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BIOGENESIS AND SIGNIFICANCE OF LIPOFUSCIN
 IN THE EQUINE THYROID GLAND.

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ABSTRACT

Yellow-brown granules of lipofuscin (age pigment) accumulate in the cytoplasm of long-lived cells of many eucaryotes, including man. The granules are derived from lysosomes, and are defined by characteristic morphology, colour, fluorescence, and histochemistry. They increase in number with age in post-mitotic cells, and are regarded as markers of the aging process. Lipofuscin is widely assumed to consist of products of peroxidised fatty acids cross-linked with amino groups of proteins, nucleic acids, and phospholipids, but this theory has not been proven.

The occurrence, histology, structure, and composition of lipofuscin in thyrocytes from horses of a wide range of ages were studied. Granules were absent at birth but were widespread by the age of 5 years. In young horses, granules were largest in follicles which contained abnormal colloid. After 5 years the amount of lipofuscin in thyrocytes was not age-related. Lipofuscin was fluorescent, and stained with PAS, Schmorl's, and Masson's Fontana stains, although the staining intensity varied between horses. Lectin histochemistry demonstrated the presence of mannose and/or glucose. Small subpopulations of granules in some sections contained iron and some granules contained DNA.

Lipofuscin granules were irregular in size and shape, and consisted of an electron-dense matrix and relatively electron-lucent 'vacuoles', which did not contain lipid. They appeared to fuse with colloid droplets, and small granules were observed within colloid droplets.

Thyroid lipofuscin granules isolated by osmotic shock and differential centrifugation showed the same ultrastructure as in situ. The protein content ranged from 15% to 77% w/w (mean = 36%). In contrast, the amino acid composition was constant and similar to that of thyroglobulin. The most distinctive feature of lipofuscin protein was the presence of four proteins of 14-18 kDa. The halide

concentration of lipofuscin was approximately twice that of thyroid tissue. Lipofuscin contained no triglycerides, and only small quantities of phospholipids (mean = 1.25% w/w). In contrast, the concentrations of cholesterol and dolichol, up to 19% and 15% respectively, were high although very variable. Small amounts of nuclear DNA were detected. The empirical formula of the rest of lipofuscin indicated that it was principally carbohydrate, which was consistent with the PAS and lectin histochemistry.

Most of the lipofuscin mass was analysed and there was no evidence that it arose from lipid peroxidation. The protein fraction of thyroid lipofuscin probably consists of proteolytic intermediates of thyroglobulin. Components from other sources, and turnover of granule contents, contribute to the chemical heterogeneity.

The composition of lipofuscin probably varies between tissues. However all lipofuscins are likely to share lysosomal functions such as storage of proteolytic intermediates, metals and dolichol. As with thyroid lipofuscin, they may be more soluble and more readily analysed than is generally assumed in the literature. They may also be active organelles of metabolic significance, rather than inert indicators of the aging process. On the basis of this study, they should be re-evaluated.

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ABBREVIATIONS

AFIP	Armed Forces Institute of Pathology
BHT	Butylated hydroxytoluene
BSA	Bovine serum albumin
DIT	diiodotyrosine
EDTA	ethylenediaminetetraacetic acid
Feulgen-NAH	Feulgen-Naphthoic acid Hydrazine
HPLC	high performance liquid chromatography
H&E	haematoxylin and eosin
IgG	immunoglobulin G
kDa	kiloDalton
LDS	Lithium dodecyl sulphate
LDS-PAGE	LDS-polyacrylamide gel electrophoresis
MIT	moniodotyrosine
NMR	nuclear magnetic resonance
PAS	periodic acid-Schiff
PBST	phosphate-buffered saline/Tween 20
PVDF	Polyvinylidene difluoride (Immobilon TM , Millipore Corporation, MA.)
TBS	Tris-buffered saline
TLC	thin layer chromatography
TRH	TSH-releasing hormone
Tris	Tris(hydroxymethyl)aminomethane
Trizma TM	Tris(hydroxymethyl)aminomethane (Sigma)
TSH	Thyroid stimulating hormone
TBBS	Tween 20/Tris + Cations
T3	Triiodothyronine
T4	Tetraiodothyronine, = thyroxine