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CHARACTERISTIC IMPREGNATION OF OSMIUM TETROXIDE IN THE MOUSE STEROID HORMONE SECRETING CELLS*)

By

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ABSTRACT

The fine structural localization of osmium impregnation in the mouse steroid hormone secreting cells, such as Leydig cell of the testis and the theca interna cell of the ovary was studied at electron microscopic level. After prolonged osmication for 40–48 hours, these two kinds of cells exhibited a common selective localization of osmium not only in the cisternae and vesicles on the cis-side of the Golgi apparatus, but also in all the cisternae of the smooth endoplasmic reticulum and that of the nuclear envelope. This fact might suggests that substances which are considered to be stored in the cisternae of the smooth endoplasmic reticulum, such as synthesized steroid hormones, their precursors or free cholesterol, which are kinds of lipids, are reacted with osmium tetroxide.

INTRODUCTION

In 1902, Kopsch[1] found that the Golgi apparatus was stained characteristically with osmium tetroxide by prolonged osmication at light microscopic level. This fact was comfirmed by the application of this technique to the electron microscopic observation by Dalton and Felix (1953)[2]. According to their observation, only the cis-side of this organelle was stained with osmium tetroxide. Since then, several papers dealing with prolonged osmication have been published (Dalton and Felix, 1954[3]; 1956[4]; Friend and Murray, 1965[5]; Friend, 1969[6]; Kurosumi, 1970[7]; Novikoff et al., 1971[8]; Paavola, 1978[9]). Recently, Sawano and Fujita (1980)[10] found out a characteristic localization of osmium impregnation in the mouse adrenal cortical cell that not only the Golgi apparatus, but also the cisternae of the smooth endoplasmic reticulum, that of the nuclear envelope and that of the characteristic lamellar bodies were stained with osmium tetroxide. In the present paper, the author wish to examine whether this characteristic stainability of osmium is a specific reaction only in the steroid hormone secreting cell or not, observing other steroid hormone secreting cells, such as Leydig cell of the testis and the theca interna cell of the ovary.

MATERIALS AND METHODS

The testis, the ovary and some other organs

*) 沢野文夫：マウスステロイド分泌細胞における特徴的オスミウム酸長時間浸漬像

such as the liver, the small intestine and the thyroid gland of about 5 male and 10 female mice (albino CF-1) of 3 months old were used for this study. Prolonged osmication was performed in two ways, one was an unfixed method and the other was a fixed method.

a) Unfixed method

Unfixed fresh tissues were cut into small pieces of about 2 mm cube and directly immersed in 1% O_sO_4 buffered at pH 7.4 with sodium cacodylate for 1-2 hours at room temperature. Then the tissues were cut into smaller pieces and immersed in 2% O_sO_4 water solution (pH 6.0-6.7) for 40-48 hours at 40°C.

b) Fixed method

The mice were fixed only for 3-5 min by cardiac perfusion with modified Karnovsky's fixative (1965)[11]. After the fixation the tissues were removed immediately and cut into small pieces and immersed in 2% O_sO_4 water solution (pH 6.0-6.7) for 12 hours at room temperature, followed for 28-36 hours at 40°C.

After prolonged osmication of each method, the tissues were rinsed 2-3 times with 0.1 M sodium cacodylate buffer (pH 7.4), dehydrated in graded concentrations of alcohol and embedded in Epon exopy resin. All the sections cut on a Porter-Blum ultramicrotome and stained doubly with saturated uranyl acetate (Watson, 1958[12]) and Millonig's lead acetate (Millonig, 1961[13]) were examined with a Hitachi HU-11D type electron microscope.

OBSERVATIONS

1) Leydig cell of the testis

All the cisternae of the smooth endoplasmic reticulum, that of the nuclear envelope, that of the characteristic lamellar bodies and the cis-side of the Golgi apparatus are stained with O_sO_4 similarly in the adrenal cortical cell, reported by Sawano and Fujita (1980)[10] (Figs. 1, 2, 3). Lipid droplets, lysosomes and mitochondria are not stained as well. But in the cells of the contortous seminiferous tubules, the cisternae of the endoplasmic reticulum and that of the nuclear envelope are not stained with O_sO_4, but the cis-side of the Golgi apparatus is stained.

2) The theca interna cell of the ovary

All the cisternae of the smooth endoplasmic reticulum, that of the nuclear envelope and the cis-side of the Golgi apparatus are selectively stained with O_sO_4 (Fig. 4). Lipid droplets, lysosomes and mitochondria are not stained. In the theca externa cells which are adjacent to these cells, no other organelles are stained with O_sO_4 except for the cis-side of the Golgi apparatus.

3) Other cells

In the parietal cell of the gastric mucosa, the cisternae of well developed smooth tubular system are also stained with O_sO_4, but the lumina of the intracellular canaliculi are not stained at all.

In the case of fixed method, some kinds of cells which are not the steroid hormone secreting cells, such as hepatocytes, absorptive epithelial cells of the duodenum, Paneth cells, plasma cells, fibroblasts and a part of the thyroid epithelial cells exhibit an interesting localization of osmium that the cisternae of the rough endoplasmic reticulum and that of the nuclear envelope are stained with O_sO_4 besides the Golgi apparatus. (Fig. 6).

DISCUSSION

It was reported by Friend and Brassil (1970)[14] and Sawano and Fujita (1980)[10] that the cisternae of the smooth endoplasmic reticulum, that of the nuclear envelope and the cis-side of the Golgi apparatus were stained with O_sO_4 by prolonged osmication in the adrenal cortical cell. In this study, the results obtained in Leydig cell of the testis and the theca interna cell of the ovary, which are the steroid hormone secreting cells, are similar to the adrenal cortical cell. When the tissues are fixed by glutaraldehyde, some other kinds of cells also exhibit the characteristic localization of osmium in the rough endoplasmic reticulum, not in that of the smooth endoplasmic reticulum. In these cases, it might be possible that the protein stored in the cisternae of the rough endoplasmic reticulum were changed to be reacted to osmium tetroxide by the action of glutaraldehyde. From this fact, the cell which shows the characteristic localization of osmium is not always limited to the steroid hormone secreting cell, but at least it is true that three kinds of the steroid hormone secreting cells, the adrenal cortical cell, Leydig cell of the testis and the theca interna cell of the ovary of mice show a com-

mon selective localization of osmium by prolonged osmication.

Osmium tetroxide has been known to be a strong oxdizing agent and a good fixative of lipids as well as protein for a long time (Schultze and Rudneff, 1865[15]). The present results show that the substance stored in the cisternae of the smooth endoplasmic reticulum which is probably a kind of lipid is reacted to osmium tetroxide. The contents of the cisternae of the smooth endoplasmic reticulum are now obscure, but it is believed that they are free cholesterol (Sharawy et al., 1979[16]), newly synthesized steroid hormones or their precursors.

There is no adequate speculation why the cisternae of the smooth tubular system of the parietal cell of the gastric mucosa stained with O_sO_4, but the lumina of the intracellular canaliculi are not stained. Although it is possible that the cisternae of all kinds of the smooth endoplasmic reticulum may be filled with osmiophilic materials, it needs the further studies to clarify the meaning of these facts.

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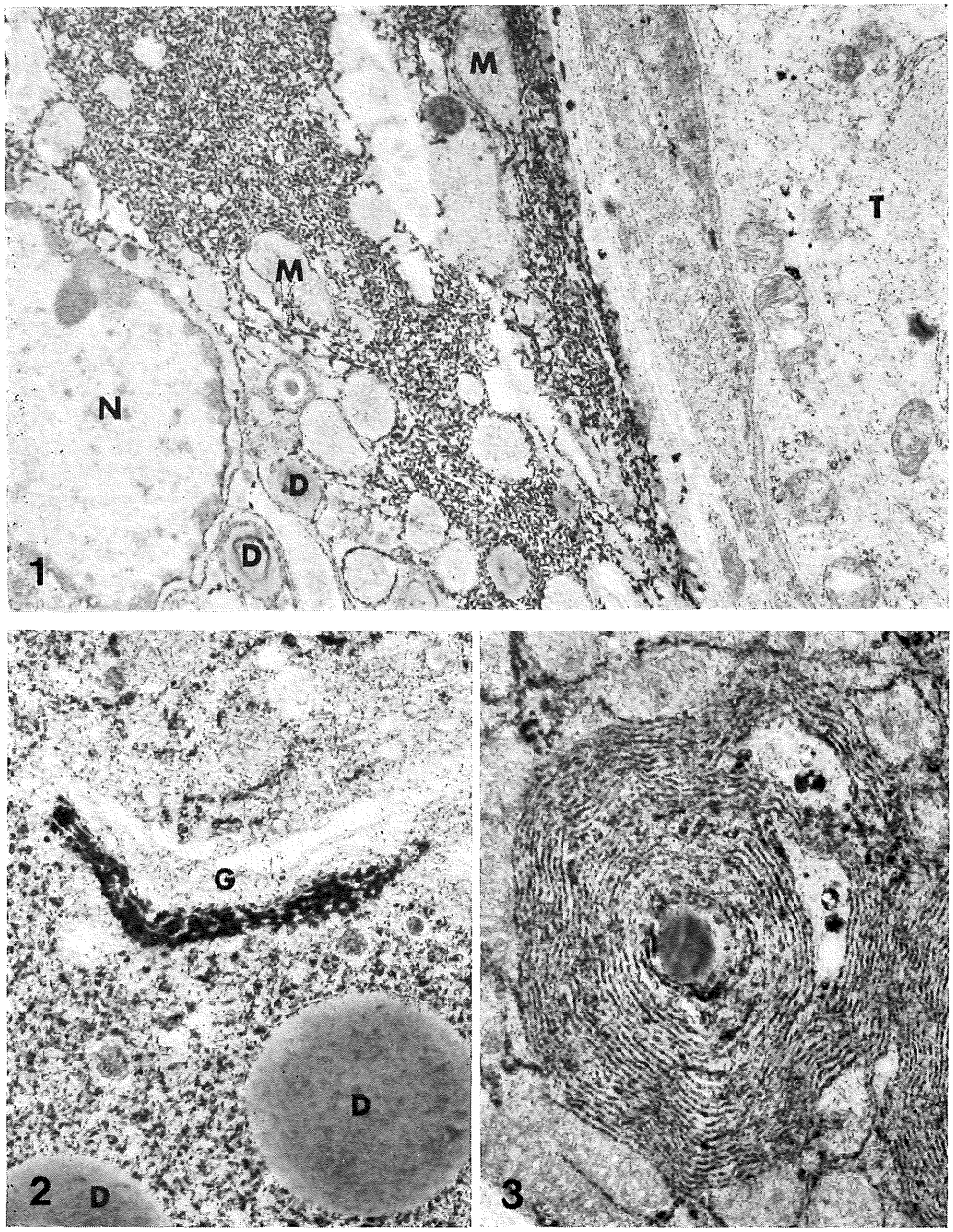
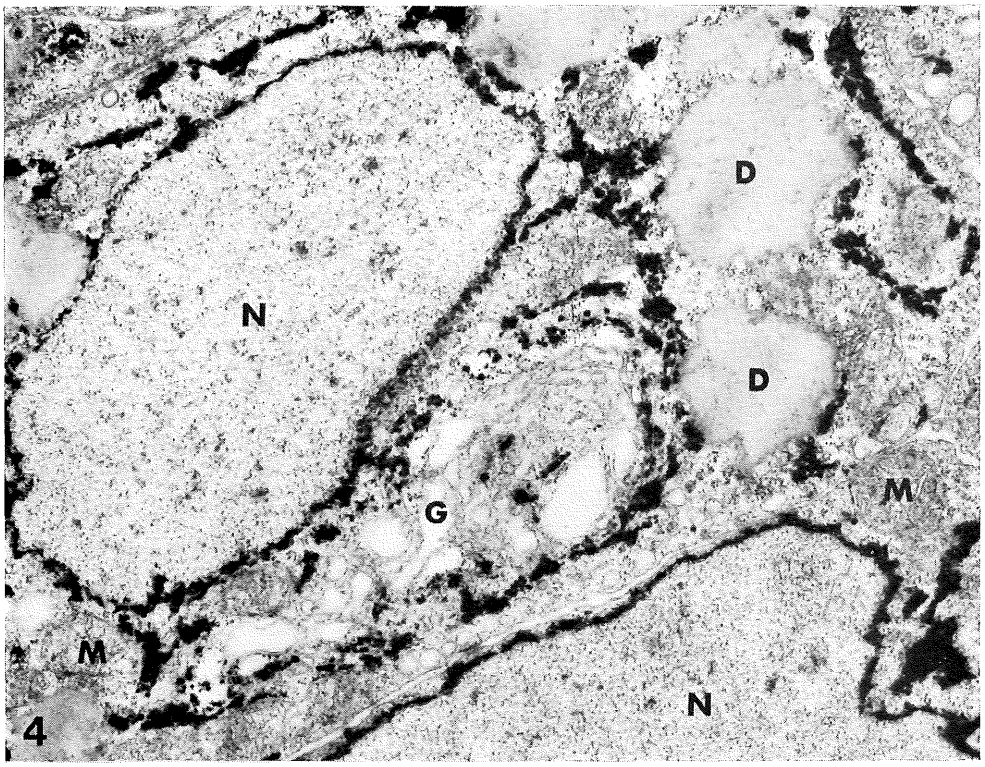


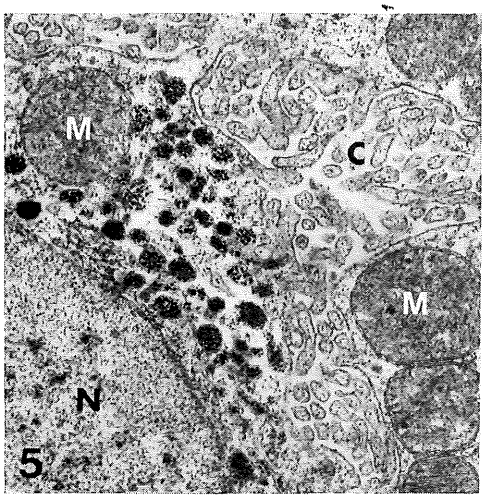
Figs. 1-3. Prolonged osmication in the mouse Leydig cells of the testis

Fig. 1. The cisternae of all the smooth endoplasmic reticulum and that of the nuclear envelope are stained with O_sO_4, but lipid droplets (D) and mitochondria (M) are not stained. The cell of the contortous seminiferous tubules (T) are not stained with O_sO_4 at all. N: nucleus ×10,000

Fig. 2. Three lamellae and vesicles on the cis-side of the Golgi apparatus and the cisternae of all the smooth endoplasmic reticulum are stained with O_sO_4. Lipid droplets (D) are not stained. ×20,000

Fig. 3. The cisternae of the characteristic lamellar body are stained with O_sO_4. ×21,000





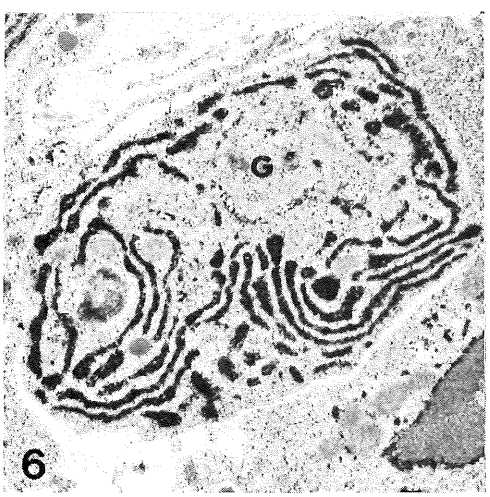


Fig. 4. Prolonged osmication in the mouse theca interna cell of the ovary. The cisterena of the smooth endoplasmic reticulum, that of the nuclear envelope and the cis-side of the Golgi apparatus (G) are stained with O_sO_4, but lipid droplets (D) and mitochondria (M) are not stained. N: nucleus ×20,000

Fig. 5. Prolonged osmication of the mouse parietal cell of the stomach. The cisternae of the smooth tubular system are stained with O_sO_4, but lumina of the intracellular canaliculi (C) are not stained. N: nucleus M: mitochondria ×15,000

Fig. 6. Prolonged osmication of the mouse plasma cell. All the cisternae of the rough endoplasmic reticulum and the cis-side of the Golgi apparatus (G) are stained with O_sO_4. ×17,000