HISTOCHEMICAL STUDY OF THE HUMAN ADRENAL CORTEX

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Studies were made on histochemical changes of the human adrenals. There was no great difference in histochemical findings between essential hypertensive patients and normotensive subjects.

In zona glomerulosa, enhanced activities of enzymes were remarkably observed in case of renal hypertension.

In primary aldosteronism, hypertrophy occurred to the non-tumorous portion in case of the zona glomerulosa and increased enzymatic activity was recognized in this zone.

Adrenocortical hyperplasia with clinical evidence of Cushing's syndrome, the zona glomerulosa exhibited too complicated histochemical changes to demonstrate any distinct conclusions.

It is recent tendency to stress the role of renin as an aldosterone stimulating factor rather than as a factor to participate in an increase in blood pressure directly. Yet no definite conclusions have been drawn on the significance of the renin-angiotensin-aldosterone system in the occurrence of hypertension. Biron and Laragh emphasized the necessity for researching the adrenal gland. But the adrenal function has not completely been clarified morphologically from postmortem autopsies. Besides, clinical experimental adrenalectomy is not always easy on account of its anatomical situation. Such being case, no sufficient investigation has been made on the adrenal function.

In order to elucidate the adrenocortical function, histochemical observations on the human adrenal glands were carried out. The paper deals with results of studies mentioned above.

I. CASE EXAMINED AND METHODS OF EXAMINATION

1. Case examined

Of total 77 cases, 34 of hypertensive and 43 of normotensive patients were examined. Hypertension was classified according to causal disease. The details of classification are indicated in Table 1.

2. Procedures for histochemical examination

A section of fresh adrenal was attached to and fixed on a wooden block, large enough to fit it. This procedure was undergone in the presence of solid carbon dioxide with acetone. By the aid of cryostat, the specimen was sliced into sections.
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10 μ in thickness at −20°C. Frozen tissues were attached to slides and allowed to leave at room temperature for 20 minutes. The dehydrated sections were incubated in reactive solutions for 30 (G-6-P DH) to 45 (3β-HSD) minutes at 37°C, gently washed in phosphate buffer solution (pH 7.0), fixed first in 10% formaldehyde-phosphate buffer solutions (pH 7.0) for 15 minutes, and then immersed in 10% ethanol for 15 minutes. The refixed sections were washed by running water and embedded with polyvinyl pirolidone. The incubation medium mentioned above are shown in Table 2 and Table 3.

### II RESULTS

Activities were investigated of 3β-hydroxysteroid dehydrogenase (3β-HSD) and glucose-6-phosphate dehydrogenase (G-6-P DH) in the adrenal cortex. The enzymatic activities were graded as follows: ++, intensely positive, +, positive, −, negative.

1. 3β-HSD activity with dehydroepiandrosterone (DHA) as substrate

   Studies were made on the relationship of the 3β-HSD activity, urinary aldosterone excretion and blood pressure. The results were shown as follows (Fig. 1~ Fig. 5)

### TABLE 1

**Distribution of Hypertensive patients**

<table>
<thead>
<tr>
<th>Hypertension, as classified according to causal disease</th>
<th>No. of male patients</th>
<th>No. of female patients</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. adrenal hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Cushing’s syndrome</td>
<td>(1) adenoma</td>
<td>(2) carcinoma</td>
<td>(3) hyperplasia</td>
</tr>
<tr>
<td>b. primary aldosteronism</td>
<td>(1) adenoma</td>
<td>(2) carcinoma</td>
<td></td>
</tr>
<tr>
<td>2. renal hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. renovascular hypertension</td>
<td>7</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>b. intrarenal hypertension</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>3. essential hypertension</td>
<td>7</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>4. normotension</td>
<td>26</td>
<td>17</td>
<td>43</td>
</tr>
</tbody>
</table>

\[\text{male: 45, female: 32/tot: 77}\]

### TABLE 2

**Incubation medium of 3β-HSD.**

<table>
<thead>
<tr>
<th>Incubation medium of 3β-HSD.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>phosphate buffer (pH 7.6, 0.1 M)</td>
<td>10 ml</td>
</tr>
<tr>
<td>NAD (10 mg/ml) 1.3 ml</td>
<td></td>
</tr>
<tr>
<td>Nitro-BT (5 mg/ml) 1.0 ml</td>
<td></td>
</tr>
<tr>
<td>Nicotinamide (40 mg/ml) 1.0 ml</td>
<td></td>
</tr>
<tr>
<td>DHA 1.44 mg</td>
<td></td>
</tr>
<tr>
<td>Acetone fluid 0.5 ml</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 3

**Incubation medium of G-6-P DH.**

<table>
<thead>
<tr>
<th>Incubation medium of G-6-P DH.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>phosphate buffer (pH 7.0, 0.005 M)</td>
<td>10 ml</td>
</tr>
<tr>
<td>NADP (0.0077 g)</td>
<td></td>
</tr>
<tr>
<td>NBT (10 mg)</td>
<td></td>
</tr>
<tr>
<td>glucose-6-phosphate (0.152 g)</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1. Correlation between urinary aldosterone excretion & 3β-HSD activity in zona glomerulosa.

Fig. 2. Comparison of 3β-HSD activity in zona glomerulosa & blood pressure of each subject.
Fig. 3. 3β-HSD activity in zona glomerulosa of normotensive and hypertensive subjects, as classified according to causal disease.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Activity (substrate pregnenolone)</th>
<th>Frequency (%)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cushing's syndrome</td>
<td>-</td>
<td>75%</td>
<td>4</td>
</tr>
<tr>
<td>Primary aldosteronism</td>
<td>-</td>
<td>100%</td>
<td>2</td>
</tr>
<tr>
<td>Renovascular hypertension</td>
<td>(++)</td>
<td>16.7%</td>
<td>6</td>
</tr>
<tr>
<td>Intrarenal hypertension</td>
<td>(++)</td>
<td>16.7%</td>
<td>6</td>
</tr>
<tr>
<td>Essential hypertension</td>
<td>(++)</td>
<td>62.5%</td>
<td>8</td>
</tr>
<tr>
<td>Normotension</td>
<td>(++)</td>
<td>48.6%</td>
<td>35</td>
</tr>
</tbody>
</table>

Fig. 4. Intensely stained cells, adjacent to the aldosterone-producing tumor, were distributed in zona glomerulosa. (3β-HSD, ×100)
2. G-6-P DH activity

Activities of G-6-P DH in zona glomerulosa were carried out just same method as 3β-HSD. The results of which were as follows. (Fig. 6,7,8,9,10,11)

![Graph](image)

Fig. 6. Correlation between urinary aldosterone excretion & G-6-P DH activity in zona glomerulosa.
Fig. 7. Comparison of G-6-P DH activity in zona glomerulosa & blood pressure of each subject.

Fig. 8. G-6-P DH activity in zona glomerulosa of normotensive and hypertensive subjects, as classified according to causal disease.
Fig. 9. Frozen section of the zona glomerulosa revealed strong enzymatic reaction. (G-6-P DH activity, \( \times 400 \)).

The subject is a female with clinical evidence of intrarenal hypertension.

Fig. 10. Intens staining for activity of G-6-P DH was remarkably observed in zona glomerulosa.

The subject is a female with clinical evidence of renovascular hypertension.
The true cause for hypertension is obscure even today. It will be clarified simply from studies on the renin-angiotensin-aldosterone system. If an increase in activity of this system was cause of hypertension, it would be observed in every type of hypertension.

But most previous investigators, except a few, are opposed to this presumption.$^{1,4,25,28}$

Interest, however, in the relationship between the adrenal cortex and hypertension has been longstanding,$^{20}$ and various methods have been used to study the glomerular zone of the adrenal cortex.$^{7,11,12,16,17}$ They include a method in which the thickness of the zone and the size of individual cells are measured,$^{26}$ the zona glomerular index,$^{17}$ and a method in which the zona glomerulosa adulta is compared with the zona glomerulosa infantum.$^{24}$ Although the conclusions has not been by any means fully clarified by these methods, it has become increasingly clear that subtle relationships do indeed exist between adrenal cortex and hypertensive disorders, however, at the present time these relationships are not so clear as to be capable of being formulated in terms of general principles.$^{9}$ By our observations previously described, there was no great difference in blood pressure or the urinary aldosterone excretion between subjects with the slightly hypertrophic zona glomerulosa and those with the normal zona glomerulosa.$^{10}$ From the pathological findings and the estimation of the steroid, we observed, it was impossible to say the accurate function of this zone. Then authors applied histochemical scrutinies in the adrenal cortex, considering the relationship between blood pressure and enzymatic activities of 3β-HSD and G-6-P DH. Rubbin and his collaborators$^{21,25,22}$ reported the distribution of the 3β-HSD in adrenals in 1957. From the early observation has

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Fig. 11. Cells of the zona glomerulosa showed marked enzymatic staining as well as that in Fig. 10. (G-6-P DH, ×400).

The subject is a female with clinical evidence of Gushing’s syndrome.

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III DISCUSSION

The true cause for hypertension is obscure even today. It will be clarified simply from studies on the renin-angiotensin-aldosterone system. If an increase in activity of this system was cause of hypertension, it would be observed in every type of hypertension.

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sprung the clear recognition of an increase in this enzymatic activity in the zona glomerulosa and zona fasciculate of the human adrenals\textsuperscript{19}. These enzymes in the zona fasciculata belong to the system of NADP-dependent dehydrogenase and are respectively increased in the soluble component of cells.\textsuperscript{9} Accordingly, 3 \( \beta \)-HSD is an enzyme spread diffusely within the cell, transmits hydrogen of the donor to tetrazolium through NADP dehydrogenase (TPN diaphorase), and forms formazan by reducing tetrazolium\textsuperscript{14}). Therefore it might be considered to be a NADP-donor-tetrazolium reductase.\textsuperscript{14,15)}

On the other hand, it is well known that G-6-P DH converts G-6-P into 6-P glucuronic acid in the pentose cycle, and that this acid changes into ribose-5-phosphate, which participates in the synthesis of RNA. It is presumed that NADPH (TPNH), produced in the pentose cycle, is utilized in the process of hydroxylation. Furthermore, aldosterone accelerates the synthesis of RNA in the nucleus of the epithelial cell. RNA acts as messenger and stimulates the synthesis of the enzyme which takes part in the supply of energy required for the transportation of sodium. Edelman\textsuperscript{8,10) suggested that an action of aldosterone through enhancing sodium entry from urine into the transport system is eminently reasonable in the light of his concerts of the sodium transport system, and proposed a hypothesis that aldosterone might give energy to that a Na pump by the aid of RNA. On the other hand, it can be stated that for most of the enzymes studied their concentrations is maximal in the regions where it can be presumed that their role in steroid hormone synthesis would be greatest. So it seemed necessary for the authors to study not only 3 \( \beta \)-HSD but also G-6-P DH in the cells synthetizing aldosterone. These studies were actually conducted and gave results shown in Fig. 6,7,8. The cases of increased enzymatic activities, observed by the authors, were found mostly in primary aldosteronism and renal hypertension. Besides, they revealed high amount of urinary aldosterone excretion. These results are of interest when compared with the morphological changes of the zona glomerulosa\textsuperscript{18). It is presumed that histochemical changes may possibly precede morphological changes.

REFERENCES