

CHEMOGRAPHY OF LYSSOSOME-LIKE STRUCTURES IN OLFATORY EPITHELIUM

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INTRODUCTION

At the light microscope level of resolution several chemicals have been shown to cause "fogging" of photographic film. Among these the principal reducing agents are glutathione, cysteine (1), hydrogen sulfide gas, and hydroquinone in animal tissue (17). The -SH groups of the first three chemicals were shown to be responsible for the effect. Chemographic reactions of any kind at the electron microscope level of resolution are yet to be reported by other investigators.

This note describes a unique application of electron microscope radioautographic techniques which demonstrated a chemical difference among three morphologically similar types of lysosome-

like structures found in rabbit olfactory epithelium. It also reports the first electron microscope observations of these lysosome-like inclusion bodies, each located in one of three cell types specific to olfactory sensory epithelium. At least one type and perhaps all three types of inclusion bodies reported here are no doubt the granules previously termed "pigment granules" by numerous investigators, beginning with the classical work of Schultze as early as 1862 (13). We have avoided applying the general term "pigment" not because we feel it to be inappropriate, but because it is our purpose to extend the characterization of these structures consistent with ultrastructural nomenclature. This extension implies a change in the concept of function as well.

MATERIALS AND METHODS

Microscope Preparations

The olfactory tissue used in these studies was obtained from New Zealand white rabbits. After anesthesia, decapitation, and exsanguination of the head, the nasal septum and endoturbinates were immediately excised and fixed at 4°C for 20 min by immersion in 1.0% OsO₄ buffered to pH 7.4 with Millonig's phosphate buffer (8). The excised tissues were dehydrated in a graded series of alcohol solutions and were cut into strips 1–2 mm wide and embedded in Epon 812 (7). After polymerization, the embedded tissue strips were cut into blocks which were reoriented for sectioning with a Porter-Blum ultramicrotome. Sections 1–1.5 μ thick were cut with glass knives and stained with a mixture of methylene blue and azure II (11) for light microscope observation. Thin sections (500–800 Å) were double stained with uranyl acetate (16) and lead citrate (10) and examined in an RCA EMU-3 electron microscope.

Liver and heart tissues were also used in these studies and were prepared in the manner described above, but without special handling for orientation.

Radioautographic (Chemographic) Preparations

The term chemography or chemographic reaction is used to describe reactions between chemical constituents of the tissue and the photographic emulsion. These reactions produce metallic or "photogenic" silver after development. A combination of electron microscope radioautographic techniques established by others (2–4, 6, 9, 12) was employed to observe the chemographic reaction. The usual application of these techniques has been to obtain the optimum localization of point sources of radioactivity in ultrathin tissue sections from labeled biological specimens. In our experiments, however, the techniques were used to demonstrate differences in chemical effect of morphologically similar cell organelles upon the silver halide crystals in the photographic emulsion. Consequently, no radiochemical labeling was employed in these experiments.

OBSERVATIONS

Microscope

At the light microscope resolving level, dark staining bodies were present at three locations in olfactory tissue. The largest and most numerous of these structures were concentrated at the base of the epithelium close to the basal cells (Fig. 1). Next in size and almost as numerous were those

in the cells of the Bowman's glands located in the lamina propria beneath the epithelial layer. Other dark staining bodies much less numerous than the two previous types were scattered throughout the nuclear band of the bipolar olfactory cells themselves. The resolution of individual Bowman's gland cell membranes permitted the only clear association between a particular cell type and dark staining bodies. Cell membranes of other cell types, olfactory, sustentacular, or basal cells, were too indistinct in the light microscope preparations to permit further associations.

An important aspect of the dark staining bodies was their variation in number. The number varied considerably from animal to animal and, to a much lesser extent, from area to area within a specimen. The most variable in number of the three types were those clustered at the base of the epithelium. In some specimens, most often the younger animals, virtually none were observed in that location while those in the Bowman's glands were significantly reduced and the number in the olfactory nuclear band remained essentially unchanged (Fig. 2). Throughout our observations the least variable in number were the latter.

Electron microscope preparations revealed the dark staining bodies of all three types to be membrane-limited, lysosome-like structures displaying certain morphological differences. Those clustered at the base of the epithelium were irregular in shape with a coarse, very electron-opaque internal matrix. The average diameter of the sectioned profiles was 0.63 μ (Fig. 3). They were located in proximal elements of the supporting (sustentacular) cells of the epithelium indicated by the presence of desmosomal intercellular connections. Desmosomes have not been observed between sustentacular and olfactory bipolar cells nor between two apposed bipolar cells. Lysosome-like structures located in the cells of Bowman's glands were more uniform in shape and size, with sectioned profiles averaging 0.47 μ in diameter (Fig. 4). Internal matrices were more homogeneous than those of the sustentacular type inclusion bodies, but were equally electron opaque. Electron micrographs of the least numerous dark staining bodies, those in the olfactory nuclear band, showed them to be located within the cell bodies of the olfactory bipolar cells themselves and most frequently distal to the nucleus (Fig. 5). Their internal structure was the most varied of the three

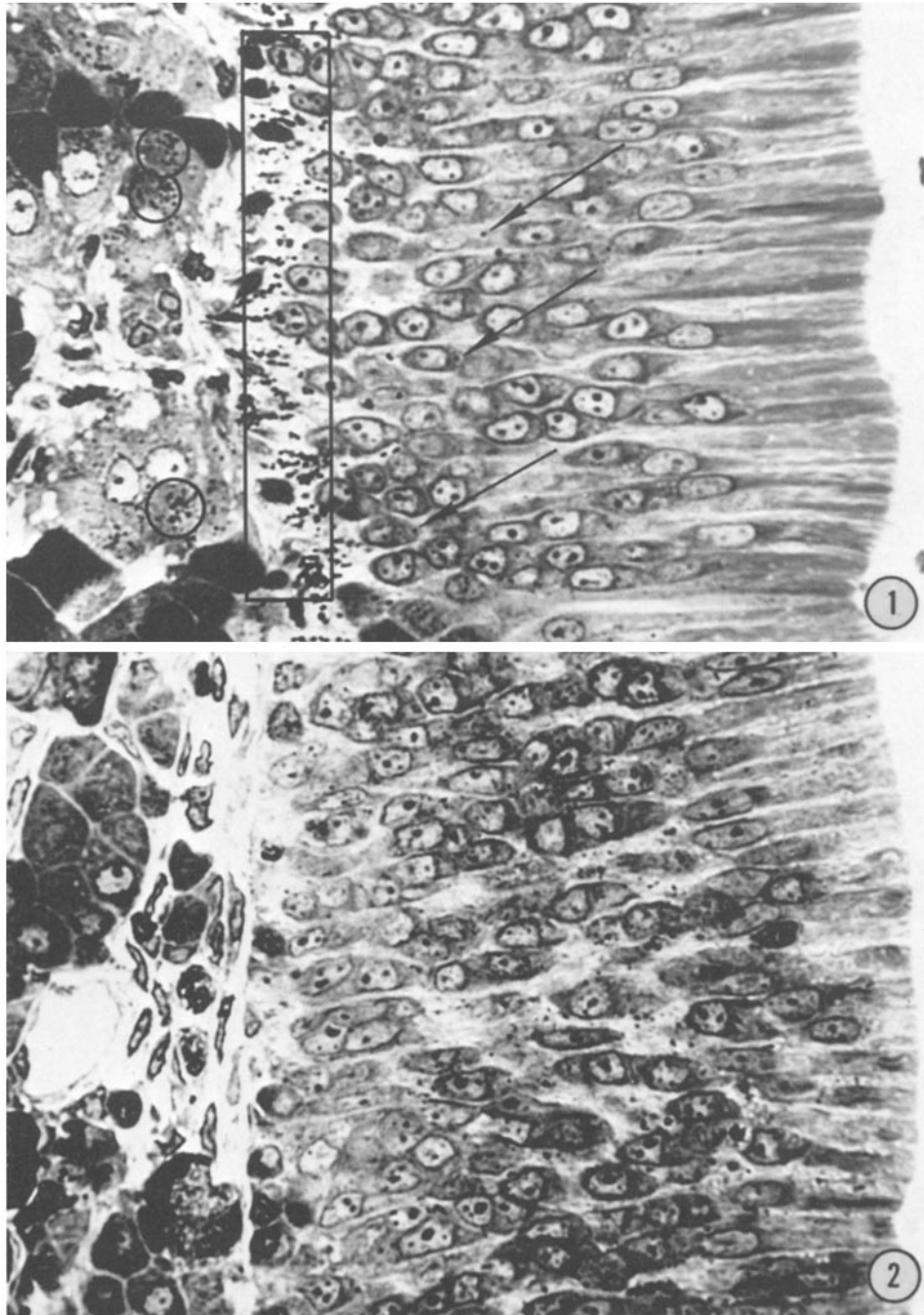


FIGURE 1 Photomicrograph of longitudinal section of olfactory epithelium from an adult rabbit. Three types of lysosome-like structures are present, classed by location. Clustered at base of epithelium (boxed area), in the Bowman's gland cells in the lamina propria (circled areas), and scattered throughout the olfactory nuclear band (arrows). $\times 1050$.

FIGURE 2 Photomicrograph of longitudinal section of olfactory epithelium from a young rabbit in which lysosome-like structures are absent at the base of the epithelium and reduced in numbers in the Bowman's gland cells. $\times 1050$.

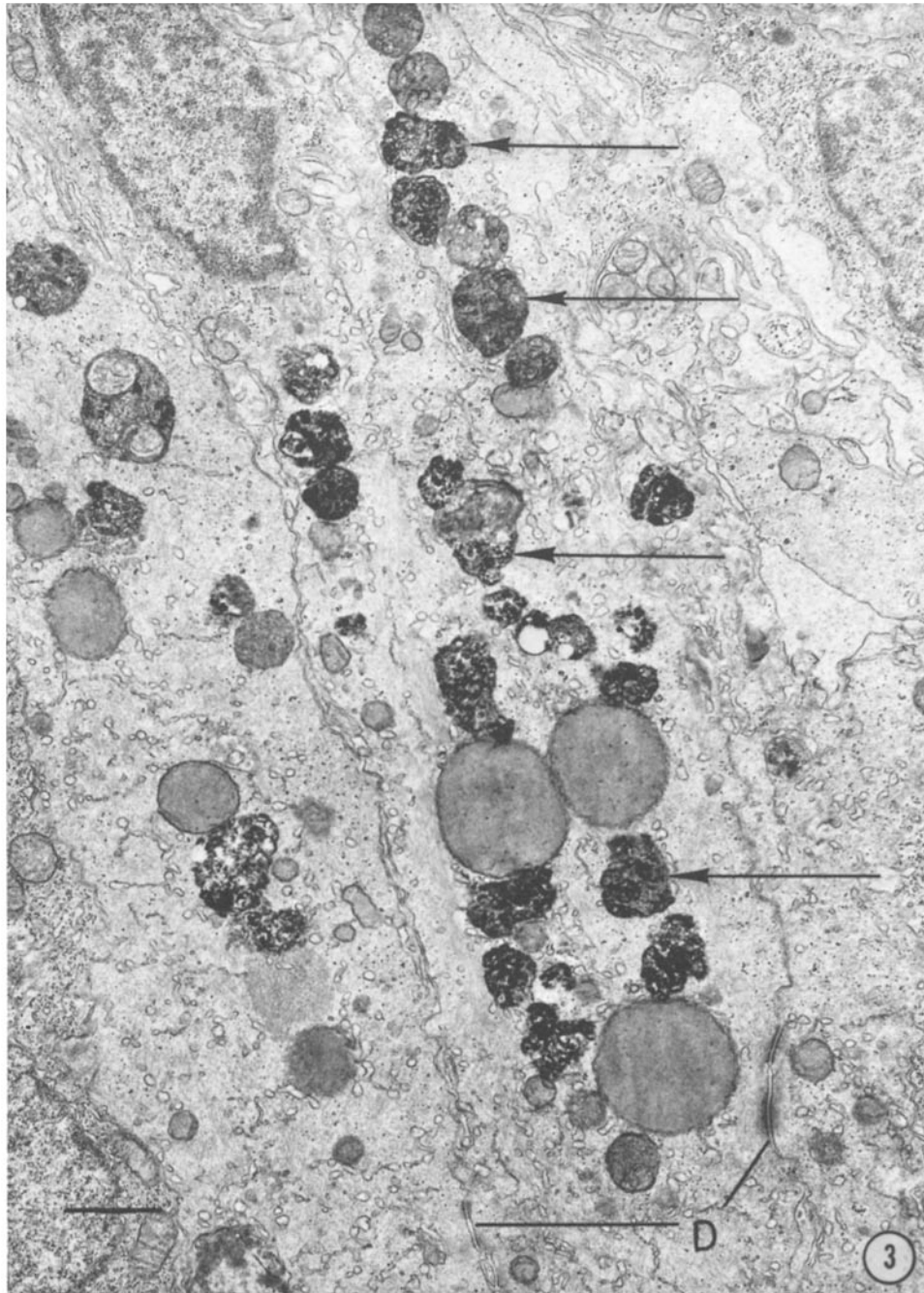


FIGURE 3 Electron micrograph of longitudinal section of olfactory epithelium. Lysosome-like inclusion bodies (arrows) located in proximal elements of the supporting (sustentacular) cells. *D*, desmosomes. Line in lower left corner denotes 1 μ in this and all succeeding micrographs. $\times 13,500$.

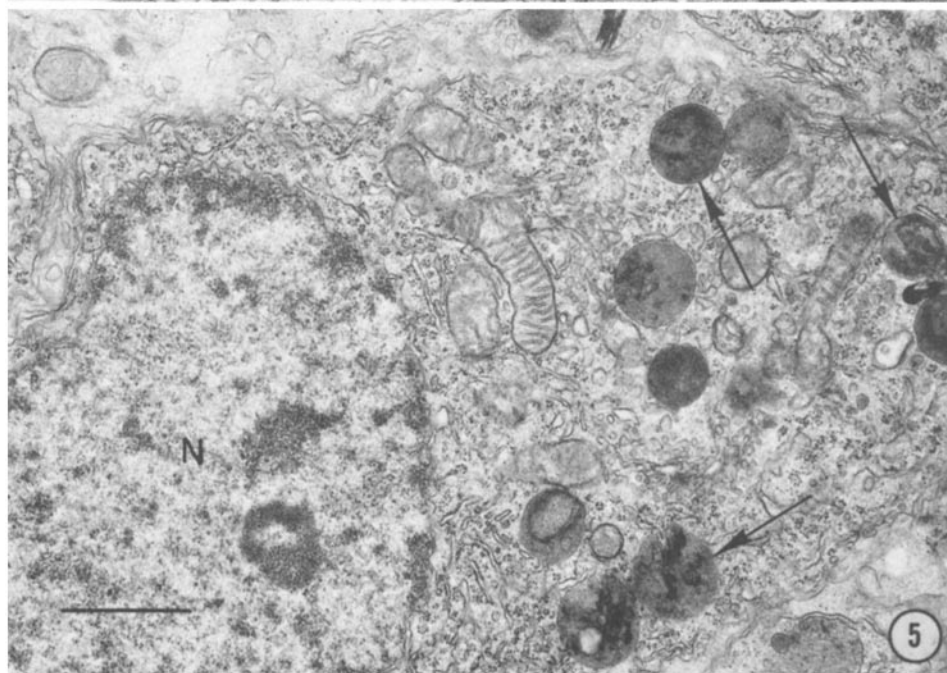
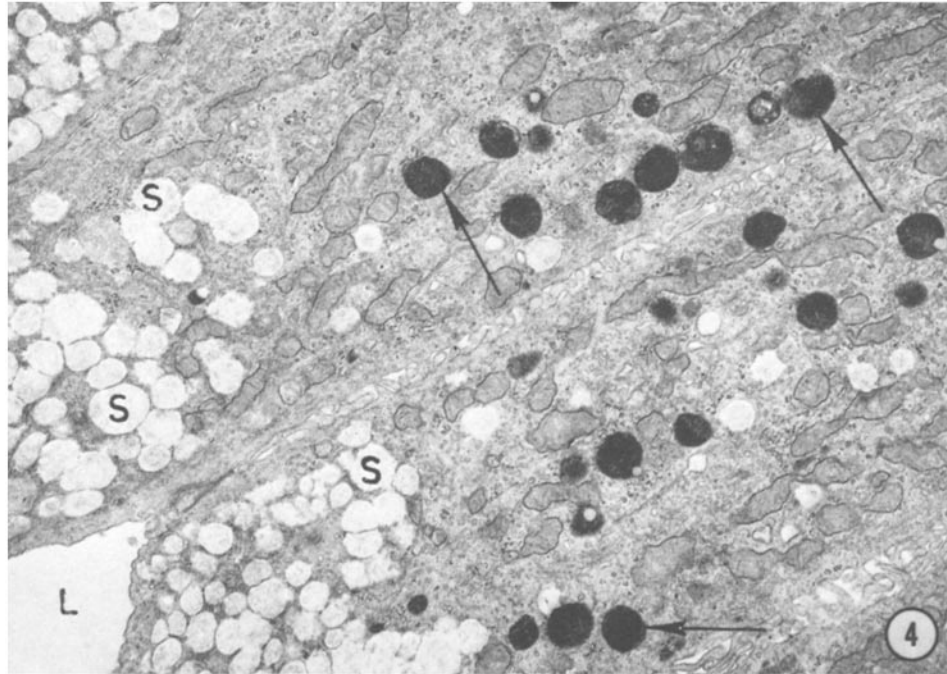


FIGURE 4 Bowman's gland cells showing lysosome-like inclusion bodies (arrows). *S*, secretory granules; *L*, lumen. $\times 12,000$.

FIGURE 5 Olfactory cell body in which several lysosome-like structures (arrows) are observed near the nucleus (*N*). $\times 16,800$.

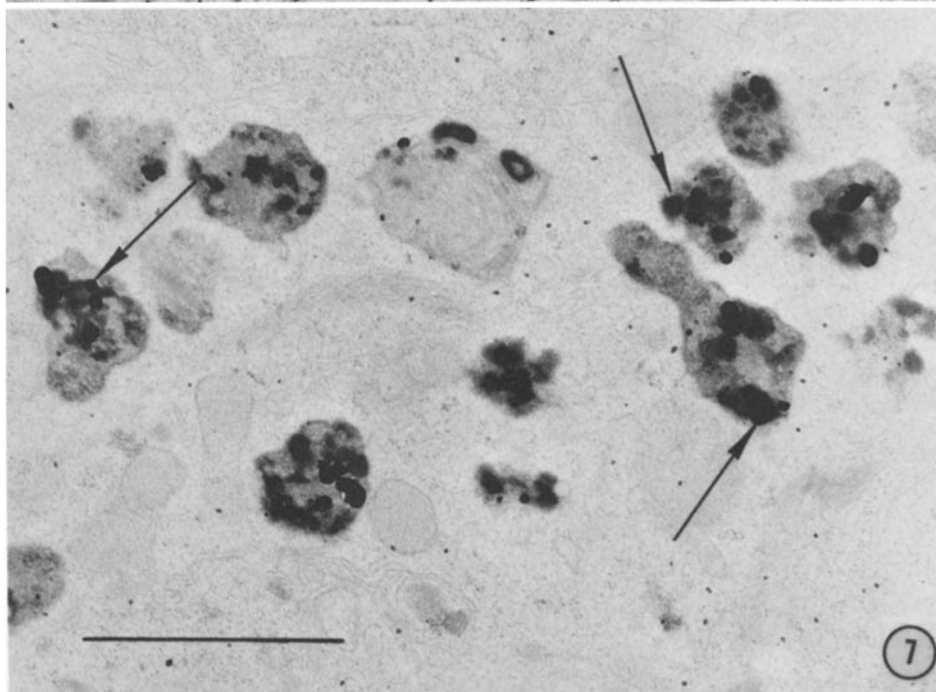
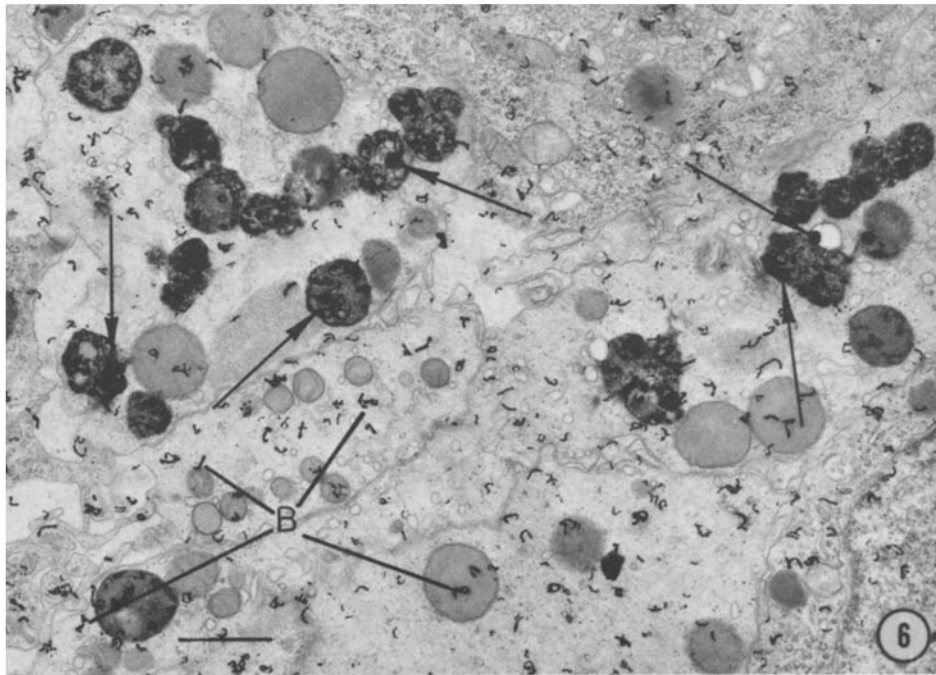


FIGURE 6 Representative positive chemograph of sustentacular lysosome-like structures. Most of the dark staining inclusion bodies are overlaid with spherical deposits of metallic silver (arrows). Substantial background (*B*) of well-developed exposed silver grains is present. Stained with uranyl acetate and lead citrate. $\times 12,200$.

FIGURE 7 Tissue section stained for a shortened period of time with uranyl acetate only, more clearly demonstrates positive chemographic reaction over sustentacular lysosome-like inclusion bodies. Silver deposits (arrows) are most frequently located over the most electron-opaque portions of the internal matrix. $\times 34,400$.

types described in this report. Some were quite homogeneous while others contained discrete lamellate structures either flat or curved. The sensory cell lysosome-like structures were generally less electron opaque than both the sustentacular and Bowman's gland cell types. The diameter of sectioned profiles averaged 0.58μ .

Chemographic

A chemograph of the sustentacular cell lysosome-like structures revealed large deposits of metallic silver over these structures (Fig. 6). When adequate tissue contrast was achieved by staining with both uranyl acetate and lead citrate, however, the presence of the silver deposits was somewhat obscured by the high electron opacity of the inclusion bodies themselves. Staining for shortened periods of time with uranyl acetate only, significantly reduced tissue contrast and allowed the silver deposits to be readily observed over this type of inclusion body (Fig. 7). Furthermore, the "label" was principally located over the most electron-opaque portions of the internal matrix.

No chemographic reaction was observed with the lysosome-like structures in both Bowman's gland and olfactory bipolar cells. Negative chemographs were also obtained with true lysosomes from liver and with lipofuscin granules from heart muscle.

DISCUSSION

The difference in chemographic reaction between the sustentacular inclusion bodies and all other dark staining bodies tested demonstrates, at most, two classes of dark staining bodies based on chemical content. It may mean that either the sustentacular type is entirely different chemically from true lysosomes or that they contain several of the known lysosomal enzymes plus additional chemical constituents which cause the chemographic reaction.

The positive chemographic reaction of only the sustentacular cell lysosome-like structures suggested that this structure is a unique chemical entity in animal tissue. Of all known dark staining bodies, these structures most closely resemble lipofuscin granules ("aging pigment") of heart and brain tissue. First, they are structurally indistinguishable and second, both types increase with the age of the animal. However, here again, the similarity between sustentacular cell inclusion bodies and lipofuscin granules stops. Lipofuscin

granules are known to autofluoresce with the use of excitation wavelengths in the ultraviolet range (5, 14, 15). Using an excitation filter which peaked at 265 nm, we observed fluorescence at approximately 550 nm of the lipofuscin granules in the heart tissue whereas no fluorescence was observed in the olfactory tissue sections.

The observed but as yet undefined chemical differences between sustentacular cell inclusion bodies and other structurally similar, dark staining bodies, their location in the olfactory epithelium, and their animal age dependence prompt us to theorize as to their function. It may be that they play an important role in the postperception fate of odorant stimuli after contact with the olfactory sensory cells. That is, odor molecules may actually enter the epithelium via the microvilli at the distal surface of the sustentacular cells to be isolated and catabolized by specialized mechanisms available for that purpose. The chemical diversity of incoming odorant molecules would demand the availability of a wide and unique spectrum of membrane-limited catabolic agents. Over time, an accumulation of certain nonmetabolites or unusable side products would occur, resulting in an increase of electron-opaque inclusion bodies.

The structural diversity of true lysosomes would permit the identification of any one or all of the three classes of dark staining bodies reported here as lysosomes. However, until definitive enzyme assay for acid hydrolase activity and additional lysosomal enzymes is performed, such identification would not be practical.

The value of the assessment of chemographic reactions as applied in this study is limited by our lack of knowledge as to the cause of such reactions. The potential value of chemographic reactions as an analytical tool at the electron microscope level of resolution, however, would appear quite high as more information is acquired. Even now in its early state of development, for instance, it will be invaluable in the positive identification of sustentacular cell inclusion bodies in cell fractions prepared by density gradient centrifugation for biochemical analysis.

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