Kawasaki Med. J. Vol. 5: No. 3·4, 131–142, 1979

SPIRONOLACTONE BODIES : A STUDY WITH TRANSMISSION ELECTRON MICROSCOPE AND FREEZE-FRACTURE REPLICATION

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Accepted for Publication on December 13, 1979

Abstract

A treatment with Aldosterone antagonist, Spironolactone, is known to produce acidophilic cytoplasmic inclusions in the zona glomerulosa cells of adrenal corteces. Transmission electron microscopic study disclosed that the inclusions were composed of concentric whorls of membranes and/or a regular aggregation of tubules, devoid of limiting membrane. The formalin-fixed tissue seemed to be an adequate material for freeze-fracture study. Freeze-fracture replication study of the inclusion body confirmed that the inclusions lacked limiting membranes and each concentric lamellae were comprised of smooth membranes with occasional membrane-associated particles. It is speculated that spironolactone body may be a partially degenerated aggregate of the smooth endoplasmic reticulum, overgrown through either direct or indirect stimulation by aldosterone antagonist.

INTRODUCTION

The presence of acidophilic cytoplasmic bodies in the zona glomeruloza cells in patients treated with aldosterone antagonist, spironolactone or aldactone, was first reported by Janigan[1] in 1963. They are generally known as spironolactone bodies (S bodies), and are frequently associated with such diseases as liver cirrhosis and congestive heart failure for which this medication is given. Hitherto, a few reports[2,3] have appeared on its ultrastructural detail, histochemical nature and histogenesis. None has examined them with freeze fracture technique.

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Our recent encounter with spironolactone bodies in a patient with liver cirrhosis treated with a long term administration of spironolactone prior to his death prompted us to carefully search the incidence of such inclusions among consecutive autopsy cases with major diagnosis of liver cirrhosis and/or hepatocellular carcinoma. In sixteen out of twenty two such cases, spironolactone bodies were found in the adrenal cortices. In all 16 cases, clinical histories were positive for the administration of spironolactone. The incidence among spironolactone users, and the relation between the dosage and the amount of the bodies will be detailed elsewhere.

The purpose of this report is two fold. Firstly, the ultrastructure of spironolactone bodies were examined by transmission electron microscope, combined with thin section and freeze-fracture replication (FFR) technique. Its histogenesis is speculated. Secondly, formalin-fixed tissues were processed for the freeze-fracture study successfully. Its significance is discussed.

MATERIALS AND METHODS

Adrenal glands of 22 autopsy cases of liver cirrhosis were originally fixed in 10 % formalin. Hematoxylin & eosin-stained sections were light microscopically reviewed for the presence of concentric lamellar inclusions. Of these glands,16 were found to have inclusion bodies. In positive cases, inclusions were studied by following special stains ; oil red O, sudan black B, luxol fast blue, PAS, alcian blue, mucicarmine, congo red, and Feulgen reaction. Portions of the tissue from formalin-fixed adrenal glands were also processed for electron microscopic and freeze-fracture replication study.

For the conventional electron microscopic study, tissues were postfixed in 1% cacodylate-buffered osmium tetroxide (pH 7.4), dehydrated with graded alcohol and propyrene oxide, and embedded in Epon 812. Sections 1 μ thick were then stained with toluidine blue for the presence of spironolactone bodies. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with Hitachi H-500 electron microscope.

For the freeze-fracture replication, tissues were washed in cacodylate buffer and immersed in 20 % glycerol in cacodylate buffer for one hour. Tissue blocks were placed on the support disc, rapidly frozen in liquid freon 22 and stored in liquid nitrogen. Tissues were fractured in a Balzers' freeze-etching device (BAF-301) at -110 °C. Using an electron-beam gun, platinum-carbon was casted at a 45° angle, followed by carbon coating. Replicas were cleaned with sodium hypochlorite, rinsed in distilled water, and mounted on 300 mesh uncoated copper grids. They were examined in a Hitachi H-500 electron microscope.

OBSERVATIONS

Light microscopic features

Round or oval, concentric lamellar intracytoplasmic inclusion bodies were present exclusively in cells of zona glomerulosa near capsule (Fig. 1). In severe cases, almost all zona glomerulosa cells had inclusions. Usually, one cell contained one to three inclusions. They measured four to 16 μm in diameter and stained faintly eosinophilic in H & E stained section. Although they were, in general, composed of concentric rings of lamellae, their central portions were often homogeneous than the periphery. There was a clear halo between the surrounding cytoplasm and the inclusion (Fig. 2). The histochemistry of the laminated inclusions is indicated in Table 1. Their staining properties are consistent with those of phospholipid compound.

Ultrastructure

The ultrastructure of zona glomeruloza cells in the present study (Figs. 3 & 4) provided characteristic features of normal zona glomerulosa cells[4,5]; lipid vacuoles, numerous smooth endoplasmic reticulum (SER), and mitochondria with tubulovesicular cristae. Spironolactone bodies were composed of concentri-

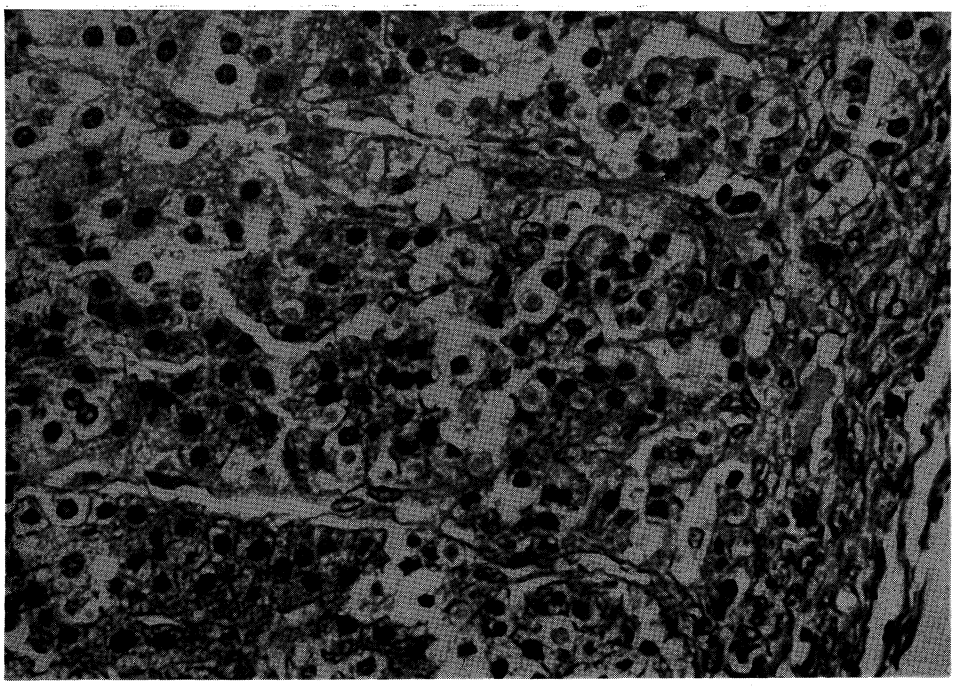


Fig. 1. Round, concentrically lamellar intracytoplasmic inclusions are present exclusively in cells of the zona glomerulosa near capsule. H & E X 100

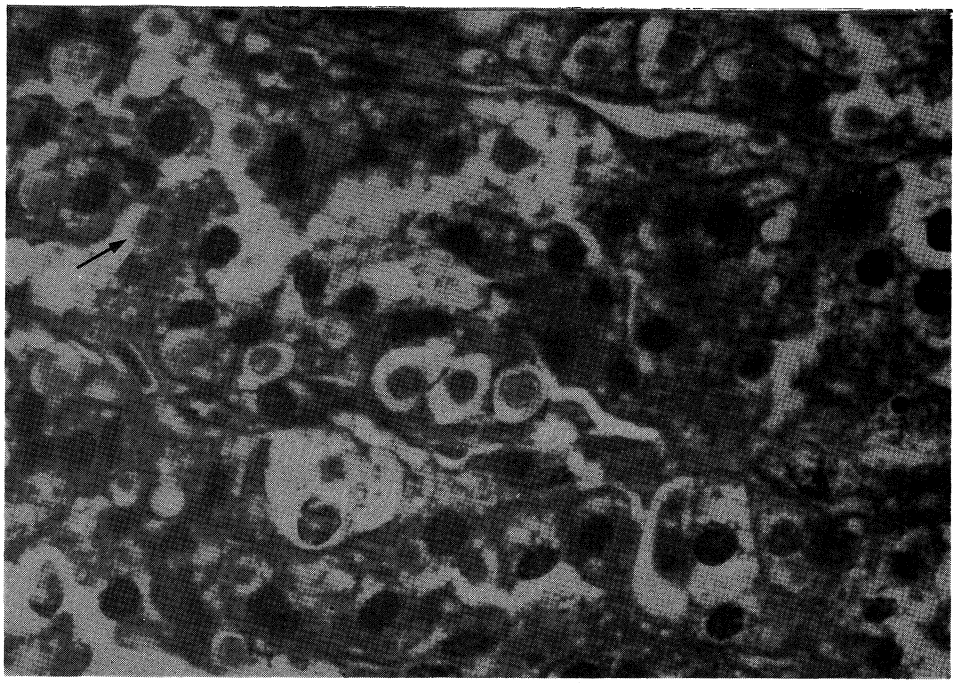


Fig. 2. In higher magnification, concentric lamellar structure is evident. Note that the central portion is often homogeneous (arrow). H & E X 400

TABLE 1.
Histochemical Study of Spironolactone Bodies

Oil red O	central portion	+++
	periphery	+
Sudan black B		+++
Luxol fast blue		+++
PAS		−
Alcian blue		−
Mucicarmine		−
Congo red		−
Feulgen reaction		−

The staining property was arbitrarily graded as negative (−), and positive (+, ++, and +++).

cally arranged membrane lamellae, tubular structure and amorphous materials (Figs 4 & 5). They usually located in the cytoplasm as round inclusion bodies, but no limiting membranes were identified at periphery. Along the margin of the body, connections were frequently seen between the smooth endoplasmic

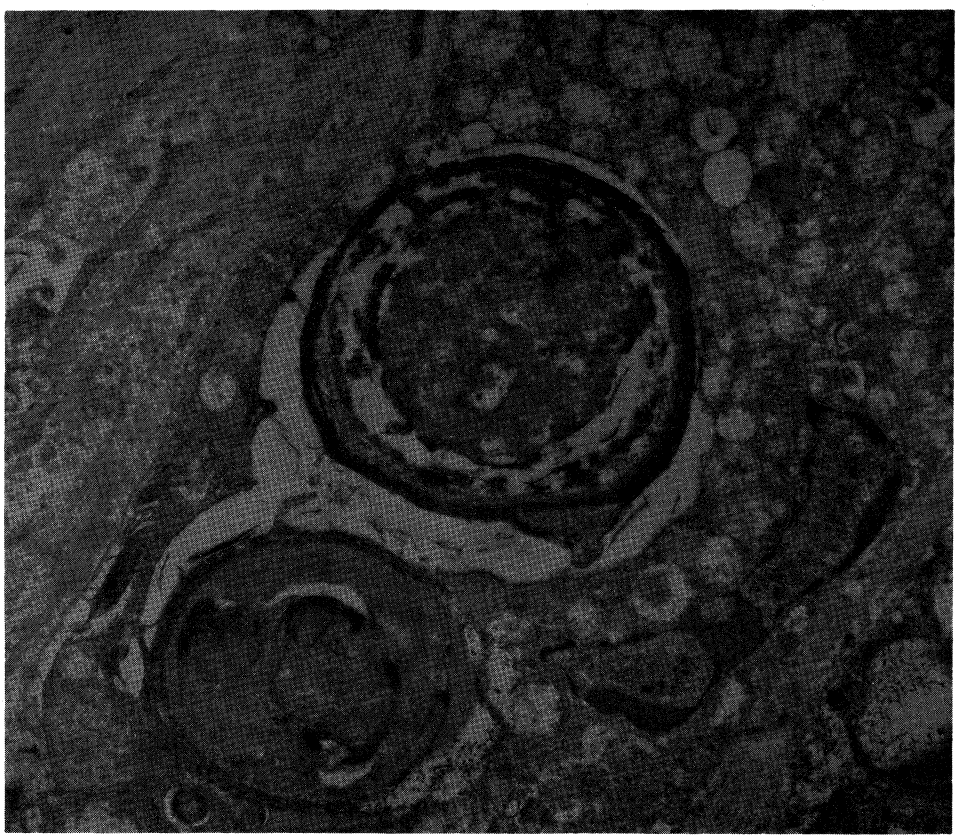


Fig. 3. Electron micrograph of zona glomerulosa cell with two spironolactone bodies. X 10,000

reticulum of the cytoplasm and lamellae and/or tubular structure of the body. Such lamellar and tubular structures also existed along the nucleus, probably within the perinuclear cistern (Fig. 6). Inner and outer karyomembranes were distinct from the above structures (Figs. 7 a,b). Cells with spironolactone bodies contained numerous swollen mitochondria which had vesicular cristae and tended to gather at its peripheral zone (Fig 4). SERs were seen increased in between mitochondria. Lipid vacuoles seemed depleted in such cells.

Freeze-fracture preparation from the formalin-fixed tissue unexpectedly provided a satisfactory preservation of ultrastructural architecture (Figs. 8 & 9). Membrane-associated particles (MAPs) of protoplasmic and nuclear membrane were present. They were usually distributed evenly, but occasionally aggregated. Spironolactone body showed stacks of fissured faces of membrane which were

Fig. 4. Higher view of spironolactone body. Aggregates of smooth endoplasmic reticula are continuous to the inclusion without intervening limiting membrane. The central portion is floccular, amorphous and tubular in this photograph. X 25,000

occasionally studded with MAPs (Fig. 8). Tubular systems of SER seemed to be continuous to the lamellae inside (Fig. 9).

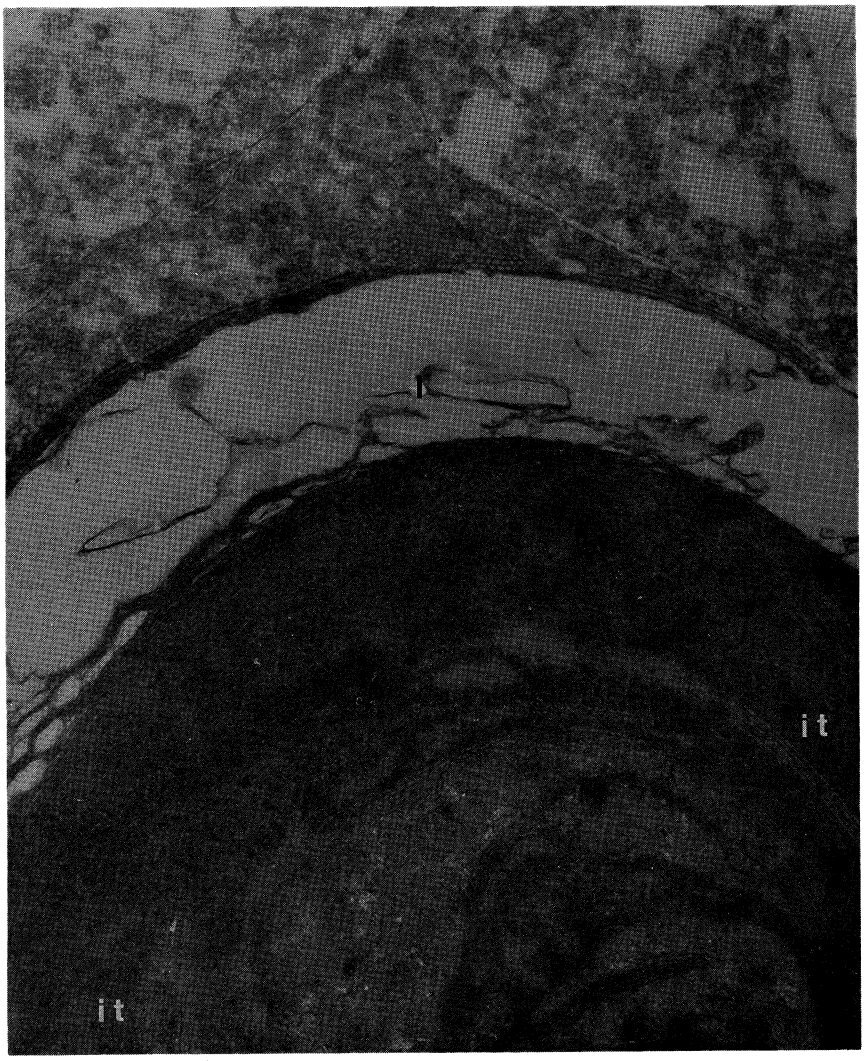
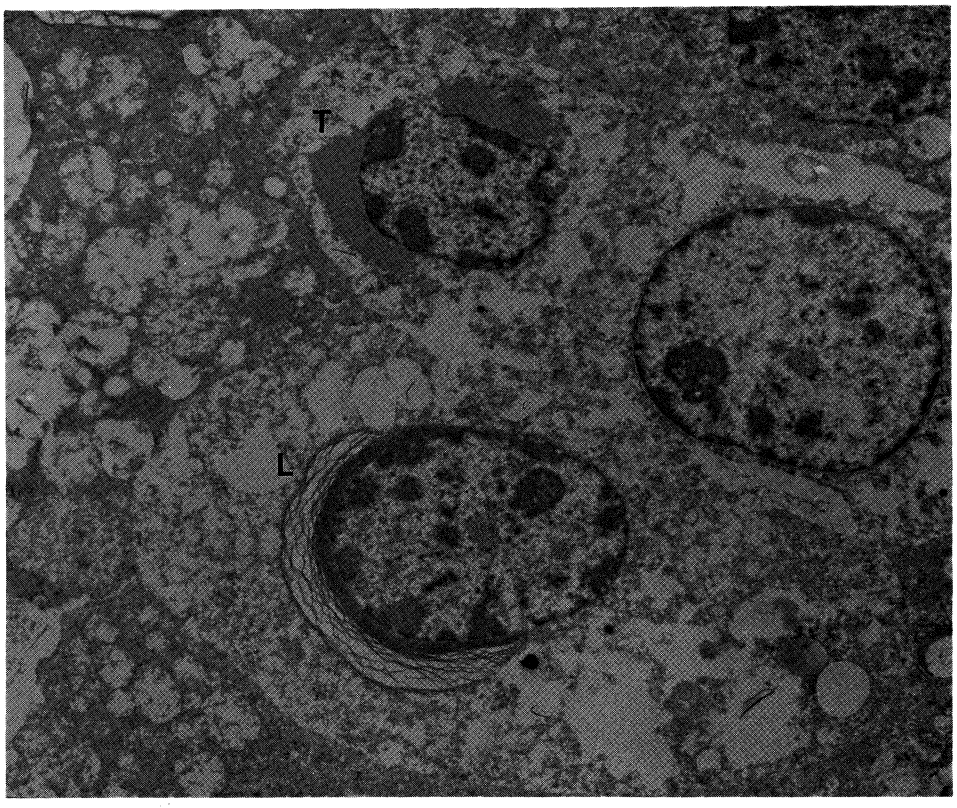


Fig. 5. The lamellar component in the inclusion (I) seems to be surrounded by the tubular one and such tubules are still observable even in between lamellae (it) and inside of the inclusion. X 20,000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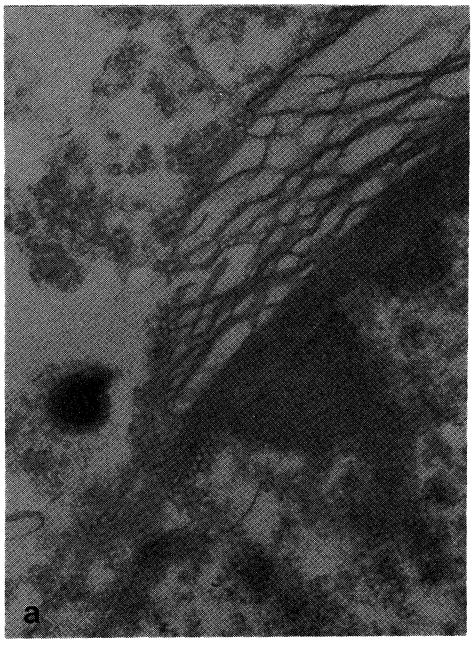
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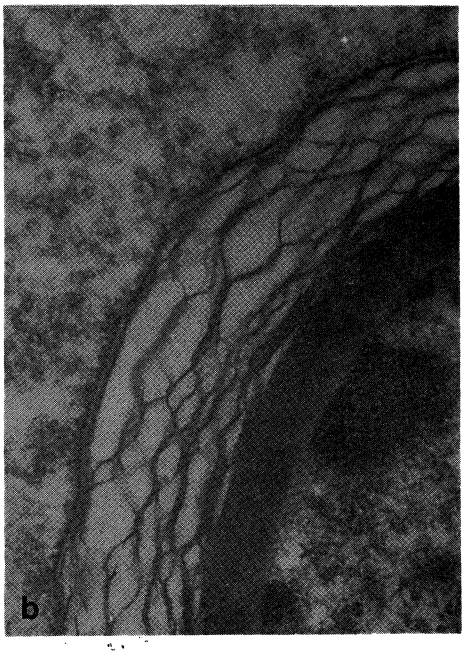
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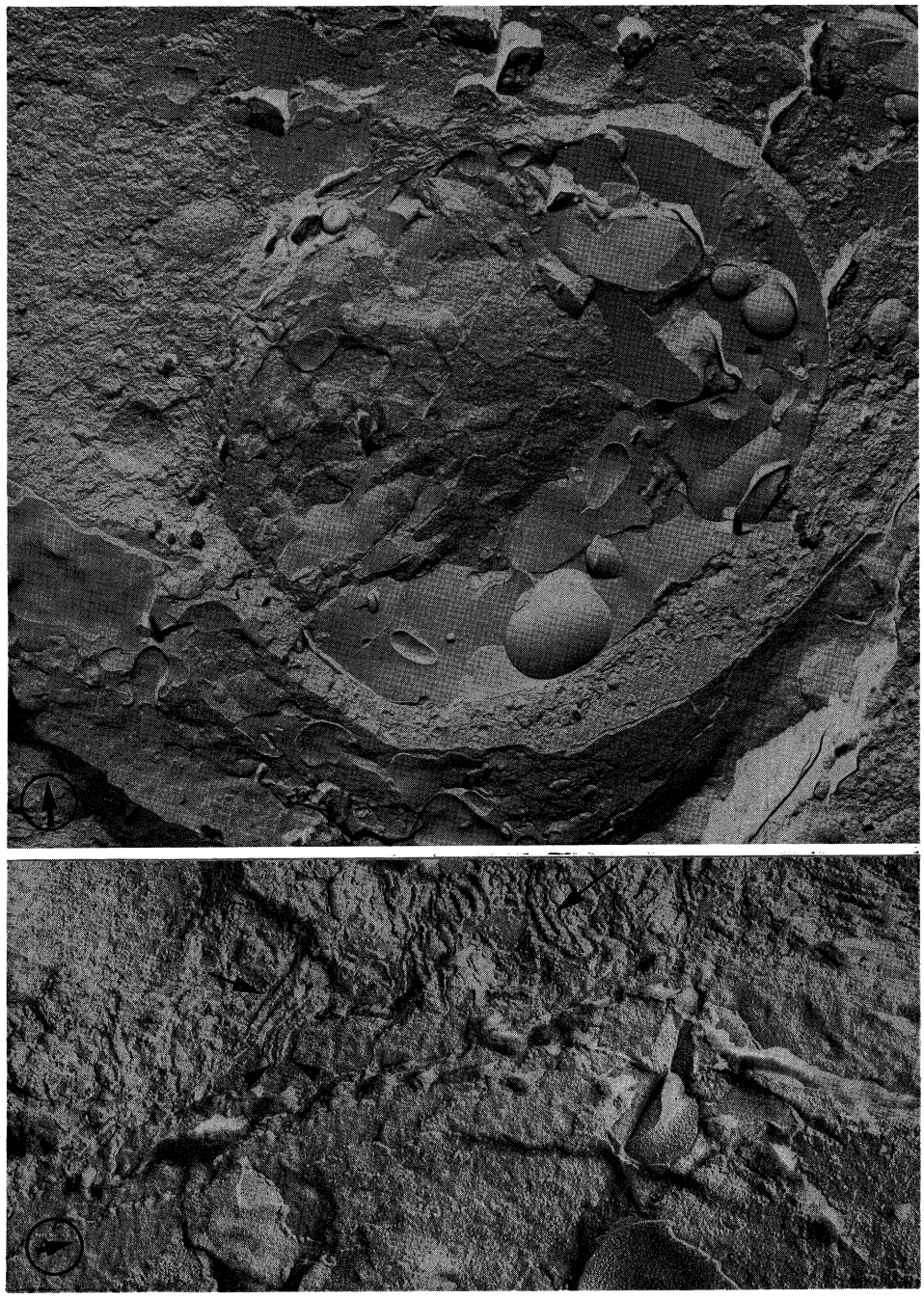

Fig. 6. Zona glomeruloza cells with perinuclear lamellar (L) and tubular (T) structure. X 6,000

Fig. 7. a, b. Higher magnification of Fig. 6. Lamellar structure is present within the perinuclear cistern. It is clear in "a" that the periphery of lamellae ends as the tubular structure. X 26,000

Fig. 8. Freeze-fracture image of the zona glomeruloza cell. Inclusion is centrally located, showing fissure-faces of lamellae. X 13,200
The encircled arrow of this and next photographs indicates the direction of shadowing.

Fig. 9. The tubular system of the smooth endoplasmic reticulum (arrows) seems to be continuous to lamellae inside (L) (arrowheads). X 45,000

DISCUSSION

Spironolactone bodies (S bodies) have been claimed to be closely associated with a prolonged administration of spironolactone[1,2,3,6]. All the cases with S bodies in our study showed positive history for its administration.

The mechanism of the S body formation still remains to be elucidated. Davis and Medline[2] assumed four possible mechanisms. They included artefact, regeneration, degeneration, and specific metabolic function. The fact that such bodies are consistently and exclusively present in the zona glomerulosa cells of the patients treated with spironolactone may exclude the first possibility. It is well known that steroid producing cells are equipped with abundant SER, lipid vacuoles, and mitochondria with tubular cristae, and that the SER in the adrenal cortical cells plays an important role in lipid metabolism[4,5]. Cholesterol biosynthesis is believed to be taken place there to produce aldosterone. Jenis and Hertzog[3] speculated that chronic administration of spironolactone and subsequent sodium diuresis results in an activation of the endogenous renin-angiotensin II, and stimulates to cause hypertrophy and/or hyperplasia of the zona glomerulosa, thereby increasing cholesterol biosynthesis. Morphologically, this event may manifest itself as hypertrophy or increase of SER. The development of agranular membranes around lipid droplets in their study suggested that precursors in the latter take part in new membrane formation and/or steroid synthesis. They also speculated another mechanism. As spironolactone is similar in structure to cholesterol and aldosterone, it may become directly incorporated into the membranes of the ER. Then, such synthetic "foreign" steroidal compound may interfere with normal membrane turnover, causing a progressive accumulation of agranular membranes. The latter speculation, however, seems to be less likely. The S bodies except for the central portion is almost exclusively of phospholipid. It is hardly conceivable that the mere addition of synthetic steroidal compound creates such complex, well-organized tubular structures rather than mere enlargement of SER. Our ultrastructural study showed that the central core of bodies were composed

of amorphous and floccular debris. In contrast to the observation by Jenis and Hertzog[3], no distinctive lipid vacuoles were usually found. Such debris was shown to have some transition from surrounding membrane substance. The lamellar structure of inclusions were always associated in both inside and outside, with tubular structure. The absence of limiting membrane around inclusion bodies, the presence of membrane continuity between the lamellar content and adjacent SERs and the presence of tubular and lamellar structure similar to S bodies in perinuclear cistern may exclude the possibility of autophagosomal origin. We speculate, therefore, that inclusions are degenerative substances derived from newly produced abundant SERs. The accumulated SERs are segregated in portions of the cytoplasm and are disintegrated and then reorganized into lamellar and floccular substances.

To our knowlege, the present report is the first to examine S bodies with freeze-fracture technique. It is generally believed that the glutaraldehyde fixation is suitable in preparation of tissue samples for this technique[7,8]. For the investigation of animal tissues, it is almost routinely employed. This is done with the intention of reducing tissue necrosis. Osmium tetroxide, on the other hand, is known to somehow affect the membrane component[9]. Membrane images such as fissure or edge-on face cannot be observed in this preparatory method for FFR. Formaldehyde is not investigated enough in this respect. Recently, Carson[10] re-emphasized the usefulness of formalin fixation for the preservation of ultrastructure. Glutaraldehyde and formaldehyde are among the group of aldehyde fixatives. Theoretically speaking, therefore, formalin fixation may give a sufficient preservation of ultrastructure and freeze-fracture image. The result of this study indicates that formalin could be a sufficient fixative for FFR provided that the rapid fixation is performed with optimal osmolarity and pH. The usefulness of formalin flxation for FFR, if indeed proved, fascinates pathologists for the retrospecitive FF study in need. In this respect, of course, further investigation is necessary.

At any rate, FFR study of spironolactone body confirmed that SERs are continuous to the lamellar structures of the inclusion at its periphery and that it is composed of membrane lamellae. Lamellae were mostly devoid of but occasionally studded with so-called membrane-associated particles. This may represent artefact or disintegration of such particles in the inclusion.

Acknowledgment

Authors wish to thank Mr. Tamotsu Takahashi, Mr. Yasuyuki Miyake, and Miss Masae Yamaguchi for their excellent technical assistance. This study was in part supported by the Research Project Grant (53-302) of Kawasaki Medical School.

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