ACTA HISTOCHEM. CYTOCHEM. Vol. 15, No. 3, 1982

COMPARATIVE STUDIES BETWEEN HORMONE CONTENTS AND MORPHOLOGICAL APPEARANCES IN HUMAN ADRENAL CORTEX

—SPECIAL REFERENCE TO NON-FUNCTIONING TUMORS (ADENOMA AND ADENOMATOUS NODULE) AND FUNCTIONING ADENOMA—

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Received for publication September 28, 1981 and in revised form November 20, 1981

The hormone content in Cushing-type non-functioning tumors of the adrenal glands was measured to be compared with those of functioning adenomas. Furthermore, the relationship between the hormone contents and the proportion of clear-type cells and compact-type cells in the tissues were also studied.

The contents of aldosterone and corticosterone were significantly higher in primary aldosteronism than in other groups. The cortisol content was significantly higher in Cushing's syndrome than in primary aldosteronism, but it was not statistically different from non-functioning adenomatous nodules. The patterns of hormone contents in non-functioning adenomatous nodules were similar to those in Cushing's syndrome.

In primary aldosteronism, there was a positive correlation between the aldosterone content and the increase of compact-type cells. Compact-type cells revealed high activities of 3β-hydroxysteroid dehydrogenase and glucose 6-phosphate dehydrogenase. These results suggest that compact-type cells play an important role in synthesis and secretion of aldosterone in primary aldosteronism.

In Cushing's syndrome, there was no significant correlation between the cortisol content and the proportion of constituting cells. The activities of 3βHSD and G6PD in both types of cells were not so different from each other. Therefore, there may be a possibility that morphological differences in the two types of constituting cells show different phases of tumor cells in Cushing's syndrome.

In the non-functioning tumors, the scattered tumor cells revealed marked activities of 3βHSD and G6PD. These results suggest that the number of constituting cells of "non-functioning" tumors have some degree of endocrinologic activity.

Tumors originating from the adrenal cortex are clinically classified as "functioning" or "non-functioning". Morphological and hormonal studies on the functioning tumors have been performed in primary aldosteronism, Cushing's syndrome, and in adrenogenital syndrome. On the contrary, non-functioning tumors originating

from the adrenal cortex, especially benign tumors, have not been fully studied yet. This is because they are mostly silent as far as clinical signs are concerned and are difficult to obtain as operation materials. According to Commons (3) and Russi (14), non-functioning tumors are found fairly frequently at autopsy and this ratio is generally considered to be 1–2% of all autopsy cases. The word "non-functioning" is a clinical criteria, and it has been shown that such silent tumors have little hormone producing capacity according to Kaplan (9) and Harada (7). In the present study, the hormone contents were measured in non-functioning adenomas and adenomatous nodules and were compared with those in primary aldosteronism and Cushing's syndrome.

Adrenocortical tumors are composed of clear-type cells with large amount of lipid droplets and compact-type cells with a few or no fine lipid granules (20, 21). Symington *et al.* (17, 18, 19) and Tsuchiyama (20, 21, 23) have suggested that clear-type cells play a role in the storage of steroid precursors and that compact-type cells

TABLE 1. *Cases of non-functioning tumor, primary aldosteronism and Cushing's syndrome*

Non-functioning tumor

Case				
No	Age	Sex	Site	Major diagnosis at autopsy or clinical diagnosis
1	72	M	left	Cholangiocarcinoma
2	56	F	both	Cerebral infarction
3	80	F	both	Carcinoma of pancreas
4	54	M	right	Non-functioning adrenocortical tumor

Primary aldosteronism

Case			Size (cm)	Plasma	
No	Age	Sex	Site	Aldosterone (ng/dl)	Renin activity (ng/dl/hr)
5	57	M	2.0×1.8×1.5 right	90-104	0.37-0.40
6	56	F	1.8×1.7×1.1 right	150	0.35

Cushing's syndrome

Case			Size (cm)	Plasma	Urine	
No	Age	Sex	Site	Cortisol (μg/dl)	170HCS	17KS (mg/day)
7	39	F	3.5×2.8×2.0 left	20.3-24.3	22.2	13.1
8	18	F	3.7×2.7×1.6 left	16.9-31.2	4.8-18.4	3-9
9	28	F	2.5×2.5×3 right	27.0-34.0	14.9-17.2	4.6-11.9
10	52	F	2.5×2.3×1.8 right	20.5-25.9	8.6-11.2	4.9-6.3
11	41	F	4×2.5×1.5 left	15.6-20.6	11.2-18.0	5.6-7.7

play a role in active synthesis and secretion of steroids. However, Harada (7) has indicated that clear-type cells store not only the steroid precursors, but also the final products in primary aldosteronism. In the present study dealing mainly with non-functioning tumors, an attempt was made to study the relationship between the hormone contents and the morphological proportion of constituting cells, which were characterized with enzyme histochemical and electron microscopic observations.

MATERIALS AND METHODS

Three cases of non-functioning adenomatous nodule obtained at autopsy, a case of non-functioning adenoma resected surgically, 2 cases of adenoma in primary aldosteronism and 5 cases of adenoma in Cushing's syndrome were examined. The clinical data of these cases are shown in Table 1. All adrenal tissues were cooled in the jar immediately after resection or at autopsy. A part was prepared for histological examination and another part of specimens of surgical cases was prepared for enzyme histochemical and electron microscopic sections. As illustrated in Fig. 1, the quantitative samples were collected by the modified method of Harada (7).

Adrenal tissue was cut into 10 μm-thick sections in a cryostat, and the proportion of clear-type cells and compact-type cells (Fig. 2) was calculated after stained with oil red O. Then, 10–30 μm-thick sections were made for the quantitative samples. After weighing, the quantitative samples were each preserved in a vial with cold acetone at −40°C until hormonal analysis could be done. Moreover, when a sufficient amount of adrenal tissue was cut off until the proportion of constituting cells changed, the quantitative samples were collected again. In this way, 7–15 quantitative samples were obtained from each one case.

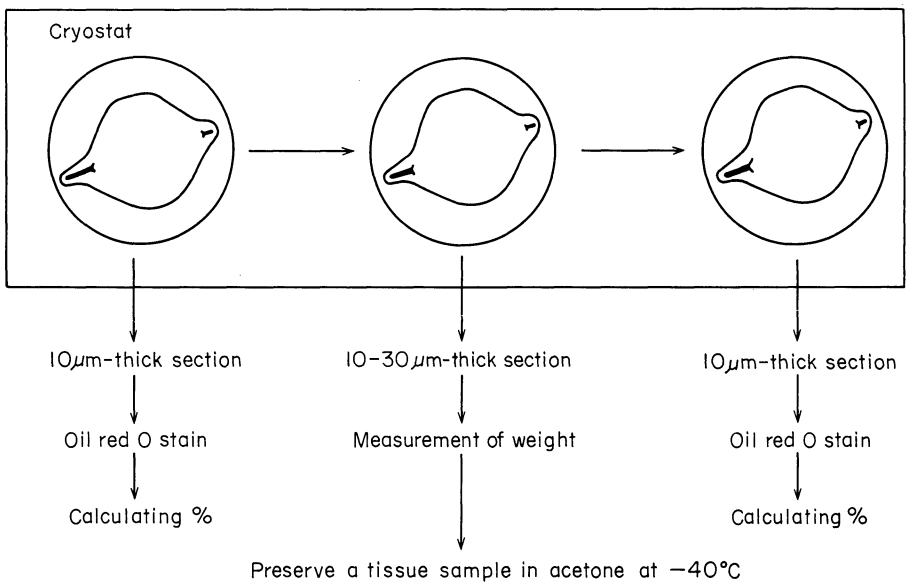

FIG. 1. Procedure for preparation of histological section and quantitative sample.

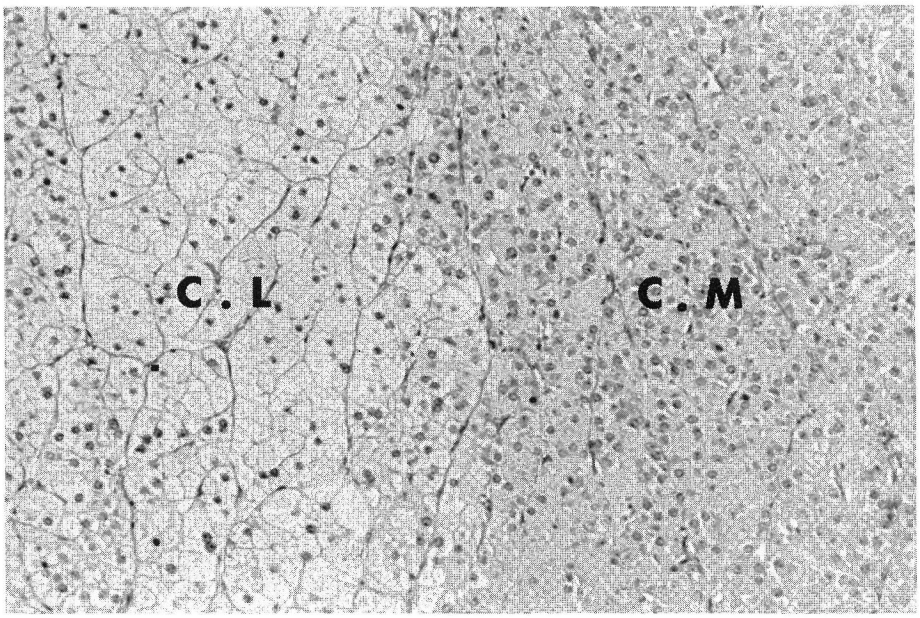

FIG. 2. The histologic appearance of adrenocortical nodule. C. L, Clear-type cells; C, M, Compact-type cells.

The contents of aldosterone, corticosterone and cortisol in adrenal tissues were measured by radioimmunoassay, based on the method of Fukuchi *et al.* (5, 6). The details of this method have been reported in the paper of Harada (7). Anti-aldosterone, -corticosterone and -cortisol-3-BSA antiserum were obtained from Japan Balcon Inc. and were stored at −70°C. Before use, they were diluted with pH 8.0, 0.05 M borate buffer, containing 0.1% gamma-venin (Hoechst Japan Co.) and methanol for optimum sensitivity to 1 : 50,000, 1 : 20,000 and 1 : 50,000, respectively. The standard and labeled corticosteroids were obtained from Sigma Co. and New England Nuclear Co.

Both histochemical activities of 3β-hydroxysteroid dehydrogenase (3βHSD) converting pregnenolone to progesterone and glucose-6-phosphate dehydrogenase (G6PD) which is considered to be an indirect parameter of the steroid synthesizing activity were examined.

Furthermore, the maximum cut surface of adrenal nodules was observed with hematoxylin-eosin and Mallory-Azan stains, and the area of round or oval nuclei which had prominent nucleoli and distinct intranuclear structure was calculated. The proportion of constituting cells and the area of nuclei were measured with Leitz's image analysis system.

RESULTS

1. MORPHOLOGICAL FINDINGS

Non-functioning adenomatous nodules continued from the surrounding adrenal cortex and were composed of clear-type cells with a large amount of lipid droplets

stained with oil red O, and compact-type cells with a few or no fine lipid granules. The adrenal cortex of the non-nodular portion was not atrophic and Cases 3 and 4 showed a cortical hyperplasia with multiple nodules. Case 4 had an adenoma sized $2.5 \times 2.0 \times 1.8$ cm with a focal fibrous capsule.

Two cases of primary aldosteronism were accompanied by adrenal adenomas. Those adenomas were lobulated into large or small alveoli by fibrovascular septa. The major part of the adenoma was occupied by clear-type cells with wide cytoplasm, and the small groups of compact-type cells were scattered around fibrovascular septa. Large or hyperchromatic nuclei were occasionally found, and some cells with a fusing appearance were also seen.

All cases of Cushing's syndrome had adrenal adenomas sized larger than 2.5 cm. The adenomas were composed of clear-type cells and compact-type cells, and their proportion was almost equal. A few compact-type cells had large nuclei. Massive infiltration of mononuclear cells was found focally.

The results of measurement of the area of nuclei in each group is shown in Table 2. There was no statistically significant difference between non-functioning adenoma or adenomatous nodules and adenoma in Cushing's syndrome. And in those groups, there was no statistically significant difference between clear-type cells and compact-type cells. The nuclei revealed a uniform size in each group, but a few compact-type cells were more than 100 μm^2. On the contrary, in primary aldosteronism, both clear-type cells and compact-type cells had large nuclei, with a

TABLE 2. *The area of nuclei*

	Nonfunctioning tumor		Primary aldosteronism		Cushing's syndrome	
μm^2	Clear	Compact	Clear	Compact	Clear	Compact
0-10						
10-20		3(0.62)	8(4.57)	8(4.12)	2(0.89)	3(0.45)
20-30	49(21.03)	163(33.82)	47(26.86)	33(17.01)	128(56.89)	253(38.28)
30-40	144(61.80)	218(45.23)	42(24.00)	42(26.65)	80(35.56)	308(46.60)
40-50	30(12.88)	68(14.11)	30(17.14)	26(13.40)	10(4.44)	84(12.71)
50-60	8(3.43)	13(2.70)	17(9.71)	17(8.76)	5(2.22)	8(1.21)
60-70	2(0.86)	11(2.82)	7(4.00)	7(3.61)		4(0.61)
70-80		3(0.62)	5(2.86)	9(4.64)		
80-90		1(0.21)	4(2.29)	2(1.03)		
90-100			6(3.43)	7(3.61)		1(0.15)
100-150		2(0.41)	5(2.86)	17(8.76)		1(0.15)
150-200			1(0.57)	13(6.70)		
200-250			1(0.57)	7(3.61)		
250-300			1 (0.57)	1(0.52)		
300-350				1(0.52)		
350-400				2(1.03)		
400-450			1(0.57)			
450-500				2(1.03)		
M±SD	35.5±7.07	34.92±1.094	48.73±45.07	75.18±75.88	30.30±6.41	33.13±7.92

( ): percentage M±SD (μm^2)

TABLE 3. *Corticosteroid contents in adrenal tissue*

Case \ Corticosteroid	Aldosterone	Cortiosterone	Cortisol
Non-functioning adenomatous nodule (4 cases)	0.05±0.01 (28)	1.89±0.21 (23)	8.55±3.86 (27) $P_1<0.02$
Primary aldosteronism (2 cases)	1.04±0.72 (12) $P_1<0.01$ $P_3<0.001$	3.18±2.12 (6) $P_1<0.001$ $P_3<0.001$	3.18±1.39 (12)
Cushing's syndrome (5 cases)	0.07±0.03 (45) $P_2<0.05$	1.23±0.36 (36) $P_2<0.01$	11.93±3.68 (42) $P_3<0.01$

Mean±SE (ng/mg tissue)

Significant by t-test
P_1: Non-functioning adenomatous nodule Vs. Primary aldosteronism
P_2: Non-functioning adenomatous nodule Vs. Cushing's syndrome
P_3: Primary aldosteronism Vs. Cushing's syndrome
( ): Number of sample

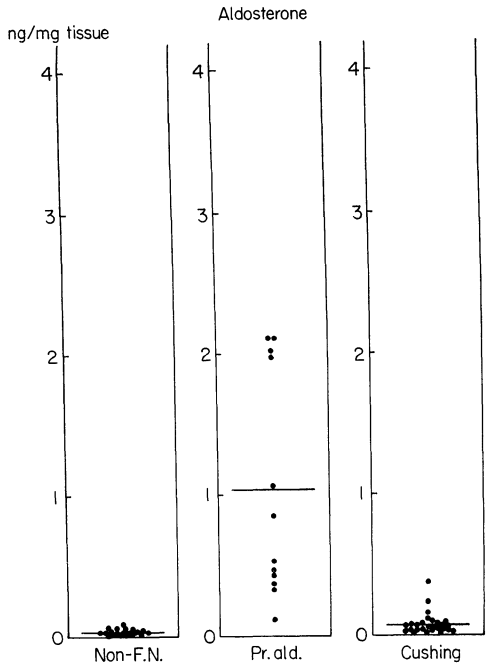

FIG. 3. Aldosterone contents (ng/mg tissue) in adrenal tissues from patients with non-functioning adenomatous nodule (Non-F. N.), primary aldosteronism (Pr. ald.) and Cushing's syndrome (Cushing). Horizontal bars indicate mean value.

mean value of 48.73 μm^2 and 75.18 μm^2, respectively, and it was significantly larger in compact-type cells ($P<0.01$) than in clear-type cells; especially nuclei more than 100 μm^2 were prominently observed in compact-type cells.

II. TISSUE CORTICOSTEROID CONTENTS

The hormone contents in adrenal tissues of each group are shown in Table 3. The contents of aldosterone and corticosterone were significantly higher in primary aldosteronism ($P_1<0.01$. $P_3<0.001$) than in other groups, with a mean value of 1.4 ± 0.72 ng/mg tissue and 3.18 ± 2.12 ng/mg tissue, respectively. The cortisol content was significantly higher in Cushing's syndrome ($P_3<0.01$) than in primary aldosteronism, with a mean value of 11.93 ± 3.68 ng/mg tissue, but there was no statistical significance between non-functioning adenomatous nodules and adenoma in Cushing's syndrome, though the cortisol content in the latter showed a tendency to higher value than in the former. The aldosterone content in the non-tumorous portion of primary aldosteronism had a mean value of 0.03 ng/mg tissue and was significantly lower than in adenoma ($P<0.001$). The mean cortisol content in non-functioning adenoma of Case 4 was 5.29 ng/mg tissue and significantly lower than in non-functioning adenomatous nodules ($P<0.01$) and adenoma in Cushing's syndrome ($P<0.001$).

Figs. 3, 4 and 5 indicate the individual values for aldosterone, corticosterone and cortisol. Wide variations were found within aldosterone in primary aldo-

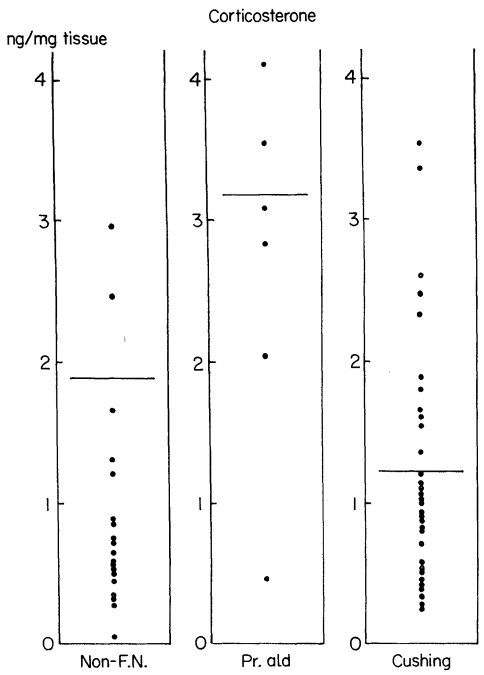

FIG. 4. Corticosterone contents (ng/mg tissue) in adrenal tissues from patients with non-functioning adenomatous nodule (Non-F. N.), primary aldosteronism (Pr. ald.) and Cushing's syndrome (Cushing). Horizontal bars indicate mean value.

steronism, corticosterone in all groups, and cortisol in non-functioning adenomatous nodules and adenoma in Cushing's syndrome. The cortisol content varied from 1.88 ng/mg tissue to 38.10 ng/mg tissue in adenoma of Cushing's syndrome and from 0.78 ng/mg tissue to 20.87 ng/mg tissue in non-functioning adenomatous nodules. Particularly, the mean cortisol content in the adenomatous portion of Cases 3 and 4 was 11.15 ng/mg tissue and 9.17 ng/mg tissue, respectively, and it was the same value as that in Cushing's syndrome.

Fig. 6 indicates the percentage of hormone content in each group. The hormone content in non-functioning adenomatous nodules was similar to those in Cushing's syndrome.

In the groups of primary aldosteronism and Cushing's syndrome, there was no correlation among tumor size, hormonal level in plasma and the tissue corticosteroid contents.

III. COMPARISON BETWEEN CORTICOSTEROID CONTENTS AND MORPHOLOGICAL APPEARANCE

Either clear-type cells or compact-type cells of adrenal nodules were chiefly distinguished with oil red O stain, and the proportion of the constituting cells was calculated compared with the hormone contents. The significant correlation between the hormone content and the proportion of constituting cells is shown in Figs. 7, 8 and 9. The abscissa indicates the percentage of clear-type cells and the longitudinal axis indicates each hormone content. There was a positive correlation

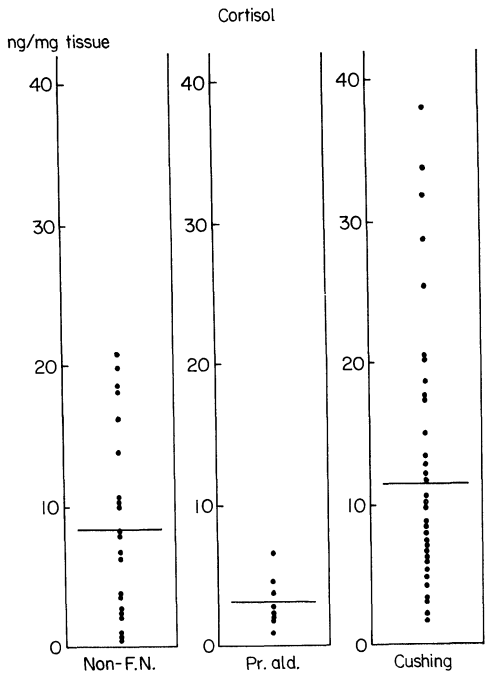

FIG. 5. Cortisol contents (ng/mg tissue) in adrenal tissues from non-functioning adenomatous nodule (Non-F. N.), primary aldosteronism (Pr. ald.) and Cushing's syndrome. Horizontal bars indicate mean value.

between the cortisol content and the increase of clear-type cells in non-functioning adenomatous nodules. In primary aldosteronism, there was a negative correlation between the aldosterone content and the increase of clear-type cells; in other words, there was a positive correlation between the aldosterone content and the increase of compact-type cells. However, there was no obvious correlation between the corticosterone content and the proportion of constituting cells. In Cushing's syndrome, there was a negative correlation between the corticosterone content and the increase of clear-type cells, but there was no obvious correlation between the cortisol content and the proportion of constituting cells.

IV. ENZYME HISTOCHEMICAL FINDINGS

In non-functioning adenoma of Case 4, the activities of 3βHSD and G6PD were generally weak but strong activity in a few compact-type cells was scattered throughout the tumor.

In primary aldosteronism, the activity of 3βHSD was slightly positive in clear-type cells and sometimes they showed negative, particularly in clear-type cells which had many lipid droplets. On the contrary, compact-type cells showed higher than moderate enzyme activity. The activity of G6PD revealed almost the same tendency and compact-type cells showed higher than moderate enzyme activity.

In Cushing's syndrome, the activity of 3βHSD was weak to moderate in clear-type cells and moderate to strong in compact-type cells. In the activity of G6PD,

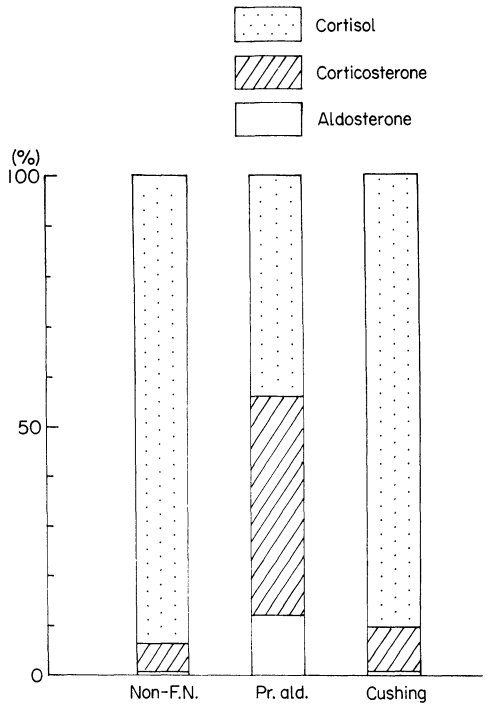

FIG. 6. The percentage of the hormone contents in non-functioning adenomatous nodule (Non-F. N.), primary aldosteronism (Pr. ald.) and Cushing's syndrome (Cushing).

compact-type cells showed higher than moderate and clear-type cells showed weak to moderate activity.

V. ELECTRON MICROSCOPIC FINDINGS

Adenoma of Case 4 contained a few cells with marked development of organellae the same as those in adenomas in Cushing's syndrome described later, but the number of these cells was less than that of Cushing's syndrome.

In primary aldosteronism, clear-type cells had a broad cytoplasm with a large amount of fat vacuoles occasionally surrounded with the membrane of endoplasmic reticulum. In most of them, a few organellae were scattered among these vacuoles. Compact-type cells had a few small vacuoles or no vacuole. The amount of smooth endoplasmic reticulum and large or small mitochondria with parallel lamellar cristae were found. The nuclei were large.

In Cushing's syndrome, clear-type cells had numerous vacuoles. The organellae were scant to rather numerous. Compact-type cells showed marked development of organellae and had many dense bodies and lysosomes. Differing from primary aldosteronism, mitochondria with tubular or vesicular cristae were abundant and the character of mitochondria in clear-type cells was also the same.

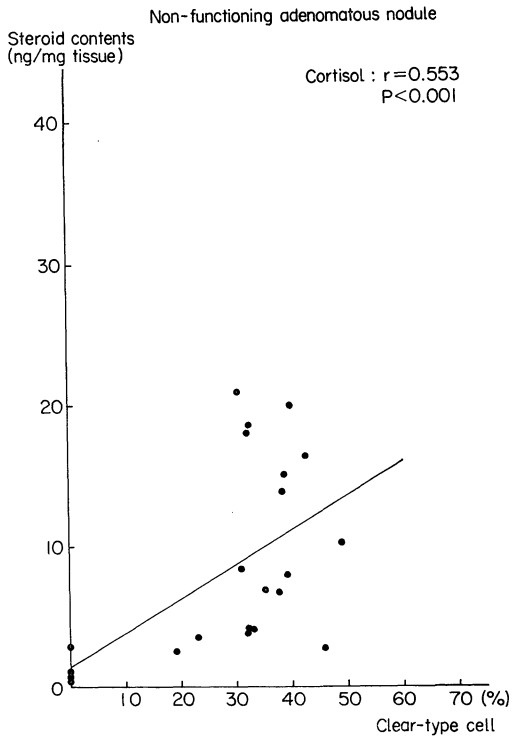

FIG. 7. Relationship between the cortisol content and clear-type cells in non-functioning adenomatous nodule ($P<0.01$).

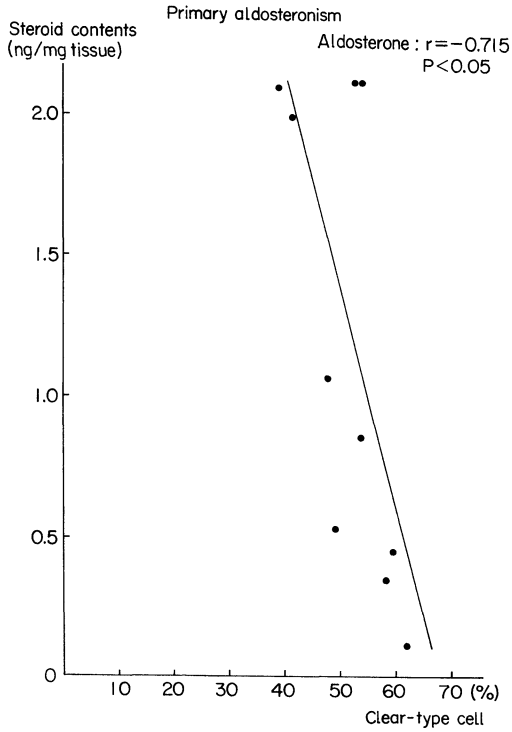

FIG. 8. Relationship between the aldosterone content and clear-type cells in primary aldosteronism ($P<0.05$).

DISCUSSION

The word "non-functioning" is a clinical criterion and does not refer to hormonal activity of natural adrenocortical nodules. Kaplan's (9) study on non-functioning nodules has been reported and the measurement of hormone content in adrenal tissues has also been reported by Harada (7). According to these reports, the hormone content in the non-functioning tumors generally revealed low values. However, in Harada's report, a case of non-functioning nodules with the same cortisol content as an adenoma in Cushing's syndrome was also found.

Concerning the hormone contents, the aldosterone and corticosterone were significantly high in primary aldosteronism and the cortisol content was high in Cushing's syndrome, as previously reported by many investigators (1, 4–7, 9, 10, 13, 16). In non-functioning adenomatous nodules, the aldosterone and corticosterone were significantly lower than those in primary aldosteronism. On the contrary, the cortisol content was significantly higher than that in primary aldosteronism. The cortisol content was also shown not to have a statistically significant difference between non-functioning adenomatous nodules and adenomas in Cushing's syndrome, though the former was lower than the latter. Furthermore, Cases 3 and 4 contained the same value of cortisol as Cushing's syndrome. A few cells in Case 4, especially compact-type cells, indicated marked activity of 3βHSD and G6PD. The fine

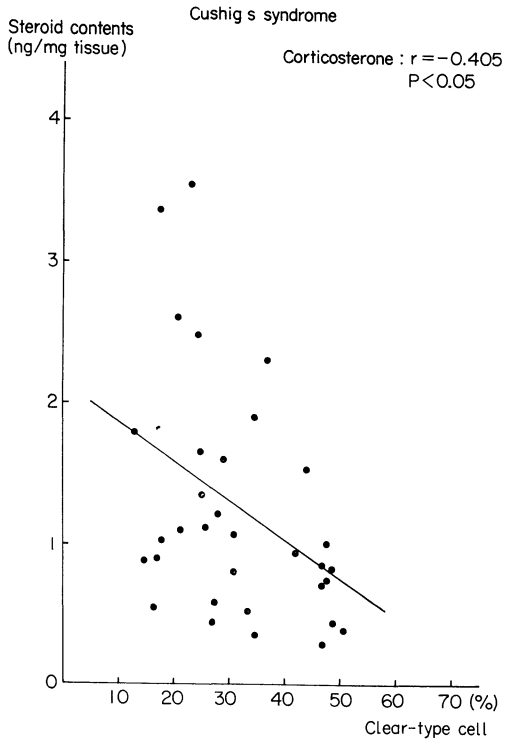

FIG. 9. Relationship between the corticosterone content and clear-type cells in Cushing's syndrome ($P<0.05$).

structure of those cells showed marked development of organellae as were found in Cushing's syndrome. Therefore, it may be possible that some kind of cells in a non-functioning tumor have hormonal activity as active as the cells in functioning tumors. However, such active cells in non-functioning tumor are less than those of functioning tumors and the areas of steroid production will be diminished. As to the mutual comparison in percentage of hormone contents, there was a similar tendency between non-functioning adenomatous nodules and adenomas in Cushing's syndrome. This fact suggests that adenomatous nodules in the present cases, especially Cases 3 and 4, have a potential of developing into Cushing's syndrome in the future under certain favorable conditions. It is necessary that the nodule becomes larger, the areas of hormone production become increased and that the receptors of tumor cells become more active in order to meet these conditions.

Case 4 had multiple adenomatous nodules besides a true adenoma. Similarities in the morphological appearance between adenoma and adenomatous nodules may support the hypothesis that nodular hyperplasia precedes the development of adrenocortical adenoma in some cases, as Cohen *et al.* (2) have suggested. However, further studies including Conn-type nodules will be necessary to prove this concept.

Next, cells constituting adrenocortical tumors can be classified into the three following types (23) : (1) Clear-type cells with clear cytoplasm containing large amount of lipid droplets. (2) Compact-type cells with acidophilic cytoplasm

containing a few or no lipid droplets. (3) Intermediate-type cells with acidophilic cytoplasm containing relatively large vacuoles. In the present study, for convenience of calculation, the constituting cells were classified into clear-type cells, compact-type cells. The intermediate-type cells were included with the compact-type cells. There was a significant positive correlation between the aldosterone content and the increase of compact-type cells in primary aldosteronism, but there was no significant correlation between the cortisol content and the proportion of constituting cells in Cushing's syndrome. According to Sasano's study (15) on primary aldosteronism, the aldosterone content showed a low value in cases with a high clear-type cell proportion and compact-type cells had a conspicuously high content. However, he indicated that those two type cells were not directly concerned with storage and secretion of aldosterone. Enzyme histochemically, compact-type cells showed higher than moderate activities of 3βHSD and G6PD. On the contrary, clear-type cells showed weak or negative activity of them. Electron microscopically, compact-type cells showed marked development of organellae. Therefore, it is suggested that compact-type cells play an important role in synthesis and secretion of aldosterone, and that clear-type cells play a role mainly in the storage of steroid precursors. These results support Tsuchiyama's speculation (20, 21, 23), and they differ slightly from the result obtained by Harada (7). This difference is probably due to the difference of methods of measuring the steroid content and cell proportion. In the author's method, there is only a 20–30 μm thickness between sections calculated, the proportion of the constituting cells, and the sections for quantitative procedure. The error of sampling is slighter than Harada's method by puncher.

In Cushing's syndrome, compact-type cells showed moderate to strong activity of 3βHSD and G6PD, but some clear-type cells also showed higher than moderate activity of those enzymes. Therefore, in Cushing's syndrome it was difficult to find the distinct enzyme histochemical difference between clear-type cells and compact-type cells, as that of an adenoma in primary aldosteronism. In this syndrome, the difference in histological appearance in these two types of cells may reflect only a difference in the phase of the cells and each of them play a role in hormone synthesis and secretion.

The area of nuclei on a maximum cut surface of nodules was also calculated in the present study. As Muto (12) and Tsuchiyama (22) pointed out, large and often hyperchromatic nuclei were prominent in primary aldosteronism and were in significantly greater numbers in compact-type cells than in clear-type cells. A few clear-type cells with an expanded cytoplasm and fused nuclei were also found. In non-functioning nodules and adenomas in Cushing's syndrome, a few large nuclei were found among compact-type cells, but the nuclei were generally uniform in size. Although the significance of large nuclei is not fully understood at the present time, it can be postulated that the large nuclei are characteristic of either an active tumor proliferation or of a degenerative process. It is desirable to perform further studies on the significance of nuclear attitude in non-functioning and functioning tumors.

ACKNOWLEDGEMENTS

Grateful acknowledgement is made to Prof. H. Tsuchiyama and Associate Prof. K. Kawai for their constant interest and guidance in this investigation. Special thanks go to Dr. T. Harada (Department of Internal Medicine, Nagasaki University School of Medicine) and Prof. T. Aikawa (Department of Physiology, Nagasaki University School of Medicine) for their technical advice on radioimmunoassay.

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