EFFECT OF HYPOPHYSECTOMY AND A LOW SODIUM DIET ON THE VOLUMES OF THE GLOMERULAR ZONE, FASCICULAR AND RETICULAR ZONES AND MEDULLA OF RATS

By
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ABSTRACT

Direct measurements of volume of the glomerular zone, fascicular and reticular zones and medulla were made in normal and hypophysectomized rats. All zones of the cortex and the medulla atrophied after hypophysectomy. However, while medullary and glomerular atrophy was proportional to the decrease in body weight, fascicular atrophy exceeded the loss in body weight.

Hypertrophy of the glomerular zone occurred in rats fed a sodium deficient diet for 5 to 28 days beginning 14 days after hypophysectomy. Hence the volume of the glomerular zone is probably independent of the pars distalis, since the glomerular zone does not atrophy after hypophysectomy and can be stimulated to hypertrophy after it and the infundibular process are removed.

Calculation of mass from areas in a mid-section by the Bahn formula and from direct measurements of volume yielded similar values for the mass of the fascicular zone. However, mean values for glomerular mass and for medullary mass were each significantly different. None the less, there was a high correlation between values, and the percent changes in mass of the glomerular zone resulting from the sodium deficiency were similar. Hence, the Bahn formula seems to be a useful shortcut procedure for detecting changes in the mass of the several regions of the cortex.

Studies of the effect of various experimental procedures on the mass or volume of the several zones of the adrenal cortex and the medulla are few, because of the difficulties in making these measurements. Eränkö (1954) showed that the formula for the volume of a rotation ellipsoid based on measurements in a mid-section yields inaccurate results. He also reviewed other attempts to estimate size of adrenal zones. More recently Bahn et al. (1960) proposed a formula for the mass of adrenal zones based upon measurements in an adrenal

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section. The present report compares the mass of the several adrenal regions as determined from the volumes in completely sectioned adrenals with that calculated from the mid-section according to the formula of Bahn. The amount of cortex and medulla was measured in hypophysectomized rats and in rats fed a sodium deficient diet, since these procedures are said to cause atrophy and hypertrophy, respectively, of the glomerular zone.

MATERIAL AND METHODS
Young rats of the Holtzman strain were employed throughout. Adrenals were preserved in Zenker-formol fixative, sectioned completely at 10 µ and stained in hema-toxylin and eosin.

The right adrenals were used for measurements. The first and every tenth section thereafter was projected at a linear magnification of 46.5 diameters and traced. In these, the area of the medulla, the fascicular and reticular zones together as a unit, and the glomerular zone was measured with a planimeter, summated, and actual volumes calculated. Where desired, the mass of the several zones was calculated from the volume percents and the weight of the adrenals.

In calculating mass according to the Bahn procedure, linear measurements were made along the major and minor axis in the largest transverse section of each adrenal. Thus, four measurements were made and a mean determined; Bahn et al. (1960) had made ten.

These same tracings of mid-sections were also used to calculate the volume, presuming the adrenal to be a rotation ellipsoid.

RESULTS
1. Effect of hypophysectomy and a sodium deficient diet

The volumes of the several divisions of the adrenal of normal rats and of rats hypophysectomized for as long as 63 days are shown in part A of Table I. Both sexes were used and in equal number. Normal rats were killed at the same time as the hypophysectomized rats, excepting only at the 41 day interval. The data indicate that the actual volume of all zones of the cortex and of the medulla decreased after hypophysectomy. However, while the atrophy of the glomerular zone and medulla was strictly proportional to the decrease in the weight of the body, atrophy of the combined fascicular and reticular zones was proportionally greater. When corrected to volume per 100 g of body weight there was no significant change in the volume of the glomerular zone or medulla; the fascicular and reticular volume, on the other hand, was diminished.

The effect of the sodium deficient diet in hypophysectomized male rats is reported in Table 1 part B. In this experiment all rats ate the standard complete diet for 14 days following hypophysectomy (pars distalis and infundibular process). Then the experimental rats were switched to the sodium deficient diet (General Biochemicals, Chagrin Falls, Ohio) for 5, 15 or 28 days, while hypophysectomized controls were continued on the standard diet.

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Table 1.
Effect of hypophysectomy and a sodium deficient diet upon adrenal weight and upon volume of the cortical zones and of the medulla.

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Body wt. g</th>
<th>Adrenal wt. mg</th>
<th>Adrenal volume mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start</td>
<td>End</td>
<td>Abs.</td>
</tr>
<tr>
<td>Normal controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>152</td>
<td>168</td>
<td>38</td>
</tr>
<tr>
<td>Hypophysectomized 5 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>160</td>
<td>136</td>
<td>20</td>
</tr>
<tr>
<td>Hypophysectomized 14 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>142</td>
<td>145</td>
<td>16</td>
</tr>
<tr>
<td>Hypophysectomized 41 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>145</td>
<td>140</td>
<td>13</td>
</tr>
<tr>
<td>Hypophysectomized 63 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>151</td>
<td>138</td>
<td>14</td>
</tr>
<tr>
<td>Normal controls, 63 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>156</td>
<td>306</td>
<td>55</td>
</tr>
</tbody>
</table>

B. Effect of low sodium diet in hypophysectomized rats (males only)

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Body wt. g</th>
<th>Adrenal wt. mg</th>
<th>Adrenal volume mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start</td>
<td>End</td>
<td>Abs.</td>
</tr>
<tr>
<td>Normal control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>142</td>
<td>179</td>
<td>32.5</td>
</tr>
<tr>
<td>Low sodium diet 5 days; hypophysectomized, 14–19 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont. 4</td>
<td>149</td>
<td>132</td>
<td>12.7</td>
</tr>
<tr>
<td>Low Na 4</td>
<td>144</td>
<td>121</td>
<td>16.3</td>
</tr>
<tr>
<td>Low sodium diet 15 days; hypophysectomized 29 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont. 2</td>
<td>149</td>
<td>145</td>
<td>11.0</td>
</tr>
<tr>
<td>Low Na 2</td>
<td>147</td>
<td>123</td>
<td>14.5</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low sodium diet 28 days; hypophysectomized 43 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont. 3</td>
<td>144</td>
<td>136</td>
<td>10.7</td>
</tr>
<tr>
<td>Low Na 4</td>
<td>145</td>
<td>117</td>
<td>13.5</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*%/g means per 100 g of body weight.

As in the previous experiment, actual adrenal weight decreased progressively in hypophysectomized controls at 14, 29 and 43 days after operation, as did
the volume of all cortical zones and the medulla. Again, only the atrophy of
the combined fascicular and reticular zones was proportionally greater than
the loss of body weight. There was no atrophy of the glomerular zone or the
medulla when these volumes were standardized per 100 g of body weight.

The adrenals of the sodium deficient groups were heavier than those of
controls (Table 1 B). Measurements showed no differences between controls
and experimental groups in the volumes of the medulla, fascicular and reticu-
lar zones. The volume of glomerular zone of the experimental rats, however,
was consistently greater than that of the hypophysectomized controls
after 5, 15 and 28 days on the low sodium diet, both actually and per 100 g
of body weight.

2. Comparison of direct and estimated calculations of mass

The thirty-five normal and hypophysectomized control male rats of the
experiments reported in Table 1 provided the data for the comparison of
values for the masses of the adrenal zones as calculated from direct measure-
ments of volume and as estimated by measurements in the mid-section. Mass
as calculated from the volume percent is plotted along the abscissa in Fig. 1
and labeled volumetric determination. Mass, as estimated from the areas in
the mid-section according to the formula of Bahn et al. (1960), is plotted
along the ordinate as areal determinations.

Fig. 1 B shows that the values for the fascicular zone were closely clustered
along the regression line; the coefficient of correlation was 0.99 and the value
for P in the comparison of the two means was 0.26. It seems, therefore, that
the two procedures yielded similar values for the mass of the fascicular and
reticular zones.

The points for the mass of the glomerular zone were not as clustered along
the regression line, but the coefficient of correlation was 0.85. Moreover, the
mass of the glomerular zone was always greater in the volumetric determina-
tions than in the areal determinations. The P value of 0.001 in the comparison
of the two means shows that the two procedures yielded values for the mass
of the glomerular zone which were significantly different.

With respect to medulla, the two methods of calculation also did not yield
the same values, but here the volumetric determinations of medullary mass
were consistently less than those estimated by the areal formula (Fig. 1 C). The
correlation coefficient, however, was 0.80.

The appropriate measurements to permit the calculation of the volume of a
rotation ellipsoid were also taken from the mid-sections of the rat adrenals
of Fig. 1. The volumes calculated from these measurements were much less
than the volumes obtained from direct measurements of serial sections. Plots
of the data showed that there was no more than a poor correlation between
the two sets of values.

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Comparisons of values for the masses of the cortical zones and the medulla as calculated from their volume percents and as estimated from their areas in a mid-section according to a formula developed by Bahn et al. (1960) in a series of intact and hypophysectomized rats.

Even though the volumetric and areal methods yielded different values for the mass of the glomerular zone, the regression line and correlation coefficients suggested that the Bahn formula might be useful for detecting experimental changes in the glomerular zone when it was not practical to employ the tedious method of planimetric measurements in complete serial sections. Accordingly, in order to test this, the data obtained in the low sodium experiment were used to calculate mass of the glomerular zone as determined by the volumetric as well as the areal method and the percent changes calculated. It appears (Table 2) that despite the differences in the actual values for the mass of the glomerular zone, the percent increase in glomerular mass resulting from the low sodium diet was approximately the same.
Table 2.

Mass (mg) of the glomerular zone per 100 g of body weight as calculated by two different procedures and the percent change in hypophysectomized rats fed a sodium deficient diet.

<table>
<thead>
<tr>
<th>Method of calculation</th>
<th>Low Na, 5 days</th>
<th>Low Na, 14 days</th>
<th>Low Na, 28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Exp.</td>
<td>% Change</td>
</tr>
<tr>
<td>Volumetric</td>
<td>1.41</td>
<td>2.05</td>
<td>+45</td>
</tr>
<tr>
<td>Areal</td>
<td>1.07</td>
<td>1.54</td>
<td>+44</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The effect of the pituitary gland, if any, upon the glomerular zone has become of some interest in connection with the larger problem of the regulation of secretion of aldosterone from that zone.

Swann (1940) originally postulated that the glomerular zone was independent of hypophyseal control, a conclusion also reached by Deane & Greep (1946) despite their finding that cells in the glomerular zone atrophied in hypophysectomized rats. However, Lane & de Bodo (1952) reported atrophy of the glomerular zone in long-term hypophysectomized dogs as have Bahn et al. (1960). The present observations, in which no change in glomerular volume per 100 g of body weight occurred in rats hypophysectomized for as long as 63 days, stand in variance with the earlier observations. The reason for this discrepancy is not obvious. Quite aside from a possibility of there being species differences in dog and rat, it might be important that the extent of the hypophysectomy was not identical. While dogs were completely hypophysectomized (Lane & de Bodo 1952) or nearly completely hypophysectomized (only a part of the pars tuberalis remained in the Bahn study), the infundibular stalk and pars tuberalis were intact in the present experiments and in the experiments of others employing rats. In an earlier study (Davis et al. 1957), Bahn found no decrease in the width and histology of the glomerular zone of hypophysectomized dogs. Microscopic remnants of the pars tuberalis were present in all of these dogs. Recently, the pars tuberalis was suggested as a possible source of growth hormone in otherwise hypophysectomized guinea pigs (Clayton & Worden 1960) to account for the slow growth observed in this animal and for the absence of the adrenal atrophy generally observed.

With respect to hypertrophic responses in the glomerular zone there are two problems: whether ACTH stimulates this zone directly and whether the zone can be stimulated in the absence of the pituitary gland and of exogenous ACTH. Long-term injections of large amounts of ACTH certainly affect the morphology of the glomerular zone (Baker 1952), but it is less certain whether shorter courses and smaller amounts of ACTH do stimulate the glomerular
zone. There is a large literature pro and con in which cytological and histochemical changes are variously interpreted, but the effect of ACTH on volume or mass of this zone has not been determined.

The effect of experimental procedures upon the morphology or size of cells in the glomerular zone of hypophysectomized rats has had slight attention. Deane et al. (1948), on the basis of five experimental rats and one control, reported that a sodium deficient diet stimulated cytochemical changes in the glomerular zone after hypophysectomy. The present work supports their conclusion in that the sodium deficient diet increased the weight of the adrenals of hypophysectomized rats; this gravimetric increase was due to hypertrophy of the glomerular zone.

The changes in volume or mass of the glomerular zone reported here are only in part consonant with changes in aldosterone secretion induced by similar experimental conditions (Davis 1962; Blair-West et al. 1963). The decreased output of aldosterone following hypophysectomy in dogs is consonant with the glomerular atrophy reported by Lane & de Bodo (1952) and Bahn et al. (1960) in dogs but not with the present observations in rats, where there was no glomerular atrophy. An enhanced release of aldosterone during sodium deficiency, however, is consistent with the hypertrophy of the glomerular zone reported here. It is difficult, however, to understand how a zone which is said to atrophy in dogs after hypophysectomy can also be stimulated to release more aldosterone in the absence of the pituitary gland.

Further measurements of changes in the volume or mass of the glomerular zone should help to harmonize the discrepant results of morphologic and physiologic experiments. In this connection, the present study indicates that the Bahn formula more accurately reflects changes in size of the glomerular zone than various other procedures which have been employed where it was impractical to make direct measurements of glomerular size.

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