SPONTANEOUS DIABETES-LIKE SYNDROME IN WBN/KOB RATS

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A strain of Wistar-derived WBN rats has been maintained at the Institute of Pathology, Bonn University, Bonn, FRG. Some of these animals were brought to Japan by Dr. Kobori in 1976 and called WBN/Kob rats. A colony of these is being maintained at the Shizuoka Laboratory Animal Center, Shizuoka, Japan.

WBN/Kob rats are smaller than the original Wistar rats, but otherwise little or nothing is known about their biological characteristics, other than that they appear to have a low incidence of spontaneous tumors.

In 1980, TOBE and KOBAYASHI[7], in an attempt to clarify this problem, carried out a long-term observation of these rats in a specific pathogen-free environment and observed that at the age of about 9 months, some male rats began to excrete an abundant and odorous urine. Further examination revealed that these rats were hyperglycemic, that some of them suffered from polyuria and glycosuria and that at 12 months of age manifested a marked glucose intolerance (tab. 1). Few female rats presented these abnormal signs.

The present experiment was conducted to confirm the occurrence of these symptoms in WBN/Kob rats and obtain information on their etiology.

MATERIALS AND METHODS

A total of 63 male WBN/Kob rats bred for several generations in the same colony, were kept under observation from the age of 3 to 21 months. During this period general health conditions, body weight, blood glucose levels (Dextrostic and Dextrometer, AMES Company, Inc.), urinary glucose excretion (Testape, Eli Lilly and Company), and serum insulin levels (IRI by two-antibody method with bovine insulin as a standard) were examined at regular intervals.

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At 19 months of age, the blood glucose responses to sulfonylurea (sodium tolbutamide, 20 mg/100 g body weight, p.o.) and insulin (bovine insulin, 300 mU/100 g body weight, s.c.), were studied.

Several rats were sacrificed simultaneously at 2-3 months' intervals. Samples of pancreas were collected for histopathologic studies, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H-E) or Gomori's aldehyde-fuchsin. Student's *t*-test was used for statistical analysis.

RESULTS

At 3 to 5 months of age, most of the animals under observation tried to escape from a restraint which imposed pressure on the abdomen as if they suffered pain. From about 12 months of age, almost all the animals gradually began to excrete soft yellow-brown feces, to show ruffled hair (transient alopecia) and to appear sluggish. These symptoms persisted up to the time of sacrifice. None of the animals recovered or became seriously ill.

Figure 1 illustrates changes in all parameters measured. Body weight increased gradually reaching an average of 425.6 ± 9.2 g at 14 months of age, when it began to drop reaching 353.6 ± 9.6 g at 21 months. In the control Wistar rats, even at 6 months of age, the mean body weight was 579.4 ± 12.4 g. Thus, the WBN/Kob rats were rather light and not obese.

In the experimental rats, up to 8 months of age, the non-fasted serum glucose levels were within the normal range (69 to 120 mg/dl; average 90.8 ± 3.4 mg/dl) as compared to a range of 81 to 123 mg/dl (96.6 ± 4.2 mg/dl) in the Wistar rat ($p > 0.01$). At about 9 months of age one of 21 rats began to show a high blood glucose level (186 mg/dl). The mean blood glucose level was 104.3 ± 4.8 mg/dl at 9 months of age. After that, the number of rats with hyperglycemia increased, so that the mean glucose levels were 207.6 ± 13.3, 328.4 ± 18.4, 372.2 ± 18.3, and 351.1 ± 23.1 mg/dl, at 14, 17, 19, and 21 months of age, respectively.

time (min)	blood glucose (mg/dl)			
	male		female	
	Wistar (n = 5)	WBN/Kob (n = 5)	Wistar (n = 5)	WBN/Kob (n = 5)
0	80.0 ± 2.4	146.2 ± 12.4 *	79.4 ± 2.8	82.8 ± 2.0
30	214.4 ± 24.6	389.5 ± 18.5 **	260.7 ± 17.7	301.0 ± 8.9
60	202.4 ± 24.2	419.4 ± 24.0 **	241.3 ± 13.7	280.2 ± 21.2
90	186.0 ± 21.6	466.6 ± 22.2 **	187.8 ± 8.6	232.7 ± 24.1
120	174.2 ± 10.7	487.4 ± 18.7 **	172.8 ± 10.5	193.2 ± 18.2

Tab. 1 - I.v. glucose tolerance tests (0.125 g/100 g body weight) in Wistar and WBN/Kob rats at 12 months of age. There were no significant differences between Wistar and WBN/Kob female rats at any time, but significant differences between males. Mean ± SEM. * $p < 0.05$; ** $p < 0.01$.

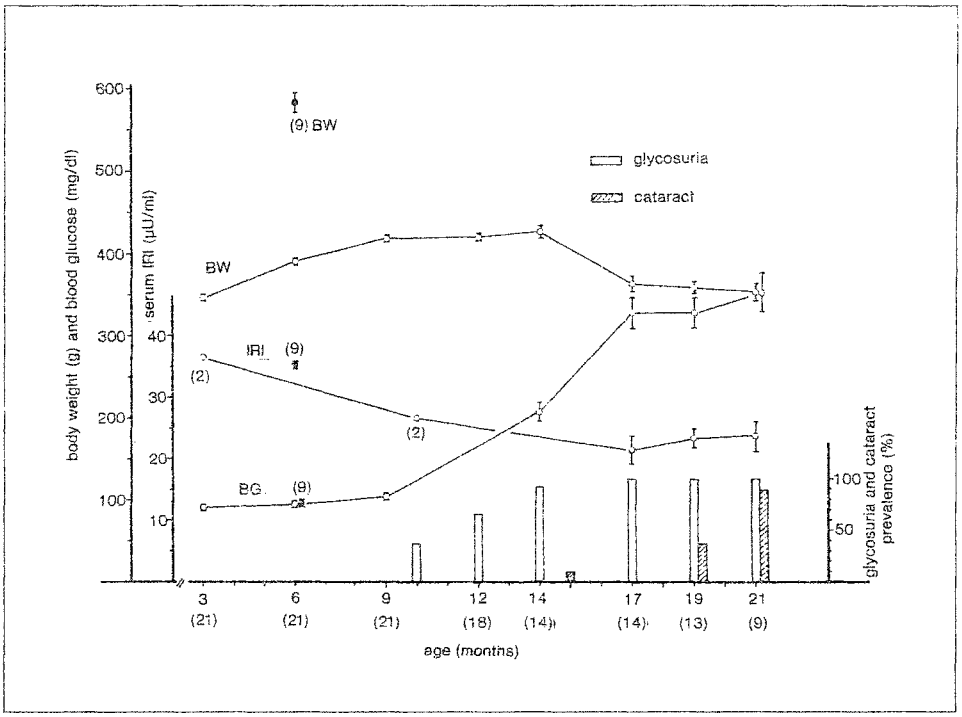

Fig. 1 - Time course of body weight (BW), blood glucose (BG), serum insulin (IRI) and frequency of glycosuria and cataract in WBN/Kob rats with age. Number of animals in parentheses. Closed circles indicate control Wistar rats at 6 months of age. Mean ± SEM.

The mean serum IRI level was within the normal range at 4 months of age, but low at all other ages (21.4 ± 2.2, 23.2 ± 1.5, and 23.6 ± 2.2 μU/ml at 17, 19, and 21 months of age, respectively).

These values were significantly different ($p < 0.01$) from the level found in the control Wistar rats (35.1 ± 2.3 μU/ml). Glycosuria was first detected 9 months after birth. Namely, it was present in 37% of the rats at 10 months and in 100% of them at 17 months of age, respectively.

There was no significant fall in blood glucose level in 19-month-old WBN/Kob rats following the oral administration of sodium tolbutamide (fig. 2-A), although the marked response to s.c. insulin (fig. 2-B) suggests that WBN/Kob rats may be highly sensitive to the hormone.

At about 15 months of age some of the rats were found to have slight bilateral lens opacities. At first, these were peripheral, but increased rapidly in severity, extending concentrically and centripetally, until total cataract was produced (fig. 3-B). These changes, although variable in severity, were noticed in 5 of 13 rats (38.5%) at 19 months of age and in 8 of 9 rats (88.9%) at 21 months of age (fig. 1).

At autopsy, macroscopically distinct brown necrotic foci were noticed in some parts of the splenic lobe of the pancreas of 3-month-old rats. With advancing age, these changes gradually extended to other pancreatic lobes. These changes were associated with an infiltration of lymphocytes and macrophages in and around

the islets, sometimes spreading to the adjacent exocrine tissue (fig. 4-A). In some rats, hemorrhages were seen inside and outside the islets.

Abundant fibrous tissue proliferated around pancreatic ducts and blood vessels, with apparent involvement of neighboring acinar cells and even some islets (fig. 4-B). At 17 months of age, these changes covered 20 to 30 percent of the whole histologic section.

With advancing age, foci of fibrosis became more extensive, invaded the islets causing severe damage so that the islets could hardly be identified, with their cells separated and broken. Nevertheless, the few remaining B-cells contained β-granules (fig. 4-C).

At 17 months of age and later, very few islets could be found even in areas that had been spared by the most severe fibrosis. In these cases, a small islet (less than 50 μ in diameter) could be detected in several low power (x 40) microscopic fields. However, the islet consisted of the cells which contained no stainable granules.

DISCUSSION

Numerous animal models of spontaneous diabetes have been described. Among them, the C57BL/6J-ob[5], the C57BL/KsJ-db[3], KK[12], NOD mouse[10], the Chinese hamster[11], the sand rat[16], the BB rat[13] and others. The BB rat in particular is a well known model of diabetes caused by insulitis.

In this animal[8], hereditary hyperglycemia occurs without obesity in about 40%, in males and females alike, at the relatively young age of 60-120 days. Insulin administration is necessary for survival.

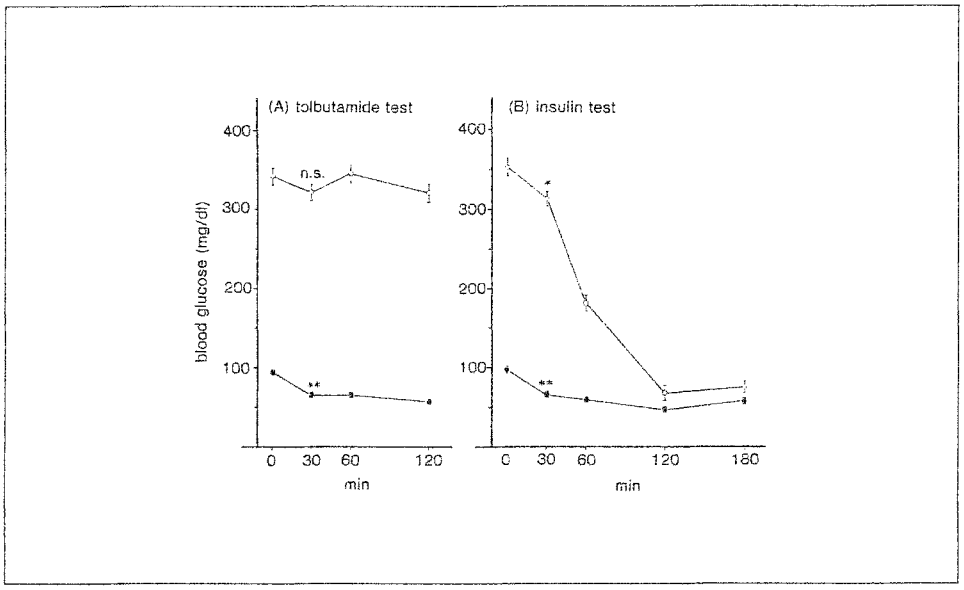

Fig. 2 - Blood glucose responses to oral sodium tolbutamide (20 mg/100 g body weight, A) and to s.c. insulin (300 mU/100 g body weight, B) in control Wistar rats (●, n = 9) and WBN/Kob rats (○, n = 11). * $p < 0.05$; ** $p < 0.01$; n.s. = not significant *vs* values at time 0. Mean ± SEM.

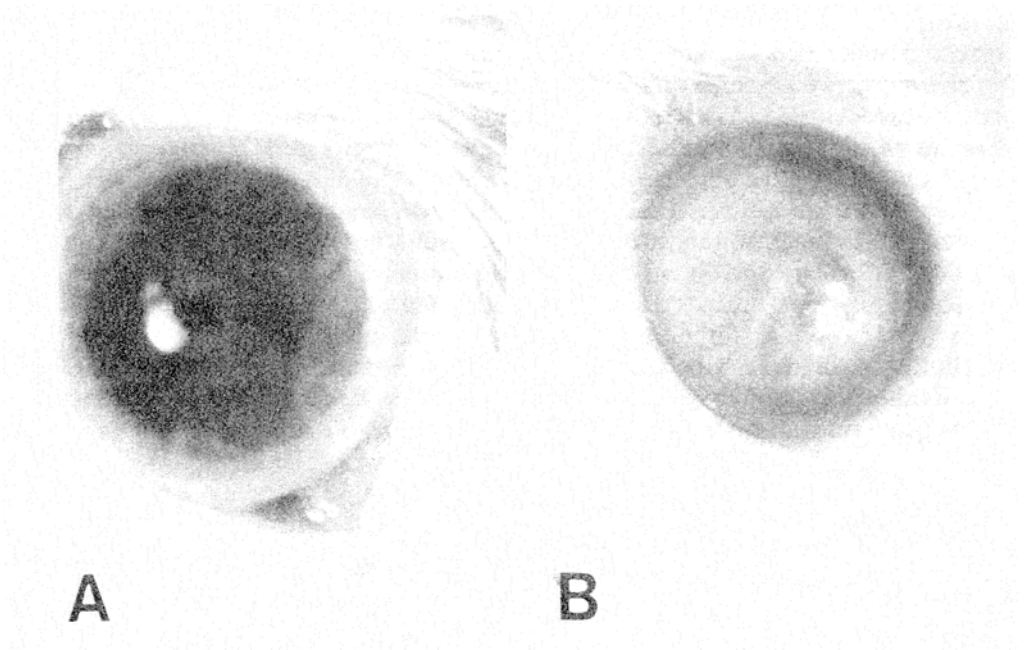

Fig. 3 - Photographs of eyes showing a transparent lens in a normal control Wistar rat in A, and complete lens opacity in a 21-month-old WBN/Kob rat (blood glucose 386 mg/dl, glycosuria ++++) in B.

In this paper we report that non-obese male WBN/Kob rats developed hyperglycemia, glycosuria, polyuria, and glucose intolerance at about 9 months of age, apparently due to deficiency of insulin caused by extensive fibrous degeneration of the pancreatic islets and exocrine parenchyma. This process started as early as 3 months of age and progressed with advancing age.

Two histopathologic patterns were observed. In one pattern, insulitis was characterized by infiltration of inflammatory cells around and into the islets leading to rapid and severe fibrotic degeneration. In the other pattern, fibrous tissue proliferated more slowly around pancreatic ducts and blood vessels and invaded the islets gradually, separating individual islet cells from one another and eventually replacing them with fibrous tissue.

Cell infiltration was observed only at an early stage (up to 6 months of age). Two distinctive histological changes, inflammatory cell infiltrations around and into the islets and periductal or perivascular fibrosis, were observed simultaneously in some rats in the early stage, thereafter fibrous degeneration of pancreas progressed with advancing age.

The etiology of this rapidly developing fibrosis, leading to extensive degeneration of the pancreas remains a matter for speculation.

In BB rats, islet infiltration by lymphocytes and macrophages has been noticed prior to the occurrence of hyperglycemia[14] and complete protection of susceptible animals has been obtained with the administration of anti-rat lymphocyte serum[9]. For these reasons, LIKE et al.[7] suggested that diabetes might be induced by autoimmune lymphocytic insulitis causing B-cell destruc-

tion; especially since BB rats also suffered from lymphocytic thyroiditis and possessed auto-antibodies against smooth muscle and thyroid colloid.

The existence of some hereditary factors in the development of the diabetes-like syndrome in WBN/Kob rats is suggested by the facts that it is limited to males, that it starts at an early age (3 months of age or possibly before) and that it involves almost all male WBN/Kob rats by the age of 17 months.

In contrast to BB rats that develop insulin-dependent diabetes mellitus (IDDM)[7], the WBN/Kob rats survive without insulin until at least the age of 21 months. This characteristic, as well as the relatively slow progression of the pathologic process, suggests a similarity with NIDDM.

In WBN/Kob rats, inflammatory changes, characterized by the infiltration of cells (mainly lymphocytes and macrophages), were noted before the appearance of the diabetes-like symptoms, suggesting a cell-mediated autoimmune mechanism, similar to that occurring in BB rats. Furthermore, in view of the

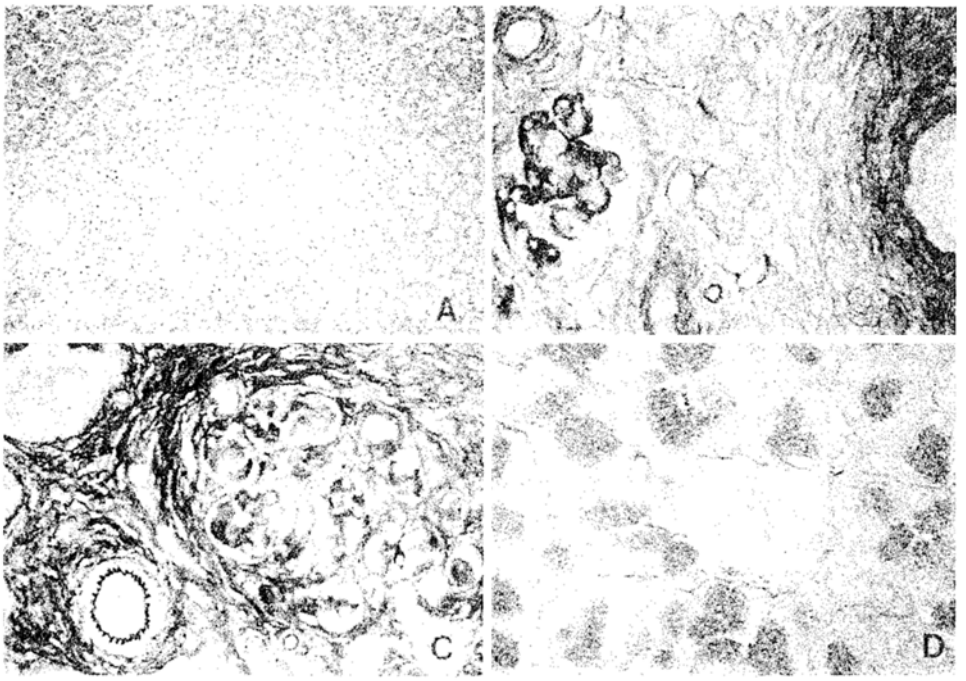

Fig. 4 - Light photomicrographs of pancreatic islets from WBN/Kob rats. (A) Pancreatic islet from an animal sacrificed at 3 months of age (blood glucose 109 mg/dl, glycosuria negative). Mononuclear inflammatory cells surround and infiltrate the islet periphery and penetrate into the immediately adjacent exocrine parenchyma. HE (x 100). (B) Another islet from the same animal. Fibrils surround the periphery of the pancreatic duct and a fibrotic process extends into the adjacent exocrine tissue and islet. B-cells are stained deeply with aldehyde-fuchsin. (x 200). (C) Islet from an animal sacrificed at 12 months of age (blood glucose 108 mg/dl, glycosuria +). B-cells are decreased in number and separated from one another by severe fibrotic processes penetrating into the interior of the islet. Gomoris' aldehyde-fuchsin (x 200). (D) Islet from an animal sacrificed at 17 months of age (blood glucose 399 mg/dl, glycosuria +++). In this animal, the islets were very few and small and consisted of degranulated B-cells, negative for aldehyde-fuchsin staining (x 400).

early development of clinical and histologic findings it seems reasonable to suggest that, in the WBN/Kob rat, acute pancreatitis develops into chronic fibrotic pancreatitis resulting in extensive degeneration of the islets of Langerhans.

This assumption must be verified by further studies in animals younger than 3 months.

Nineteen-month-old WBN/Kob rats responded well to exogenous insulin, suggesting that there is no insulin resistance. However, they hardly responded to the administration of sodium tolbutamide. Judging from the pathological findings in the pancreas of WBN/Kob rats at 19 months, this was clearly due to deficiency of additional secretion of insulin.

Cataract began to be noticed in some WBN/Kob rats from about 15 months of age. Diabetes complicated with cataract in animal models and human beings has been reported in a number of papers. In the polyol (sorbitol) theory on the cause of diabetic cataract[6,18], the etiological factor of this disorder is considered to be related to high concentrations of blood glucose[1,2,15]. It has, however, been stated[4] that diabetes was complicated by cataract in only 30 to 40% of human patients with diabetes. In the present experiment, WBN/Kob rats were kept under observation for a long time, without taking any step to control the blood glucose level. As indicated above, a hyperglycemia which persisted for at least 5 months was necessary for the occurrence of cataract. Moreover, this complication was very frequent in WBN/Kob rats. Therefore, it is assumed that WBN/Kob rats can be a suitable model for hyperglycemia complicated by cataract.

It is regrettable that no retina was examined in the present experiment. It was therefore impossible to discuss how the hemorrhage in the aqueous humor observed in only one 21-month-old rat (no data presented) was related to retinal or vitreous hemorrhage.

Detailed examination of the renal glomeruli is under way. However, preliminary data reveal signs of glomerulosclerosis, such as thickening of the glomerular basement membrane and mesangium. We are also examining the reasons for the resistance of female WBN/Kob rats to the development of the syndrome.

SUMMARY

Spontaneous hyperglycemia, glycosuria, hypoinsulinemia, and glucose intolerance were observed in some WBN/Kob rats, at about 9 months of age, and in all at the age of 17 months. Females did not present this pathology. Histopathologic examination of the pancreas revealed severe changes in male rats at the age of 3 months. Between 3 and 6 months of age a distinct infiltration of inflammatory cells was found around islets and among adjacent acinar cells. At the same time, marked fibrosis was seen around the pancreatic ducts and blood vessels. With advancing age the fibrous tissue gradually invaded extensive areas of the pancreas where also the islets became involved in fibrotic degeneration. At 17 months of age and later, an obvious decrease in islet number and size (less than 50 μ in diameter) was observed, even in relatively unaffected areas of the organ. Frequent bilateral cataracts began to appear at about 15 months of age. Opacities were first observed in the periphery of the lens, then increased rapidly in intensity and extended centripetally. Nineteen-month-old male rats were hypersensitive to exogenous insulin, but showed no significant decrease in blood glucose level when treated with oral tolbutamide. These results suggest that these rats suffered from a decreased insulinogenic response.

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