Lipid Histochemistry of Surgically Resected Adrenal Glands in Hyperadrenocorticism

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Recently, fresh adrenal tissue of operation material has become available and many histochemical investigations in the adrenal cortex have been recorded. The hyperplastic adrenal glands cause three syndromes such as Cushing’s, adrenogenital and aldosteronism, and the adrenal cortex in each case has a characteristic histochemical change. In the present paper histochemical comparison of lipids in surgically resected adrenal glands are described relating to these three syndromes.

Material and Methods

Fresh adrenal glands were obtained from 3 patients with Cushing’s syndrome, 8 patients with aldosteronism and one patient with adrenogenital syndrome. Control biopsy specimens were obtained from 5 patients of operations for renal stone, tuberculosis and tumors. Immediately after operation each adrenal tissue was cut into three blocks. Two blocks were kept in formaldehyde and Tellyesniczky’s acetic bichromate. Unixed cryostat sectioning was performed from one block of the adrenal glands. Many sections were made at each block and stained with the following histochemical procedures:

Modified Schultz method by Weber; Cryostat sections cut at 20μ and washed for 24 hours in distilled water. Sections were then treated with 2.5

References

per cent ferric ammonium sulphate in 0.2 M-acetate buffer at 37° for 7 days. After treatment sections were washed for 1 hour each in 3 changes of acetate buffer and rinsed in distilled water. They were transferred to 5 per cent. formalin for 10 minutes and mounted on slides.

Nile blue method for phospholipid by Menschik: After fixation in formol-calcium for 6 hours cryostat sections were cut at 6µ. These sections were stained for 90 minutes at 60° in saturated aqueous Nile blue sulphate 500ml. with 50 ml. 0.5 per cent. H₂SO₄ and rinsed in distilled water. They were placed in aceton heated to 50°, and after aceton was removed from source of heat sections were allowed to remain in for 30 minutes. Differentiation was done in 5 per cent. acetic acid for 30 minutes.

Heidenhain's iron hematoxylin-sudan double stain by Maeda: One block of adrenal tissue was immersed in Tellyesniczky's acetic bichromate for 2 days and immersed in 3 per cent. potassium bichromate for 1 day. Tissue block was embedded in paraffin, sections were cut at 4µ, and stained with Heidenhain's iron hematoxylin for 3 hours. Sections were double stained with sudan III for 30 minutes. During the Heidenhain-sudan double staining sudanophilia were clearly visualized against hematoxylin blue.

Oil red O stain for general lipid; Sudan black B-propylene glycol method for lipid by Chiffele and Putt; Smith-Dietrich's method for phospholipid; Okamoto's method for cholesterol and their esters. Detail of these methods have been fully described elsewhere.

Results

In other paper, we have reported histochemical figures of the adrenocortical adenoma in primary aldosteronism. In this report, therefore, lipid histochemistry in the hyperplastic adrenal cortex will be discussed.

Adrenocortical hyperplasia causing Cushing's syndrome (Fig. 1, 3 and 5): The zona glomerulosa was not prominent except for partial proliferation of this zone in one case. Sometimes the fascicular elements extended directly to the capsule. The outer portion of the zona fasciculata was composed of clear cells with vacuolated cytoplasm and a broad reticularis which consists of compact cells was noted in the inner portion of the cortex. In some area micronodule of clear cells was present. Occasionally large lipid spaces were scattered throughout the zona fasciculata and reticularis. With oil red O stain, a considerable quantity of lipid was observed in the zona glomerulosa. Most of these cells contained small or medium-sized droplets of lipid. In clear cells of the outer fasciculata large conglomerate droplets of lipid were often found. The zona reticularis and the inner half portion of the zone fasciculata were characterized with very small or no lipid content. The positive lipids for Schultz method tended to concentrate in the zona fasciculata, especially in the outer portion. Micronodules in the cortex were composed predominantly of the fascicular elements and lipid distribution of each nodule varied slightly from case to case. Some micronodule was lipid-free and other was lipid-rich. In addition, micronodules could be often demarcated from extranodular cortex
which showd different distribution of lipids. Phospholipids and stainable granules were generally abundant in compact cells. The reduction of general lipids, especially decrease in cholesterol, in the inner fascicular and reticular zones was striking appearance in Cushing's syndrome.

In the two large nodules of the adenomatous hyperplasia the cells were arranged large alveoli or sheets. Many large cells with abundant cholesterol but poor phospholipid and few compact cells which contained dense stainable granules were intermingled in the small nodule. The cells of the larger nodule were mostly compact in type, uniform in size and often they had small granules of lipofuscin. Phospholipid and stainable granules were prominent compared with small one.

**Adrenocortical hyperplasia causing aldosteronism** (Fig. 2 and 4): In the hyperplastic cortex widening of the zona glomerulosa and micronodular proliferation of the outer cortical cells were observed. The prominent zona glomerulosa were rich in stainable granules including mitochondria, and phospholipid. The glomerulosa cells containing no lipid with oil red O stain were frequent. Demonstrable cholesterol showed marked depletion in the zona glomerulosa. Histochemical findings in the micronodular hyperplasia were similar to those observed in the zona glomerulosa. The presence of considerable lipid in the zona fasciculata and reticularis was seen. Cholesterol tended to concentrate in the zona fasciculata and made up a small proportion of total general lipids in the inner fascicular zone. Phospholipid was abundant in the zona reticularis.

The cells forming the adenomatous nodule differed from histochemical appearances of the surrounding tissues. Two cases of adenomatous hyperplasia exhibited slight different fashion to positive reactive lipids. In one, large clear cells with lipid-rich cytoplasm were present throughout the nodule. The distribution of Schultz positive lipids was similar, but the intensity of the reaction often varied. In another one, many acidophilic compact cells were seen. Some of them contained small vacuoles in the cytoplasm. The nodule reacted positively to phospholipid and stainable granules.

**Adrenocortical hyperplasia causing adrenogenital syndrome** (Fig. 6): Al-
though prominent zona glomerulosa was noted in some area, acidophilic large cells with finely granular cytoplasm often extended directly to the capsule from the medulla. A distinct differentiation between the zona reticularis and zona fasciculata could not be recognized. Occasionally these cells formed micronodules surrounding with infiltration of lymphocyte like cells. A few small vacuolated cells also scattered. In sections with oil red O stain, the prominent granular compact cells contained small or no lipid droplets. When positively reactive lipids were present granules were very fine and colored with yellowish brown. Large conglomerate droplets of lipid were not seen. On the other hand, zona glomerulosa showed fairly remarkable distribution of lipid droplets in medium size. Demonstrable cholesterol with Schultz and Okamoto's methods was mostly similar to the distribution with oil red O stain. Micronodules of the reticular and fascicular zones revealed strongly positive reaction to phospholipid. This was sharp contrast to those of weak reaction in the zona glomerulosa. The cytoplasm of compact cells was packed with many fine stainable granules for Heidenhain's iron hematoyxlin-sudan double stain.

Discussion

The zona glomerulosa in aldosteronism was most prominent among three types of disease in hyperplastic adrenals. It may suggest that depletion of lipids, particularly cholesterol, and an increase in phospholipid in hyperplastic
zona glomerulosa is an indication of active action in aldosteronism. Similar
tendency was noticed in micronodules which composed predominantly of the
glomerular cells. In Cushing's syndrome zona glomerulosa was often narrowed
and occasionally absent. The cells of zona glomerulosa have scanty lipid-
containing cytoplasm which gives a weak positive stain for cholesterol. These
distribution of lipids may be included within normal limits.

The cells of the outer fasciculata in aldosteronism were filled with large
lipid droplets. Transition from these lipid rich cells of the zona fasciculata to
the lipid-poor cells of the inner portion of the zona fasciculata was fairly
clear. There was no striking difference in lipid distribution between the zona
fasciculata in aldosteronism and in the control cases. Histochemical changes
in the zona fasciculata were most characteristic in Cushing's syndrome. A
marked increase in width of the zona fasciculata was seen histologically.
From the midportion to the inner portion of the hyperplastic zona fasciculata
concentrations of lipid were often remarkably reduced and stainable granules
were generally abundant. In addition, many cells of zona reticularis appeared
no concentration of lipid, especially cholesterol. Distribution of lipids in the
cells forming the micronodule often differed from these of the surrounding
cortex. In adrenogenital syndrome a distinct transition between the reticular
and fascicular zones could not be found. The distribution of lipid was also
same fashion in the both zones. The cells of these uniform zones contained
abundant stainable granules and phospholipid but scanty cholesterol. This
fact seemed to suggest that broad compact cell layer may play an important
role of hormonal activity.

Adenomatous hyperplasia in aldosteronism revealed two types of lipid
content. One type was similar to those found in the true adenoma which
consisted of many clear cells with scanty phospholipid and abundant cholesterol
in the cytoplasm. In other type, small droplets of phospholipid were distrib-
uted throughout the cytoplasm of most cells in the nodule. In a case of
Cushing's syndrome two adenomatous nodules were found. Small one was com-
posed predominantly of the fascicular element with lipid-poor cytoplasm. Large
nodule showing abundant phospholipid and stainable granules in their cells was
considered as unusual response for hormone activity.

Summary

Histochemical study was made of surgically resected adrenal glands with
hyperadrenocorticism. Characteristics of lipid distribution were described in
each hyperplastic adrenals in Cushing's syndrome, aldosteronism and adreno-
genital syndrome. In these investigations some relationship between the
histochemical change and hormone secretion in the hyperplastic cortex was
also discussed.

References

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**Explanation of Plates**

Fig. 1 Case 10. Cushing's syndrome. Compressed zona glomerulosa and widening of the zona fasciculata with abundant cholesterol. Schultz reaction by Weber's modification.

Fig. 2 Case 5. Aldosteronism. No cholesterol in the hyperplastic zona glomerulosa is seen. Schultz reaction by Weber's modification.

Fig. 3 Case 11. Cushing's syndrome. In the outer fascicular zone large conglomerate droplets of lipid are found. Inner portion of the zona fasciculata shows very small or no lipid content. Oil red O stain.

Fig. 4 Case 4. Aldosteronism. A sharply demarcated nodule in adenomatous hyperplasia characterized by scanty or no lipid for Smith-Dietrich's reaction. The surrounding tissue contained considerable positive droplets. Smith-Dietrich's reaction.

Fig. 5 Case 9 Cushing's syndrome. Small adenomatous nodule is composed predominantly of lipid-rich cells. Sudan black B-propylene glycol stain.

Fig. 6 Case 12. Adrenogenital syndrome. Sudanophilic and no sudanophilic cells are intermingled in the micronodule. Sudan black B-propylene glycol stain.

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"Histochemical Studies on the Adrenocortical Hypofunction of Rats with Long-Term Administration of Corticoids."

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The problems of adrenocortical atrophy in treatment with corticoid has been repeatedly discussed by several authors. 1-4) The purpose of this paper is to show the histochemical findings of the adrenal hypofunction, which was induced by long-term treatment of exogenous corticoids, and to compare the inhibiting power of the three kinds of corticoids upon the adrenals.

**Materials and Methods**

1) Animals; Wistar strain male rats of ca. 120g. in weight.
2) Drugs and their daily doses
   (1) Hydrocortisone (HC.) 1mg./100g. + Acromycin 1mg./100g.
   (2) Paramethasone (PM.) 0.05mg./100g. + Acromycin 1mg./100g.