Electron Microscopic Studies of the Pancreatic Islets and Some Other Organs in Experimental Congenital Diabetic Rats

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Synopsis

Nineteen rats were obtained by the mating of two spontaneous diabetic males, that had diabetes for over one month, with three nondiabetic females, all of which were born from alloxan diabetic rats in the same successive generations. General observations on these animals and light and electron microscopic studies on their pancreatic islets as well as some other organs were performed. Among 19 rats, 11 developed diabetes spontaneously without any treatment, 5 of which showed long persistent diabetes. (a) Before the onset of diabetes, the pancreatic B-cells showed hypoplasia (reduced in number and size). Electron microscopically, some B-cells in the granular stage showed degranulation and an increased number of ribosomes, some B-cells in the predominantly agranular stage irregular dilatation of granular endoplasmic reticulum. (b) After the onset of diabetes, some pancreatic B-cells showed hydropic degeneration and vacuolar formation, other B-cells pyknosis and atrophy. Under electron microscopy, marked degranulation, swelling and vacuolation of mitochondria, and a decreased number of ribosomes were observed in all pancreatic B-cells, and in addition, marked irregular dilatation of granular endoplasmic reticulum in the predominantly agranular stage. The adrenal gland showed slight hypertrophy and in the cells of the fascicular zone an increased number of ribosomes and abundant dilated agranular endoplasmic reticula both before and after the onset of diabetes were observed. In the kidney, the glomerulus showed no obvious changes, but the renal tubules demonstrated deposition of glycogen particles in the diabetic stage and they did not show any degenerative lesions in their organelles.

In short, in experimental congenital diabetic rats, the pancreatic B-cells work excessively to maintain the animals free of diabetes, during the early prediabetic stage; however, with the aging process their growth disturbance becomes severe, they are exhausted and show degeneration and finally spontaneous diabetes occurs. The presumed hyperfunction of their adrenal cortex accelerates the onset of diabetes.

Since Okamoto (1952) found hypoplasia of the pancreatic B-cells in the descendants of alloxan diabetic rabbits, many investigations on these animals have been made, with many interesting results. That is, the diabetic animals (rabbits, rats and guinea pigs) in which diabetes was induced by the administration of alloxan or other diabetogenic substances, were mated after being kept diabetic for more than one month, to obtain F₁ offspring and this process was repeated to obtain F₂ and several subsequent generations. On examination it was revealed that these descendants had hypoplasia of the pancreatic B-cells (reduced in number and in size), increased percentage of acidophiles in the adenohypophysis and hypertrophy of the adrenal cortex (especially zona fasciculata). These changes gradually

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aggravated from generation to generation and finally diabetes developed spontaneously in $F_4$ and $F_5$ descendants of serially diabetic parents and in $F_7$ descendants of diabetic fathers. Spontaneous diabetes also developed in the descendants of spontaneously diabetic animals obtained by the above-mentioned procedure. Okamoto (1960) designated this spontaneous diabetes occurring in the descendants of successively diabetic ancestors as “experimental congenital diabetes.” Okamoto et al. have already reported the morphological and pathophysiological findings of the experimental congenital diabetic animals (Okamoto, 1964; 1965), but electron microscopic studies have not yet been reported. Meanwhile, from the viewpoint that hereditary factors play an important role in the development of diabetes, many investigations have recently been made on prediabetes in man but there are few experimental studies on prediabetic animals. This report gives details of the electron microscopic appearance of the pancreatic islets, adrenal cortex and kidney of experimental congenital diabetic rats before the onset of diabetes (analogous to prediabetes in man) and in the diabetic stage.

Materials and Methods

$F_8$ rats, which were produced by Dr. Yoshikawa (1969) in our Department of Pathology, were used in this experiment. (He obtained $F_1$, $F_2$ rats from alloxan diabetic parent Wistar rats, $F_3$ and thereafter from alloxan diabetics on the male side only). His male $F_8$ rats No. 5051 and No. 5061 with spontaneous diabetes were mated on the 35th, 40th and 54th days after the onset of diabetes with non-diabetic female $F_8$ rats No. 5058, No. 571 and No. 5062, and 19 offspring of $F_8$ rats were obtained as shown in Figure 1. Controls were normal Wistar rats of the corresponding age and sex, supplied by the Animal Center Laboratory, Kyoto University.

Animals were kept at constant temperature and humidity, and were fed Oriental stock chow diet (NMF, MF and CMF: Oriental Yeast Co. Japan). Tap water was given ad libitum. They were weighed once a week and urine was tested for sugar using Benedict’s reagent every morning from one month after birth. Blood sugar level was estimated by Somogyi-Nelson method (Nelson, 1944) on samples from the caudal vein just before sacrifice. Five $F_8$ rats with spontaneous diabetes were sacrificed by decapitation 94, 98, 128, 131 and 139 days after the onset of diabetes, respectively, and their 5 litter mates which had not yet developed diabetes were sacrificed 86, 90, 90, 90 and 90 days after birth, respectively. They were all used for light and electron microscopy of the pancreatic islets and some other organs. Immediately after sacrifice, the organs were dissected, cleaned and weighed on a torsion balance or a lever balance. The organ weight was recorded as the ratio of organ weight to body weight: 

\[ \text{organ weight (gm)} \times 10^6 \div \text{body weight (gm)} \]

for the pituitary, thyroid and adrenal gland and 

\[ \text{organ weight (gm)} \times 10^6 \div \text{body weight (gm)} \]

for the testis. For light microscopy, the pancreas was divided into the head, body and tail, each of which was fixed in Bouin’s solution, embedded in paraffin and stained with Gomori’s chrome-alum hematoxylin phloxine stain (Gomori, 1939; 1941). For the demonstration of glycogen, the pancreas and the kidney were fixed in absolute alcohol and their paraffin sections were stained with periodic-acid-Schiff (PAS) (McManus, 1948) with and without prior digestion with deastase; the other organs were fixed in 10% formalin and their paraffin sections were stained with hematoxylin and eosin. Histometrical examinations on the pancreatic islets were performed in the following manner. Twenty or more islets were selected at random from each of the 3 parts. The mean number of A-and B-cells per islet was estimated. The mean size of the islets was calculated using Abbe’s drawing instrument and planimeter. Twelve or more A-and B-cells were taken from each of the 3 parts and mean cellular size was similarly calculated. For electron microscopy, small fragments of the pancreas, adrenal gland and kidney were fixed in chilled 5% glutaraldehyde solution adjusted to pH 7.4 with 0.1 M phosphate buffer for two hours. After three successive washings in a buffer solution, the pieces were submitted to a postfixation in chilled 1% osmium tetroxide solution with sucrose, buffered with veronal acetate at pH 7.4, for two hours (Sabatini et al., 1963). All tissues were rapidly dehydrated in a graduated series of ethanol solutions and embedded in a mixture of Epon 812 and Epon 815. Sections cut at 1 micron were stained with 1% toluidine-blue (Chandra and Skelton, 1964) for light microscopic identification of the pancreatic islet, zona fasciculata of adrenal cortex and glomerulus of kidney. Other sections of the kidney were stained with PAS to identify the glycogen in the tubular epithelium (Cardno and Steiner, 1965). These sections were cut with a diamond knife, using a Porter-Blum...
Table 1. Male rats sacrificed for electron microscopic examination

<table>
<thead>
<tr>
<th>Experimental congealional diabetic or control rats</th>
<th>Generation</th>
<th>Age in days</th>
<th>No. of animals</th>
<th>Blood-sugar level at sacrifice (mg/dl)</th>
<th>Duration of glycosuria (days)</th>
<th>Body weight at sacrifice (gm)</th>
<th>Organ weight/body weight (gm/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-diabetic stage</td>
<td>F9</td>
<td>86-90</td>
<td>5</td>
<td>116</td>
<td>0</td>
<td>246</td>
<td>Pituitary × 10⁻² Thyroid × 10⁻⁸ Adrenal × 10⁻⁸ Testis × 10⁻⁸</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>85-92</td>
<td>5</td>
<td>113</td>
<td>0</td>
<td>264</td>
<td>2.5 5.5 11.9 9.4</td>
</tr>
<tr>
<td>Diabetic stage</td>
<td>F9</td>
<td>225–235</td>
<td>5</td>
<td>401</td>
<td>94–139</td>
<td>283</td>
<td>3.3 6.0 21.5 7.8</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>230–238</td>
<td>5</td>
<td>118</td>
<td>0</td>
<td>383</td>
<td>2.4 4.6 12.3 7.8</td>
</tr>
</tbody>
</table>

Servall MT-1 ultramicrotome, mounted on 100 or 150 mesh copper grids, double stained with acetone solution of uranyl acetate followed by Millonig's lead acetate (Millonig, 1961) and examined with a Hitachi HS-7 electron microscope.

Result

As shown in Table 1, the mean body weight of experimental groups was lower than that of controls; especially the animals with long persisting severe diabetes showed marked weight loss.

In 5 rats of 19 F₉ offspring, spontaneous and persistent glycosuria occurred 95 to 146 days after birth, and in 6 other F₉ rats spontaneous and transient glycosuria occurred 98 to 148 days after birth. The remaining 8 F₉ rats, including 5 rats used as prediabetics in this experiment, and all controls, had no glycosuria.

The 5 rats with persistent glycosuria (spontaneous diabetes) had blood sugar levels of 258–495 mg/dl just before sacrifice.

Five rats in the prediabetic stage showed no significant difference in blood sugar level as compared with controls.

The ratios of organ weight to body weight of pituitary, thyroid gland and adrenal gland in experimental groups were higher than those of controls. However, no significant difference was observed in that of the testis. The sizes of pancreatic islets and B-cells of experimental groups were smaller than those of controls.

Light microscopic examination of the pancreatic islets demonstrated hypoplasia of B-cells in the prediabetic stage. After the onset of diabetes, marked degranulation, hydropic degeneration, pyknosis and vacuolar formation of B-cells were observed in remarkably small and irregularly shaped pancreatic islets. On the other hand, no significant changes were observed in A-cells.

In the adrenal gland, an increase of the size of the cells in zona fasciculata was observed in experimental groups. Adrenal medulla showed no definite change.

In the kidney of rats with spontaneous diabetes, cytoplasmic vacuolization was observed in the thin portion of Henle's loop, distal and collecting tubules, and this was attributed to PAS stainable diastase-digestible material (glycogen) in alcohol fixed-paraffin embedded sections. No remarkable abnormality of glomerulus was found, even in rats with long persistent diabetes.

The results of histometric studies on pancreatic islets are shown in Table 2. Hypoplasia of pancreatic B-cells (reduced in size and in number) was observed.

Electron microscopic examination of pancreatic islets revealed that, in the prediabetic
Table 2. Histometrical measurements of islet cells

<table>
<thead>
<tr>
<th>Generation</th>
<th>No. of animals</th>
<th>Body weight at sacrifice (gm)</th>
<th>Number of cells per islet</th>
<th>Area of cells in islet (μ²)</th>
<th>Area per pancreatic islet (×100 μ²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F9</td>
<td>10</td>
<td>265</td>
<td>17.1</td>
<td>60.6</td>
<td>96.1</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>324</td>
<td>17.8</td>
<td>65.5</td>
<td>135.7</td>
</tr>
</tbody>
</table>

Fig. 1. Diagram of mating of F₈ rats.

Stage, some B-cells showed abnormal findings but others showed a normal appearance. The abnormalities, which were found in the granular stage (Herman et al. 1964), were increased quantities of granular endoplasmic reticulum, plenty of attached and unattached ribosomes, in addition to an increased number of both B granules and empty membranous sacs. Agranular endoplasmic reticulum and Golgi complexes showed no significant findings (Fig. 3). Figure 2 shows a normal appearance of a B-cell of the control rat. In the predomi-nantly agranular stage (Herman et al. 1964) some B-cells, which were considered to be abnormal, showed irregularly distended granular endoplasmic reticulum with partial detachment of ribosomes. The Golgi complexes were moderately hypertrophied. Presumed lysosomes showed no abnormal findings in the granular or predominantly agranular stage.

In the diabetic stage, very few normal B-cells were observable. They firstly showed marked degranulation. Most of the remaining B-granules showed marginal irregularity and
lower electron densities. Frequently there was swelling of mitochondria, most of which showed disrupted and fragmented cristae, and terminal vacuolization (Fig. 4).

In the predominantly agranular stage, irregularly dilated granular endoplasmic reticulum with partial detachment of ribosomes was observed. The Golgi complexes were hypertrophied and showed vesicular swelling. On the whole, ribosomes were decreased,
especially the unattached ones. Presumed lysosome showed no significant changes. No remarkable changes were observed in A-cells. On adrenal cortex, an increased number of ribosomes, dilatation and an increased number of agranular endoplasmic reticulum were observed in the cells of zona fasciculata both before and after the onset of diabetes. Golgi complexes, mitochondria, presumed lysosome and lipid droplet showed no significant changes (Fig. 6, 7). Figure 5 shows a normal appearance of a cell of zona fasciculata of the control rat.

On kidney, the glomerulus showed no remarkable findings in the basement membrane and mesangial cells before or after the onset of diabetes. On renal tubules, electron microscopic studies were performed on the thin sections in which glycogen was previously demonstrated by PAS staining. In the epithelial cells of the thin portion of Henle's loop, distal and collecting tubules, the glyco-

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Fig. 3. Pancreatic B-cell of prediabetic rats in the granular stage (86 days after birth), showing abundant B-granules (BG) and empty membranous sacs (ES). Granular endoplasmic reticulum (GR) and ribosome particles (RP) appear considerably increased. (×20,000).
Fig. 4. Pancreatic B-cell of spontaneous diabetic rats in the granular stage (128 days after the onset of diabetes) (blood sugar level at sacrifice: 479 mg/dl), showing a decreased number of B-granules (BG) and distorted, swollen and vacuolated mitochondria (M). (×20,000)

Discussion

There are now in existence several strains of animals which exhibit spontaneous diabetes (Lazarus and Volk, 1962; Warren et al. 1966). Okamoto and his co-workers succeeded in the experimental induction of diabetic disposition and spontaneous diabetes in the descendants of diabetic animals. The author also succeeded in producing 11 spontaneous diabetic rats including 5 with long persistent diabetes among 19 F_{0} offspring by a method similar to that adopted by Okamoto (1952) and Okamoto and Fukutome (1954), and made light and electron microscopic examinations of pancreatic islets and some other organs of
these animals with long persistent diabetes and their siblings which had not yet developed diabetes. Light microscopic examinations of pancreatic islets revealed hypoplasia of B-cells before the onset of diabetes, and after the onset of diabetes, hydropic and vacuolar degeneration as well as atrophy and pyknosis was observed in them. In hereditary diabetic animals such as obese mice (Bleisch et al., 1952), NZO mice (Bielschowsky and Bielschowsky, 1956), yellow obese mice (Hellerström and Hellman, 1963) and KK mice (Nakamura, 1965), the hypertrophy of pancreatic islets are observed. On the other hand, Chinese hamsters (Carpenter et al., 1967) and sand rats (Hackel et al., 1965) show a reduced number of pancreatic islets and various degenerative lesions which are similar to those of the experimental congenital diabetic rat.

Histometric examinations on pancreatic islets of the experimental congenital diabetic rats revealed that the number and size of pancreatic B-cells were reduced not only after but also before the onset of diabetes. These findings suggest that the B-cell changes are not secondary to hyperglycemia but primary,
congenital ones. That is, congenital factors may be operative at the B-cell level.

One of the characteristic findings in these animals was the hypertrophy of adrenal glands, especially zona fasciculata, the individual cells which showed hypertrophy and hyperplasia. Such a finding is also recognized in obese mice (Hellerstrom et al., 1962) and NZO mice (Bielschowsky and Bielschowsky, 1956) of spontaneous hereditary diabetic animals. In obese mice it is regarded as the result of an obese-hyperglycemic stage (Hellerström et al., 1962). On the other hand, in experimental groups, it is already observed before the onset of diabetes. Therefore, the mechanism which causes hypertrophy of the adrenal gland in experimental congenital diabetic animals may be different from that in obese mice. The hypertrophy of the adrenal gland is suggestive of hyperfunction, which may play an important role in the occurrence of diabetes in this animal.

In this experiment, the glomerulus showed no significant findings, probably because of the short duration of diabetes mellitus. On the other hand, glycogen was demonstrated in the thin portion of Henle’s loop, distal tubules and partially collecting tubules of diabetic animals.
In alloxan diabetic rats, high blood sugar level over 350 mg/dl causes glycogen nephrosis and is a reversible lesion (Robbins, 1950). Among spontaneously diabetic rodents, only the sand rat has shown glycogen nephrosis (Hackel et al. 1965).

Since Williamson and Lacy (1959) reported their electron microscopic studies on pancreatic islets of alloxan treated rabbits, many electron microscopic examinations on experimental diabetes have been performed. The author also performed electron microscopic examinations on pancreatic islets and some other organs of F₃ rats before and after the onset of diabetes and obtained some interesting results. Before the onset of diabetes, some B-cells showed normal appearance and others showed various degrees of changes, that is, degranulation and a slight increase of ribosomes in the granular stage, and a marked and irregular dilatation of granular endoplasmic reticulum and a slight decrease of...
Fig. 8. Portion of a cell of collecting tubule of kidney of rats with spontaneous diabetes (131 days after the onset of diabetes) (blood sugar level at sacrifice: 489 mg/dl) showing the deposition of glycogen particles (GLY) which are not related to any particular cytoplasmic organelles and appear to displace and compress the cytoplasmic structures. (×20,000).

ribosomes in the predominantly agranular stage. After the onset of diabetes, marked degranulation, irregularly sac-shaped dilatation of granular endoplasmic reticulum as well as swelling and vacuolation of mitochondria were observed. Degranulation and an increased number of ribosomes have been observed in the pancreatic B-cells of alloxan diabetic rabbits, cats which had received intraperitoneal administration of glucose (Williamson, 1960), glucagon treated rats which were fed high carbohydrate diets (Lacy et al. 1959), and spontaneous diabetic rodents, namely, obese mice (Björkman et al. 1963), KK mice (Nakamura and Yamada, 1969), diabetic hybrid mice (Like et al. 1965) and sand rats (Like and Miki, 1967). These findings suggest the increased synthesis and secretion of insulin. So, in experimental congenital diabetic rats, the individual pancreatic B-cells, which are decreased in number and in size, may work excessively during the
prediabetic stage in order to supply sufficient insulin to prevent the onset of diabetes. On the other hand marked vesiculation of granular endoplasmic reticulum of B-cells has been observed in tolbutamide treated rats (Williamson et al. 1961), cortisone-alloxan induced subdiabetic rabbits (Volk et al., 1965), and in growth hormone treated dogs (Volk and Lazarus, 1963). This remarkable and irregular dilatation of endoplasmic reticulum was considered to be evidence of an imbalance of insulin secretion and synthesis, or inability either to synthesize or to store insulin, and possible evidence of cell damage. In other words, before the onset of diabetes, some B-cells work excessively to supply sufficient insulin to prevent the onset of diabetes and others may be already becoming exhausted. The swelling of mitochondria, distortion of their cristae and the dilatation of the profiles of endoplasmic reticulum were observed in alloxan treated rabbits (Wellmann et al. 1967), growth hormone treated dogs (Volk and Lazarus, 1963) and sand rats (Like and Miki, 1967), all of which were suffering from severe diabetes. It may be considered that marked and irregular dilatation of endoplasmic reticulum and the swelling and vacuolation of mitochondria observed in the pancreatic B-cells of the spontaneous diabetic F₉ rats are evidences of cell damage resulting from exhaustion due to excessive work of each individual B-cell because of congenital hypoplasia.

Recently, enzyme histochemical examination on the pancreatic islets of experimental congenital diabetic rats revealed an increase in activity of the various enzymes in the prediabetic stage and decrease in the diabetic stage (Yoshikawa, 1969). From the results of the electron microscopical and enzyme histochemical studies, it may be hypothesized that in the early prediabetic period, the pancreatic B-cells of this animal have the ability to respond to the demand for insulin output by means of excessive work of the individual B-cells in spite of their growth disturbance, but with the aging process, become gradually unable to supply sufficient insulin to meet the amount required and finally are exhausted, leading to the onset of spontaneous diabetes.

The electron microscopic examination of adrenal cortex of the experimental groups revealed that most cells of zona fasciculata showed an increase in number, dilatation of the agranular endoplasmic reticulum, and plenty of ribosomes compared with those of the controls, throughout the prediabetic and diabetic stages. An increased number of agranular endoplasmic reticula and ribosomes are observed in ACTH treated Syrian hamsters (Yates, 1965) and rats (Ashworth et al., 1959; Yamori et al., 1961), together with mitochondrial changes. Such findings suggest the hypersynthesis of adrenocortical steroid. Biochemical (Okamoto, 1964) and enzyme histochemical studies (Yoshikawa, 1969) in our laboratory also showed hyperfunction of the adrenal cortex of experimental congenital diabetic animals. That is, the hyperfunction of this gland in these animals is already recognized before the onset of diabetes and persists after the onset of diabetes.

The electron microscopy of the glomerulus of experimental groups showed no obvious findings before or after the onset of diabetes. On the other hand, the electron microscopy of renal tubules in the diabetic stage revealed deposition of glycogen particles in the thin portion of Henle's loop, distal and collecting tubules, but did not show any degenerative lesions of the cytoplasmic organelles. The fact that the cytoplasmic organelles of renal tubules of spontaneous diabetic rats suffering from glycogen nephrosis showed no degenerative findings supports the previous report (Robbins, 1950) that glycogen nephrosis is reversible and not an irreversible degenerative lesion.
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References
