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Morphometric Study of the Human Adrenocortical Adenomas (Functioning Adenomas)

Junji IRIE

Department of Pathology, Nagasaki University School of Medicine

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SUMMARY

Morphometric studies on the light microscopic level were carried out on 11 cases from adrenocortical adenomas associated with primary aldosteronism (P. A.), 7 cases from those with Cushing's syndrome (C. S.) and as control, 6 cases from adrenal cortex at autopsy. Perimeter and area of cytoplasm and nuclei, form factor, nuclear contour index and nuclear cytoplasmic ratio were measured as parameters.

A great variety of each parameter was characterized in the adrenocortical adenomas compared with that of control. This pleomorphism in adenoma cells may reflect an active state in steroidogenesis or a sign of active proliferation of tumor cells. Rather than compact-type cells, clear-type cells contributed to the pleomorphism of C. S.

INTRODUCTION

Morphometry is a method of objectivity and reproducibility in morphological descriptions. Recently, this method has been applied to pathologic diagnosis and research [1)5)16)] and quantitative histopathology has been of great value.

Since SYMINGTON and NEVILLE[25)26)] established the histopatholgy of the human adrenal cortex, electron microscopic[2)] and biochemical studies[4)] have been reported. In animal experiments,[20)] especially in rats, many reports on the adrenal cortex have been also published.[3)7)8)14)15)19)] However, there are only few reports concerning morphometric study of human adrenocortical adenomas.[24)29)]

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The present study is aimed at the morphometric analyses in cell level of human adrenocortical adenomas associated with primary aldosteronism (P. A.) and Cushing's syndrome (C. S.).

MATERIALS AND METHODS

The cases examined in the present study are shown in Table 1. Eighteen adrenocortical adenomas were surgically removed from patients with primary aldosteronism and Cushing's syndrome. These adenomas were fixed in 10% formalin and embedded in paraffin. The paraffin embedded material was sectioned at 4-6 micron-thick were stained with hematoxylin and eosin (H. E.). As controls, six cases of adrenal cortices were obtained at autopsy (Table 2). They were treated in the same manner as the adenomas.

In each adenoma case, ten to twenty areas were selected randomly and photographed at the megnification of 400 times. The photographs were printed at the final magnification of 2800 times. The size of 200 cells in each case was measured. From each cell the contour of the nucleus and the cytoplasm was delineated by using a Leitz A. S. M. semiautomatic image analysis system. The following parameters were determined:

Measured

CP: Cytoplasmic perimeter

CA: Cytoplasmic area

NP: Nuclear perimeter

NA: Nuclear area

Calculated

$N/C\ ratio = NA/(CA-NA)$: Nuclear cytoplasmic ratio

$FF = 4\pi NA/NP^2$: Form factor of nucleus

$NCI = NP/(\text{Square root of } NA)$: Nuclear contour index

FF and NCI act as markers of nuclear contour irregularity. FF value of a circle is 1.0. According to SCHREK *et al*,[22] the lowest theoretical index, that for a circle, is 3.54. PAYNE *et al*.[17] described that the relationship between FF and NCI was as follows:

$FF = 12.571/(NCI)^2$

In the controls, the size of 200 cells in each adrenocortical zone, that is, zona glomerulosa (Z. G.), zona fasciculata (Z. F.) and zona reticularis (Z. R.), was examined as well as the adenomas. In each zone, well-preserved cells were selected.

Statistical analysis: The data obtained from each case were averaged and the

Table 1. Cases of Functioning Adenomas

	No.	Age	Sex	Site	Size (cm)	Plasma aldosterone (pg/ml)[2]
P.A.[3]	1	40	F	Left	-	-[1]
	2	42	F	-	-	-
	3	39	M	Left	1.5 × 1.5 × 1.5	-
	4	41	F	Left	-	-
	5	30	F	Left	-	-
	6	44	M	Right	1.3 × 1.2 × 0.9	-
	7	51	F	Left	2.5 × 1.7 × 1.4	-
	8	49	F	Left	2.0 × 1.9 × 0.5	311 - 396
	9	35	F	Left	2.0 × 1.6 × 1.2	257 - 442
	10	32	F	Right	1.0 × 1.0 × 0.7	142 - 283
	11	31	M	Left	1.2 × 1.1 × 1.4	118 - 208
C.S.[3]						Plasma cortisol (ng/dl)[2]
	1	30	M	Right	-	-
	2	30	F	Left	-	-
	3	45	F	Right	-	-
	4	27	F	Right	2.5 × 1.5 × 1.5	-
	5	32	F	Right	3.0 × 3.0 × 1.5	22.4 - 25.4
	6	34	F	Left	2.8 × 3.0 × 2.3	22.1 - 25.4
	7	55	F	Right	3.4 × 3.2 × 2.8	22.4 - 27.0

1. "-" : Data not available
2. Normal range:
   plasma aldosterone: 10.6 - 62.7
   plasma cortisol : 5.0 - 15.0
3. P.A.: Primary aldosteronism
   C.S.: Cushing's syndrome

Table 2. Control (Adrenal cortices of autopsy cases)

Case	Age	Sex	Diagnosis at autopsy
1	42	M	Cardiomyopathy
2	57	M	Liver cirrhosis
3	37	M	Aortic regurgitation
4	46	M	Liver cirrhosis
5	56	M	Duodenal ulcer
6	56	F	Mitral stenosis

standard deviation from the mean was calculated. Student's t-test was used for the statistical evaluation of the data. The difference between two mean values was considered to be significant if the probability of error (p) was found to be less than 0.05.

RESULTS

Light microscopic findings: The adenomas associated with primary aldosteronism (P. A.) showed an alveolar pattern in which each lobule was separated by fine or thick fibrous septa. Most of the tumors were composed of the lipid-rich clear cells with markedly vacuolated cytoplasm similar to those of the zona fasciculata in appearance. Scattered throughout the tumors were small groups of compact cells with granular cytoplasm (Fig. 1). Pleomorphism was usually present, and occasional giant or bizzare nucleated forms could be detected (Fig. 2 and 3). According to TSUCHIYAMA[27)31)], cells constituting adenomas can be classified into four kinds as follows: (1) clear-type cells, (2) compact-type cells, (3) intermediate-type cells, and (4) zona glomerulosa-type cells. They were detected among the cases in the present study.

The adenomas associated with Cushing's syndrome (C. S.) showed a cord or small alveolar pattern. Most of the adenomas were mixed cell adenoma composed of clear-type cells and compact-type cells (Fig. 4 and 5). Case 5 of C. S. was composed predominantly of compact-type cells. They were arranged in small alveoli, cords, or columns separated by delicate fibrovascular trabeculae. Some of the compact-type cells often had lipofuscin

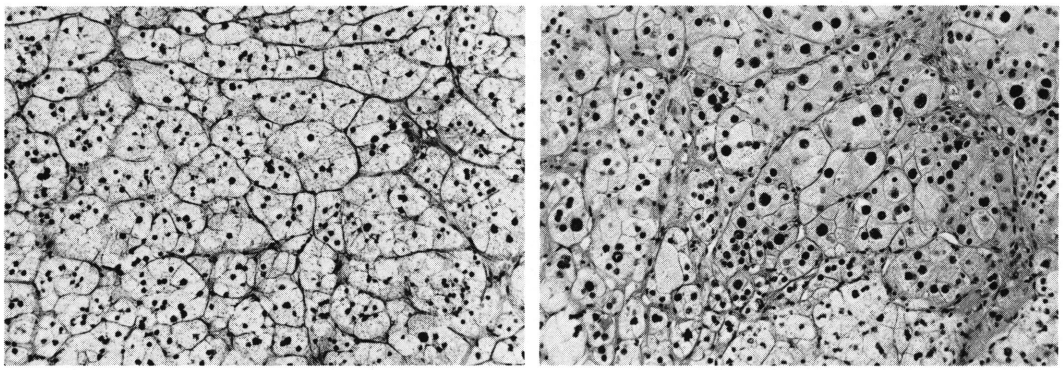

Fig. 1. Adrenocortical adenoma associated with primary aldosteronism (Case 8). The tumor is chiefly composed of clear-type cells with lipid-rich cytoplasm. They are arranged in small cords or acini. X 100, H. E.

Fig. 2. Adrenocortical adenoma associated with primary aldosteronism (Case 2). The cells of the compact-type showing acidophilic granular cytoplasm and pleomorphic nuclei. X 100, H. E.

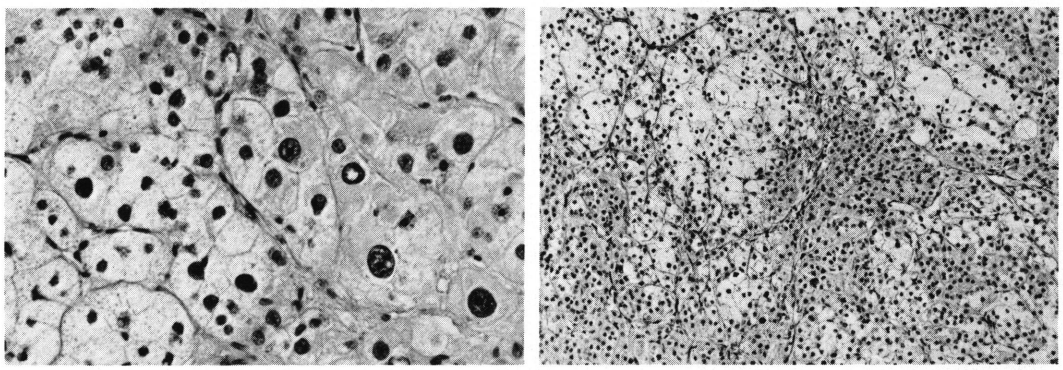

Fig. 3. Adrenocortical adenoma associated with primary aldosteronism (Case 2). Higher magnification from Fig. 2. Marked pleomorphism of the cells and nuclei is found, but nuclear irregularity is not obvious. X 200, H. E.

Fig. 4. Adrenocortical adenoma associated with Cushing's syndrome (Case 3). The tumor is mainly composed of clear-type cells and compact-type cells. The relative proportion of clear and compact-type cells varies between different areas of one tumor. X 100, H. E.

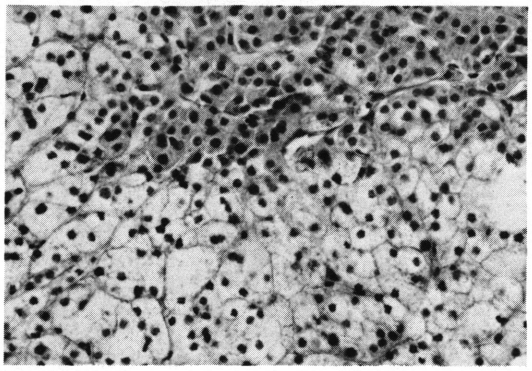

Fig. 5. Adrenocortical adenoma associated with Cushing's syndrome (Case 3). In the compact-type cells (upper) lipofuscin pigment is shown. X 200, H, E.

granules.

Morphometric analysis : All data obtained are shown in Table 3 and 4, and expressed as means ± standard deviation (S. D.). They are further summarized in Table 5 and 6. The perimeter and area of the adenoma cells of P. A. and C. S., as well as FF, NCI and N/C ratio were not significantly different between P. A. and C. S. This differed from the subjective impression that the nucleus and cytoplasm in P. A. had greater values than C. S. None of the zones of adrenal cortex showed a significant differences with morphometric parameter. Between P. A. and each zone of the adrenal cortex, however, N/C ratio of P. A. was significantly smaller than that of Z. G. and Z. R. There were no significant differences in the other parameters. In comparison C. S. with each zone of adrenal cortex, N/C ratio of C. S. was only significantly smaller then that of Z. R. Further studies on clear-type cells (CL) and compact-type cells (CM) of C. S. were performed in the same manner, but the size of 100 cells in CL and that in CM in each case of C. S. were measured. The perimeter of cytoplasm of CL was significantly greater than that of CM. No significant differences were found in the other parameters (Table 7).

Coefficient of variation in each data was calculated (Table 8). The values in almost all of the parameters of adrenocortical adenomas were greater than those of control, that is, Z. G., Z. F, and Z. R.

The relationship between variance of histopathology and level of plasma hormone cannot be clarified because of incomplete data on laboratory record in the present cases.

Table 3. Synopsis of adrenocortical adenomas

		Cytoplasmic perimeter (μm)	Cytoplasmic area (μm^2)	Nuclear perimeter (μm)	Nuclear area (μm^2)	Form factor	Nuclear contour index	N/C ratio
P.A.	1	31.70 ± 7.27	66.46 ± 31.47	9.980 ± 2.313	7.556 ± 5.003	0.9048 ± 0.0566	3.733 ± 0.141	0.1476 ± 0.0943
	2	39.65 ± 13.58	108.74 ± 88.17	13.803 ± 4.981	15.131 ± 13.43	0.8982 ± 0.0769	3.778 ± 0.388	0.1769 ± 0.0959
	3	34.64 ± 8.27	82.13 ± 38.29	9.855 ± 1.520	7.289 ± 2.575	0.9227 ± 0.0396	3.693 ± 0.081	0.1159 ± 0.0574
	4	32.86 ± 8.26	69.34 ± 36.74	10.207 ± 1.540	7.214 ± 2.179	0.8563 ± 0.0715	3.844 ± 0.179	0.1493 ± 0.0970
	5	40.67 ± 14.93	116.21 ± 97.08	13.922 ± 4.940	15.638 ± 16.45	0.8899 ± 0.0548	3.744 ± 0.157	0.1774 ± 0.0889
	6	35.84 ± 12.14	87.75 ± 63.91	11.866 ± 3.312	10.614 ± 7.251	0.8776 ± 0.0587	3.786 ± 0.148	0.1742 ± 0.1020
	7	33.19 ± 8.11	69.87 ± 37.31	11.183 ± 2.972	9.406 ± 6.474	0.8835 ± 0.0531	3.773 ± 0.120	0.1783 ± 0.01
	8	35.04 ± 10.44	87.26 ± 53.10	10.845 ± 2.826	8.940 ± 5.003	0.9132 ± 0.0658	3.725 ± 0.295	0.1571 ± 0.1581
	9	34.69 ± 10.36	84.17 ± 55.35	10.969 ± 2.636	9.172 ± 6.016	0.9087 ± 0.0446	3.724 ± 0.098	0.1438 ± 0.0640
	10	28.99 ± 8.73	56.77 ± 35.79	10.645 ± 3.139	8.797 ± 6.629	0.8957 ± 0.0515	3.748 ± 0.118	0.2202 ± 0.0122
	11	42.65 ± 14.76	121.30 ± 94.78	13.403 ± 4.253	13.694 ± 10.978	0.8684 ± 0.0636	3.812 ± 0.151	0.1481 ± 0.0781
C.S.	1	25.95 ± 6.26	46.18 ± 21.39	9.786 ± 2.204	7.279 ± 5.099	0.9290 ± 0.0476	3.682 ± 0.105	0.2420 ± 0.2126
	2	24.75 ± 8.81	43.88 ± 30.46	8.548 ± 1.168	5.161 ± 1.435	0.8757 ± 0.0860	3.803 ± 0.151	0.2189 ± 0.1616
	3	27.04 ± 8.68	51.55 ± 33.10	9.484 ± 1.365	6.375 ± 1.930	0.8710 ± 0.0671	3.803 ± 0.151	0.2126 ± 0.1480
	4	30.41 ± 7.35	62.03 ± 30.19	11.103 ± 1.769	8.958 ± 3.021	0.8904 ± 0.05	3.758 ± 0.111	0.2017 ± 0.0964
	5	22.44 ± 4.99	36.54 ± 20.17	10.309 ± 1.461	7.887 ± 2.554	0.9186 ± 0.0346	3.755 ± 0.815	0.3183 ± 0.1237
	6	24.97 ± 5.71	43.09 ± 21.02	9.243 ± 1.082	6.296 ± 1.472	0.9128 ± 0.0436	3.711 ± 0.007	0.2104 ± 0.0995
	7	30.87 ± 12.35	68.74 ± 59.47	10.204 ± 1.428	7.479 ± 3.333	0.8855 ± 0.0529	3.772 ± 0.008	0.2079 ± 0.1389

Mean ± S.D.

Table 4. Synopsis of controls

		Cytoplasmic perimeter (μm)	Cytoplasmic area (μm²)	Nuclear perimeter (μm)	Nuclear area (μm²)	Form factor	Nuclear contour index	N/C ratio
Z.G.	1	19.85 ±3.39	27.74 ± 9.80	9.241 ± 1.161	6.551 ± 1.650	0.9195 ± 0.0447	3.743 ± 0.590	0.3656 ± 0.1970
	2	19.96 ± 3.08	27.24 ± 7.91	9.210 ± 1.031	6.246 ± 1.401	0.1407 ± 0.9239	3.711 ± 0.101	0.3337 ± 0.1360
	3	19.28 ± 3.15	25.35 ± 8.29	9.438 ± 1.205	6.378 ± 1.611	0.8944 ± 0.0557	3.798 ± 0.630	0.3832 ± 0.1640
	4	20.41 ± 3.28	28.48 ± 9.07	9.343 ± 1.116	6.370 ± 1.569	0.9056 ± 0.0469	3.736 ± 0.139	0.3310 ± 0.1819
	5	24.87 ± 5.87	40.06 ± 18.67	9.633 ± 1.202	6.743 ± 1.596	0.8975 ± 0.0557	3.737 ± 0.184	0.2556 ± 0.1308
	6	24.07 ± 5.10	39.10 ± 16.47	10.069 ± 1.4	7.376 ± 2.072	0.8975 ± 0.0480	3.746 ± 0.103	0.2819 ± 0.1476
Z.F.	1	23.02 ± 4.35	37.96 ± 14.92	10.160 ± 1.339	7.800 ± 2.316	0.9332 ± 0.0361	3.672 ± 0.075	0.2933 ± 0.1192
	2	23.93 ± 3.52	39.59 ± 10.81	10.228 ± 1.127	7.867 ± 1.794	0.9331 ± 0.0332	3.672 ± 0.068	0.2720 ± 0.1020
	3	25.79 ± 4.11	45.70 ± 14.24	9.640 ± 0.843	6.795 ± 1.182	0.9122 ± 0.04	3.714 ± 0.083	0.1982 ± 0.0806
	4	28.39 ± 7.30	55.39 ± 28.86	9.744 ± 1.101	7.003 ± 1.568	0.9110 ± 0.0458	3.717 ± 0.098	0.1891 ± 0.0980
	5	30.45 ± 5.23	61.17 ± 20.28	10.516 ± 1.125	8.146 ± 1.677	0.9129 ± 0.06	3.704 ± 0.077	0.1727 ± 0.0663
	6	30.30 ± 5.52	61.44 ± 22.59	10.187 ± 1.425	7.544 ± 2.124	0.8948 ± 0.05	3.752 ± 0.110	0.1576 ± 0.0663
Z.R.	1	25.09 ± 5.70	43.67 ± 20.66	10.346 ± 1.515	7.924 ± 2.029	0.9208 ± 0.0574	3.702 ± 0.208	0.2591 ± 0.1131
	2	25.85 ± 4.40	46.39 ± 15.12	10.661 ± 0.936	8.563 ± 1.546	0.9394 ± 0.0316	3.659 ± 0.065	0.2578 ± 0.1030
	3	25.10 ± 4.17	42.97 ± 14.39	10.058 ± 1.255	7.466 ± 2.076	0.9145 ± 0.0447	3.711 ± 0.096	0.2318 ± 0.0837
	4	22.33 ± 3.06	33.87 ± 9.34	9.664 ± 0.877	6.893 ± 1.189	0.9213 ± 0.0424	3.696 ± 0.087	0.2858 ± 0.11
	5	25.28 ± 5.89	43.71 ± 20.49	10.049 ± 1.495	7.480 ± 2.264	0.9141 ± 0.0412	3.711 ± 0.087	0.2406 ± 0.0938
	6	25.45 ± 4.51	43.19 ± 15.35	9.842 ± 1.319	7.109 ± 1.947	0.9053 ± 0.0469	3.729 ± 0.10	0.2188 ± 0.0837

Mean ± S.D.

Table 5. Summarized data from Table 3.

Parameter	P.A.	C.S.	p
Cytoplasmic perimeter (μm)	31.75 ± 10.95	26.63 ± 8.07	n.s.
Cytoplasmic area (μm²)	86.36 ± 62.25	50.29 ± 33.33	n.s.
Nuclear perimeter (μm)	11.516 ± 3.329	9.811 ± 1.538	n.s.
Nuclear area (μm²)	10.314 ± 8.570	7.062 ± 2.804	n.s.
Form factor	0.8926 ± 0.0588	0.8976 ± 0.0568	n.s.
Nuclear contour index	3.760 ± 0.192	3.755 ± 0.329	n.s.
N/C ratio	0.1629 ± 0.0996[a]	0.2303 ± 0.1449[b]	n.s.

Mean ± S.D.

a: $p < 0.05$, vs. Z.G. and Z.R.

b: $p < 0.05$, vs. Z.R.

n.s.: not significant

Table 6. Summarized data from Table 4.

Parameter	Z.G.	Z.F.	Z.R.
Cytoplasmic perimeter (μm)	21.41 ± 4.12	26.98 ± 5.15	24.85 ± 4.72
Cytoplasmic area (μm²)	31.33 ± 12.45	50.21 ± 19.57	42.30 ± 16.36
Nuclear perimeter (μm)	9.489 ± 1.191	10.084 ± 1.175	10.103 ± 1.258
Nuclear area (μm²)	6.611 ± 1.662	7.526 ± 1.815	7.573 ± 1.877
Form factor	0.9064 ± 0.0736	0.9162 ± 0.045	0.9192 ± 0.0447
Nuclear contour index	3.745 ± 0.369	3.705 ± 0.088	3.701 ± 0.117
N/C ratio	0.2652 ± 0.1613	0.2138 ± 0.0908	0.2490 ± 0.15

Mean ± S.D.

Table 7. Clear-type cell and compact-type cell

Parameter	CL	CM	p
Cytoplasmic perimeter (μm)	32.68 ± 8.04	21.98 ± 3.72	<0.05
Cytoplasmic area (μm²)	71.06 ± 38.53	34.10 ± 11.82	n.s.
Nuclear perimeter (μm)	9.908 ± 1.848	9.608 ± 1.134	n.s.
Nuclear area (μm²)	7.105 ± 3.625	6.744 ± 1.662	n.s.
Form factor	0.8752 ± 0.0566	0.9129 ± 0.0442	n.s.
Nuclear contour index	3.798 ± 0.148	3.711 ± 0.095	n.s.
N/C ratio	0.1421 ± 0.1260	0.2891 ± 0.1223	n.s.

1. n.s.: not significant
2. Case 5 of C.S. was excluded.

Table 8. Coefficient of variation (%)

Parameter	P.A.	C.S.	CL	CM	Z.G.	Z.F.	Z.R.
Cytoplasmic perimeter	34.49	30.30	24.60	16.92	19.24	19.09	18.99
Cytoplasmic area	72.08	66.28	54.22	34.66	39.74	38.98	38.68
Nuclear perimeter	28.91	15.68	18.65	11.80	12.55	11.65	12.45
Nuclear area	83.09	39.71	51.02	24.64	25.14	24.12	24.79
Form factor	6.587	6.328	6.467	4.842	8.120	4.910	4.863
Nuclear contour index	5.106	8.762	3.897	2.560	9.853	2.375	3.161
N/C ratio	61.14	62.92	88.67	42.30	60.82	42.47	60.24

Coefficient of variation : S.D./ mean X100

DISCUSSION

Human adrenocortical adenomas have been investigated by using light and electron microscopy. TSUCHIYAMA and MUTO[29] studied on the comparison of nuclear diameter between P. A. and nonfunctioning adenomas. In animals under non-neoplastic conditions, especially in rats, morphometric studies of the adrenal cortical cells have been reported by many investigators.[3][7][8][14][15][19] However, there is no report of detailed cell morphometry in regard to P. A. and C. S. A part of objective results in the present study is consistent with the subjective impression at observation by light microscopy as mentioned above. However, some results are different from such impression. This is probably due to the large value of variance from the mean. In other words, it is the striking pleomophism of adrenocortical adenomas. Especially in P. A., the morphologic parameters are greater than those of C. S. Although it has been well known that cellular and nuclear pleomorphism reveal a sign of malignant cells, which is accepted generally in oncology and exfoliative cytology,[18] these cases with primary aldosteronism and Cushing's syndrome in the present study took a benign clinical course. Therefore, it may be considered that pleomorphism of adenomas reflects an increase of functional activity in steroidogenesis. Furthermore, it is also to be speculated a manifestation of cellular proliferation in neoplastic growth. The nuclear irregularity approaches to a circle according to the indices. Therefore, nuclear enlargement seems to be the morphological feature in adrenocortical adenomas.

The coefficient of variation of CL and CM in C. S. suggests that CL participates in pleomorphism of C. S. rather than CM. According to TSUCHIYAMA[27][31], CL is rather inactive in steroidogenesis and many lipid droplets may be storage sites of steroid precursors. On the other hand, CM might indicate active steroid hormone synthesis and subsequent secretion. MURAKOSHI *et al.*[9][10] proved these possibilities by enzyme histochemical methods. It is nesessary to inquire further into this interesting point.

The present study is limited to pure morphology. It is necessary to investigate the adrenocortical adenomas by systemic research, including hormone content of tumor tissue[23] and microphotometry of nucleic acid.[6]

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