

## BRIEF REPORTS

### Xanthogranuloma of the Choroid Plexus in the Fat-Tailed Dwarf Lemur (*Cheirogaleus medius*)

JASON T. LEE<sup>1</sup>, CAROL A. MILLER<sup>1,3</sup>, COLIN T. MCDONALD<sup>2</sup>, AND JOHN M. ALLMAN<sup>1</sup>

<sup>1</sup>Division of Biology, California Institute of Technology, Pasadena, California; <sup>2</sup>Department of Neurology, Massachusetts General Hospital, Boston, Massachusetts; and <sup>3</sup>Department of Pathology, University of Southern California, School of Medicine, Los Angeles, California

This report documents the death of two fat-tailed dwarf lemurs (*Cheirogaleus medius*) maintained over 6 years each in our laboratory. Postmortem studies revealed xanthogranuloma of the choroid plexus, a mass replete with stored lipids, including cholesterol crystals. Six months prior to their deaths, both animals developed a peculiar head tilt and signs suggestive of neurological dysfunction. At autopsy, each had masses projecting into the lateral and IVth ventricles and an associated obstructive hydrocephalus. Cryostat sections of the brains of both lemurs showed histological features consistent with xanthogranuloma of the choroid plexus, a histologically benign and usually asymptomatic lesion found in up to 7% of human autopsies. This case is of special interest because of the unique feeding strategies in the fat-tailed dwarf lemur. Since *C. medius* remains in torpor for 6 months out of the year during the time of food scarcity in the forests of Madagascar, the animal must accumulate large reserves of fat during its active period. In the laboratory, however, dwarf lemurs do not normally go into torpor, and the accumulated fat is not used. The finding of this tumor, therefore, suggests that the combination of a captive high-fat diet and the unusual fat-storage mechanisms utilized by *C. medius* contributed to the buildup of lipids and might be etiologically related to the development of these lesions. © 1996 Wiley-Liss, Inc.

**Key words:** brain tumor, lipids, diet, captivity

#### INTRODUCTION

The nocturnal dwarf lemurs, *Cheirogaleus*, are interesting in their unique lipid metabolism and related feeding strategies. The most characteristic feature of the fat-tailed dwarf lemur, *Cheirogaleus medius*, is that it goes into torpor during the dry season, remaining inactive for at least 6 months [Petter, 1978]. Torpor, though functionally similar to hibernation, does not require the inactivity to take place in the winter months as seen in northern mammals. During the dormant period of *C. medius*, which usually occurs from May to November, the lemurs retire to deep holes within tree trunks, forming groups of 3–5. They do not emerge from

Received for publication September 2, 1994; revision accepted September 17, 1995.

Address reprint requests to Jason T. Lee, c/o John Allman, Caltech 216-76, Pasadena, CA 91125.

this state until the beginning of the rainy season, when there is maximal food availability in the forests of Madagascar [Hladik, 1980].

During this transient period of surplus food, the animals will provide for their annual nutritional requirements. *C. medius* prepares for the next dormancy by storing large fat reserves under the skin and inside the tail. The accumulation of lipids in their body is crucial to the overall feeding strategy, allowing them to survive during the following 6 months when food is scarce. This period of torpor in the dry season is therefore thought to be an adaptive measure for survival [Wright, 1994]. Field studies reveal that the mean body weight of *C. medius* increases from 145 g to 220 g from November to March, and mean volume of the tail triples, increasing from 15 cc to 42 cc in the same time frame [Harcourt, 1990]. In nature *C. medius* eats mainly fruit supplemented by nectar and insects when available; undigested invertebrate parts have been found in 50% of fecal samples but never constituting more than 10% of the sample [Hladik et al., 1980].

Similar body weight fluctuations in *C. medius* have been recorded in laboratory environments [Russell, 1975]. But in these captive animals, the period of torpor was not clearly defined, and there was never a period of genuine inactivity. In a separate study [Petter-Rousseaux, 1980], definitive torpor in the lab was not observed until the animals were subjected to winter temperatures as low as 12°C. Only under those experimental conditions did the animals become inactive for 6 months of the year. In our laboratory, we never observed a period of torpor or significant body weight fluctuations during the 6 years we housed the two lemurs.

## MATERIALS AND METHODS

### Case History

**Housing conditions.** Both dwarf lemurs were housed inside 0.8 m × 0.8 m × 0.8 m stainless steel cages with climbing perches and nest boxes. Each dwarf lemur had a mate. Their mates did not develop this condition. The temperature was maintained year round between 26 and 28° Celsius. Between 8 AM and 12 PM every morning, each pair of lemurs was fed approximately 100 g each of Iams brand dry cat chow #7250 (two small handfuls) and 40 g fruit supplement (two pieces of bananas, apples, pears, peaches, or nectarines). The composition of the chow was as follows: 32% protein, 21% fat, and 3% fiber per daily serving. Water was available at all times.

**Lemur #1.** A male fat-tailed dwarf lemur was one of seven obtained from Duke University Primate Center (DUPC) in July, 1988. Born in October, 1987, at DUPC, lemur #1 displayed no remarkable behavior or neurological signs in the first 6 years of his life indicative of brain dysfunction. The light cycle for lemur #1 was 12/12 hr (day/night) without any seasonal variations. His feeding habits remained constant throughout most of his lifetime, slowly gaining weight as the years progressed. Six months prior to death, lemur #1 developed an occasional, peculiar, sustained head tilt to one side for periods of up to a day. The animal's decline was also marked by lethargic behavior and a reduction in appetite. The lemur then expired. At the time of autopsy, lemur #1 weighed 283 g.

**Lemur #2.** A second male fat-tailed dwarf lemur, obtained at the same time as lemur #1, was born at DUPC in September, 1987, and he too displayed no abnormal behavior or neurological changes during his lifetime. The light cycle for Lemur #2 was varied on an annual cycle between 10 and 14 hr day and the balance night. Two months after lemur #1 died, lemur #2 began to develop and exhibit the same intermittent head tilt and sluggish behavior for 3 months until he also expired. At the time of autopsy, lemur #2 weighed 265 g.

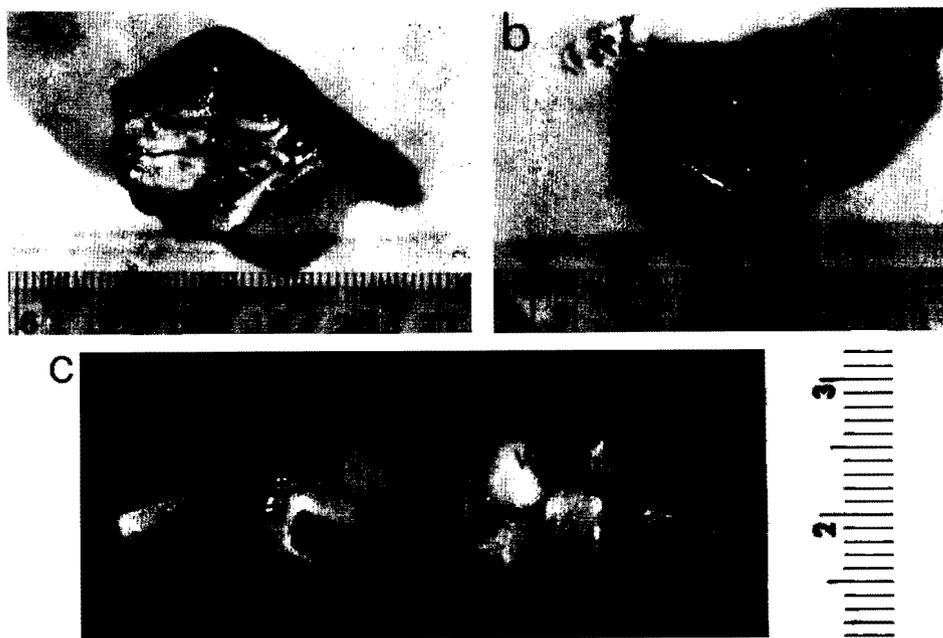


Fig. 1. a: Ventral view of the entire lemur #1 brain after removal. Notice the great enlargement of the left hemisphere. b: Dorsal view of the enlarged left hemisphere of lemur #1 with view of the tumor (arrow). c: Coronal sections of right hemisphere brain from lemur #2. The mass (arrow) projects into the dilated ventricle (V).

### Preparation of Brain Tissue

Both brains were removed and immersed overnight in standard 0.1 M sodium phosphate buffer containing 3% glutaraldehyde and 10% sucrose at 4°C. The sucrose concentration was then increased to 30% for cryoprotection for approximately 6 hr, until the brain was saturated and sunk to the bottom of the flask. Cryostat sections (40  $\mu$ ) were cut and stained with hematoxylin and eosin.

### RESULTS

The brain from lemur #1 was markedly asymmetric with enlargement of the left hemisphere (Fig. 1a). The cerebellar hemispheres were also enlarged. The brain was bisected in the parasagittal plane through the corpus callosum, revealing a large, yellow-orange, mottled mass (Fig. 1b) extending into and distending the left lateral ventricle and a second mass effacing the IVth ventricle. Cryostat sections (40  $\mu$ ) were then cut sagittally from the left half, and coronal sections from the right half.

Upon gross inspection of the brain from lemur #2, the right cerebral hemisphere was markedly dilated. Coronal sections revealed hydrocephalus with diffuse bilateral thinning of the cortices, the right greater than the left. A 4  $\times$  4 mm mass extended from the thalamic region into the ventricle and obstructed the foramen of Monroe (Fig. 1c). The cut surface of this mass showed a yellow-tan, variegated coloration. In the cerebellum, a small 3  $\times$  3 mm mass extended from the roof of the IVth ventricle, but did not occlude the ventricle.

Microscopically, the lesions from both lemurs showed a cellular pattern con-

sistent with xanthogranuloma of the choroid plexus. Each lesion projected as a mass, partially effacing the ventricular cavity and in continuity with the brain parenchyma (Fig. 2a). Viewed in an EDGE high-definition microscope, the mass contained numerous, crescent-shaped clear spaces, or "cholesterol clefts," lined by flattened, foreign body giant cells and macrophages (Fig. 2b,c). Some of these structures were rimmed by a focal, calcified capsule-like structure. Within some spaces, there was amorphous, acellular, granular debris. The intervening neuropil and the base of each mass contained areas of dense gliosis with bands of collagen, scattered astrocytes, and mineralized deposits. Entrapped choroid plexus epithelium was not present. No mitotic figures were seen. There was no nuclear atypia indicative of malignancy. The adjacent cerebral cortex and cerebellum showed normal neuronal layering and organization. The underlying white matter was thinned and there was loss of the ependymal lining of the ventricles.

## DISCUSSION

Xanthogranulomas of the choroid plexus of the human CNS occur asymptotically in 1.2–7% of autopsies [Ayres & Haymaker, 1960]. Lesions producing neurological symptoms are, however, rare and can occur throughout life. Characteristically, these histologically benign lesions arise within the glomus of the trigone of the lateral ventricle [Brück et al., 1991]. Although infrequent in humans, they commonly occur in aged horses [Innes and Saunders, 1962], leading to a condition called "Dummkoller" (literally "dumb staggers"). No documentation of these particular brain lesions in lemurs has been found in the previous literature [Benirschke et al., 1985], although lipid-related tumors have been found in other nonhuman primates.

A lipoma was incidentally found in the choroid plexus of the lateral ventricle of an intrathalamically inoculated rhesus monkey [Weston, 1965]. A lipoma occurring in the abdominal cavity led to the death of a previously pregnant *Cerco-pithecus diana* that had been in captivity for 15 months [Ratcliffe, 1942]. The tumor proved to be continuous with the adjacent subcutaneous fat, and the liver and kidneys of the monkey also contained unusually large quantities of fat within the cytoplasm of epithelial cells. Another lipoma was reported in the omentum of an 8-year-old *Macaca silenus* [Kronberger & Rittenbach, 1968] that subsequently led to the animal's death. The unique occurrence of this particular CNS lesion causing death in our lemurs therefore leads to speculation concerning the impact that captivity and diet have on the health of these animals.

When symptomatic, these lesions produce obstructive hydrocephalus by mass effect, resulting in headache, visual disturbances, and papilledema. Brainstem dysfunction, produced by the increased intracranial pressure, was observed in both lemurs prior to death, as evidenced by the peculiar head tilt. The hydrocephalus and resultant brainstem compression are the likely cause of death in both lemurs. Because xanthogranulomas do not arise in the IVth ventricle, the occurrence at that site in these dwarf lemurs is therefore unusual and distinct from that in the human CNS [Hicks et al., 1993].

The etiology of xanthogranulomas is unclear. Possibilities include processing of erythrocyte membranes to cholesterol after small, intra-choroidal hemorrhages, or deposition of plasma lipids in the choroid plexus matrix, a site lacking a blood-brain barrier. Positive correlation between serum hyperlipidemia or elevated cholesterol and the occurrence of these lesions has not been observed. However, xanthomatous disease of a genetic basis can involve the brain [Flach & Weinkelman, 1986; Giller et al., 1988]. Considering the fat reserves built up during the feeding period in a normal fat-tailed dwarf lemur, we believe the lack of torpor in captive



Fig. 2. a: Parasagittal cryostat section of the brain of lemur #1 shows two tumors (arrows): one projects into the lateral ventricle (LV) and a second into the IVth ventricle (IV) (5 $\times$ ). b: At low magnification, nests of cholesterol clefts (clear areas) are surrounded by glial stroma. Each cleft is enveloped in a cellular syncytium (arrows). The cleft is devoid of stainable material as cholesterol is removed by lipid solvents during histologic processing. A dense, calcific deposit lies in the adjacent stroma (arrowheads) (300 $\times$ ). Bar measures 5  $\mu$ M. c: At higher magnification, the syncytium (arrows) is multinucleated and lines the cleft (750 $\times$ ). Bar measures 1  $\mu$ M (hematoxylin and eosin).

animals is a strongly contributing factor to accumulation of lipids and subsequent development of these lesions. Both animals had remained active and alert throughout the years, without any significant fluctuations in activity level or appetite until the gradual decline in health approximately 6 months before death. The weight of both lemurs at the time of death was above the average maximum at the end of the feeding season for sample dwarf lemurs living in Madagascar (283 and 265 vs. 220 g), showing that our lemurs were overweight.

A previous study [Birt et al., 1992] has shown that dietary calorie restriction of carbohydrates and fats inhibits the development of papillomas and carcinomas in the skin of rats. The direct relationship between high dietary fats and tumor formation indicates that unmonitored or nonutilized high-fat diets (like those in our lemurs) can have direct consequences related to neoplasia. Because of the fat storage adaptation of this species, these lemurs may be at a higher risk for fatty tumor production. Other studies have documented high-fat diets enhancing carcinogenesis in a number of tumor types, including breast [Welsch, 1987], colon [Newberne et al., 1986], and pancreas [Roebuck et al., 1989].

A future dietary study in this type of lemur could elucidate the role of high-fat diets in the development of these lesions. Because of the lipid nature of the tumor, we believe that the rich captive diet was a contributing factor, and when considered with the lack of torpor in laboratory lemurs may have fostered the formation of the tumor and subsequent death. The Iams brand cat chow that comprised the lemurs' diets for 6 years obviously had more protein and fat than the lemurs in captivity required, as evidenced by the constant weight gain throughout the years. We have since switched, as a precautionary measure, to the Purina brand New World Primate Chow (#5040) that contains 20% protein, 9% fat, and 5% fiber per daily serving. This regimen essentially halves the amount of calories from fat contributed by chow in the diet. We have also increased fruit availability, now serving about 75 g of chow (one large handful) with about 75 g of fruit (4–5 pieces) to each pair, because fat calories from a predominantly carbohydrate (fruit and nectars) food source have been shown to not be as tumor inducing as fat calories associated with a protein source [Tannenbaum, 1942]. We hope that such changes in diet will prevent xanthogranuloma formation in future lab animals.

These tumors might therefore provide a model system for the development of lipid-related brain tumors. Further studies of the unusual fat metabolism and storage in the fat-tailed dwarf lemur might lead to the elucidation of lipid mobilization pathways in the brain. Such mechanisms would be especially important in the relation between the effects of hypercholesterolemia or hyperlipidemia and the formation of xanthogranulomas.

## CONCLUSIONS

1. Multiple xanthogranulomas of the choroid plexus, a lipid-related, benign lesion that is usually asymptomatic in the human CNS, produced obstructive hydrocephalus and ultimately death in two fat-tailed dwarf lemurs.
2. The high-fat captive diet of the lemurs may have contributed to the development of the tumors.
3. Coupled with the lack of definitive torpor, the unusual fat-storage mechanisms of this species of lemur may also be etiologically related to the accumulation of lipids and subsequent lesion formation.

## ACKNOWLEDGMENTS

We thank Dr. Janet Baer for providing veterinary support and Ms. Mimi Sengupta for research assistance. Funding was provided by the Human Brain

Program (DA/MH 08944), the Shannon Award (NIA IR55-AG10283-01A1 and NIMH 5-R37-MH39145) to C.A.M., and the Caltech Summer Undergraduate Research Fellowship (SURF 1993) to J.T.L.

## REFERENCES

- Ayres, W.W.; Haymaker, W. Xanthoma and cholesterol granuloma of the choroid plexus. Report of the pathological aspects in 29 cases. *JOURNAL OF NEUROPATHOLOGY AND EXPERIMENTAL NEUROLOGY* 19:280-295, 1960.
- Benirschke, K.; Miller, C.; Ippen, R.; Heldstab, A. The pathology of prosimians, especially lemurs. *ADVANCES IN VETERINARY SCIENCE AND COMPARATIVE MEDICINE* 30:167-208, 1985.
- Birt, D.F.; Kris, E.S.; Choe, M.; Pelling, J.C. Dietary energy and fat effects on tumor promotion. *CANCER RESEARCH* 52: 2035s-2039s, 1992.
- Brück, W.; Sander, U.; Blanckenberg, P.; Friede, R.L. Symptomatic xanthogranuloma of choroid plexus with unilateral hydrocephalus. *JOURNAL OF NEUROSURGERY* 75:324-327, 1991.
- Flach, D.B.; Winkelmann, R.K. Juvenile xanthogranuloma with central nervous system lesions. *JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY* 14:405-411, 1986.
- Giller, R.H.; Folberg, R.; Keech, R.V.; Piette, W.W.; Sato, Y. Xanthoma disseminatum. An unusual histiocytosis syndrome. *AMERICAN JOURNAL OF PEDIATRICS, HEMATOLOGY, AND ONCOLOGY* 10:252-257, 1988.
- Harcourt, C. LEMURS OF MADAGASCAR AND THE COMOROS. THE IUCN RED DATA BOOK. Gland, Switzerland, IUCN, 1990.
- Hicks, M.J.; Albright, S.; Trask, T.; Byrne, M.E.; Narayan, R.K.; Goodman, J.C. Symptomatic choroid plexus of the lateral ventricle. *CLINICAL NEUROPATHOLOGY* 12:92-96, 1993.
- Hladik, A. The dry forest of the west coast of Madagascar: Climate, phenology, and food available for prosimians. Pp. 3-40 in *NOCTURNAL MALAGASY PRIMATES*. D.M. Rumbaugh, ed. New York, Academic Press, 1980.
- Hladik, A.; Charles-Dominique, P.; Petter, J.J. Feeding strategies of five nocturnal prosimians in the dry forest of the west coast of Madagascar. Pp. 41-73 in *NOCTURNAL MALAGASY PRIMATES*. D.M. Rumbaugh, ed. New York, Academic Press, 1980.
- Innes, J.R.M.; Saunders, L.Z. *COMPARATIVE NEUROPATHOLOGY*. New York, Academic Press, 1962.
- Kronberger, H.; Rittenbach, P. Tumours in primates. *DER ZOOLOGISCHE GARTEN (LEIPZIG)* 35:205-217, 1962.
- Newberne, P.M.; Nauss, K.M. Dietary fat and colon cancer: Variable results in animal models. Pp. 311-330 in *PROGRESS IN CLINICAL AND BIOLOGICAL RESEARCH*. C. Ip; D.F. Birt; A.E. Rogers; C. Mettlin, eds. New York, Alan R. Liss, Inc., 1986.
- Petter, J.J. Ecological and physiological adaptation of five sympatric lemurs to seasonal variations in food production. Pp. 211-223 in *RECENT ADVANCES IN PRIMATOLOGY*. D.J. Chivers; J. Herbert, eds. New York, Academic Press, 1978.
- Petter-Rousseaux, A. Seasonal activity rhythms, reproduction, and body weight variations in five sympatric nocturnal prosimians, in simulated light and climatic conditions. Pp. 137-152 in *NOCTURNAL MALAGASY PRIMATES*. D.M. Rumbaugh, ed. New York, Academic Press, 1980.
- Ratcliffe, H.L. Deaths and important diseases. *REPORT OF THE PENROSE RESEARCH LABORATORY*, pp. 11-25, 1942.
- Roebuck, B.D.; Longnecker, D.S.; Birt, D.F. Role of dietary fat in experimental pancreas carcinogenesis. Pp. 135-150 in *CARCINOGENESIS AND DIETARY FAT*. S. Abraham, ed. Boston, Kluwer Academic Publishing, 1989.
- Russell, R.J. Body temperatures and behavior of captive cheirogaleids. Pp. 193-206 in *LEMUR BIOLOGY*. I. Tattersall; R.W. Sussman, eds. New York, Plenum Press, 1975.
- Tannenbaum, A. The genesis and growth of tumors. Effects of caloric restriction per se. *CANCER RESEARCH* 2:460-467, 1942.
- Welsch, C.W. Enhancement of mammary tumorigenesis by dietary fat: Review of potential mechanisms. *AMERICAN JOURNAL OF CLINICAL NUTRITION* 45:192-202, 1987.
- Weston, J.K. Spontaneous lesions in monkeys. Pp. 351-381 in *PATHOLOGY OF LABORATORY ANIMALS*. W.E. Ribelin; J.R. McCoy, eds. Springfield, Charles C. Thomas, 1965.
- Wright, P.C.; Martin, L.D. Predation, pollination, and torpor in two nocturnal primates, *Cheirogaleus major* and *Microcebus rufus*. In *CREATURES IN THE DARK*. M.K. Izard; L. Alterman, eds. New York, Plenum Press, in press.

