HISTOLOGICAL, HISTOCHEMICAL AND MORPHOMETRIC CHANGES OF THE CONTRALATERAL ADRENAL GLAND AFTER UNILATERAL ADRENALECTOMY

DR: SOAD EL-SAYED DR: SAMIR N, IBRAHIM DR: MOHAMED K, TAWFIK DR: EL-ARIAN Y: EKLADIOUS

"FROM HISTOLOGY AND ANATOMY DEPARTMENTS, FACULTY OF MEDICINE, AIN-SHAMS UNIVERSITY"

ABSTRACT

Three weeks after unilateral adrenalectomy, the adrenocortical thickness was increased, the lipid content of the cortical zones was markedly decreased, the alkaline phosphatase reaction of the outer part of the zona fasciculata was decreased, the acid phosphatase activity showed an increased reaction in zona glomerulosa and outer part of the zona fasciculata and the succinic dehydrogenase activity was increased in the zona fasciculata and zona reticularis.

Eight weeks after the operation, the hypertrophied zones regained their normal thickness, the fasciculata cells with their remarkable accumulation of fat droplets reappeared. The phosphatase activity (acid and alkaline) regained its normal intensity seen in the control sections, and the reaction of succinic dehydrogenase enzyme was similar to that of the control.

In the contralateral adrenal medulla the pattern of distribution of adrenaline and noradrenaline secreting cells was not affected by the operation.
INTRODUCTION

The changes in the contralateral adrenal gland after unilateral adrenalectomy were extensively studied by a number of investigators in the acute, subacute and chronic cases up to 7 days after the operation (Omoto, 1969). In this study we are going to extend the work to see the changes that occur after 3 weeks from the operation in order to know more details about the prolonged effect of unilateral adrenalectomy on the contralateral adrenal.

MATERIAL and METHODS

Thirty adult male albino rats were used in this study.

They were classified into two groups of animals; a control group and an experimental group to which unilateral adrenalectomy was performed. The animals were sacrificed 3 and 8 weeks after operation and the glands were dissected. Some of the adrenal glands from both groups were used for paraplast embedding and serial sections (4-6 µm thick) were stained with haematoxylin and eosin and amoniacal silver nitrate intensification of the chromaffin reaction for the demonstration of adrenaline and nor adrenaline secreting cells (Culling, 1974).

Fresh cryo-cut sections were obtained from both the control and the experimental group and used for demonstration of the following:

a) Lipid by Sudan III stain.

b) Alkaline phosphatase by using the calcium phosphate method of Gomori.

c) Acid phosphatase by using the azo dye coupling method (Bancroft, 1975)

d) Nachla’s technique for succinic dehydrogenase.

The thickness of the different cortical zones was measured in both experimental and control sections stained with haematoxylin and eosin. The results were calculated and statistically analysed and the final results were obtained by the computer.
RESULTS

(A) Histological results:

(I) CONTROL GROUP:

The gland was covered by a relatively thick connective tissue capsule. Each gland consisted of an outer cortex and an inner medulla. The suprarenal cortex consisted of three concentric zones. Immediately beneath the capsule was the zona glomerulosa; this was a thin layer composed of clusters of columnar cells with deeply stained nuclei and the cytoplasm containing some lipid droplets. A subglomerular sudanophilic zone was seen. Next was the zona fasciculata, which was a thick layer composed of radially arranged columns of cells.

The cells were polyhydral with basophilic cytoplasm rich in lipid droplets (Figs. 1 & 2). The innermost layer was the zona reticularis, which consisted of an anastomosing network of cells adjacent to the medulla. Their cytoplasm was acidophilic of few lipid droplets.

The suprarenal medulla was composed of groups of epithelial cells supported by a delicate connective tissue and separated by blood sinusoids. A few parasympathetic ganglion cells were also present.

(II) EXPERIMENTAL GROUP:

Three weeks after unilateral adrenalectomy the adrenocortical thickness was increased (Table 1). No significant changes could be observed in the thickness of the zona glomerulosa. The most prominent alteration encountered was in
### Table. (1)
Adrenocortical thickness in control and experimental groups

(\textit{valus are the means in um \pm S.E.})

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>3w</th>
<th>8w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>536.41</td>
<td>1060.3\textsuperscript{0}</td>
<td>556</td>
</tr>
<tr>
<td>±</td>
<td>18.83</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>±</td>
<td>32.45</td>
<td>±</td>
<td>21.31</td>
</tr>
</tbody>
</table>

(\(p > 0.001\))

### Table. (2)
The thickness of adrenocortical zones in control and experimental groups

(\textit{valus are the means in um \pm S.E.})

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>3W</th>
<th>8W</th>
</tr>
</thead>
<tbody>
<tr>
<td>zona glomer.</td>
<td>49.3 ± 1.89</td>
<td>53.1 ± 4.88</td>
<td>51.23 ± 3.21</td>
</tr>
<tr>
<td>zona fascic.</td>
<td>305.11 ± 19.31</td>
<td>629.49 ± 7.21\textsuperscript{0}</td>
<td>314.23 ± 17.32</td>
</tr>
<tr>
<td>zona reticu.</td>
<td>183.14 ± 9.36</td>
<td>381.56 ± 28.32\textsuperscript{0}</td>
<td>194.61 ± 11.25</td>
</tr>
</tbody>
</table>

(\(p > 0.001\))
the zona fasciulata. The depth of the zona fasciulata was double that of the control (Table 2), with general compactness of their cells due to decrease of lipid (Fig. 3) Also the zona reticularis showed an increase in thickness and the lipid vacioles were reduced when compared with the control.

In general the lipid content of the cortical zone was markedly decreased. The sudanophobic zone disappeared (Fig. 4).

Eight weeks after unilateral adrenalectomy, the hypertrophied zones regained their normal thickness and their lipid content and became non-significantly different from that of the control.

(B) Histochemical results:

(I) CONTROL GROUP:

In control group the capsule of the adrenal gland showed negative alkaline phosphatase reaction. The zona glomerulosa always gave a stronger alkaline phosphatase reaction than that of the fasciulata and reticularis. In the glomerulosa, the strong alkaline phosphatase activity was noticed in the endothelial cells lining the blood capillaries.

The parenchymatous cells showed a weak to moderate reaction. In zona fasciulata and reticularis a moderate reaction was observed in the blood capillaries, while a weak reaction was noticed in the parenchymatous cells (Fig. 5) The reaction of the nuclei varied, some showed weak reaction and others had a moderate reaction.

In the control group a negative acid phosphatase reaction was seen in the capsule. A moderate reaction was observed in the zona glomerulosa and the outer part of the zona fasciulata. The inner part of the zona fasciulata and zona reticularis showed a faint acid phosphatase reaction (Fig. 6)

In control group the capsule of the adrenal gland did not show succinic dehydrogenase activity. The zona glomerulosa revealed a moderate succinic dehydrogenase reaction.

A strong reaction was observed in the zona fasciulata and reticularis (Fig. 7).

(II) EXPERIMENTAL GROUP

Three weeks after unilateral adrenalectomy the alkaline phosphatase reaction was decreased in the outer part of the zona fasciulata (Fig. 8). The acid phosphatase re-
action of the zona fasciculata (particulary its outer part) and zona glomerulosa was increased (Fig. 9). The succinic dehydroge-
nase activity was markedly in-
creased in the inner part of the zona
fasciculata and zona reticularis
(Fig. 10).

Eight weeks after the operation
the phosphatase activity (acid and
alkaline) regained their normal pat-
tern of distribution seen in the con-
trol group. The reaction of the suc-
cinic dehydrogenase was similar to
that of the normal section.

(III) ADRENAL MEDULLA :

In the control group with the use
of ammoniacal silver nitrate method
for staining the adrenal medulla, the
noradrenaline cells were stained
dark brown while the adrenaline
cells took a yellow colour. The nor-
adrenaline cells were found in
groups scattered between the lighter
adrenaline cells (Fig. 11).

At 3 weeks after the operation
no evident changes could be ob-
served in the pattern of adrenaline
and noradrenaline (Fig. 12).

DISCUSSION

The trophic effect of the unilater-
al adrenalectomy on the cortical
thickness could be attributed to
stress or increased ACTH secre-
tion. Miller (1953), demonstrated
an increase in cortical thickness
follwing ACTH administration.

Following stress or ACTH se-
cretion the lipid content of the corti-
cal zones decreased and as stress or
hormonal level diminished the lipid
content was restored and the corti-
cal cells changed from compact to
clear. Clik and Ochs (1955), ob-
served loss of cholestrol in the ad-
renal cortex after stimulation by
ACTH or stress condition. Since
cholestrol is the main precursor of
the steroid hormone biosynthesis it
can be assumed that lipid depletion
from adrenal cortexmay indicate an
increased functional activity. How-
ever Fortier et al. (1950), stated
that the loss of cholestrol from the
adrenal may be unaccompanied by
loss of other lipids.

Three weeks after the operation
the alkaline phosphatase reaction
was decreased in the outer part of
the zona fasciculata. Since the alka-
line phosphatase was mainly dis-
tributed in the endothelium of the
blood capillaries, it might play a
role in the transport of secretion
from the cells to the blood. According to the result of Clik and Ochs (1955), the cholesterol ester disappeared completely from the outer part of the fascicular zone following stimulation by ACTH or stress condition. From this data we could attributed the loss of the alkaline phosphatase activity from the outer part of the zona fasciculata to loss of cholesterol ester and subsequently decreased secretory activity.

In the present study we noticed an increase in acid phosphatase reaction 3 weeks after the operation in the zona glomerulosa and zona fasciculata. Mietkiewski et al. (1970) attributed this enzymatic activity to the increase in secretory activity of the cells. The acid phosphatase is considered as a component of the content of lysosomes, so we could suggest that the lysosomal system could play a role in the synthesis or the release of cortical hormones.

In the present study, unilateral adrenalectomy increased the succinic dehydrogenase reaction in the zona fasciculata and zona reticularis. This activity represent the mitochondria content and subsequently the secretory activity of the cells. This data was confirmed by the data obtained by Kadioglu and Harrison (1971) on their electron microscopic study of the adrenal gland after unilateral adrenalectomy. They observed marked enlargement and increase in number of the mitochondria.

The data of the histochemical studies including decrease of the lipid of the inner zones, and increased acid phosphatase as well as succinic dehydrogenase activity of these zones indicated increased secretory activity of the adrenal cortex 3 weeks after the operation. This increased activity can be explained by increased secretion of ACTH as a compensatory mechanism to the unilateral adrenalectomy.

In the contralateral adrenal medulla 3 weeks after the operation no evident changes could be observed in the adrenaline and noradrenaline secreting cells. However degranulation of these cells in addition to the small granule chromaffin cells was noticed to occur in rat exposed to stress for 8-22 hours by Kobayashi and Serizawa (1979).
REFERENCES


Fig. 1: Part of the adrenal cortex of a control animal showing that the cells of the zona glomerulosa and fasciculata are clear and vacuolated.
(H. & E. X 100)

Fig. 2: Part of the adrenal cortex of a control animal showing that the zona fasciculata was heavily loaded with lipoid droplets. Notice the subglomerular sudanophobic zone.
(Sudan III X 100)

Fig. 3: Part of the adrenal cortex of an experimental animal 3 weeks after adrenalectomy. The cells of zona glomerulosa and fasciculata showed general compactness due to depletion of lipid.
(H. & E. X 100)

Fig. 4: Part of the adrenal cortex of an experimental animal 3 weeks after adrenalectomy showing that the lipid content of the cortical zones was markedly decreased. The sudanophobic zone disappeared.
(Sudan III X 100)
Fig. 5: Part of the adrenal cortex of a control animal showing strong alkaline phosphatase reaction in the zona glomerulosa.

(Gomori method X 100)

Fig. 6: Part of the adrenal cortex of a control animal showing a moderate acid phosphatase activity in zona glomerulosa and outer part of zona fasciculata.

Notice the intense acid phosphatase reaction in the medulla.

(Azo dye coupling method X 100)
Fig. 7: Part of the adrenal cortex of a control animal stained for succinic dehydrogenase activity. Notice the strong reaction in the zona fasciculata and reticularis. The zona glomerulosa showed a less intense reaction.

(Nachla's technique X 250)

Fig. 8: Part of the adrenal cortex of an experimental animal 3 weeks after adrenalectomy showing diminished alkaline phosphatase reaction in the outer part of the zona fasciculata.

(Gmori method X 100)
Fig. 9: Part of the adrenal cortex of a control animal showing a marked increased acid phosphatase reaction in the zona glomerulosa and outer part of the zona fasciculata.

(Azo dye coupling method X 100)

Fig. 10: Part of the adrenal cortex of an experimental animal 3 weeks after adrenalectomy showing a marked increase in succinic dehydrogenase reaction of the inner part of zona fasciculata and reticularis.

(Nachla's technique X 250)
Fig. 11: Adrenal medulla of a control animal stained with ammoniacal silver nitrate method. Notice the dark noradrenaline secreting cells and the pale adrenaline secreting cells.

(X 100)

Fig. 12: Adrenal medulla of an experimental animal 3 weeks after adrenalectomy stained with ammoniacal silver nitrate. The pattern of reaction was non significantly different from that of the control.

(X 100)
دراسة هستولوجية وهيستوكيميائية وتقييم
تأثير استنصال احدي الفخذين الكليتين على الفخذ الآخر

بحث مقترح من
سمير نوروز إبراهيم محمد
كامل ترابيق
المريان يوسف
من أقسام الهستولوجيا والتشريح - كلية الطب - جامعة عين شمس

تتم دراسة تأثير استئصال احدي الفخذين الكليتين على الفخذ الآخر بعد ثلاثين وثمانية أسابيع من استئصالهما باستخدام الطرق الهستولوجية والهستوكيميائية وقد تم قياس سمك الفصيرة الكريستن بعد استئصال الفخذ الآخر وقد لوحظ زيادة في السمن بمقدار مرتين من القيمة الأصلية ولاحظ ان المنطقة الحزمة والمنطقة
الشبكية كانتا مستوثقتين تماماً على هذه الزيادة في السمن.

بعد ثلاثين وثمانية من العملية لوحظ استئناف الاحتواءات الدهنية في المنطقة الحزمة في المجموعة المختبرة.
وفي نفس الوقت لوحظ تقلص في نشاط الفوسفاتيزات القاعدية في المنطقة الحزمة كما لوحظ زيادة نشاط الفوسفاتيز السكيتي في المنطقة الحزمة أما ذا نشاط السكستيك ديهيدروجينيز فقد لوحظ ازدياد كافأ
التفاعل في المنطقة الحزمة ونقطة الشبكية.

وبعد ثلاثين وثمانية من العملية رجح أن القشرة الكريستنية استعاد سمكها الطبيعي تقريباً كما استعاد الفوسفاتيز القلوي والحامضي توزيعهم الطبيعي في الفخذ الأخرى وتناقص نشاط السكستيك ديهيدروجينيز
في المنطقة الحزمة والشبكية لعدمها الطبيعي.