outer border being the most hydrated and the center of the preparation remaining dry. Zones in between contain more and more water as one approaches the outside border. This simple method is helpful in determining the number and texture of the phases found in a detergent water mixture, provided the ambient temperature is above the critical micellar or penetration temperature of the detergent (*17*).



We hypothesize that crustacean detergent is essential for the intestinal micellization of endogenous and ingested sterols and thus permits their efficient solubilization and absorption. In order to examine this possibility we have studied the interactions between the sodium salt of *N*-(*N*-dodecanoylsarcosyl)taurine (DST), a representative model for the class of crustacean detergents, and cholesterol in water. For this purpose, a mixed anhydride of the benzyloxycarbonyl derivative of sarcosine was reacted with taurine, and, after removal of the blocking group and crystallization from ethanol, the resultant sarcosyltaurine was reacted with dodecanoic acid (*13*). The synthesized product was purified by reversed-phase column chromatography, and no traces of dodecanoic acid, sarcosine, taurine, or sarcosyltaurine were discovered by thin-layer chromatography (TLC), proton titration, or amino acid analysis. The melting point of the sodium salt of DST was 190° to 192°C. The critical micellar temperature (*14*) was below 0°C, as indicated by the fact that a 1 percent solution showed no trace of turbidity on prolonged cooling (0°C). The apparent *pK* (by titration with HCl, 1 percent solution) was 2.2 (*15*). DST exhibited lyotropic mesomorphism (liquid crystalline phases) comparable to that demonstrated by soaps and synthetic straight-chain detergents (*16*). Polarizing microscopy (*17*) revealed that, upon hydration of dry samples of DST, alternating sharply delineated, texturally characteristic, birefringent and isotropic bands corresponding to crystalline hydrates, neat (possibly lamellar), viscous isotropic (cubic), and middle (possibly hexagonal) liquid crystalline phases, and a micellar phase were demonstrable at increasing distance from the unhydrated material (Fig. 1).

Cholesterol solubilization by DST micellar solutions was demonstrated in binary mixtures in an excess of aqueous solvent (Fig. 2). At concentrations of 20 m*M* DST or less, there was no solubilization of cholesterol. Increasing amounts of cholesterol were solubilized at concentrations of