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Adrenal Adenomata Causing Primary Aldosteronism

An Ultrastructural Study of Twenty Five Cases

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Summary. Adenomata taken from 25 patients with primary aldosteronism were observed by electron microscopy. The cells in the adenoma had a well developed agranular endoplasmic reticulum but granular endoplasmic reticulum was not prominent. Most of the mitochondria resembled those in the cells of the zona glomerulosa, suggesting that the adenomata which caused primary aldosteronism are derived from this zone. Spironolactone bodies were found in the cells of the adenoma from a patient who received spironolactone. Their appearance was identical to that descrived in previous reports.

Key words: Primary aldosteronism – Adrenal adenomata – Ultrastructure

Introduction

It is well known that primary aldosteronism is caused by excessive aldosterone secretion from an adrenal cortical tumor. This is an uncommon disease but has been more commonly recognized. The clinical and pathological features of the disease have been well documented, but only a few electron microscopic studies have been made (Propst, 1965; Cervós-Navarro et al., 1965; Luse, 1967; Tsuchiyama, 1967; Mackay, 1969; Reidbord and Fisher, 1969; Sommers and Terzakis, 1970; Tannenbaum, 1973;Kovacs et al., 1974; Beskid et al., 1978). Histologically the cells of aldosteronoma are similar to those of the zona fasciculata, but ultrastructural features of the adenoma cells reveal variable architecture. The organelles, including agranular endoplasmic reticulum and mitochondria show variable changes consistent with hyperfunction: Cytoplasmic inclusions have also been observed in the cells of the zona glomerulosa or adenomata of patients treated with spironolactone. These inclusions are called spironolac-

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tone bodies, and close correlation between their appearance and aldosterone production has been suggested (Conn and Hinerman, 1977).

In this paper the ultrastructural characteristics of adrenal adenomata causing primary aldosteronism are reported and discussed with particular reference to changes in agranular endoplasmic reticulum and the mitochondria which are involved in aldosterone biosynthesis, and to inclusions bodies formed by administration of spironolactone.

Materials and Methods

Twenty five patients with primary aldosteronism due to adrenal adenomata (8 male and 17 female) aged between 25 and 60 years were included in the study. They were operated on during the period from January 1, 1966 to January 31, 1977 at Niigata University Hospital. All adenomata were histologically verified. Data for the patients and their tumors are shown in Table 1.

Table 1. Data of twenty five patients with adenoma

Case	Age	Sex	Weight of tumor (g)	Plasma K (mEq/l)	Plasma renin activity (ng/dl)		Spironolactone therapy	
							Total dosage (mg)	Duration (days)
1	40	F	2.0	2.2	71.0	+	4,475	41
2	44	F	(7.7)	2.2	43.9	+	not clear	not clear
3	27	F	(8.7)	2.2	51.7	+	1,350	18
4	36	F	(6.8)	3.7	50.0	+	3,075	24
5	43	F	(4.0)	2.7	0	+	4,650	31
6	42	M	(5.0)	1.8	0	+	6,450	43
7	43	M	1.2	2.9	80.0	+	6,000	40
8	30	F	(4.5)	2.6	67.0	+	3,750	25
9	43	F	2.0	1.8	0	+	25,950	263
10	34	M	/	2.7	0.03	++	5,250	35
11	37	M	(6.0)	1.8	0.1	++	4,375	22
12	49	F	1.6	2.3	0.18	++	1,275	17
13	49	M	1.2	2.3	0	++	3,750	25
14	39	F	2.0	2.1	0	++	4,500	30
15	38	F	(7.0)	1.6	0	++	6,375	25
16	28	F	2.0	2.7	0	++	8,625	62
17	45	F	2.0	2.5	0	++	2,550	17
18	31	F	2.1	2.3	0.08	++	3,100	33
19	49	F	3.0	2.3	0	++	6,150	41
20	60	M	(4.6)	2.8	0	++	13,950	159
21	48	F	(8.6)	2.6	0.05	++	5,000	37
22	47	M	1.1	2.4	0.15	++	3,750	25
23	46	F	0.6	3.2	0.22	++	1,600	16
24	29	F	3.8	2.8	0.10	++	9,500	95
25	25	M	1.7	2.0	0.03	++	1,650	19

+ Bioassay normal range 177 ± 34 ng/dl
++ Radioimmunoassay normal range 0.51–4.05 ng/ml/h
( ) Together with adrenal tissue

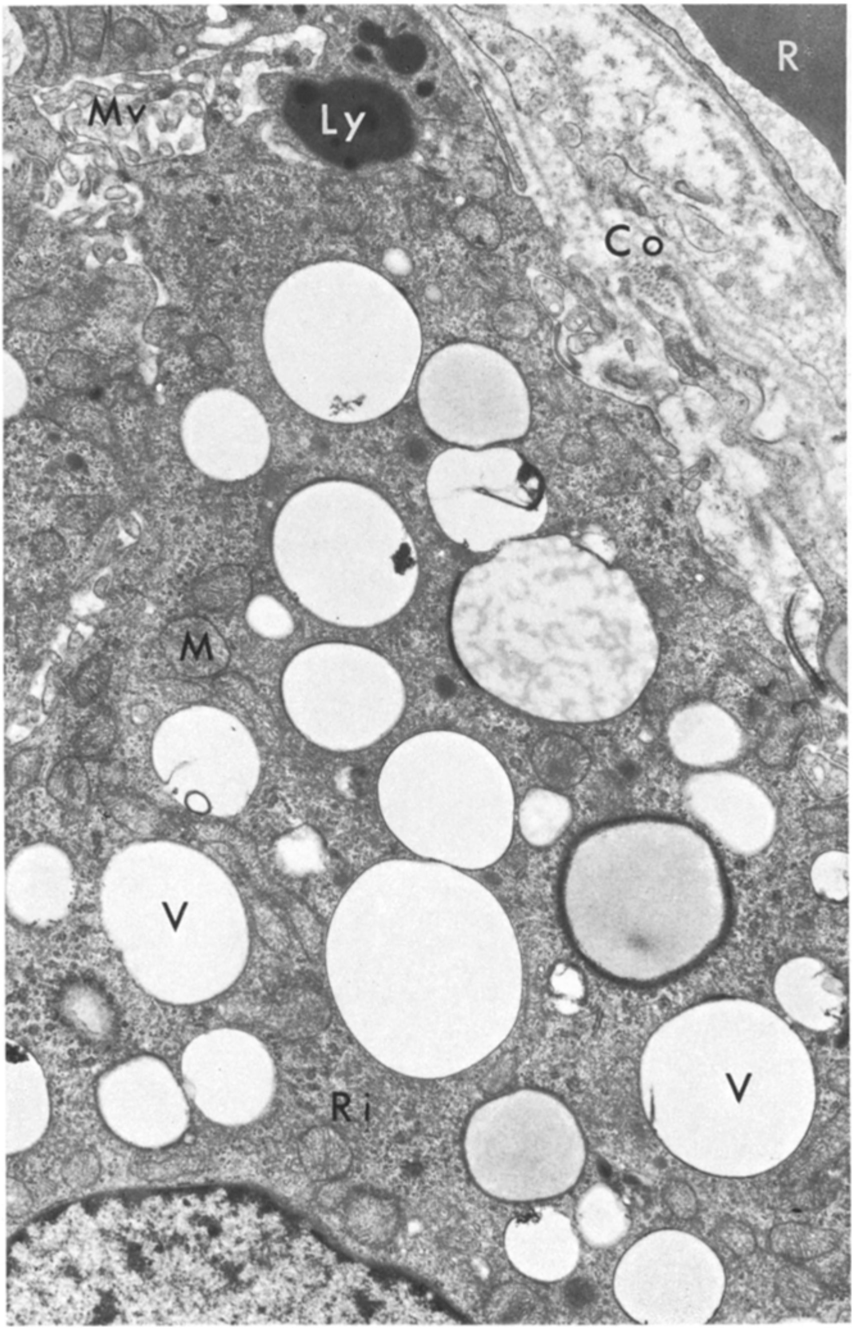

Fig. 1. Adenoma cells with numerous lipid vacuoles (*V*). Mitochondria (*M*) and free ribosomes (*Ri*) are scattered among them. Microvilli (*Mv*) project into intercellular space. *R*: Erythrocyte. *Co*: Collagenous fibers. *Ly*: Lysosome. × 12,000

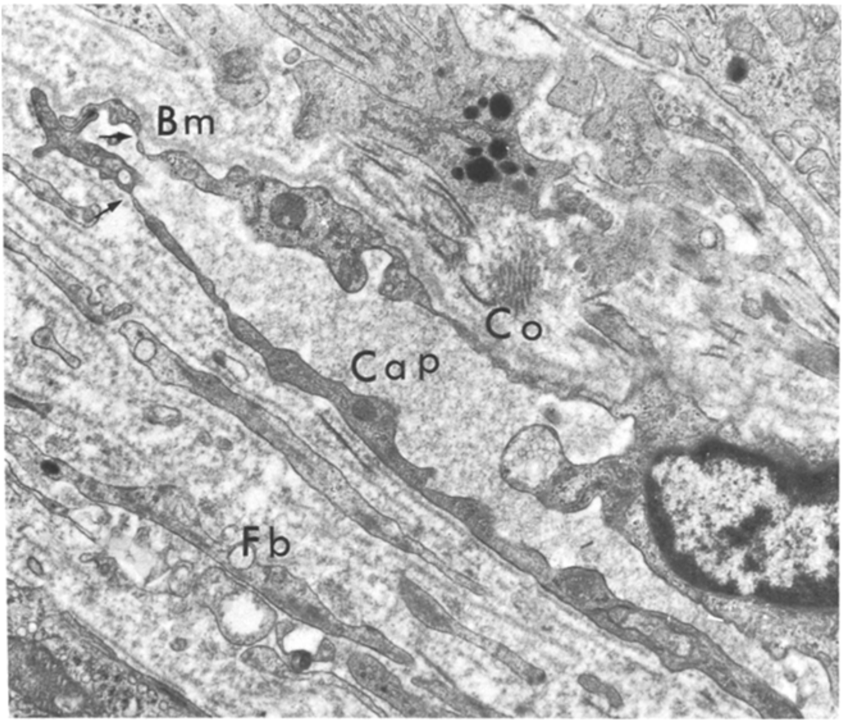

Fig. 2. Blood capillary (*Cap*) in the adenoma. Basement membrane (*Bm*) surrounds the endothelial cells with pores (*arrows*). Thin cytoplasmas of fibroblasts (*Fb*) and collagenous fibers (*Co*) are seen in perivascular space. ×5,000

The specimens were fixed immediately either in 2% osmium tetroxide in cacodylate buffer or in 2.5–4% glutaraldehyde in cacodylate or phosphate buffer followed by 2% osmium tetroxide. After dehydration with graded concentrations of ethanol, the specimens were embedded in Epon epoxy resin. Ultrathin sections were made with glass knives on a Porter-Blum MT-1 microtome, and stained with lead or uranyl acetate and lead. Electron micrographs were taken with a Hitachi HS-7S, HS-125DS or HS-9 electron microscope.

Results

Basement membrane surrounded clusters of adenoma cells and those cell surfaces which faced toward the perivascular and/or intercellular space were frequently provided with microvilli (Fig. 1). Interdigitation was occasionally encountered and showed a comparatively simple structure. Collagenous fibers and fibroblasts were often found between the capillaries and the adrenal tumor cells (Figs. 1, 2), and the fibers extended into the intercellular spaces between the cells. The nuclei of the tumor cells were spherical and occasionally irregularly shaped. Chromatin was condenced at the periphery of the nucleus and the nucleolus was usually prominent. The endothelium of the capillaries showed pores, as is usual in the adrenal cortex (Fig. 2).

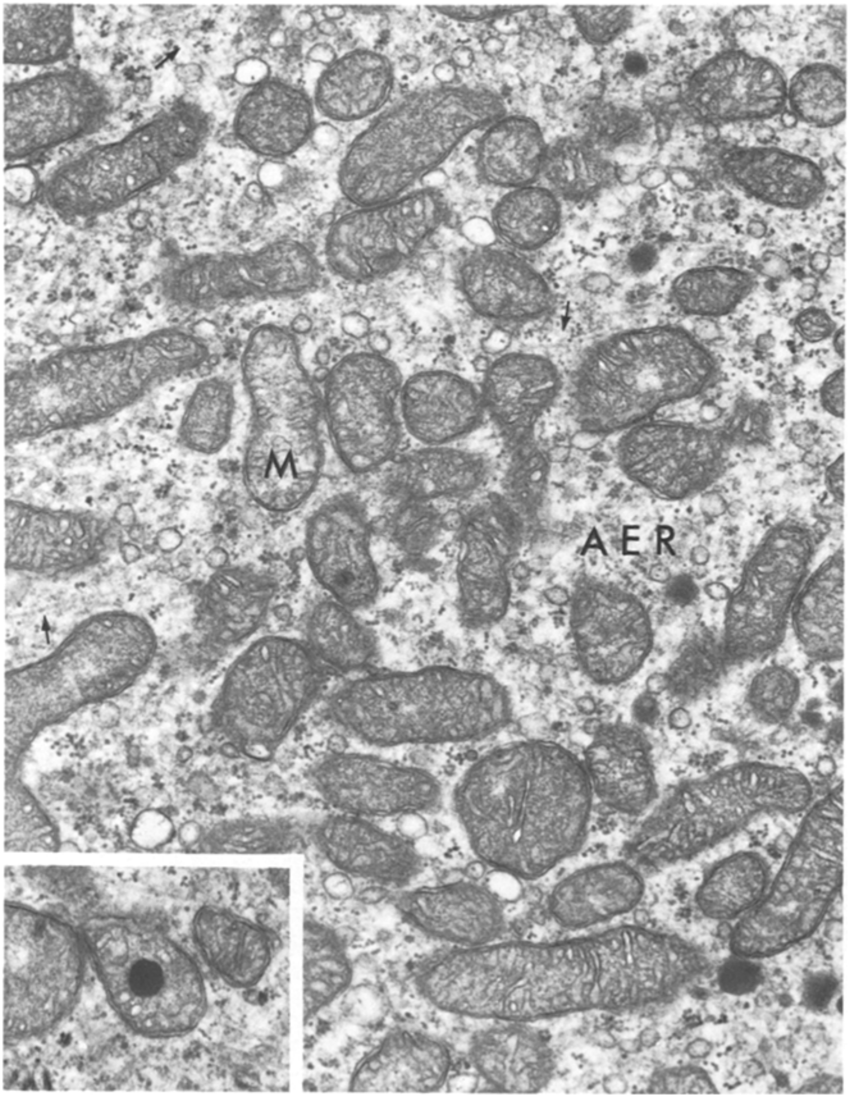

Fig. 3. Many mitochondria (*M*) and scarce agranular endoplasmic reticulum (*AER*) are located in an adenoma cell. Microtubules (*arrows*) run irregulary in the cytoplasm. ×23,000. *Insert:* Mitochondria with electron dense droplet in the matrix.

One prominent feature of the adenomata cells was the existence of many vacuoles which occupied the larger part of the cytoplasm. They varied in size and were generally electronlucent, but some contained homogenous, low electron dense material which occurred either peripherally or in patches (Fig. 1).

Developement of agranular endoplasmic reticulum varied in extent from cell to cell. This organell was usually prominent in cells with few lipid vacuoles and showed vesicular or tubular profiles (Fig. 4). Granular endoplasmic reticu-

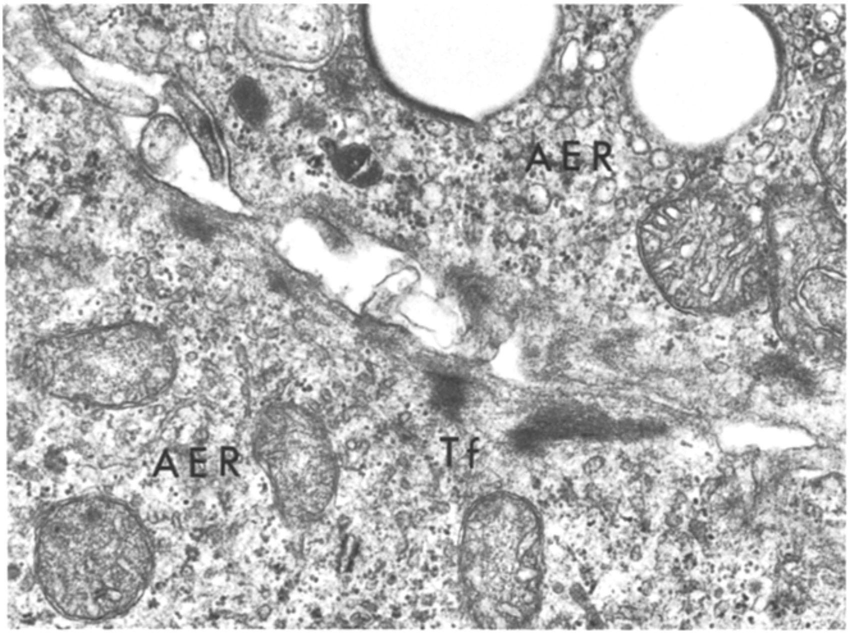

Fig. 4. Several bundles of tonofilaments (*Tf*) run parallel with the cell menbrane. Agranular endoplasmic reticulum (*AER*) is prominent. ×27,000

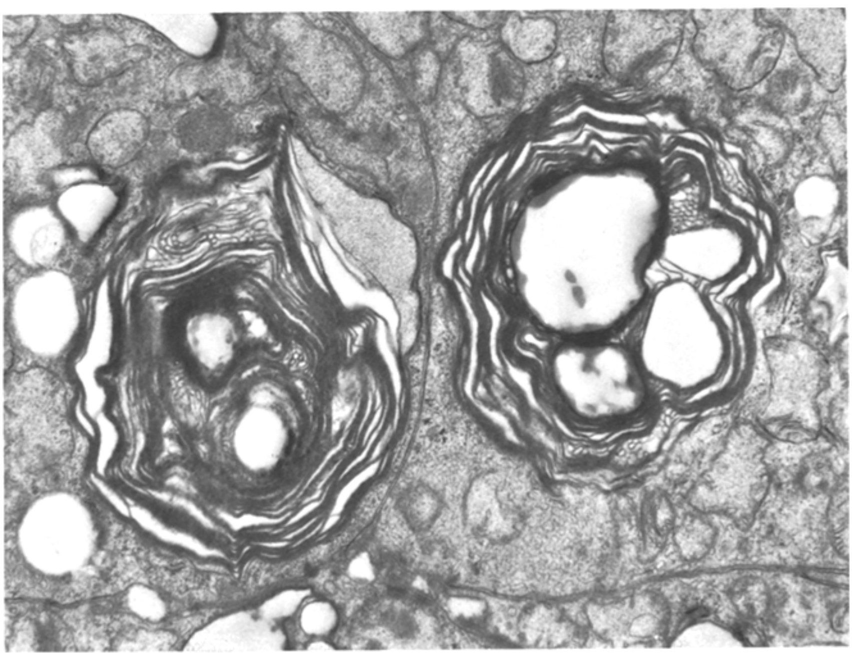

Fig. 5. Two spironolactone bodies. They have vacuoles in their central portions. ×9,500

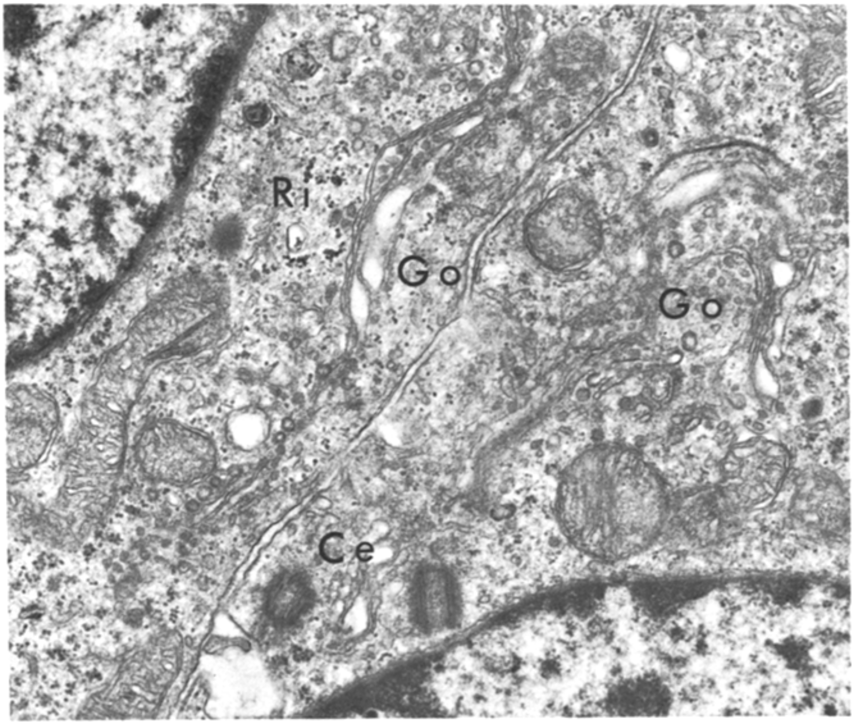

Fig. 6. Golgi complex (*Go*) and centrioles (*Ce*) occur in the vicinity of the nucleus. Free ribosomes (*Ri*) are scattered in the cyptoplasm. ×24,000

lum was not well developed, with a few exceptions. In these cases it assembled in a parallel arrangement in several parts. Continuity between agranular and granular endoplasmic reticulum was occasionally found.

A large number of mitochondria were seen, with sarcotubular cristae. They were spherical, oval or slightly elongated in form and similar to those found in the zona glomerulosa cells (Figs. 1, 3). Others resembled those in the zona fasciculata or reticularis cells. In addition mitochondria which were unlike those in the cells of the three zones were observed in several cases. Almost all of the mitochondria were small, and occasionally an electron dense droplet was situated in the matrix (Fig. 3, insert).

Spironolactone bodies, measuring 2–8μ in diameter, took the form of whorl-like structures and contained one or more lipid droplets in their central portions; most of them were electronlucent. These inclusions also had a few clusters of vesicles which were situated between concentric agranular membranes (Fig. 5). Spironolactone bodies were encountered frequently in only one case (Case 1). Continuity between these bodies and agranular or granular endoplasmic reticulum was not demonstrated. Myelin figures occurred in all cases and were simple in structure.

Bundles of tonofilaments, measuring 0.5–1.0 μ in length and 0.2–0.5 μ in width, ran parallel the cell membrane and occasionally came in contact with it (Fig. 4).

The Golgi complex was not generally well developed; in a few cases, however, it was prominent (Fig. 6). Microtubules, measuring 250–300Å in diameter and 0.5–1.5μ in length, ran irregularly in the cytoplasm in several cases (Fig. 3). Free ribosomes were scattered in the cytoplasm (Figs. 1, 6). Centrioles were only rarely found near the nucleus (Fig. 6).

Discussion

The mitochondria and microsomal fraction of the cells, which is mainly composed of membranes derived from agranular endoplasmic reticulum, contain several enzymes taking part in steroid hormone biosynthesis in the adrenal cortex. In a hyperfunctional state such as primary aldosteronism the agranular endoplasmic reticulum and mitochondria in the adenoma cells would be expected to be prominent, however, the findings obtained from the adenomata studied here not always reach our expectation.

A prominent agranular endoplasmic reticulum characterizes the cell of the human adrenal cortex (Long and Jones, 1967). It is generally accepted that several enzymes in the synthetic pathway from cholesterol to aldosterone are contained in these organells and accumulation of agranular endoplasmic reticulum may be histological evidence that aldosterone is being actively produced.

In the adenomata causing primary aldosteronism, agranular endoplasmic reticulum is generally abundant (Reidbord and Fisher, 1969; Tannenbaum, 1973; Kovacs et al., 1974; Beskid et al., 1978), but this finding is not invariable (Sommers and Terzakis, 1970). According to our observations, the amount of agranular endoplasmic reticulum in adenoma cells varied from cell to cell and generally more developed than in the cells of the zona glomerulosa, but was less than that seen in the cells of the adenomata causing Cushing's syndrome (Kano and Sato, 1977).

In well preserved specimens of human adrenal cortex, agranular endoplasmic reticulum takes the form of a tubular network (Long and Jones, 1967). In our laboratory, even in material fixed immediately after removal, it takes a vesicular rather than tubular form which we attribute to an artifact in fixation.

Aldosteronoma have been classified into three groups the zona glomerulosa type (Cervós-Navarro et al., 1965; Luse, 1967), the zona fasciculata type (Propst, 1965; Sommers and Terzakis, 1970) and the hybrid type (Reidbord and Fisher, 1969; Beskid et al., 1978). One similarity between the cells of the adenoma and the adrenal cortex is mitochondrial size, shape and inner structure. A characteristic feature of the mitochondria in the zona glomerulosa cell is their relatively small size, their round to slightly elongated shape and the presence of sarcotubular cristae. Mitochondria in the zona fasciculata cell, however, are large and spherical and possess tubular cristae. Thus, the architecture of the mitochondria may be a usefull finding on which to base a classification of the tumor cells. The aldosteronoma cells contained mitochondria of different types but most of them were identical to those found in the zona glomerulosa cells. It therefore seems likely that the adenoma which caused primary aldosteronism are derived from the zona glomerulosa.

Since Janigan (1963) described the occurrence of cytoplasmic bodies in the zona glomerulosa cells of patients who were treated with spironolactone during life time, the number of reports dealing with spironolactone bodies have been increasing. Several authors discussed the fine structure of the bodies in the cells of the zona glomerulosa (Jenis and Hertzog, 1969; Mackay, 1969; Davis and Medline, 1970; Fisher and Horvat, 1971; Okano et al., 1972; Kovacs et al., 1973) and in the cells of the aldosterone-producing adenoma (Cain et al., 1974; Shrago et al., 1975; Conn and Hinerman, 1977) of autopsy cases or from patients receiving spironolactone. Spironolactone bodies are variable in size (from 2 to 25μ) and are composed of whorled highly packed membrane. They are continuous with the agranular or granular endoplasmic reticulum and occasionally contain one or more lipid cores in the central portion (Jenis and Hertzog, 1969; Devis and Medline, 1970; Kovacs et al., 1973; Cain et al., 1974). Janigan (1963) reported that variation in the number of the cytoplasmic bodies did not correlate with either total dosage or interval between the last dose of spironolactone and time of death. On the other hand, Conn and Hinerman (1977) suggested that spironolactone bodies appeared to be morphological expression of a block in aldosterone biosynthesis, because they were rapidly formed in the tumor cells during inhibition of aldosterone biosynthesis by administration of spironolactone. As this effect lessened or disappeared, these bodies diminished or disappeared. However, in this study we were unable to clarify the correlation between occurrence of the bodies and the duration or total dosage of spironolactone.

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