# Lipofuscin: Identification and localization in monkey uterus

CHARLES E. GRAHAM, PH.D.

*Atlanta, Georgia*

*Large quantities of lipofuscin were identified histochemically in the uterus of squirrel monkeys (Saimiri sciurea). In the myometrium, the pigment was contained in macrophages, but in the endometrium it was chiefly present as extracellular aggregates. There was no evidence of an influence of ovarian hormones on the quantity or distribution of the pigment.*

LIPOFUSCIN is a pigment of uncertain origin and mixed composition, and some of the literature describing its occurrence in several organs in a variety of mammalian species has been enumerated by Strehler[7] and by Miyawaki.[3] The gradual accumulation of the pigment throughout life has made it of interest in gerontology,[1, 5, 8] and the suggestion that the pigment has a lysosomal origin[2] demands the attention of cytologists and enzyme histochemists. It is the purpose of this communication to report the occurrence and peculiar distribution of lipofuscin in the uterus of the squirrel monkey *(Saimiri sciurea).*

## Materials and methods

Paraffin sections of the uterine corpus of 32 squirrel monkeys were prepared. Six of these were studied one month after ovariectomy and 14 following ovariectomy and 10 days to 3 months' continuous stimulation with estradiol-17$\beta$ at physiologic and hyperphysiologic levels. Hormone was administered daily in oil (0.0023 to 0.23 mg. per day) for 10 days or in a subcutaneously implanted pellet (12 to 100 mg.) for longer periods.

Unstained sections were examined with normal transmitted light and ultraviolet illumination using a Carl Zeiss Standard Universal Microscope. Ultraviolet studies were conducted under the following conditions: light source—high pressure mercury lamp HBO 200W; exciter filters BG 38/2.5 and B 912 giving a spectral transmission of 325 to 500 m$\mu$; barrier filters No. 47 transmitting wavelengths in excess of 450 m$\mu$ and No. 53 transmitting wavelengths in excess of 525 m$\mu$.

Sections were stained with the following techniques according to methods described by Pearse[6]: Periodic acid–Schiff with diastase control; Mowry's Alcian blue technique[4]; Sudan black B in 70 per cent alcohol; tetrazolium reaction; the Turnbull blue test for ferric iron; the Lillie and Hueck Nile blue methods for lipofuscin; the long Ziehl-Neelsen method; chrome-alum hematoxylin; the Schmorl reaction; Millon's test; Gmelin's reaction; and plasmal reaction. Extraction, with cold and hot acetone and ether, three changes of boiling methanol/chloroform mixture, or pyridine, was also carried out on sections and followed by staining with Sudan black B.

## Results

Varying quantities of yellowish brown pigment were observed in unstained sections of the corpus uteri in 26 of 32 animals. The age of the animals was unknown, but those in which the pigment was absent were suspected to be immature, on the basis of the development of the reproductive tract. The

*From Yerkes Regional Primate Research Center, Emory University.*
*This study was supported by National Institutes of Health Grant FR-00165.*

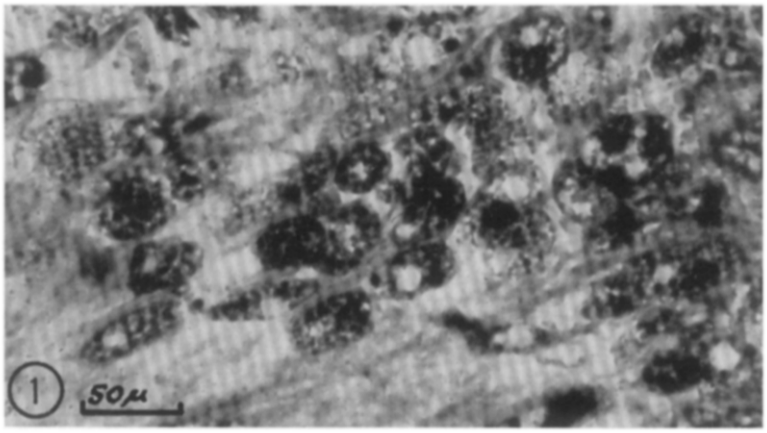


**Fig. 1.** Macrophage-like cells in myometrium containing numerous lipofuscin granules. (Sudan black B.)

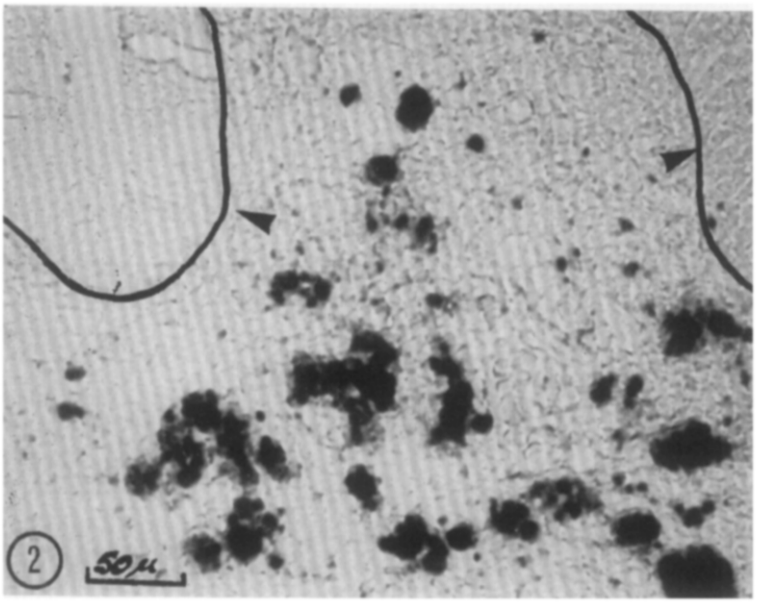


**Fig. 2.** Extracellular aggregates of lipofuscin in endometrium. Arrows and dark lines delineate endometrial glands. (Nile blue.)

pigment was usually positive to all histochemical reactions performed except the Millon, Gmelin, and plasmal reactions, which were consistently negative; however, not all pigment granules were uniformly positive to the Nile blue methods, the long Ziehl-Neelsen method, chrome-alum hematoxylin, and the Schmorl reactions.

The pigment was not extracted by routine paraffin embedding techniques, hot acetone or hot ether; but following extraction with the methanol/chloroform mixture or pyridine for 24 hours, sudanophilia was eliminated, although granules of pigment could still be identified in unstained sections.

The pigment and associated histochemical reactions were observed both in the endometrium and in the myometrium. In the myometrium the pigment was always contained in scattered and often numerous cells

thought to be macrophages (Fig. 1). In the endometrium, on the other hand, the pigment was chiefly but not exclusively in an extracellular location (Fig. 2), being deposited in conspicuous aggregates in the mucosa a short distance below the luminal epithelium.

In unstained sections the pigment was observed to fluoresce blue when aggregated in large granules but yellow when dispersed in the cell cytoplasm. It was shown by examining identical areas under fluorescent illumination and then under normal illumination following staining with Sudan black B that fluorescence and sudanophilia were coincident in the same pigment particles.

Variations in quantity and histochemical reactions of the pigment which could be related to the stage of the cycle at which the animals were killed were sought but not identified. There were no evident effects on the pigment following ovariectomy with or without subsequent estrogenic administration.

## Comment

The morphology, physical properties, histochemical reactions, and incidence in older animals all indicate that the pigment described in this study is lipofuscin. Variation in the histochemical reactions of lipofuscin is common and is thought to be due to the pigment having reached varying stages of oxidation.[6] The unusual distribution of lipofuscin in the uterus may provide an important clue to its mechanism of accumulation. The tentative identification of extra- and intracellular lipofuscin in the brain of squirrel monkeys[1] suggests that the pigment has a rather general distribution, and further studies are presently in progress to determine if this is the case. Whether there is any special significance in the fact that the uterus is a site of lipofuscin deposition has yet to be determined.

I thank Dr. T. R. Shantha for valuable discussions during this study.

## REFERENCES

1. Creswell, G. F., Reis, D. J., and MacLean, P. D.: Am. J. Anat. **115:** 543, 1964.
2. Essner, E., and Novikoff, A. B.: J. Ultrastructure Res. **3:** 374, 1960.
3. Miyawaki, M.: J. Nat. Cancer Inst. **34:** 601, 1965.
4. Mowry, M.: J. Histochem. Cytochem. **14:** 800, 1966.
5. Novikoff, A. B.: *In* Brachet, J., and Mirsky, A. E., editors: The Cell, New York, 1959, Academic Press, Inc., vol. II, p. 423.
6. Pearse, A. G.: Histochemistry, Theoretical and Applied. ed. 2, Boston, 1960, Little Brown & Company.
7. Strehler, B. L.: Time, Cells and Aging, New York and London, 1962, Academic Press, Inc., pp. 181-184.
8. Sulkin, N. M., and Strivani, J. P.: J. Gerontol. **7:** 533, 1960.