OVERWEIGHT IN OFFSPRING
OF CORTISONE-TREATED PREGNANT RATS

By
Lennart Angervall and Alf Martinsson

ABSTRACT

Pregnant albino rats were given 0.75 mg cortisone intramuscularly twice daily from the 12th to the 22nd day of pregnancy. The gestation period was 522 hours. The offspring of these rats were significantly heavier and longer than control offspring. The maternal adrenals and thymus were atrophied and the foetal adrenals were hypoplastic owing to the passage of cortisone through the placenta. For chemical analysis the offspring were divided into groups weighing more than and less than 4.50 g. It turned out that the heavier offspring in the cortisone group had significantly more total lipids and neutral fat than the controls at equal body weights. The lighter offspring in the cortisone group had significantly less neutral fat at equal body weights. No significant differences were found in cholesterol, phospholipids and water content at equal body weights. The total nitrogen content was similar at equal body weights and proportional to the weight excess of the offspring in the cortisone group. Possible mechanisms responsible for the overweight are discussed. This mechanism could be similar to that responsible for foetal overweight in diabetic pregnancy.

The pathogenesis of overweight in infants of diabetic mothers is still unsettled. The present investigation is one of a series designed to elucidate why diabetic pregnancy produces overweight offspring.

Overweight offspring of non-insulinized alloxan-diabetic rats have been found to be abnormally long as well as abnormally rich in neutral fat suggesting obesity, but a normal total body water content (Angervall 1959; Angervall et al. 1965). In experiments with subdiabetogenic doses of alloxan to pregnant rats, Lazarow et al. (1960) obtained abnormally heavy offspring.

The significance of the maternal endocrine environment for the foetal growth
both normally and in diabetes is imperfectly understood despite much work. It has been suggested that the pituitary growth hormone (STH) increases the foetal growth but the results of experiments involving administration of STH to pregnant animals have been conflicting (litt. see Angervall & Lundin 1963). Administration of crystalline STH to pregnant rats did not result in significant changes in birth length or weight nor in total body water content compared with these parameters of offspring of hypophysectomized non-STH-treated rats (Angervall & Lundin 1963).

Schnürer (1963) among others found that the offspring of formalin stressed, pregnant rats were abnormally heavy and showed a length increase proportional to their excess weight. With respect to the pathogenesis of overweight in infants of diabetic mothers, his results are especially interesting because enhanced adrenocortical function indicated by maternal adrenal hyperplasia and thymus involution and foetal adrenal hypoplasia may occur both in diabetic pregnant women (litt. see Kyle 1963) and in alloxan-diabetic pregnant rats (Angervall 1959).

There is evidences indicating that maternal adrenal corticosteroids are factors in foetal growth. Indirect evidence is given by the observation that the birth weight of the offspring is reduced following adrenalectomy of the mother (Davis & Plotz 1954; Christiansen & Chester Jones 1957; Angervall 1962). Observing hypoplasia of the pancreatic islets in these offspring, Angervall (1962) suggested that the somewhat lowered birth weight was due to hypoglycaemia associated with adrenalectomy and this might tend to inhibit the production of growth promoting insulin in the foetuses. Several experiments have shown that high doses of cortisone given to pregnant rats inhibit the growth of their foetuses (cf. Mercier-Parot 1957). In experiments with the primary aim of studying the sensitivity of foetal lymphoid tissue to cortisone, Angervall & Lundin (1965) noted a tendency to increased birth weight of offspring of rats given cortisone, 0.5 mg twice daily, during the second half of pregnancy.

In this paper experiments are presented with pregnant rats given relatively small doses of cortisone during the second half of pregnancy resulting in overweight offspring. Endocrine organs of mother and foetuses were studied morphologically as well as the body composition of their offspring by estimating birth length, total water and nitrogen content, and different lipid fractions.

**MATERIAL AND METHODS**

21 newborns from 10 cortisone-treated pregnant rats (Co group) and 22 newborns from 19 controls (C group) were used. Each series was divided into two subgroups,
one with newborns weighing more than 4.50 g and the other with newborns weighing less than 4.50 g (groups Co1, C1, Co2 and C2).

The experiments were performed on unmated female albino rats bred for many years in the Department of Pathology, Gothenburg. The rats were fed commercial rat pellets and tap water. The rats were followed by examination of vaginal smears every morning. When it was evident that a rat was in preoestrus (Long & Evans 1922), it – as a rule together with another rat in preoestrus – was transferred to a cage in which a fertile male rat was allowed to remain between 9 – 9.30 p.m. Conception was assumed to have taken place if next morning's vaginal smear contained spermatozoa.

The pregnant rats were divided into the following groups:

- Controls, i.e. untreated pregnant rats – 19 rats.
- Pregnant rats given cortisone, 0.75 mg twice daily, from the 12th to 22nd day of pregnancy – 10 rats.

If not born spontaneously all the rats were removed on the 22nd day of gestation at 8 p.m., i.e. 522 hours after copulation. Immediately after parturition, the young were weighed and measured for length as described previously (Angervall 1959). Some of them were decapitated, frozen to –20° C and then used for chemical analysis. The remainder were decapitated and, after the large body cavities had been opened, fixed whole.

Shortly after parturition the mother rats were killed by decapitation. The adrenals, thymus, kidneys and spleen were excised at once, weighed and fixed in 10% formalin.

After fixation for 18 days, the adrenals, thymus and spleen of the young were excised, trimmed under a dissection microscope and weighed as described in a previous paper (Angervall & Lundin 1965). For histological examination of the foetal pancreas, 5 µ thick sections were stained according to Weigert-van Gieson.

The frozen carcasses were homogenized in 5 ml of redistilled water for five minutes with a Bühler homogenizer. The homogenization vessel was cooled by running tap water. The homogenate was poured into weighed vessels and the homogenization vessel carefully rinsed with redistilled water and immediately frozen to –40° C, transferred to a freeze-drier for 48 h and further desiccated under vacuum over phosphorus pentoxide to constant weight. The total body content of water then was the difference between the wet weight and the dry weight. From the dried tissue fractions were taken for determinations of lipids and nitrogen. Lipids were extracted from 100 mg dry tissue with 15 ml chloroform – methanol, 2:1 (v/v) at 50° C for one hour. The extract was then processed as described by Folch et al. (1954).

Aliquots of the washed chloroform infranatant were taken for determination of the weight of total lipids, Liebermann – Buchard chromogens (Cramér & Isaksson 1959) and lipid phosphorus (Svanborg & Svennerholm 1961). Cholesterol was estimated as Liebermann – Burchard chromogens, and lipid phosphorus was converted to phospholipids by multiplying by 25. The residual weight after subtraction of the weights of cholesterol and phospholipids was assumed to represent glycerides. Nitrogen was determined by means of the micro-Kjeldahl method as described by Clark (1956).

The statistical methods are the same as in a previous investigation (Angervall et al. 1965).

**RESULTS**

**Mother rats**

Mean conception, parturition, adrenal, thymus and spleen weights for the mother rats are given in Table 1. The rats themselves (without foetuses)
Table 1.
Conceptions weights, parturition weights and organ weights for the mother rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of rats</th>
<th>Body weight at conception (g)</th>
<th>Body weight at parturition (g)</th>
<th>Absolute organ weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Adrenals</td>
</tr>
<tr>
<td>Controls</td>
<td>19</td>
<td>177 ± 3°</td>
<td>196 ± 3°</td>
<td>61.8 ± 2.2°</td>
</tr>
<tr>
<td>Cortisone treated</td>
<td>10</td>
<td>182 ± 3</td>
<td>181 ± 2</td>
<td>50.8 ± 1.8</td>
</tr>
</tbody>
</table>

* Mean and standard error of the mean.

increased significantly in weight, on average 19 g during the pregnancy ($P < 0.001$), while there was no change in body weight of the cortisone treated rats. The mean weights of the adrenals and thymus were significantly lower in the cortisone treated rats than in the controls ($P < 0.001$ and $P < 0.01$). There was a tendency to lower mean spleen weight ($0.05 < P < 0.1$). No rat died during the experiment.

**Offspring**

Birth weights and lengths for the offspring are given in Table 2. The 19 controls gave birth to 169 offspring with a mean birth weight of $4.01 ± 0.04$ g, and the 10 cortisone-treated rats gave birth to 89 offspring with a mean birth weight of $4.41 ± 0.03$ g, the difference between the means being significant ($P < 0.001$). The mean litter weights, $4.02 ± 0.10$ g and $4.42 ± 0.06$ g, respectively, also differ significantly ($P < 0.01$). The differences remain significant after taking into account mother weights and litter sizes, i.e. factors capable

Table 2.
Birth weights, litter weights, birth lengths and litter lengths.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of young</th>
<th>No. of litters</th>
<th>Birth weight (g)</th>
<th>Length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean birth weight</td>
<td>Mean litter weight</td>
</tr>
<tr>
<td>Controls</td>
<td>169</td>
<td>19</td>
<td>$4.01 ± 0.04^*$</td>
<td>$4.02 ± 0.10^*$</td>
</tr>
<tr>
<td>Cortisone treated</td>
<td>89</td>
<td>10</td>
<td>$4.41 ± 0.03$</td>
<td>$4.42 ± 0.06$</td>
</tr>
</tbody>
</table>

* Mean and standard error of the mean.
of influencing birth weight (Angervall 1959). The mean birth length and litter length in the Co group rats were also higher than in the C group \((P < 0.001\) for both differences). All offspring were alive at parturition.

The absolute mean weights of the adrenals, thymus and spleen are given in Table 3. The adrenals were significantly lighter in the Co group \((P < 0.001)\) but there was no difference in thymus and spleen weights after taking into account differences in birth weights.

No qualitative histological difference was observed between pancreatic islets from C offspring and Co offspring. No quantitative morphological analysis, however, was performed.

The total body water content was determined in 23 C offspring and in 20 Co offspring. The mean birth weights for these offspring were 4.35 ± 0.10 and 4.36 ± 0.07 g. The mean water content was 88.10 ± 0.10 and 88.22 ± 0.23%. Thus there was no difference in water content.

Table 4 presents mean and standard error of the mean for the weight of total lipids, phospholipids, cholesterol, neutral fat, total nitrogen and water for the subgroups. Table 4 also gives the mean birth weights and lengths for the samples used for these determinations of weight. The equations for the regressive relationships between nitrogen and the various lipid fractions and birth weight were studied. Statistically significant regression were found only between cholesterol and birth weight in the C01-group \((\bar{y}_x = 3.57 x - 11.24; s_b = 0.83; t_b = 4.30; P < 0.01)\), between nitrogen and birth weight in the C02-group \((\bar{y}_x = 50.55 x - 164.63; s_b = 16.39; t_b = 3.08; P < 0.01)\) and between cholesterol and birth weight in the C2-group \((\bar{y}_x = 2.17 x - 3.20; s_b = 0.45; t_b = 4.83; P < 0.001)\). The relationship between the weights of body contents of nitrogen, total lipids, neutral fat and the birth weights are given in Figs. 1, 2 and 3 with the estimated regression lines.

**Table 3.**

Organ weights in offspring of controls and cortisone treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Absolute organ weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adrenals</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.40 ± 0.10*</td>
</tr>
<tr>
<td></td>
<td>(n = 147)</td>
</tr>
<tr>
<td>Cortisone treated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.60 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>(n = 69)</td>
</tr>
</tbody>
</table>

* Mean and standard error of the mean.
<table>
<thead>
<tr>
<th>Group</th>
<th>Total lipids (mg)</th>
<th>Phospholipids (mg)</th>
<th>Cholesterol (mg)</th>
<th>Neutral fat (mg)</th>
<th>Total nitrogen (mg)</th>
<th>Water content (%/w)</th>
<th>Birth weight (g)</th>
<th>Birth length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co group 1</td>
<td>60.29 ± 5.75</td>
<td>24.21 ± 3.09</td>
<td>6.06 ± 0.40</td>
<td>30.02 ± 3.67</td>
<td>67.06 ± 8.04</td>
<td>87.40 ± 0.47</td>
<td>4.84 ± 0.10</td>
<td>36.00 ± 0.58</td>
</tr>
<tr>
<td>Co group 2</td>
<td>41.94 ± 2.90</td>
<td>19.76 ± 1.35</td>
<td>5.10 ± 0.29</td>
<td>17.08 ± 1.24</td>
<td>50.06 ± 4.02</td>
<td>88.25 ± 0.29</td>
<td>4.24 ± 0.04</td>
<td>55.40 ± 0.37</td>
</tr>
<tr>
<td>C group 1</td>
<td>44.54 ± 3.36</td>
<td>21.64 ± 1.66</td>
<td>6.16 ± 0.14</td>
<td>16.74 ± 3.99</td>
<td>65.44 ± 1.49</td>
<td>88.18 ± 0.17</td>
<td>4.84 ± 0.07</td>
<td>54.56 ± 0.49</td>
</tr>
<tr>
<td>C group 2</td>
<td>45.78 ± 2.01</td>
<td>18.15 ± 1.03</td>
<td>5.67 ± 0.24</td>
<td>22.07 ± 1.63</td>
<td>50.81 ± 3.02</td>
<td>88.02 ± 0.50</td>
<td>4.11 ± 0.07</td>
<td>51.10 ± 0.59</td>
</tr>
</tbody>
</table>

* The groups 1 comprise newborns weighing more than 4.50 g, the groups 2 newborns weighing less than 4.50 g.

† One sample lost.
Groups 1 (birth weights 4.50 g). The mean birth weights for the offspring used for the chemical analysis was 4.84 ± 0.10 g in the Co group and 4.84 ± 0.07 g in the C group. The total body nitrogen content was 67.06 ± 8.04 in the Co group and 65.44 ± 1.49 mg in the C group. Thus no difference in means was demonstrated. Total lipids were significantly higher in the Co group than in the C group (P < 0.05). There were no significant differences in phospholipids and cholesterol either between the means or between the levels of the lines. The mean neutral fat content was significantly higher in the Co group (P < 0.05). P for the difference between the levels of the lines was 0.01. The water content was somewhat higher in the C group than in the Co group but the difference was not significant. P for the difference between the levels of the lines was ≈ 0.2.

Groups 2 (birth weights < 4.50 g). The mean birth weight was 4.24 ± 0.04 g in the Co group and 4.11 ± 0.07 g in the C group, the difference not being significant. The total body content of nitrogen was similar in the two groups, 50.06 ± 4.02 and 50.81 ± 3.02 mg in the Co and C groups, respectively. There
were no significant differences in the weights of total lipids, phospholipids, or cholesterol between the groups. The mean neutral fat content was significantly lower in the Co group \( (P < 0.05) \), the difference in neutral fat being significant even when the difference in the levels of the lines was tested \( (P < 0.05) \). No significant difference was demonstrated between the mean water content in the two groups.

**DISCUSSION**

The present investigation has revealed that administration of a comparatively small daily dose of cortisone to pregnant rats during the latter half of the gestation period gives rise to significantly increased mean birth weight and birth length.

For analysis of their chemical composition the young were divided into groups heavier than and lighter than 4.50 g (slightly over 2 standard deviations from the mean birth weight of the controls) for the following reasons: (i) the body composition of the heavy offspring is particularly relevant to a study of the causes of overweight in diabetic pregnancy, which in the rat may produce either underweight or overweight offspring (cf. Angervall 1959); (ii) cortisone doses larger than those used here produce abnormally high offspring, so the
lighter offspring in this investigation could be lighter owing to a contributory weight reducing action of cortisone.

The dose of cortisone adopted had distinct repercussions on the pregnant rats: the normal weight increase during pregnancy failed to materialize, the adrenals and thymus became atrophic, and, owing to the passage of cortisone through the placenta, the foetal adrenals became hypoplastic (cf. Angervall & Lundin 1965).

The investigation showed that the heavier offspring in the cortisone group had significantly higher total lipids and neutral fat contents than the corresponding controls at equal body weights, while the lighter young in the cortisone group had a significantly lower neutral fat content than the controls. Neither light nor heavy young in the cortisone group exhibited any significant differences in cholesterol and phospholipids compared with the corresponding controls at equal body weights.

Considering that the depot fat is composed almost wholly of neutral fat (e.g. Björntorp 1960), the increased neutral fat content observed in the heavier young in the cortisone group suggests that these were more obese. Though the mechanism for this neutral fat increase is not known, it could be similar to that in diabetic pregnancy, which in the rat has been shown to give rise to young under the influence of increased maternal corticosteroids and with a similar neutral fat excess (Angervall et al. 1965). Since cortisone, via hyperglycaemia (cf. Williams 1962) is capable of stimulating production of insulin which is lipogenetic and protein anabolic (for references see Angervall et al. 1965) the two experimental conditions could have in common an increased
lipogenesis induced by the enhanced endogenous insulin production in offspring of both diabetic and cortisone-treated rats. The fact that the lighter offspring in the cortisone group tended to have subnormal neutral fat could imply that they were more influenced by the lipolytic action of the cortisone.

Foetal overgrowth following cortisone administration may, however, be explained otherwise. It is well-known that cortisone mobilizes tissue proteins. An indication of such action in the present investigation was the cortisone-treated rats’ failure to gain weight during pregnancy. Thus, since it has been established, moreover, that the serum protein level rises (Clark 1953; Silber & Porter 1953) and serum albumin and amino acids seem capable of transplacental passage and, in addition, amino acids are absorbed against the gradient (Dancis 1960), it seems reasonable to assume, according to an hypothesis suggested by Schnürer (1963) that cortisone administration to the pregnant rat mobilizes maternal tissues proteins which pass to the foetuses and thereby promote their growth. Such an hypothesis receives support from the fact that the foetal total nitrogen content and birth length both increased proportionately to their excess weight (cf. Fig. 1).

ACKNOWLEDGEMENTS

The skilful technical assistance by Mrs. Anita Nilsson is highly appreciated.

This work was supported by the Swedish Medical Research Council (Project no. 12X-719-02).

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Received on December 27th, 1967.