Changes in the Cholesterol Level in the Tissues of Streptozotocin-Induced Diabetic Mother Rats and Their Infants During the Early Postpartum Period

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Synopsis

A diabetes-like state was induced by intravenous injection of a single dose of streptozotocin (SZ), 45 mg/kg, in pregnant rats and the cholesterol level in the tissues of the mothers and their infants was examined during the early postpartum period.

The following results were obtained.
1) The infants of the diabetic mothers were smaller than the infants of normal mothers and were markedly hyperglycemic.
2) Serum cholesterol was markedly increased on day 21 of pregnancy both in normal and diabetic groups. The concentration, however, declined nearly to the nonpregnant normal level on the 1st day after delivery in normal mothers, while it decreased stepwise until day 7 in diabetic mothers.
3) The cholesterol contents in the adrenal, liver and serum of infants of normal and diabetic groups increased progressively during the early postpartum period. However, the cholesterol level in these tissues was always lower in the infants of diabetic mothers than in normal infants; the concentration in the liver was considerably lower.
4) The adrenal cholesterol concentration decreased abruptly in the normal mother on day 1 after delivery and it returned to approximately normal level on day 7. On the other hand, no alteration in the cholesterol concentration was observed in the diabetic mother after parturition. In addition, the adrenal weight of the diabetic mother was heavier than that of the normal control mother at all stages examined.

These results suggest impairment of the steroidogenesis in the maternal adrenal in response to endogenous ACTH during the parturition in a diabetic state. Moreover, it was clearly demonstrated that SZ-induced hyperglycemia in pregnant rats affected the cholesterol metabolism in the adrenal and liver in newborns.

Streptozotocin has been considered to induce a mild diabetic state in rats as compared with the diabetic state induced by alloxan; less mortality, no incidence of ketosis or no elevation of plasma free fatty acid concentration has been reported when 50 mg/kg was injected intravenously (Mansford and Opie, 1968). In our previous study (Fujimoto, 1973), however, severe hypercholesterolemia was found in diabetic pregnant rats which were made diabetic by injection of a single dose of 45 mg/kg of streptozotocin. Moreover, growth retardation of fetuses born to diabetic mother rats was observed.

Maternal lipid and cholesterol metabolism is an important factor in relation to the growth and development of the fetus since cholesterol serves as a membrane component of biological cells. Furthermore, the changes in cholesterol metabolism during the pregnancy may be reflected in the changes in the hormonal regulation in mother and fetus because cholesterol is known to serve as a precursor for steroid hormones.
The present experiment was therefore undertaken to investigate the distribution pattern of cholesterol in various tissues of mother rats which were injected with streptozotocin on the 5th day of gestation and of their infants during the first week after delivery.

Materials and Methods

Sprague-Dawley adult female rats, weighing from 230 to 250 g, were mated and their vaginal smears were examined every morning for evidence of impregnation. The day of presence of sperm in the vaginal smears was counted as day 0 of pregnancy. The animals were kept under conditions of constant temperature and humidity with a lighting schedule of 14 hr light (6:00 am to 8:00 pm) and 10 hr darkness. They were fed a commercial stock diet (CA-1, Japan Clea) and water ad libitum. On the 5th day of gestation, the rats were injected with SZ (Upjohn Company) in a single dose of 45 mg/kg body weight into the femoral vein. SZ solution was freshly prepared in 0.01M citrate buffer, pH 4.5, (Junod, et al., 1967) immediately before injection.

The urine on the 3rd day after injection was tested for glucose with glucose oxidase paper (Combistex, Ames), and if positive, the animal was considered diabetic. The blood glucose level was determined by the micromethod described by Sasaki (1967).

On the 1st, 3rd and 7th day after delivery, the venous blood of the mother or of newborn rats was collected under light ether anesthesia and the serum was isolated by centrifugation within 30 min. The animals were then killed by decapitation. The ovary, adrenal, uterus, pituitary, kidney and liver of mother rats, and the adrenal and liver of infants were dissected out, freed from the surrounding tissues, rinsed in ice-cold saline if necessary, blotted and weighed on a scale or torsion balance up to 0.1 mg.

On adrenal of each mother and a portion of the liver tissue from mothers and infants were homogenized in 99% ethanol. The final volume of ethanol was adjusted to 5 or 8 ml and the mixture was left at room temperature for approximately 1 hr with occasional stirring and then the supernatant was separated by centrifugation at 3,000 rpm for 10 min. An aliquot of the serum was mixed with 99% ethanol and cholesterol was extracted as described above. Fetal adrenals were pooled for the extraction of cholesterol. The total cholesterol amount was determined by the method of Glick et al., (1964).

Results

Body Weight and Blood Glucose Level

The body weight of lactating diabetic mothers was lighter than that of normal mothers. Body weight gain in the newborns of diabetic mothers was apparently small at all ages examined. The concentration of maternal serum glucose in the intact group was 89.5 ± 12.5 mg/100 ml on the 1st day after delivery. This value did not differ from the values obtained on the 21st day of pregnancy. Mother rats which were injected with SZ on the 5th day of gestation were markedly hyperglycemic on days 1, 3 and 7 after delivery (Table 1). The blood glucose level in normal

<table>
<thead>
<tr>
<th>Days after delivery</th>
<th>No. of rats</th>
<th>Body wt. (g)</th>
<th>Blood glucose (mg/100 ml)</th>
<th>Blood glucose (mg/100 ml)</th>
<th>Body wt. (g)</th>
<th>No. of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>3</td>
<td>308 ± 25</td>
<td>89.5 ± 12.5</td>
<td>36.5 ± 9.5</td>
<td>6.16 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>4</td>
<td>288 ± 18</td>
<td>409.8 ± 80.0**</td>
<td>176.0 ± 52.0</td>
<td>5.94 ± 0.10</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>3</td>
<td>350 ± 5</td>
<td>76.0 ± 10.0</td>
<td>55.5 ± 10.5</td>
<td>7.25 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>3</td>
<td>292 ± 17</td>
<td>322.6 ± 69.8**</td>
<td>118.8 ± 29.7</td>
<td>6.23 ± 2.97</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>2</td>
<td>320 ± 17</td>
<td>122.0 ± 37.7</td>
<td>84.5 ± 1.5</td>
<td>8.99 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>3</td>
<td>261 ± 19</td>
<td>352.0 ± 51.0*</td>
<td>126.8 ± 20.0**</td>
<td>7.93 ± 0.22*</td>
</tr>
</tbody>
</table>

*Mean ± S.E.; **, P<0.025 vs. Controls; *, P<0.05.
Table 2. Organ weights of control and SZ-injected diabetic mother rats and of their infants during the first week after delivery.

<table>
<thead>
<tr>
<th>Days after delivery</th>
<th>No. of rats</th>
<th>Liver</th>
<th>Kindney</th>
<th>Adrenal</th>
<th>Ovary</th>
<th>Pituitary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mothers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>3</td>
<td>3.60±0.10*</td>
<td>0.53±0.05</td>
<td>21.7±2.9</td>
<td>32.4±3.6</td>
</tr>
<tr>
<td>1</td>
<td>Diabetic</td>
<td>4</td>
<td>4.48±0.31</td>
<td>0.81±0.09**</td>
<td>37.8±3.8**</td>
<td>33.6±2.3</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>3</td>
<td>4.31±0.08</td>
<td>0.60±0.01</td>
<td>26.1±1.0</td>
<td>27.9±2.8</td>
</tr>
<tr>
<td>3</td>
<td>Diabetic</td>
<td>3</td>
<td>4.64±0.11</td>
<td>0.83±0.08**</td>
<td>37.3±6.5**</td>
<td>30.6±1.8</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>2</td>
<td>4.56±0.28</td>
<td>0.65±0.03</td>
<td>21.6±0.5</td>
<td>29.9±1.0</td>
</tr>
<tr>
<td>7</td>
<td>Diabetic</td>
<td>3</td>
<td>4.54±0.23</td>
<td>0.96±0.02**</td>
<td>31.5±0.9**</td>
<td>27.7±2.8</td>
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<tr>
<td>Infants</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>25</td>
<td>3.64±0.13</td>
<td>0.53±0.05</td>
<td>37.6±1.55</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Diabetic</td>
<td>26</td>
<td>4.43±0.17</td>
<td>0.81±0.09**</td>
<td>34.2±1.32</td>
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<tr>
<td>3</td>
<td>Control</td>
<td>23</td>
<td>4.02±0.15</td>
<td>0.53±0.03</td>
<td>35.4±1.96</td>
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<tr>
<td>3</td>
<td>Diabetic</td>
<td>24</td>
<td>3.86±0.11†</td>
<td>0.83±0.08**</td>
<td>29.4±1.46</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>11</td>
<td>3.32±0.09</td>
<td>0.53±0.05</td>
<td>28.9±1.33</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Diabetic</td>
<td>16</td>
<td>2.66±0.05†</td>
<td>0.96±0.02**</td>
<td>23.7±2.21</td>
<td></td>
</tr>
</tbody>
</table>

*a/g/100 g body wt.; *mg/100 g body wt.; *Mean±S.E.; **P<0.01 vs. controls; †P<0.05 vs. 1-day-old diabetic infants; ††P<0.01 vs. 1-day-old diabetic infants.

neonatal rats was lower than that in their mother and increased gradually toward the maternal level by the time they were 7 days of age (Table 1). On the other hand, hyperglycemia was found in the newborns of diabetic mothers 1, 3 and 7 days after birth; 1-day-old rats had a relatively higher blood glucose level than 3- and 7-day-old rats.

**Organ Weights**

A significant increase in the relative weights of the kidney and adrenal was noted in the diabetic mothers (Table 2). The liver weight in diabetic mothers was also heavier than that in normal mothers on day 1 after delivery, but was approximately the same on days 3 and 7 (Table 2). No significant difference in the weights of the ovary and pituitary between normal and diabetic mothers was demonstrated (Table 2).

The weight of the adrenal relative to body weight in infant rats in both normal and diabetic groups tended to decrease with advancing ages. One-day-old newborns of diabetic mothers had a relatively heavier liver, and the weight tended to decrease below the control level by 7 days of age, whereas no significant alteration in the liver weight in the normal infants was seen during the 1st week after birth (Table 2).

**Cholesterol Concentration**

**Serum**

The serum cholesterol concentrations in the mothers and newborns are presented in Figure 1. The serum cholesterol level in normal pregnant rats at day 21 was higher than that in normal nonpregnant rats (83.2±12.2 mg/100 ml, unpublished data, Fujii) and a rapid decline toward the control level in a nonpregnant state occurred after delivery. The serum cholesterol level in SZ-induced diabetic mothers was much higher than that in normal pregnant rats at day 21 of pregnancy and showed a stepwise decrease by day 7 after delivery, reaching the control level.

The serum cholesterol level in the one day old infants of normal mothers was slightly lower than that in their mothers, but tended to increase progressively and exceeded the maternal level when determined on the age of 7 days (Fig. 1). Changes in the serum...
cholesterol level in the infants of diabetic mothers during the 1st week after birth were closely paralleled with those observed in normal infants, showing a gradual increase by the 7th day after birth, but the level was always lower than that in normal controls (Fig. 1).

**Liver**

There was no significant alteration in the cholesterol concentration in the maternal liver in a normal or in diabetic state before and after parturition (Fig. 2). In contrast to mother rats, the liver cholesterol concentration in the infants born to normal mothers increased progressively by 7 days of age. The values at the age of 3 and 7 days markedly exceeded those of their mothers. The liver cholesterol concentration in the fetus of diabetic mothers was significantly higher than that in the fetus of normal mothers at day 21 of pregnancy. However, the liver cholesterol concentration in the infants born to diabetic mothers was significantly lower than that in normal infants at all ages examined, though a gradual elevation was shown (Fig. 2).

**Adrenal**

On the first day after delivery, the adrenal cholesterol concentration in normal mother rats decreased remarkably from the level at 21 days of pregnancy; it reduced to one-third, and was still below the normal level on day 7 (Fig. 3). The cholesterol concentration in normal nonpregnant rats was 55.5±2.4 mg/g (Fujii and Fujii, 1972). In contrast there were no significant changes in the adrenal cholesterol concentration in the SZ-diabetic mothers before and after delivery. The pattern in the sequential alteration in the total cholesterol content in the adrenal was quite similar to that in the concentration since there was no significant variation in the adrenal weight in the animals during the...
Fig. 2 Liver cholesterol concentrations in control and diabetic mother rats and in their infants at the term of pregnancy and during the first week after delivery *, P<0.05 vs. controls; **, P<0.01.

Fig. 3 Cholesterol concentrations and total cholesterol contents in the maternal adrenal of control and streptozotocin-injected diabetic rats. *, P<0.05 vs. controls; **, P<0.01.
Fig. 4 Cholesterol concentrations and total cholesterol contents in the adrenal of infant rats born to normal and diabetic mothers. **, P<0.01 vs. controls.

Discussion

It has been observed that the near-term fetus is capable of independently regulating the glucose level in the blood during maternal hypoglycemia (Goodner et al., 1969). In the present experiment, the glucose level in 1-day-old rats born to diabetic mothers was higher than that in infants born to normal mothers and even that in 3- and 7-day-old rats of diabetic mothers. These results may indicate that hyperglycemia of the 1-day-old infants was largely due to the direct transmission of the maternal glucose to the fetus through a placental transport system.

The functional activity of the pancreas in secreting and/or producing insulin increases with advancing age of animals (Golob et al., 1970a). In the present experiment the significantly higher blood glucose level was shown in infants born to diabetic mothers at the age of 3 and 7 days, suggesting that the development of the regulatory mechanism for the blood glucose was impaired when the mother was hyperglycemic from the early stage of pregnancy.

Increased fetal weight has been observed in several of these studies of experimental diabetes in pregnancy (Lenwart, 1959; Golob et al., 1970b; Pitkin et al., 1971), particularly when the diabetes was of mild degree. On the other hand, the retardation of fetal growth was also evident in diabetic mother rats (Sybulski and Maughan, 1971; Love et al., 1964). In the present experiment, the degree of diabetes was relatively severe, at least as judged by maternal weight gain and blood

period of 7 days after delivery (Fig. 3).

The adrenal cholesterol concentration in infants of normal and diabetic mothers increased at the age of 3 days, and retained the similar level at 7 days of age (Fig. 4). The total cholesterol content in the adrenals of infants born to both normal and diabetic mothers increased progressively by 7 days of age, but the level was always low in the newborns of diabetic mothers as compared with that of newborns of normal mothers.
glucose level. This is probably reflected in the retarded growth of infants born to diabetic mothers.

Hypercholesterolemia is a frequent complication of diabetes in man. An increase in the serum cholesterol concentration during pregnancy has also been reported (Green, 1966; Ohmori, 1970). In the present experiment, the serum cholesterol concentration fell rapidly on the 1st day of delivery in both normal and diabetic mothers, but the level in diabetic mothers remained elevated on days 1 and 3 following delivery than in normal mothers, and then it returned to the control level on day 7. Thus, hypercholesterolemia which had resulted from pregnancy plus SZ-induced diabetes disappeared during the 1st week of lactation. The mechanism by which the cholesterol level returned to the normal range even in a diabetic state is uncertain. A progressive increase in the serum cholesterol level was demonstrated in infant rats of both normal and diabetic mothers but the level was always low in the diabetic group. It has been suggested that the hepatic cholesterol as well as cholesterol synthesized in the small intestine contributes to the circulating cholesterol level in rats (Morris et al., 1957; Wilson, 1968). It is possible to state that the lower serum cholesterol level in infants of diabetic mothers was due in part to the decreased biosynthesis of cholesterol in the liver; a marked reduction in the liver cholesterol content was demonstrated in the infants of diabetic mothers.

The limited transport of cholesterol or free fatty acid through the placental barrier has been reported by many investigators (Goldwater and Dew, 1947; Davis et al., 1956; Koren and Shafrir, 1964) and the liver and adrenal in fetus are able to synthesize their own cholesterol (Carroll, 1964; Givner and Jaffe, 1971). A rapid increase in the total cholesterol content in the neonatal adrenal during the 1st week after birth in both normal and diabetic mothers may suggest the enhancement of enzyme activities involved in the cholesterol synthesis. However, the relatively lower level of the adrenal cholesterol in infants of diabetic mothers than in infants of normal mothers suggests an evidence of a reduced functional activity for cholesterol biosynthesis.

Impaired reproductive functions in diabetic state have been well known. The reduced ovarian weight in alloxan diabetic rats was considered to be the results from reduced pituitary gonadotropin output (Shipley and Danley, 1947). However, it has been recently suggested that ovarian response to exogenous gonadotropin was reduced in alloxan diabetic rats, though the gonadotropin level was in a sufficient range (Lie et al., 1972; Farina et al., 1971). In the present experiment, no significant difference in the ovarian weight was obtained.

A striking difference in the adrenal cholesterol level between the diabetic mother and normal mother was demonstrated after delivery. In normal mothers, the adrenal cholesterol content decreased very sharply on day 1 following delivery and returned to nearly the normal level on day 7, whereas no significant fluctuation in the adrenal cholesterol level was seen in diabetic mother rats during the 1st week after delivery. These results indicate that the adrenal cholesterol might be utilized very rapidly for steroidogenesis during the course of delivery in normal state. Adrenal cholesterol depletion has been considered to be a good index of the activity of ACTH (Glick and Ochs, 1955; Riley, 1963). The pituitary-adrenal system may play an important role during the period of pregnancy and parturition. Particularly an increase in the production and secretion of adrenal steroids must be implicated in the stressful situation of parturition, reflecting the relative low level of cholesterol in the adrenal. However, no decline in adrenal cholesterol in diabetic mothers was demonstrated in the present experiment. It can be caused by either 1) a greater rate of biosynthesis of cholesterol than its utilization for steroidogenesis, 2) a reduced utilization of cholesterol due to the decreased rate.
of steroidogenesis in the adrenal, or 3) an acceleration of the uptake of cholesterol from the circulating blood.

In general, an increase in adrenal weight is considered to be the result of stimulation by ACTH. Evidence of the significantly large adrenal and no fluctuation in the adrenal cholesterol concentration after delivery in SZ-diabetic mother rats may suggest that the response of the adrenal to endogenous ACTH is reduced, and this might result in a reduction of the circulating adrenal steroid level and subsequently lead to an increase in ACTH stimulation via feedback mechanism. The reason why the adrenal of diabetic rats did not respond to ACTH is uncertain. It is known that insulin has diverse biologic effects, such as alterations in cell membrane permeability, lipolysis and protein synthesis. Insulin might be required for the suitable response to ACTH stimulation in the adrenals.

Acknowledgements

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References