ADMINISTRATION OF CORTISONE TO THE PREGNANT RAT. EFFECTS ON THE LYMPHOID TISSUE OF THE OFFSPRING

By

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ABSTRACT

The lymphoid tissue of newborn rats from mothers treated during pregnancy by adrenalectomy or various doses of cortisone was studied morphologically. The size of the adrenal glands of the newborn was used as a morphological indicator of the amount of cortisone circulating in the foetal blood. Only the highest dose used – 4.5 mg cortisone daily to the pregnant rat from the 13th day of pregnancy to parturition significantly reduced the size of the thymus in newborn animals. Adrenalectomy induced a slight but significant hyperplasia of the thymus of the newborn. Histologically the thymus cortex appeared to be thinner after the highest dose of cortisone and thicker after adrenalectomy. This high dose of cortisone increased the size of the spleen of the newborn but no definite histological changes were observed in any of the experimental groups as compared with the spleen of the controls. It is concluded that the rat foetal lymphoid tissue is less sensitive to cortisone than that of the adult rat.

Previous studies suggest that the lymphoid tissue of the foetal rat is remarkably resistant to corticosteroids. Thus no significant morphological changes were demonstrated in the thymus of the newborn of alloxan diabetic rats which probably had high levels of circulating corticosteroids obtained from the mother rat (Angervall 1959) or in the thymus and spleen of newborn animals of formalin stressed rats (Schnirer 1963). Nor did adrenalectomy or hypophysectomy of the pregnant rat significantly influence the thymus volume of the newborn (Angervall 1962; Angervall & Lundin 1963). On the other hand, Hultquist (1950) noted significant increases of so-called relative thymus and adrenal volumes in the newborn of pancreatectomized diabetic rats. Fur-
thermore Engfeldt & Hultquist (1953) demonstrated similar changes in the foetal thymus after STH administration to the pregnant rat.

In preliminary studies it was found that corticosteroids can modify the growth of the foetal thymus and spleen (Angervall & Lundin 1964). In the present paper further experiments are presented which demonstrate effects on the foetal thymus and spleen of cortisone given in various doses to the pregnant rat.

MATERIAL AND METHODS

The experiments were performed on albino rats, pregnant for the first time and of the same Wistar strain, which have been bred for many years at the pathological institute. They were divided into the following groups:

C group: 10 control rats, i.e. untreated rats.
A group: 6 rats adrenalectomized on the 12th day of pregnancy.

In the three following groups cortisone was given twice daily intramuscularly from the 13th day of pregnancy to parturition.

Co1 group: 7 rats given 0.5 mg cortisone daily.
Co2 group: 6 rats given 1.5 mg cortisone daily.
Co3 group: 7 rats given 4.5 mg cortisone daily.

When the females had been mated they were inspected twice daily, mornings and afternoons, to see whether a vaginal plug had formed. The presence of such a plug was taken as evidence that conception had occurred. Adrenalectomy was performed by the dorsolumbar approach. Throughout the experimental period the rats were fed a special rat bread (for composition see Angervall 1959, p. 12) and drank tap water ad libitum, except those in the A series which after adrenalectomy drank freely of physiological saline.

If not born spontaneously on – or before – the 22nd day of pregnancy, the young were removed at the end of this day, so as to allow comparison after the same gestation period. Immediately after parturition, the young were weighed, decapitated and immersed in 10 per cent formaldehyde solution. In order to promote fixation of internal organs, the abdomen and thoracic cavity were opened before the body was immersed into the fixative. Shortly after parturition, the mother rats were killed by decapitation. The lymphoid organs and the adrenal glands were excised, immediately weighed and fixed in 10 per cent formaldehyde solution.

After fixation for a week, the adrenal glands, thymus and spleen of the young were excised and trimmed under a dissection microscope. The organs were weighed in a bottle containing formaldehyde solution. The organ was withdrawn from the bottle, which was then weighed again, the difference between the weights of the bottle with and without the organ representing the weight of the organs.

The histology of spleen, thymus and lymph nodes were studied on specimens from single animals from each litter. The cytology of the thymus, the thickness of its cortex, the development of follicles and the amount of myeloid tissue in the spleen were estimated on coded slides by both authors separately.

Fatty tissue from the neck, axillae and hepatic hilus of the newborn animals was dissected out and prepared in the usual manner for microscopical examination.
Statistics

Means and standard errors of mean for organs of the mothers and newborn and birth weights as well as the regression of the organ weight and birth weight of the newborn were computed for each group. The regression was used for comparing the organ weights in the groups at equal birth weights. The comparison was performed at the birth weight \( \frac{1}{2} (\bar{x}_o + \bar{x}_\epsilon) \) where \( \bar{x}_o \) denotes the mean birth weight in the experimental group and \( \bar{x}_\epsilon \) that of the control group. If the regression is written

\[
\hat{y}_x = a + b (x - \bar{x})
\]

where \( \hat{y}_x \) is the mean weight of organ weights and \( \bar{x} \) is the mean birth weight within the group, then the standard error of the expected value will be

\[
s_{\text{ew}} = \frac{s_{yx}^2}{n} + s_b^2 (x_i - \bar{x})
\]

where \( s_{yx} \) is the residual variation and \( s_b \) the standard error of the regression coefficient \( b \). The difference between the expected organ weight values in the experimental groups and the control group is t-tested in the usual manner with \( n_1 + n_2 - 4 \) degrees of freedom.

RESULTS

Mothers. (See Table 1). The body weight increase in the A, Co1 and Co2 groups is less than in the C group. In the Co3 group there is a body weight decrease. The adrenal weights are lower in the cortisone treated groups and lowest in the group with the highest cortisone dose. A marked and significant increase in thymus and spleen weights is found in the A group as compared with the C group. All cortisone groups show significant thymus involution, the larger the dose the more marked the involution. In these groups too a decrease in the weight of the spleen is observed, though this is not statistically significant.

Offspring. Birth weights, weights of the adrenal glands, thymus and spleen are given in Table 2. In the cortisone groups there is a gradual decrease in birth weight with increasing doses of cortisone.

The correlation between organ volume and birth weight was analyzed. Significant correlations were demonstrated for the adrenal glands, thymus and spleen in all groups.

The regressive relations between the adrenal glands and birth weights are given in Fig. 1. The adrenal weight in the A group is significantly higher in the Co1 and Co3 groups and significantly lower than in the C group after correction for the difference in birth weight \( (P < 0.001 \) for all t-tests). The regressive relation between the thymus and the birth weights are given in Fig. 2. By comparison at equal body weight there is a significantly higher thymus weight in the A group than in the C group \( (t = 2.56; P < 0.02) \). No difference is demonstrated between the Co1 group and the control group after correction for the difference in birth weight \( (t = 0.04) \). By comparison at equal body weights, there is a decrease which is, however, not statistically significant.
Table 1.
Weights of the body, adrenals, thymus and spleen of the mother rats (means ± standard error of the means).

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Body weight at conception (g)</th>
<th>Body weight at parturition (g)</th>
<th>Absolute organ weight (mg)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Adrenals</td>
</tr>
<tr>
<td>C</td>
<td>9</td>
<td>194 ± 6</td>
<td>213 ± 8</td>
<td>58.5 ± 3.5</td>
</tr>
<tr>
<td>A</td>
<td>8</td>
<td>195 ± 11</td>
<td>206 ± 17</td>
<td>104 ± 9</td>
</tr>
<tr>
<td>Co1</td>
<td>6</td>
<td>190 ± 10</td>
<td>201 ± 13</td>
<td>46.4 ± 3.5</td>
</tr>
<tr>
<td>Co2</td>
<td>5</td>
<td>188 ± 7</td>
<td>192 ± 10</td>
<td>48.3 ± 3.7</td>
</tr>
<tr>
<td>Co3</td>
<td>7</td>
<td>188 ± 6</td>
<td>170 ± 7</td>
<td>42.8 ± 4.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Spleen</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td>718 ± 82</td>
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<td></td>
<td>1108 ± 121</td>
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<td></td>
<td>508 ± 40</td>
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<td></td>
<td>612 ± 48</td>
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<td></td>
<td></td>
<td></td>
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<td>520 ± 61</td>
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</tbody>
</table>
Table 2.
Weights of the body, adrenals, thymus and spleen of the newborns (means ± standard error of the means).

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of young</th>
<th>No. of litters</th>
<th>Weights of newborns (g)</th>
<th>Absolute organ weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean birth weight</td>
<td>Mean litter weight</td>
</tr>
<tr>
<td>C</td>
<td>58</td>
<td>9</td>
<td>4.79 ± 0.07</td>
<td>4.93 ± 0.17</td>
</tr>
<tr>
<td>A</td>
<td>58</td>
<td>8</td>
<td>4.71 ± 0.08</td>
<td>4.88 ± 0.21</td>
</tr>
<tr>
<td>Co₁</td>
<td>41</td>
<td>6</td>
<td>5.20 ± 0.06</td>
<td>5.24 ± 0.11</td>
</tr>
<tr>
<td>Co₂</td>
<td>33</td>
<td>5</td>
<td>5.08 ± 0.08</td>
<td>5.07 ± 0.11</td>
</tr>
<tr>
<td>Co₃</td>
<td>59</td>
<td>7</td>
<td>4.62 ± 0.04</td>
<td>4.63 ± 0.06</td>
</tr>
</tbody>
</table>
Fig. 1.
The regression of adrenals' weight (mg) on body weight (g) in control and experimental groups.

C  \( y = 0.3448 x + 1.530, \quad s_b = 0.1191 \)

A  \( y = 0.4354 x + 1.931, \quad s_b = 0.1179, \quad S_w, \) for C group 0.0649 for A group 0.0709 \( P \) for \( Y_A - Y_C < 0.001 \)

Co1  \( y = 0.5382 x + 1.530, \quad s_b = 0.1271, \quad S_w, \) for C group 0.0700 for A group 0.574 \( P \) for \( Y_C - Y_{Co1} < 0.001 \)

Co2  \( y = 0.2405 x + 1.111, \quad s_b = 0.1772, \quad S_w, \) for C group 0.0665 for A group 0.0803 \( P \) for \( Y_C - Y_{Co2} < 0.001 \)

Co3  \( y = 0.2868 x + 0.1880, \quad s_b = 0.1177, \quad S_w, \) for C group 0.0657 for A group 0.0402 \( P \) for \( Y_C - Y_{Co3} < 0.001 \)

in the Co2 group \( (t = 1.97; \quad P \sim 0.05) \). A higher, statistically significant decrease \( (t = 9.67; \quad P < 0.001) \) is demonstrated in the Co3 group by comparison at equal body weight.

The regressive relations between the spleen and birth weight are given in Fig. 3. No difference is demonstrated between the spleen weight in the A, Co1 or Co2 groups and the control group. An 11.6 per cent significant increase \( (t = 2.57; \quad P = 0.01) \) of the mean spleen weight is found in the Co3 group as compared with that in the control group group at equal birth weight.

The thymus cortex appeared thinner in the Co3 group and definitely
thicker at least in some newborns of adrenalectomized rats. Thus the difference between the cortisone treated and adrenalectomized animals was very clear. No definite difference in the cytology of the thymus could be observed. In some newborn in the Co group, the medulla seemed to be more crowded with lymphocytes than that of the controls (Figs. 4 and 5).

There was some variation in the follicle development of the spleen in all groups. In some animals no follicles could be observed, in others fairly distinct follicles were seen. These were composed of large lymphoblast-like cells and showed no reaction centres. There seemed to be an inverse relation between the amount of myeloid tissue and the development of follicles: the more developed the follicles the less the amount of myeloid tissue (Figs. 6 and 7).
The regression of spleen weight (mg) on body weight (g) in control and experimental groups.

C: $y = 4.0821 x - 9.093$, $s_b = 0.6065$, for C group 0.2035

A: $y = 3.3797 x - 5.534$, $s_b = 0.3605$, $s_w$, for A group 0.2250

Co1: $y = 2.3920 x - 0.3150$, $s_b = 0.7481$, $s_w$, for Co1 group 0.3386

Co2: $y = 3.8265 x - 7.577$, $s_b = 0.5298$, $s_w$, for Co2 group 0.2403

Co3: $y = 3.3836 x - 4.634$, $s_b = 0.6420$, $s_w$, for Co3 group 0.2198

$P$ for $Y_{Co2} - Y_A < 0.01$

The small lymph nodes seen in the tissue from the neck, axillary tissue and the hepatic hilus all showed an immature lymphoid tissue without any follicles and no difference could be observed between the groups.

DISCUSSION

In the present investigation the changes in the maternal adrenal glands and the thymus indicate that the maternal corticosteroid level is decreased after adrenalectomy and increased after cortisone treatment. The changes in the adrenal weights of the newborn indicate that the foetal corticosteroid blood level parallels that of the mother rat and that corticosteroids are transferred
Fig. 4.
Thymus of a newborn from an adrenalectomized rat. × 93.

Fig. 5.
Thymus of a newborn from a rat treated with largest cortisone dose (C03). The cortex is thinner than in Fig. 4, and the medulla seems to be more crowded with smaller lymphocytes. × 90.

via the placenta. These findings are in agreement with previous observations (ref. see Angervall 1962; Angervall & Lundin 1963).

The effect of cortisone on the thymus and spleen of the pregnant rat is in principle the same as in the male rat. As in Lundin's (1958) experiments on adult male rats, the effect on the thymus, particularly of cortisone, is much more pronounced than that on the spleen.

Adrenalectomy of the mother resulted in a slight hyperplasia of the foetal thymus apparently as a consequence of the reduced amounts of circulating corticosteroids in the foetal blood. However, our observations on the thymus
Fig. 6.
Spleen of a newborn from the control group. A fairly distinct demarcated follicle composed of medium sized lymphocytic cells can be seen. × 225.

Fig. 7.
Spleen of a newborn from the control group. No definite follicles can be seen. The tissue is dominated by erythromyeloid cells with some large megakaryocytes. × 225.

show that only a marked increase in circulating foetal corticosteroids, such as is formed after excessive doses of cortisone to the mother rat, results in hypoplasia of the thymus.

No significant effect on the spleen of the foetus was observed after adrenalectomy in this experiment, but previous observations (Angervall & Lundin 1964) invariably showed hypoplasia of the foetal spleen after adrenalectomy of the mother. This may be analogous to the findings of an increased spleen weight after the largest dose of cortisone in these experiments. At the time of parturition the foetal rat spleen consists essentially of myeloid or erythro-
poid tissue and it is possible that the growth of these tissues are dependent on circulating foetal corticosteroids.

It is concluded that the foetal lymphoid tissue is less sensitive to cortisone than the adult tissue.

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