Volume Changes of the Pancreatic Islets during Perinatal Days in the Rat*

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Synopsis

Morphologic and volume changes of the pancreatic islets during perinatal days in the rat were studied. During 3 days at the end of fetal life, the islet cells containing Gomori's aldehyde-positive $\beta$ granules gradually increased in number and in degree of granulation. The total volume of islets sharply increased at term.

After birth, the total volume of the islets did not increase. The size of islet cells was markedly reduced. The number of $\beta$ granules in islet cells markedly increased. These observations would support the view of some limitations of insulin secretion under a normal physiologic condition at least during the first 3 days after birth in the rat.

In the development of the pancreatic islets in the rat, insulin-like activity has been demonstrated from the 14th day of fetal life (Grillo, 1964). Insulin content in the islet gradually increases toward term (Dixit et al., 1964). The total volume of pancreatic islets also gradually increases toward term (Esterhuizen, 1959). After birth, some morphologic and functional changes may occur in the pancreatic islets as an adaptation to cessation of supply of maternal nutrients via the placenta. However, little information is available concerning morphologic changes in perinatal days.

The present study was designed to extend the volume determinations and histologic observations to the early postnatal stages and to assess how the pancreatic islets in the neonatal rat are adapted to the event of birth.

In addition, blood sugar levels during perinatal days were determined.

Materials and Methods

Rats of the Wistar strain were used. They were given a commercial diet (Oriental pellets NMF) and water, both ad libitum. Days of gestation were counted from the day following an overnight mating as the 1st day of gestation.

Fetuses on the 20th, 21st and 22nd days of gestation and newborn rats on the 1st, 2nd and 3rd days after birth were used. On each day, 6 males and 6 females were selected at random from among 3 litters. These young rats were further divided into 2 groups. In the 1st group, the pancreatic tissue was removed singly from the surrounding tissues. In the 2nd group, the pancreas was removed en bloc with neighboring intestines and the spleen. Pancreases of both groups were fixed in Bouin's fluid and embedded in Paraplast (Sherwood Inc.).

The tissue blocks in the 1st group were cut at 5$\mu$ and stained by a modification of Gomori's aldehyde fuchsin technique (Scott, 1952) or with hematoxylin and eosin for histologic and cytologic observation.

The blocks in the 2nd group were cut at 7$\mu$ serially, stained with aldehyde fuchsin as described above.

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and used for the histometric determination of the total volume of pancreatic islets by the method of Chalkley (1943). The basis of the method was the multiplication of the volume of pancreas by random "hitting" percent of islets with pointers placed in the ocular of a microscope by viewing every 15th section. Hittings in each pancreas were 3,000 to 5,000 according to the age of animals observed. The volume of the whole pancreas was determined by the paper weight method: the outline of the pancreas (including the capsular tissues) of every 15th section was drawn on ledger paper at a magnification of × 30 by the use of a projector.

Blood sugar levels in fetuses on the 20th, 21st and 22nd days of fetal life, their mothers and newborn rats on the 1st, 2nd and 3rd days after birth were determined by the method of Hagedorn-Jensen. The blood of the mother and her young was obtained from the neck after decapitation. Since the volume of the blood was small in younger fetuses, samples were pooled from 2 or 3 fetuses.

**Results**

The microscopic observations of fetal pancreatic islets were essentially confirmatory of those recorded by previous authors (Hard, 1944; Nerenberg, 1954; Frye, 1959). The size of islets was gradually increased toward term. Aldehyde positive granulated β cells were increased in number together with an increased degree of granulation particularly prominent on the 22nd day of gestation, when about 50% of the cells in each islet were granulated (Fig. 1).

After birth, the size of islets seemed somewhat decreased. Granulated β cells in the islets were remarkably increased in number (about 90% or more of the cells in each islet) along with a highly increased degree of granulation in each β cell (Fig. 2). Individual islet cells appeared shrunken as compared with fetal islet cells in sections stained with hematoxylin and eosin (Figs. 3 and 4); in sections stained with aldehyde fuchsin, such a difference in cell size was obscure by the presence of plenty of granules in each cell.

Changes in the total volume of islets during perinatal days are shown in Table 1. The volume gradually increased toward term, markedly increasing between the 21st day and the 22nd day of fetal life, confirmatory of the observation of Esterhuizen (1959). However, the volume determinations extended to postnatal days showed no increase of the volume from the 22nd day of fetal life to the 3rd day after birth.

Incidentally, the volume of the whole pancreas gradually decreased after birth as shown in Table 1. Though detailed studies of the total pancreas were beyond the scope of the present study, such neonatal shrinkage of

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>No. of animals</th>
<th>Vol. of pancreas (mm³)</th>
<th>% hits of islet cells (Mean)</th>
<th>Total vol. of pancreatic islets (× 10⁻³ mm³)</th>
<th>No. of determinations</th>
<th>Blood sugar Mean ± S.E. (mg%)</th>
</tr>
</thead>
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<tr>
<td>20</td>
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<td>7.5</td>
<td>2.5</td>
<td>187 ± 22</td>
<td>20</td>
<td>66 ± 2</td>
</tr>
<tr>
<td>21</td>
<td>5</td>
<td>9.3</td>
<td>2.3</td>
<td>216 ± 30</td>
<td>13</td>
<td>87 ± 4</td>
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<tr>
<td>22</td>
<td>6</td>
<td>14.8</td>
<td>2.4</td>
<td>354 ± 30</td>
<td>19</td>
<td>135 ± 8</td>
</tr>
<tr>
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<td>359 ± 26</td>
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</tr>
<tr>
<td>2</td>
<td>6</td>
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<td>3.1</td>
<td>353 ± 25</td>
<td>12</td>
<td>122 ± 9</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>10.2</td>
<td>3.3</td>
<td>338 ± 22</td>
<td>12</td>
<td>120 ± 3</td>
</tr>
</tbody>
</table>

Table 1. Changes in the total volume of pancreatic islets and the blood sugar level in late fetal and early postnatal days.
the pancreas might be related to changes in exocrine portions and connective tissues. At any rate, the acini and acinar cells were considerably smaller than those seen in prenatal days. Perhaps, this is caused by the rapid secretion of pancreatic juice according to the commencement of feeding.

The blood sugars during perinatal days are shown in the extreme right column in Table 1. The blood sugar rose steeply 2 days before term. Maternal blood sugar level was $113 \pm 6$ (S.E.) mg% (average of 16 rats) on the 20th, 21st and 22nd days of gestation, with no significant fluctuation according to gestational days. The fetal blood sugar level was lower than the maternal level on the 20th and the 21st days, but was slightly higher than the maternal level on the 22nd day. Just after birth, the blood sugar tended to decline somewhat and remained constant thereafter.

Discussion

The observed increase of number of granulated $\beta$ cells and degree of their granulation points to a gradual increase of insulin storage in the islets toward term, in line with biochemical assays carried out by Dixit et al. (1964).

The development of the early postnatal islets differed very much from that of the prenatal islets: (a) the total volume of the islets did not increase, (b) the size of islet cells was reduced, (c) the number of $\beta$ granules in islet cells increased significantly. These observations would support the view of some limitations of insulin secretion under a normal physiologic condition at least for the first 3 days after birth, in good agreement with the report of Blázquez et al. (1970) who found that the level of plasma insulin decreased precipitously just after birth in the rat. Regarding the blood sugar, the level dropped somewhat just after birth, remaining constant thereafter, a finding in line with the observations recorded by Kim et al. (1960). A probable decline of blood sugar level just after birth by the cessation of supply of maternal sugar would soon be overcome by degradation of glycogen stored in the newborn’s own liver (Shelly, 1961; Dawkins, 1963) and by suckled milk.

The newborn rat does not seem to need high secretion of insulin which would cause excess energy supply by increased peripheral utilization of glucose, since it does not maintain its own body temperature by itself. Thus, limitations of insulin secretion just after birth in such a poikilothermal animal as the newborn rat constitute a sharp contrast to the recorded observations in a homoiothermal animal such as the newborn lamb in that both the neonatal level of the blood sugar and the secreting rate of insulin are much higher than those found in the prenatal period (Willes et al., 1969).

References

Figs. 1 and 2 are photomicrographs of sections of the fetal and newborn rat pancreases, taken at a magnification of × 470. These sections were stained with Gomori's aldehyde fuchsin, counter-stained with phloxine and fast green FCF (Scott, 1952).

Fig. 1. Islet of a fetus on the 22nd day of fetal life. About 50% of the islet cells are granulated.

Fig. 2. Islet of a newborn rat on the 2nd day of life. About 90% or more of the islet cells are granulated. Degree of granulation in each cell is increased as compared with that in fetal days.

Figs. 3 and 4 are photomicrographs of sections of the fetal and newborn rat pancreases, taken at a magnification of × 1,000. These sections were stained with hematoxylin and eosin.

Fig. 3. Islet of a fetus on the 22nd day of fetal life. The islet cells are large with smaller cells in the periphery.

Fig. 4. Islet of a newborn rat on the 2nd day of life. The islet cells are clearly smaller than those seen in the fetal day, which is readily understood by comparing the numbers of cell nuclei per unit area of section with those in Fig. 3.