Characteristic Reaction of the Mouse Adrenal Cortical Cell to Osmium Impregnation*

Fumio Sawano and Hisao Fujita
Department of Anatomy (Prof. H. Fujita), Osaka University School of Medicine, Osaka, Japan

Received February 27, 1980

Summary. The fine structural localization of osmium impregnation in the mouse adrenal cortical cell was studied using the electron microscope. After osmication for 40-48 hrs all kinds of adrenal cortical cells of the mouse exhibited a selective localization of osmium impregnation in almost all the cisternae of the smooth endoplasmic reticulum in addition to 1-2 or 3 stacks and vesicles of cis-side of the Golgi apparatus. This fact might be implicated with the steroid hormones which may be localized in the cisternae of smooth endoplasmic reticulum. Moreover, the cisternae of the nuclear envelope, and of the characteristic lamellar body closely related to the smooth endoplasmic reticulum, were also stained with OsO₄.

It is well known that the cisternae and vesicles on one side of the Golgi apparatus are stained with OsO₄ after prolonged osmication in several kinds of cells, especially in the epithelial cells of the mouse epididymis as Dalton and Felix (1953) first recognized using the electron microscope. The present paper concerns the characteristic localization of osmium impregnation in the mouse adrenal cortical cells. Almost all the cisternae of the smooth endoplasmic reticulum, of the characteristic lamellar body of the nuclear envelope and of 1-2 or 3 stacks of the Golgi apparatus of this cell are intensely and selectively stained with OsO₄.

MATERIALS AND METHODS

Observations were made on the adrenal cortex from five normal, 3-month old, male albino CF-1 mice. The materials were used for osmium impregnation and for thiamine pyrophosphatase reaction.

Osmium impregnation: Mice were killed and their adrenal glands were removed immediately. They were cut into small pieces and fixed with 1% OsO₄ buffered at pH 7.4 with 0.1M sodium cacodylate for 1-2 hrs at room temperature. After fixation the tissues were immersed in 2% OsO₄ water solution (pH 6.0-6.7) for 40-48 hrs at 40°C. After 24 hrs, the 2% OsO₄ solution was renewed by a fresh one.

* This study was supported by grants from the Japan Ministry of Education.
were then rinsed 2–3 times with 0.1M sodium cacodylate buffer (pH 7.4), dehydrated in graded concentrations of alcohol and embedded in Epon epoxy resin.

**Thiamine pyrophosphatase reaction:** Some adrenal cortical tissues were fixed by cardiac perfusion with a modified Karnovsky’s fixative (KARNOVSKY, 1965), containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.065M sodium cacodylate buffer (pH 7.4) added 0.025% CaCl₂ for 5 min at room temperature. The tissues were cut into small pieces and immersed in the same fixative for another 30–40 min at 4°C, and rinsed overnight in 0.1M sodium cacodylate buffer (pH 7.4) with 5% sucrose at 4°C. Then the tissues were incubated for 2 hrs at 37°C in the medium employed by NOVIKOFF and GOLDFISCHER (1961), containing 50mg of Na-thiamine pyrophosphate (Sigma), 14 ml of distilled water, 20 ml of 0.2M Tris-maleate buffer (pH 7.4), 10 ml of 0.025M manganese chloride and 6 ml of 1% lead nitrate with 5% sucrose. After incubation the tissues were postfixed for 1 hr at 4°C in 1% OsO₄ solution buffered at pH 7.4 with sodium cacodylate added 5% sucrose, dehydrated in graded concentrations of alcohol and embedded in Epon epoxy resin.

All the sections cut on a Porter-Blum ultramicrotome and stained doubly with saturated uranyl acetate (WATSON, 1958) and Millonig's lead acetate (MILLONIG, 1961), were examined with a Hitachi HU-11D type electron microscope.

**OBSERVATIONS**

All kinds of the mouse adrenal cortical cells have been well known to be characterized by mitochondria with tubulovesicular cristae, well-developed tubular smooth endoplasmic reticulum, and lipid droplets.

In the present study, the Golgi apparatus, the smooth endoplasmic reticulum, the nuclear envelope and the lamellar body showed interesting reactions to the osmium impregnation.

1. **Golgi apparatus**

The Golgi apparatus in all zones of the mouse adrenal cortical cells consists of 4–6 Golgi stacks and some vesicles around them. In general, the parallel stacks of the Golgi cisternae are curved and the outer surface of the stacks is called the convex face, forming face, or cis-side, while the inner surface of the stacks is called the concave face, maturing face, or trans-side. In the present study, the cisternae of 1–2 or 3 stacks and vesicles of the cis-side of the Golgi apparatus were stained intensely with OsO₄, but 2 or 3 stacks of the trans-side were not stained. The intensity of OsO₄ staining in the cisternae of the Golgi stacks was gradually diminished from cis to trans.

The reaction products of thiamine pyrophosphatase were recognized in the cisternae of 1–2 or 3 stacks of trans-side of this apparatus.

The OsO₄-stained cisternae were almost negative for this enzyme reaction. Though the localization of these two reaction products might be more or less overlapped, main sites for both reactions were obviously different from each other.

2. **Smooth endoplasmic reticulum**

As mentioned above, the mouse adrenal cortical cells have characteristically abun-
dant tubular smooth endoplasmic reticulum in their cytoplasms. In this study, all the cisternae of the smooth endoplasmic reticulum of all kinds of the cortical cells were intensely stained with OsO₄. Similar findings have been reported by Friend and Brassil (1970).

3. **Nuclear envelope**

The cisternae of the nuclear envelope of all kinds of cortical cells were also well-stained with OsO₄. However, the intensity of this reaction in the cisternae was somewhat weaker than that of the Golgi apparatus and of the smooth endoplasmic reticulum.

4. **Characteristic lamellar body**

Highly organized lamellar structures have been encountered in the mouse adrenal cortical cells, especially in the reticular zone cells (Sato, 1967; Shelton and Jones, 1971; Moore and Callas, 1975; Fujita, 1977; Setoguchi et al., 1979). As Setoguchi

**Fig. 1.** OsO₄ impregnation of the fasciculata cell of the mouse adrenal cortex. Cisternae of Golgi apparatus, smooth endoplasmic reticulum and nuclear envelope are stained with OsO₄. But mitochondria and plasma membrane are not stained. ×12,000
et al. (1979) mentioned, this structure consists of closely piled electron dense plates which are continuous with the smooth endoplasmic reticulum. The authors agree with their opinion that this structure might represent a part of the smooth endoplasmic reticulum. In the present study, all the cisternae of the lamellar bodies were stained with OsO₄.

5. **Lysosome**

Lysosomes which are usually positive for acid phosphatase reaction, were not stained with OsO₄ at all.

**DISCUSSION**

Since the fine structural observation of osmium impregnation of the Golgi apparatus in the epithelial cells of the mouse epididymis and duodenum performed by DALTON and FELIX (1953), several papers dealing with this reaction were published for the epithelial cells of mouse or rat epididymis (DALTON and FELIX, 1954, 1956; FRIEND and MURRAY, 1965; FRIEND, 1969; KUROSUMI, 1970), epithelial cells of the mouse Brunner's gland, plasma cells in the lamina propria of the mouse intestine (FRIEND and
Murray, 1965), adrenal cortex of rat (Friend and Brassil, 1970) and luteal cells of the guinea pig (PaaVola, 1978). The present authors attempted to apply the OsO₄ impregnation for the mouse adrenal cortex. Two or three stacks and vesicles of cis-side of the Golgi apparatus were well-stained with OsO₄, while the reaction products for thiamine pyrophosphatase were localized in the cisternae of trans-side of the Golgi apparatus. This finding is similar to that reported for the epithelial cell of mouse or rat epididymis.

In addition, the authors wish to emphasize that the cisternae of the smooth endoplasmic reticulum, of the nuclear envelope and of the lamellar structures were also stained with OsO₄. For a long time, osmium tetroxide has been used for the investigation of cytology, especially for the studies of myelin of the neuron, because of its ability to fix proteins as well as lipids. The mouse adrenal cortical cells release steroid hormones. It has been believed that the hormones are synthesized with the aid of mitochondria and smooth endoplasmic reticulum and localized in the cisternae of smooth endoplasmic reticulum to be released by a diacrine secretion. The fact that the cisternae of the smooth endoplasmic reticulum of the mouse adrenal cortical cells were stained with OsO₄ might suggest the possibility of an existence of lipids in the cisternae of smooth endoplasmic reticulum. If so, it might be true that synthesized steroid hormones which are kinds of lipids are produced in the smooth endoplasmic reticulum of the mouse adrenal cortical cells.

In general, the smooth endoplasmic reticulum is considered to be derived from

Fig. 3. TPP-ase reaction in the fasciculata cell of the mouse adrenal cortex. Reaction products are localized in the cisternae of more trans-side of Golgi apparatus than the OsO₄ stained cisternae of cis-side. × 46,000
Fig. 4. The cisternae of all the smooth endoplasmic reticulum intensely stained with OsO$_4$ in the fasciculata cell of the mouse adrenal cortex. ×16,000

Fig. 5. Legend on the opposite page.
the rough endoplasmic retitulum in the adrenal cortical cell (McNutt and Jones, 1970: Fujita and Ihara, 1972), and the rough endoplasmic reticulum has been known to be continuous with the outer nuclear membrane of the nuclear envelope. From these facts, it seems reasonable that the cisternae of the nuclear envelope are also stained with OsO₄.

The cisternae of characteristic lamellar bodies which appear sometimes in the reticular zone cell of the relatively old mouse adrenal cortex are also positive for osmium impregnation. According to Setoguchi et al. (1979), the lamellar bodies are originated from the endoplasmic reticulum, because their ends are continuous with the cisternae of smooth endoplasmic reticulum as well as of rough endoplasmic reticulum. It is reasonable that the cisternae of the lamellar bodies are stained with OsO₄.

Fig. 5. OsO₄ impregnation of the reticularis cell of the mouse adrenal cortex. All the cisternae of the lamellar body which might be continuous with those of smooth endoplasmic reticulum are intensely stained with OsO₄. ×23,000

REFERENCES


長時間オスミウム酸浸漬染色法によるマウス副腎皮質細胞の特徴的反応

沢野文夫 と 藤田尚男

マウスの副腎を 2 %オスミウム酸に40°Cで40-48時間浸漬した。その結果、マウスの副腎皮質全層の細胞のゴルジ装置は、他の器官の細胞と同じように、外帯 (cis 側) の 1 - 3 層の層板の内腔と、cis 側のゴルジ小胞において陽性を示すが、特記すべきことは、ゴルジ装置の他に管状の滑面小胞体の全内腔が強染することである。さらに核膜腔と層板小体の内腔も強い陽性を呈するが、このことは層板小体の起源が滑面小胞体であるという説を裏書きするものである。なお滑面小胞体が陽性を示す事実は、副腎皮質細胞において合成されたステロイドホルモンが、滑面小胞体腔内に局在する可能性を暗示するものと思われる。


