

“Non-functioning Adrenocortical Adenomas”
—Enzyme Histochemistry, Ultrastructure and Steroid Contents—

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SUMMARY

Quite rare enzyme histochemical and ultrastructural observations on non-functioning adenoma (NFA) are presented and discussed in detail together with the steroid contents since we obtained NFAs at surgery from two patients. Ultrastructurally, each adenoma was composed of 3 different types of cells; type 1, type 2 and type 3 cells, most of which in both cases showed weak activity of 3β -hydroxysteroid dehydrogenase (3β -HSD) but cells with strong activity were sporadically scattered throughout the adenomas. Some of the type 3 cells contained well-developed organelles resembling those in functioning adenoma (FA) though fewer in number, indicative of having an ability to produce corticosteroids although the amount would be less than that of FA. No essential qualitative difference lies between NFA and FA in cellular components or in organellar features except for a decrease in number of the cells with well-developed organelles in NFA. The mean values of corticosteroid contents were lower than those in FA.

Key words : Adenoma-Adrenal cortex diseases-Adrenal cortex neoplasma- 3β -Hydroxysteroid dehydrogenase

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INTRODUCTION

Most of non-functioning adrenocortical adenomas (NFA) have been found incidentally at autopsy and its frequency ranges from 1.45% up to 8.7%³⁾¹⁰⁾¹⁸⁾ reflecting diverse criteria upon which adrenocortical adenomas are defined.²⁾ There have been no detail reports on histochemical or ultrastructural observations on them or on the measurements of the steroid hormone contents except for two ultrastructural studies on NFA.¹⁷⁾²²⁾ Some of non-functioning adrenocortical tumors (NFT) were proven to produce biochemically inactive precursor steroids resulting in defects of the enzymic system in a certain course of the steroid biosynthesis.⁵⁾⁸⁾¹³⁾¹⁶⁾¹⁹⁾²⁵⁾ The majority of these reports, however, deal with non-functioning adrenocortical carcinoma but not adenoma (NFA).

Therefore, it would be quite interesting to investigate whether or not the cells of NFA may have any capability of producing biologically active steroid hormone resembling those of FA, and whether or not any essential morphological difference may exist between NFA and FA cytochemically and ultrastructurally.

The present enzyme histochemical and electron microscopic studies revealed morphological features of NFA. In addition, the contents of steroids in the NFA tissue were measured by radio-immunoassay. We discuss the hormonal activity in the NFA cells and the difference between NFA and FA in cellular components and ultrastructural findings.

Case Report (1)

A 54-year-old Japanese man had been complaining of general malaise and anorexia. He was suspected of chronic pancreatitis and diabetes mellitus (DM). Upper abdominal CT scanning revealed a tumorous mass of the right adrenal gland, which was confirmed to be an adenoma by right adrenal arteriography (Fig. 1). The adrenal function of the patient was within normal limit (Table. 1), and then the right adrenal gland was totally resected.

Case Report (2)

A 74-year-old Japanese woman had been suffering from lumbago while receiving therapy for cataracta and DM. Upper abdominal CT scans disclosed a tumor mass of the right adrenal gland, which was surgically removed. The adrenal function of the patient is shown in Table 1.



Fig. 1. Selective right adrenal arteriography demonstrates a round tumorous mass in the right adrenal gland of case 1.

Table 1. Steroids in Serum and Urine, and Plasma Electrolyte

	Serum Aldosterone (ng/dl)	Serum Cortisol (ng/dl)	17-OHCS in Urine (mg/day)	17-KS in Urine (mg/day)	Plasma Potassium (mEq/l)	Plasma Sodium (mEq/l)
Case 1	*59 (Not in serum)	8.3	4.6	5.2	4.1	143
Case 2	7	6.5-11.3	2.1-4.2	4.5-6.3	4.0	145
Normal	(2-12)	(4.9-14.7)	(1.9-6.1)	(3.1-8.8)		

*Measured in adrenal vein (Normal value : 20-100 ng/dl)

MATERIAL AND METHODS

The adrenal adenomas surgically removed from the two patients with non-hormonal activity were prepared for enzyme histochemical and electron microscopic examinations,

and the content of steroid hormone was measured. Frozen sections were cut by a cryostat for enzyme histochemical examination to investigate the activity of 3β -hydroxysteroid dehydrogenase (3β -HSD).¹²⁾ Then sliced tissues for electron microscopic examination were immediately minced in a solution of 2.5% glutaraldehyde in 0.1M phosphate buffer and postfixed in 1% osmium tetroxide. The tissues were dehydrated in a series of acetone, embedded, sectioned and stained by the routine manners. Small pieces of the adenomas for hormonal assay were preserved in a solution of cold acetone at -40°C until the analysis. The content of cortisol and aldosterone of the adenomas was measured by radioimmunoassay based on the method of FUKUCHI.⁶⁾⁷⁾ For comparative study, the following 10 cases of adrenal adenomas with hormonal activity were examined; 5 from Cushing's syndrome and 5 from primary aldosteronism.

RESULTS

Gross findings: The surgically removed right adrenal glands weighing 14 gm. in case 1 and 23.4 gm. in case 2, each combined with a tumor which was encapsulated and golden yellow on section, measuring $2.5 \times 2.0 \times 1.5\text{cm.}$ and $4.4 \times 3.5 \times 2.8\text{cm.}$ respectively (Fig. 2). There was no evidence of hemorrhage or necrosis but the adrenal cortices adjacent to the tumors were atrophied in both cases.

Microscopic findings:

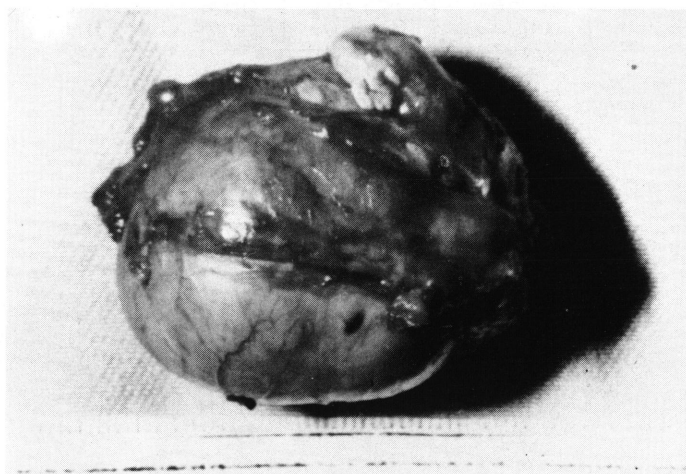


Fig. 2. The surgically removed right adrenal tumor of case 2 is encapsulated and weighs 23.4 gm. measuring $4.4 \times 3.5 \times 2.8\text{ cm.}$

Case 1-The adenoma was demarcated from the surrounding cortex by fibrous capsule or dilated vascular sinuses, and was predominantly composed of clear-type cells varying in size, with foamy, clear cytoplasm. Occasionally there were compact-type cells with cytoplasm which stained eosinophilic. These findings of the cells resembled those observed in Cushing-type adenoma.

Case 2-The adenoma was mainly composed of various sized clear-type cells with foamy, clear cytoplasm and in some areas intermediate-type cells were noted. The feature of the cell was similar to that of the adenoma in primary aldosteronism (PA adenoma).

Both of the two cases showed cells with broad and dense eosinophilic cytoplasm called "oncocyte" (oxyphilic cell) in the other organs, forming aggregations (Fig. 3). There was no evidence of malignant invasion in either cases.

Enzyme histochemical findings :

The activity of 3β -HSD was generally weak but areas in which strong activity was present were sporadically recognized throughout the adenomas in both cases (Fig. 4). As a notable finding in case 2, the nodular portion of the adenoma demonstrated strong activity of 3β -HSD.

Electron microscopic findings :

Each adenoma was composed of 3 different types of cells, i. e. type 1, type 2 and type 3. The type 1 cells (Fig. 5) showed broad cytoplasm filled with numerous, large and small lipid vacuoles, among which scanty organelles were observed. The lipid vacuoles ranged from $4\ \mu$ to $500\ m\mu$ in diameter and some of them were covered with membrane of smooth endoplasmic reticulum (SER) (Fig. 6), which occasionally displayed stacked arrays of ribosomes. Mutual fusion of the lipid vacuoles was noted. A small number of vesicular SER were scattered and rough-surfaced endoplasmic reticulum (RER) was poor in development. The Golgi apparatus was inconspicuous.

The type 2 cells (Fig. 7) contained a moderate amount of lipid vacuoles and relatively well-developed organelles. The development of SER and mitochondria varied considerably by cells; SER was well-developed and most prominent in the cells with few mitochondria, while the cells with poorly-developed SER revealed numerous mitochondria with tubular or tubulo-lamellar cristae (Fig. 8). Exceptionally, however, several cells possessed well-developed SER and many mitochondria. The Golgi apparatus was moderately developed in type 2 cells. Type 3 cells (Fig. 9) contained few lipid vacuoles or none of

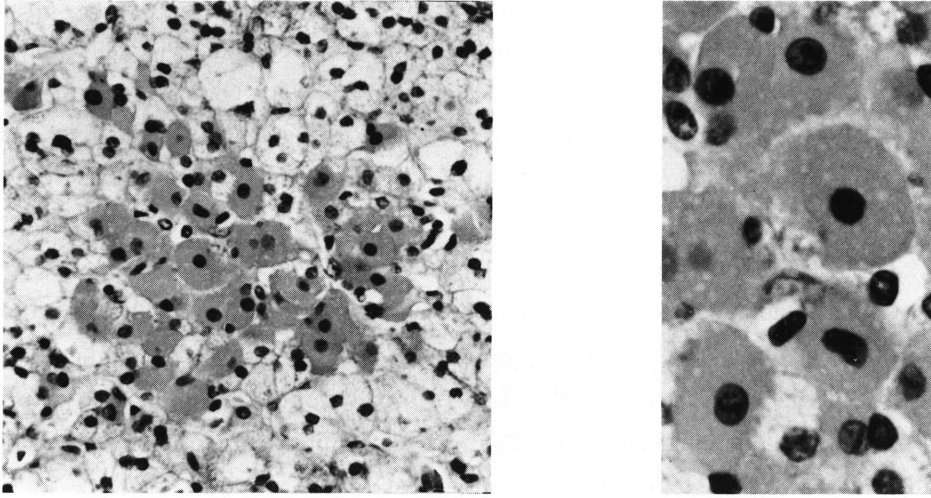


Fig. 3. a) Aggregation of cells with broad and densely stained cytoplasm (case 2) resembling "oncocyte" is observed. H & E, $\times 170$
b) Higher power view of "oncocyte" [Fig. 3 a)] . H & E, $\times 350$

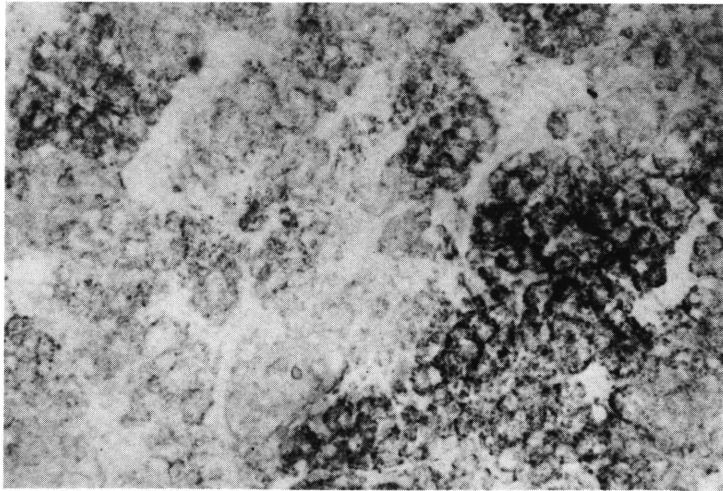


Fig. 4. The activity of 3β -HSD is generally weak but occasionally strong activity (case 1) is recognized scattered throughout the adenoma. $\times 170$

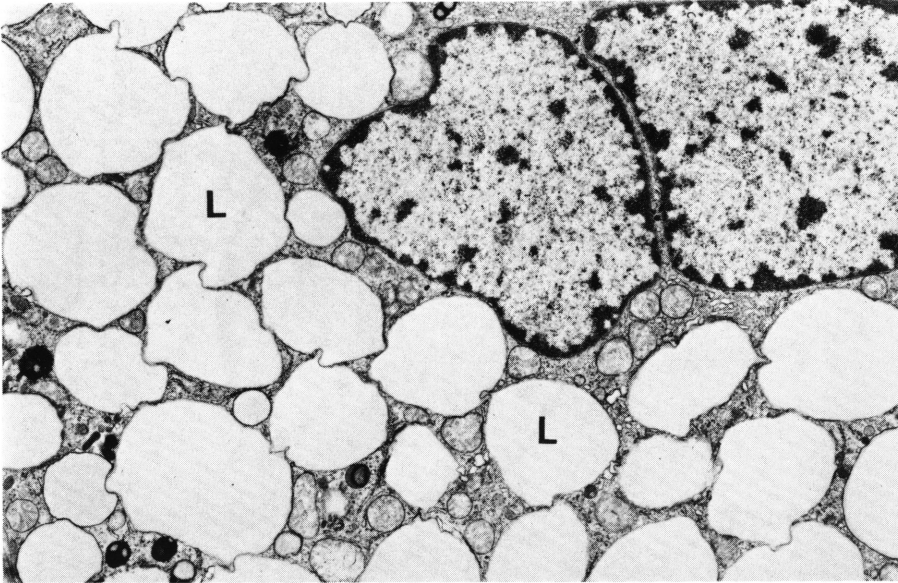


Fig. 5. The type 1 cells show broad cytoplasm with numerous lipid vacuoles (L) among which scanty organelles are scattered. $\times 8,100$

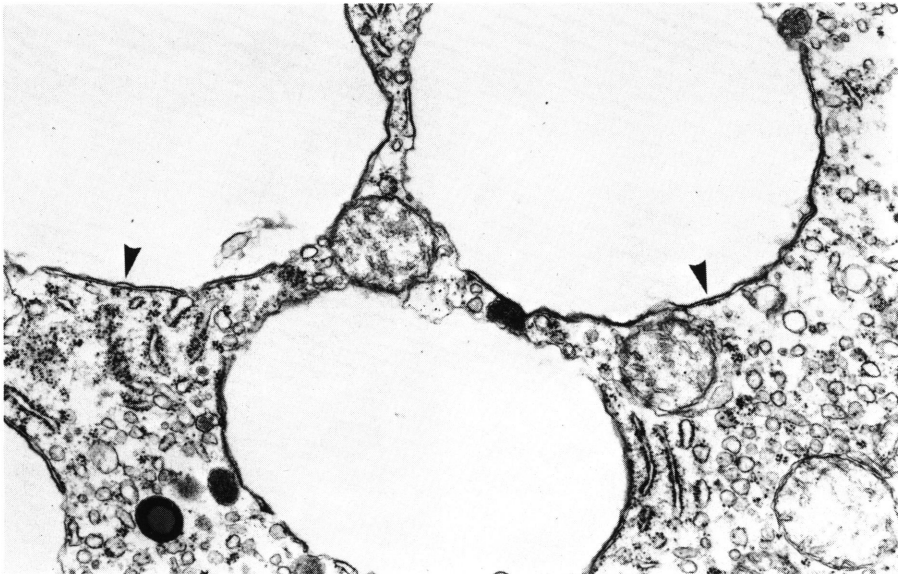


Fig. 6. Some of the lipid vacuoles in the type 1 cells are covered with the membrane of SER (Arrows). SER, RER and mitochondria are sparse. $\times 24,500$

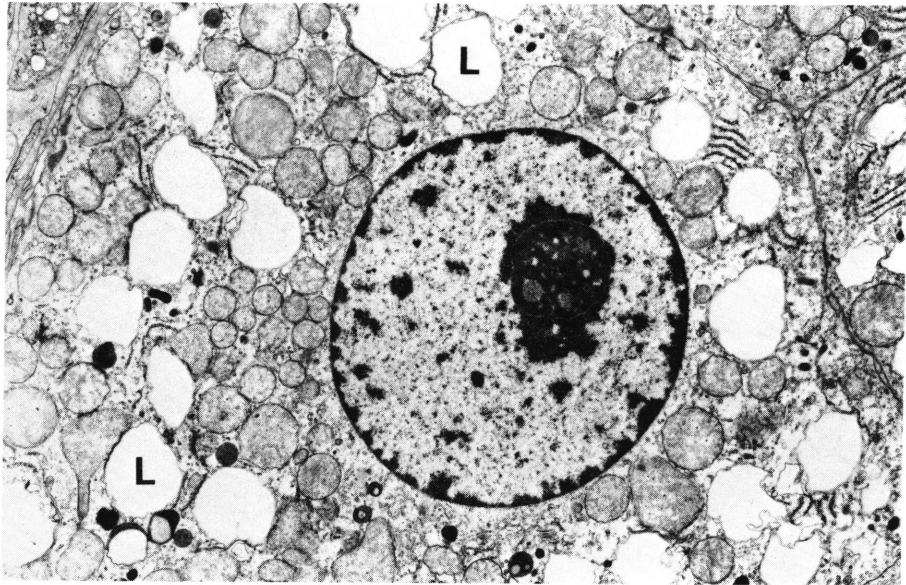


Fig. 7. The type 2 cells contain numerous mitochondria with tubular cristae and several lipid vacuoles (L). RER is not so prominent. $\times 8,100$

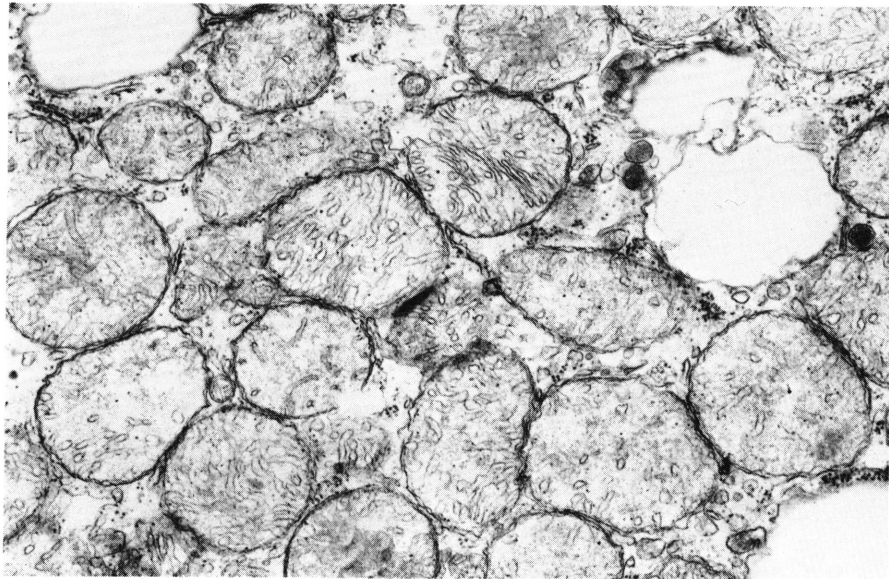


Fig. 8. Mitochondria with tubulo-lamellar cristae are observed in the type 2 cells. $\times 27,200$

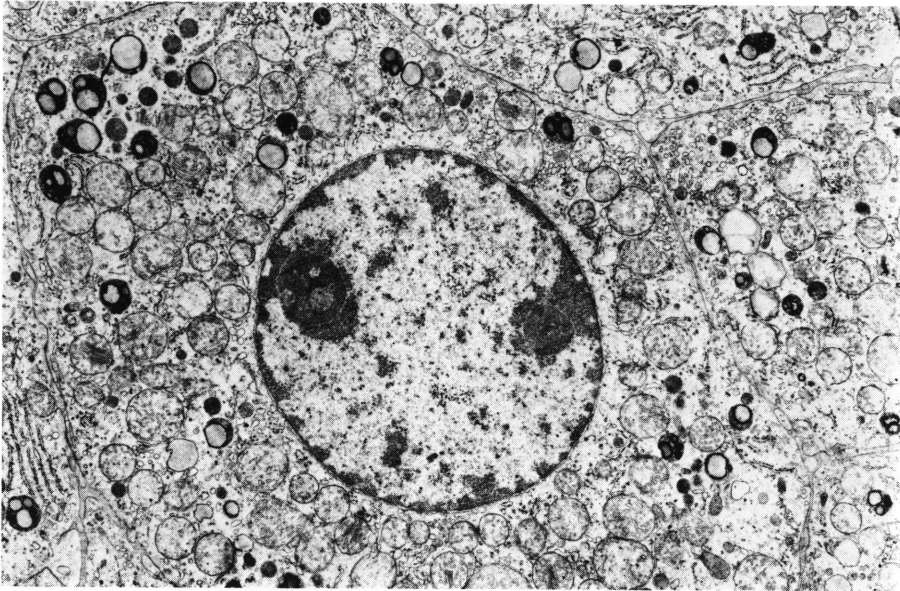


Fig. 9. The type 3 cells disclose no lipid vacuoles but numerous mitochondria and lysosomes are present. Vesicular SER is not so conspicuous. $\times 8,100$

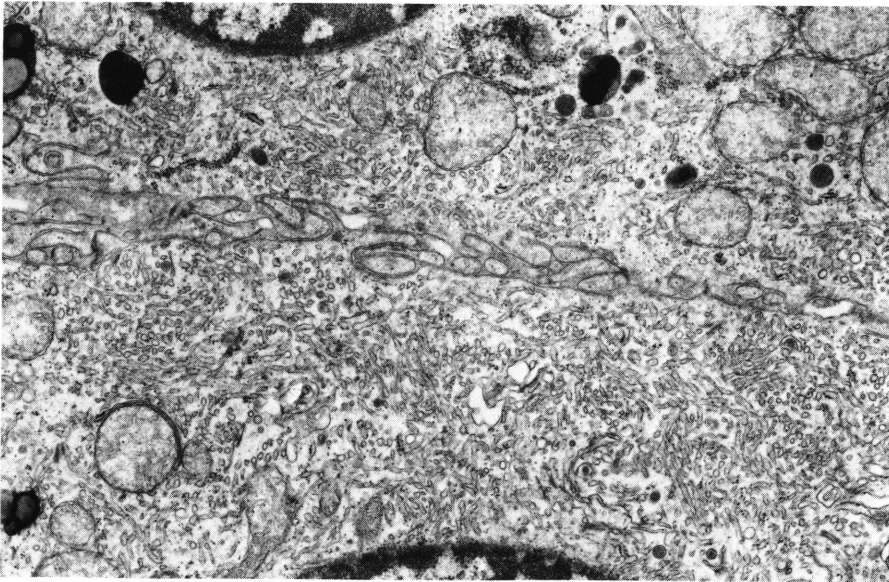


Fig. 10. Tubular SER is tightly packed like a network in the cytoplasm. $\times 12,300$

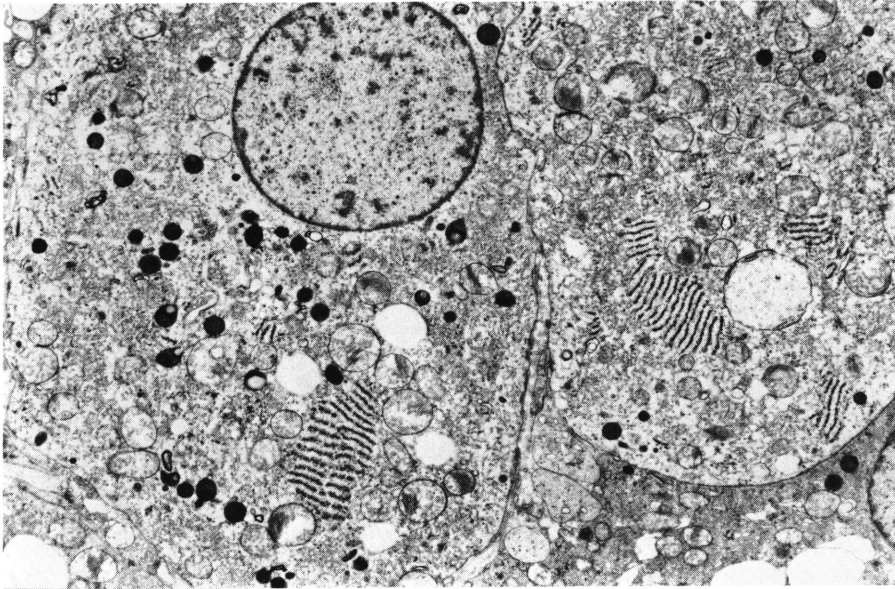


Fig. 11. Vesicular SER, RER and mitochondria are well-developed in the functioning adenoma cells of Cushing's syndrome. Lamellar arranged-RER is outstanding. $\times 6,800$

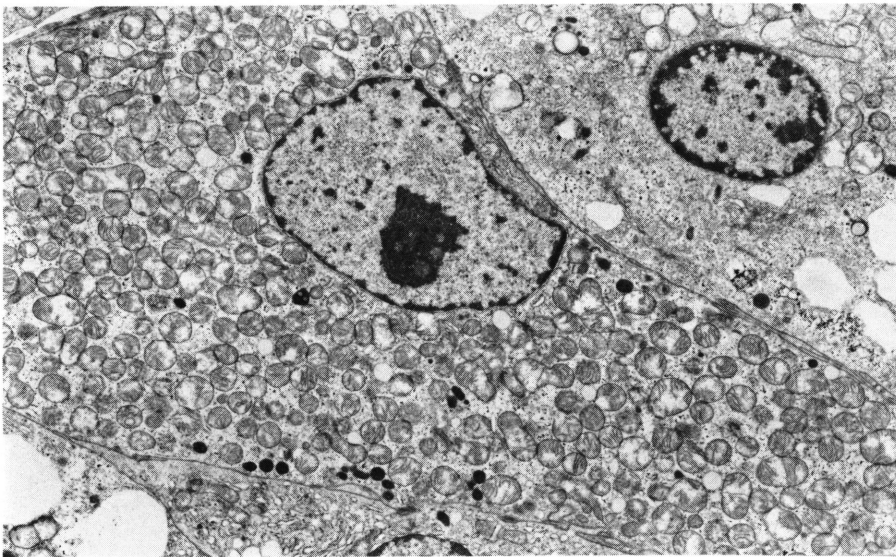


Fig. 12. The cytoplasm is occupied with numerous mitochondria which are oval to elongated with tubular to lamellar cristae. $\times 6,600$

them, but the development of both SER and RER was well with numerous mitochondria. SER was tubular and vesicular. The tubular SER was tightly packed like a network in the cytoplasm (Fig. 10). RER was prominent showing the several-layered lamellar structure with stacked-arrays by numerous ribosomes on each surface of the layers. Among them mitochondria were present varying in size with scanty cristae which were mainly tubular or tubulo-lamellar in shape but rarely tubulo-vesicular. The developmental ratio of organelles differed by cells: some cells had well-developed SER with tubular to vesicular structures and others had numerous mitochondria. Occasionally there were cells with well-developed SER and also with numerous mitochondria. Only a few cells demonstrated almost normally-balanced developmental ratio of SER, RER and mitochondria as seen in the cells of functioning adenoma (FA). Generally, the development of organelles was not so conspicuous in non-functioning adenoma (NFA) cells as in FA cells. Particularly, vesicular SER and lamellar arranged-RER were less than FA cells (Fig. 11). It is interesting that the cytoplasm of some cells were occupied with numerous mitochondria and glycogen particles scattered (Fig. 12). Mitochondria were oval to elongated with tubular to lamellar cristae. These cells corresponded with "oncocyte" (oxyphilic cell) in other organs.

Microvilli were well-developed on the surface of the cells as well as the Golgi apparatus was. Lipofuscin granules and dense bodies were numerous in the cells where the development of SER, RER and mitochondria was well-balanced.

The ratio of the three types of the cells composing NFA varied by cases. The case 1 adenoma consisted of type 1 and type 3 cells, and case 2 adenoma was composed of type 1 and 2 cells. In both cases a few cells in NFA showed marked development of organelles as noted in FA. Besides the three types of cells, zona-glomerulosa-like cells were observed in the case 1 adenoma.

Steroid contents in tissues :

Table 2. Corticosteroid Values of Adrenal Tissue

	Aldosterone	Cortisol
Case 1	0.13±0.01 ng/mg tissue	5.29±2.66 ng/mg tissue
Case 2	0.06±0.10 ng/mg tissue	3.05±0.59 ng/mg tissue
Cushing's Syndrome	0.07±0.03 ng/mg tissue	11.93±3.68 ng/mg tissue
Primary Aldosteronism	1.04±0.72 ng/mg tissue	3.18±1.39 ng/mg tissue

The measurements of the steroid hormones in the individual tissues were given in Table 2. The cortisol value of NFA was significantly lower in both cases than that in Cushing's syndrome whose mean value of cortisol was 11.9 ± 3.68 ng/mg tissue. As well the aldosterone value was lower than that of PA adenoma whose value of the aldosterone was 1.04 ± 0.72 ng/mg tissue.

DISCUSSION

Non-functioning adrenocortical tumors (NFT) including both adrenocortical adenoma and carcinoma demonstrate no clinical signs or symptoms due to endocrine dysfunction, neither do they show over production nor of biochemically active hormones. It is known, however, that some of them produce biochemically inactive precursor steroids,⁸⁾¹³⁾¹⁶⁾ and in such case the metabolites of pregnenolone increase in the urine, while enzyme defects involving Δ^5 -3 β -HSD, 17 α -hydroxylase and C₁₇₋₂₀ desmolase system were proven in some of NFT.⁵⁾⁸⁾¹³⁾¹⁶⁾ But these phenomenons have been recognized only in adrenocortical carcinoma not in adenoma. It remains questionable whether or not both adrenocortical carcinoma and adenoma should be categorized as one entity of NFT in spite of their morphological differences.

The fact that a few cells in the present two adenomas demonstrated a strong activity of 3 β -HSD indicates that the cells of NFA might have an enzyme capable of converting pregnenolone into progesterone. The tissues from the present two adenomas were confirmed to have produced cortisol and aldosterone. Furthermore, the cultured NFA cells of case 2 responded to exogenous ACTH and produced cortisol and aldosterone¹⁴⁾ as reflecting the definite presence of certain steroidogenetic activity in the NFA cells. TANNENBAUM²²⁾ noticed that SER and RER were very sparse in the cells of NFA and mitochondria were morphologically not identical to the features seen in the steroid-producing tissue.

Occasionally, the type 3 cells in the present cases showed marked development of organelles analogous to those of FA, but the development of vesicular SER and lamellar-arrayed RER were less prominent than that of FA. Since SER and mitochondria are known as the important sites in which the enzymes localize to synthesize steroid hormones, significant development of SER and mitochondria indicates the hyperfunction of the cellular steroid synthesis. The type 3 cells corresponded to compact-type cells in the zona reticularis by a light microscope,¹⁵⁾²³⁾²⁴⁾ and the steroid synthesis appeared active though the

enzymes and organelles observed were fewer than in FA cells. The type 1 cells corresponding to clear-type cells⁵⁾²³⁾²⁴⁾ were abundant in lipid vacuoles showing poorly-developed SER and mitochondria, which might be deeply responsible for the storage of steroid precursor hormones. Some of the type 1 cells possessed double membrane-bound lipid vacuoles surrounded by SER. Mitochondria scattered sparsely among the lipid vacuoles revealed tubular to tubulo-lamellar cristae. These findings are included in those aldosteronoma.¹⁷⁾ The presence of clear-type cells in NFA accounts for the enzymic defects,⁵⁾ a deficiency of C-20=22 desmolase system prevented the release of cholesterol which accumulated in the cytoplasm and thus, the tumor cells gradually became clear-type cells.⁵⁾¹³⁾

The type 2 cells corresponding to intermediate-type cells over light microscopy²³⁾²⁴⁾ were suspected of having an ability of steroidogenesis to some degree judging from both the SER and mitochondrial development. Therefore, it is considered that the type 2 cells may have the capability of producing an insufficient amount of corticosteroid for demonstrating clinical signs or symptoms.¹⁾²⁵⁾ No essential qualitative difference is found between NFA and FA in cellular components or in organellar features. On the other hand, it can be assumed that NFA cells may have an ability of transform themselves into FA cells in the long course if a certain effective stimulator would be given such as ACTH¹⁴⁾ or any other factor²⁰⁾ until the area producing the hormone extends wider.

Small adenomas of the adrenal cortex have been found more frequently in the patients with DM than in the normals.¹⁰⁾¹⁸⁾ Since such adenoma increases in incidence along with the age as well as in the patients with hypertension,¹⁰⁾¹⁸⁾ the cause of the adenoma in such case may be related to arteriopathy.²¹⁾

It is quite interesting that the present two cases had suffered from DM and showed a large adrenal cortical adenoma each. Ultrastructural and quantitative findings suggest that the adrenocortical adenoma cells of the patients with DM may not have sufficient hormonal activity to demonstrate typical symptoms or signs of hypercorticalism like those with Cushing's syndrome have. Consequently, further studies would be necessary to make the above suggestion fruitful.

As far as we have investigated, the oncocytes in the adrenal cortex was first reported by KARSNER,¹¹⁾ but the present study confirmed those oncocytes in the adrenocortical tumor for the first time over an electron microscope, although the oncocytes are known to occur in several organs.⁹⁾ In spite that any significance or histogenesis of the oncocytes has not been clarified, it would be of great interest to study forward on the presence of the oncocytes in the adrenal gland.

REFERENCES

- 1) BERTAGNA C, Orth DN (1981) Clinical and laboratory findings and results of therapy in 58 patients with adrenocortical tumors admitted to a single medical center (1951 to 1978). *Am J Med* 71 : 855-875.
- 2) COHEN RB (1969) Observations on cortical nodules in human adrenal glands: Their relationship to neoplasm. *Cancer* 19 : 552-556.
- 3) COMMONS RR, CALLAWAY CP (1948) Adenomas of the adrenal cortex. *Arch Int Med* 81 : 38-41.
- 4) DLUHY RG, BARLOW JJ, MAHONEY EM, SHIRLEY RL, WILLIAMS GH (1971) Profile and possible origin of an adrenocortical carcinoma. *J Clin Endocrinol* 33 : 312-317.
- 5) FANTL V, BOOTH M, GRAY CH (1973) Urinary pregn-5-en-3 α , 16 α , 20 α -triol in adrenal dysfunction. *J Endocrinol* 57 : 135-142.
- 6) FUKUCHI S (1961) Contents of adrenocortical hormones in adrenal tissue in various endocrine disorders. *Tohoku J Exper Med* 76 : 365-373.
- 7) FUKUCHI S, NAKAJIMA K, SASANO N, NAKAMURA K (1978) Microdetermination of corticosteroids in adrenocortical zones in various adrenal tissues. *Folia Endocrinol Jap* 54 : 898-908 (In Japanese with English abstract).
- 8) FUKUSHIMA DK, GALLAGHER TF (1963) Steroid production in "non-functioning" adrenal cortical tumor. *J Clin Endocrinol Metab* 23 : 923-927.
- 9) HAMPERL H (1962) Benign and malignant oncocytoma. *Cancer* 15 : 1019-1027.
- 10) HEDELAND H, ÖSTBERG G, HÖKFELT B (1968) On the prevalence of adrenocortical adenomas in an autopsy material in relation to hypertension and diabetes. *Acta Med Scand* 184 : 211-214.
- 11) KARSNER HT (1950) Tumors of the adrenal. Atlas of Tumor Pathology. Sect. 8 fasc 29, Armed Forces Institute of Pathology p 16, Washington, D. C.
- 12) LEVY H, DEANE HW, RUBIN BL (1959) Visualization of steroid 3 β -O1-dehydrogenase Activity in Tissues of Intact and Hypophysectomized Rats. *Endocrinol* 65 : 932-943.
- 13) LEWINSKY BS, GRIGOR KM, SYMINGTON T, NEVILLE AM (1974) The clinical and pathologic features of "nonhormonal" adrenocortical tumors. *Cancer* 33 : 778-790.
- 14) MATSUO K, SHIGEMATSU K, IRIE J, KAWAI K, TSUCHIYAMA H (1984) Morphological and quantitative pathological observations of non-functioning adrenocortical adenoma in cell culture. *Tr Soc Pathol Jpn Liber* LXXIII.
- 15) NEVILLE AM, SYMINGTON T (1966) Pathology of primary aldosteronism. *Cancer* 19 : 1854-1868.
- 16) NEVILLE AM, O'HARE MF (1982) 'Non-functioning (Non-hormonal)' adrenocortical tumors. The human adrenal cortex. Springer-Verlag Berlin Heiderberg New York pss. 242-247.
- 17) REIDBORD H, FISHER ER (1969) Aldosteronoma and nonfunctioning adrenal cortical adenoma. *Arch Pathol* 88 : 155-161.
- 18) RUSSI CS, BLUMENTHAL HT, GRAY SH (1945) Small adenomas of the adrenal cortex in hypertension and diabetes. *Arch Int Med* 76 : 284-291.
- 19) SASANO N, OJIMA M, MASUDA T (1980) Endocrinologic pathology of functioning adrenocortical tumors. *Path Annual Part 2* 15 : 105-141.

- 20) SHIGEMATSU K (1982) Comparative studies between hormone contents and morphological appearances in human adrenal cortex. *Acta Histochem et Cytochem* 15 : 386-400.
- 21) SYMINGTON (1969) Functional pathology of the human adrenal gland. E & S. Livingstone, Edinburgh and London.
- 22) TANNENBAUM M (1973) Ultrastructural pathology of the adrenal cortex. *Path Annual* 8 : 109-156.
- 23) TSUCHIYAMA H (1967) Pathological findings on corticosteroids producing tumor (In Japanese) *Saishin Igaku* 22 : 2433-2441.
- 24) TSUCHIYAMA H, MUTO Y (1970) Pathological study of adrenocortical adenoma and hyperplasia. *Nishinihon Hinyōki Gakkai Zasshi* 32 : 8-15.
- 25) WIELAND RG (1982) Adrenocortical adenomas: Nonfunctioning? *Am J Med* 72 : 829-830