Diabetologia 9, 413–421 (1973)

Structural Changes of Pancreatic Islets in Genetically Obese Rats

A. Shino, T. Matsuo, H. Iwatsuka and Z. Suzuoki

Biological Research Laboratories, Central Research Division. Takeda Chemical Industries Co. Ltd, Osaka, Japan

Received: February 9, 1973, and in revised form: June 7, 1973

Summary. Light and electron microscopic observations were performed on pancreatic islets from genetically obese rats, (Zucker, "fatty"), from 5 to 52 weeks of age. At 5 weeks of age, islets were moderately hypertrophied. After that age, hypertrophy of islets became more prominent, until 24 weeks of age, with accompanying degranulation of B cells. The plasma insulin level also continued to increase during this period, but the glucose level was normal. Degranulated B cells contained a highly developed Golgi complex, numerous vesiculated, granular endoplasmic reticulum and a small number of secretory granules, but no glycogen deposits. Emiocytosis and microtubule formation were very remarkable with these B cells. Frequently, mixed or intermediate cells, such as exocrine-endocrine or ductural-endocrine cell, were observed in pancreas with hypertrophied islets. At 52 weeks of age, both the plasma insulin and triglyceride levels decreased. In the pancreas, there were observed proliferation of fibrous tissue and well granulated B cells in hypertrophied islets. Hence, in fatty rats, pancreatic islets were in an active state during the period of development of obesity and hyperlipaemia (from 5 to 24 weeks of age). These correlates of obesity and hyperinsulinism disappeared at 52 weeks of age.

Key words: Pancreatic islets, obese rats, ultrastructure, obesity, insulin, B cell, hyperlipaemia.

Zucker or "fatty" rats have genetically determined metabolic disorders such as obesity, hyperlipaemia and hypercholesterolaemia [25, 26]. Generally, obesity is associated with hyperinsulinism in humans and animals. York *et al.* [24], Lemonnier [10] and Zucker and Antoniades [27] confirmed increases in the plasma insulin level and insulin content of pancreas of these obese rats.

Structural changes of pancreatic islets were remarkable in spontaneously obese and hyperglycaemic animals — db/db mice [13], Wellesley hybrid mice [11], sand rat [12], spiny mice [16] and yellow KK mice [18]. Fatty rats can be distinguished from these animals by the blood glucose level, because fatty rats are normoglycaemic [26].

In hyperglycaemic animals, there has been consistently observed a stimulation of B cells secondary to hyperglycaemia, although other factors may be also involved. It is therefore important for physiological studies of the pancreas to compare B cells of normoglycaemic fatty rats to those of obese and hyperglycaemic animals. In the present study, we observed pancreatic islets from fatty rats at different ages to elucidate structural changes of B cells in relation to the advance of obesity.

Materials and Methods

Fatty rats, introduced from Zucker's laboratory, were made free from specific pathogens by Caesarean section in our laboratory. Male fatty rats (*fa/fa*) and their lean littermates (*fa/+*, *+/+*) were fed water and laboratory chow diet (CE-2, Japan Clea. Inc., Tokyo) in an individual metal cage. The blood samples were obtained by heart puncture under anaesthesia with ether. Plasma glucose and plasma insulin were determined by the glucose oxidase method [8] and immunological procedures [23] with Sephadex-antibody complex (an assay kit from Pharmacia Comp., and human insulin as the standard), respectively. Plasma triglyceride was determined as glycerol by triglyceride kit [20] (Wako pure chem. Ind., Osaka). Epididymal adipose tissue weight was measured as an index of adiposity.

The pancreas was rapidly removed and fixed in Bouin's or Rossman's fixative, embedded in paraffin and stained with Gomori's aldehyde fuchsin trichrome [4] and periodic acid Schiff (PAS), respectively. Specimens for PAS staining were treated with 1% collodion solution to prevent loss of glycogen during staining procedures. For electron microscopy, small blocks of pancreas were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C, washed in the same buffer and then fixed in 1% osmium tetroxide. Some specimens, fixed at room temparature by the above procedures, were used for observation of microtubular systems. They were dehydrated in ethanol, cleared with propylene oxide and embedded in Epon 812 [14]. Thin sections were cut with glass knives on an LKB ultrotome, mounted on unsupported copper grids, and stained by uranyl acetate and lead citrate [17]. Observations were performed with an electron microscope (JEM-T7s, Japan electron optics lab. Co., LTD).

The size of the pancreatic islets was measured on fatty and lean rats at 5 weeks of age using two specimens from each rat. The longest diameter of each islet was used as an index of islet size.

Results

Body weight and epididymal fat pad weight were much greater in fatty rats than in lean littermates at the same ages (Table 1). The range of plasma glucose values was 95 to 150 mg% in fatty rats, corresponding to that of lean littermates. In fatty rats, the plasma IRI and triglyceride levels were elevated even at 5 weeks, continued to increase until 24 weeks and then decreased. These observations clearly demonstrated that fatty rats were obese, hyperlipidaemic and hyperinsulinaemic and that these syndromes advanced with increasing age from 5 to 24 weeks. Upon fasting of two fatty rats at 8 weeks, the plasma IRI level decreased but plasma triglyceride and glucose levels hardly altered.

Table 1. *Biochemical data from genetically obese rats and their lean littermates*

	Age (weeks)	N	B.W (g)	Epididymal adipose tissue wt. (g)	Plasma glucose (mg/dl)	Plasma IRI (μU/ml)	Plasma TG (mg/dl)
Fatty	5	5	142± 3	1.2±0.1	102± 5	41± 3	92± 8
	8	5	335±12	6.3±0.6	95±11	81± 8	150± 17
	8 (a)	2	315, 320	5.3, 5.6	84,91	19,48	137, 167
	16	4	540±17	12.1±1.2	142± 5	135±13	305± 37
	24	5	609± 8	18.0±0.5	120± 5	252±26	1080±252
	52	5	784±37	23.1±3.1	150±16	87±17	657±117
Lean	5	5	125± 5	0.5±0.02	118± 5	13± 1	49± 5
	8	5	261±11	1.4±0.3	83± 5	18± 6	63± 6
	16	4	402± 5	3.9±0.1	144± 5	35± 5	81± 8
	52	4	504±29	5.6±0.5	110±19	—	—

All values represent mean ± S.E.M.

[a] Rats were fasted for 18 h.

Table 2. *Histological findings on the pancreatic islets from fatty rats*

Age (weeks)	5	8	16	24	52
Degranulation of B cell	−	++	++	+	−
Glycogen deposition in B cell	−	−	−	−	−
Hypertrophy of islets	+	++	+++	+++	+++
Increaese of fibrous tissue	−	−	+	+	++

* Percentage of pale granules in B cells.
lean: 12.4± 4.5 (n: 10)
fatty: 36.2±13.0 (n: 9)

* Quantitative determinations of pale granules were performed at 8 weeks of age.

Histological observations: (Table 2)

Histological examinations were performed on the pancreas from all of the rats cited in Table 1. At 5 weeks of age, the size of islets was increased in fatty rats (Fig. 1). However, the appearance of the B cells was completely normal in fatty as compared with those of lean rats. Eight week old fatty rats showed marked alterations in the islets besides advanced hypertrophy. Light microscopically, most of the B cells in islets were degranulated (Fig. 2). Ultrastructurally, in these B cells, there were observed a decreased number of secretory granules, vesicularization of granular endoplasmic reticulum and highly developed Golgi apparatus (Fig. 3). Quantitative observation of the B cell granule population showed that 36.2% of total granules were low in electron density (pale granules), while only 12.4% of granules were pale in B cells from control littermates (Table 2).

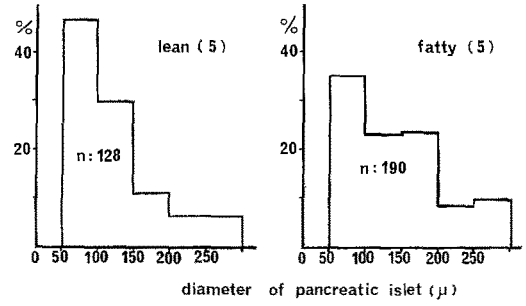

Fig. 1. Size distribution patterns of pancreatic islets from fatty rats and their lean littermates at 5 weeks of age. Perpendicular: Number of islet (% of total observed islets). Horizontal: Diameter of islets(μ)

Frequently, in the fatty rats, B granules, regardless of differences in density, were attached to each other or to the cell membrane by fusion of limiting membranes (Fig. 4). In pancreas fixed at room temperature, microtubules were prominent throughout the cytoplasm of B cells from fatty animals (Fig. 5). In fasted fatty rats, whose plasma insulin level was decreased, degranulation of B cells observed in the fed state was reversed and B cells were well granulated. Thus, the changes mentioned above may reflect an active state of insulin synthesis and secretion.

These changes observed in B cells became more prominent in 16 and 24 weeks old fatty rats. At these ages, there were extremely hypertrophied and degranulated islets, some of which exhibited proliferation of fibrous tissue (Fig. 6). Under the electron microscope, B cells of these rats showed not only profiles suggesting active synthesis and secretion of insulin, but also characteristic changes indicating formation of B cells from extra-insular cells. Some exocrine cells containing endocrine granules were observed around pancreatic islets. In these cells, exocrine granules coexisted with A and/or B granules and the lamella structure of endoplasmic reticulum was as remarkable as in acinar cells (Fig. 7). Another type of unusual cell was observed in the epithelial layer of pancreatic ducts. These cells were stained by aldehyde fuchsin (Fig. 8).

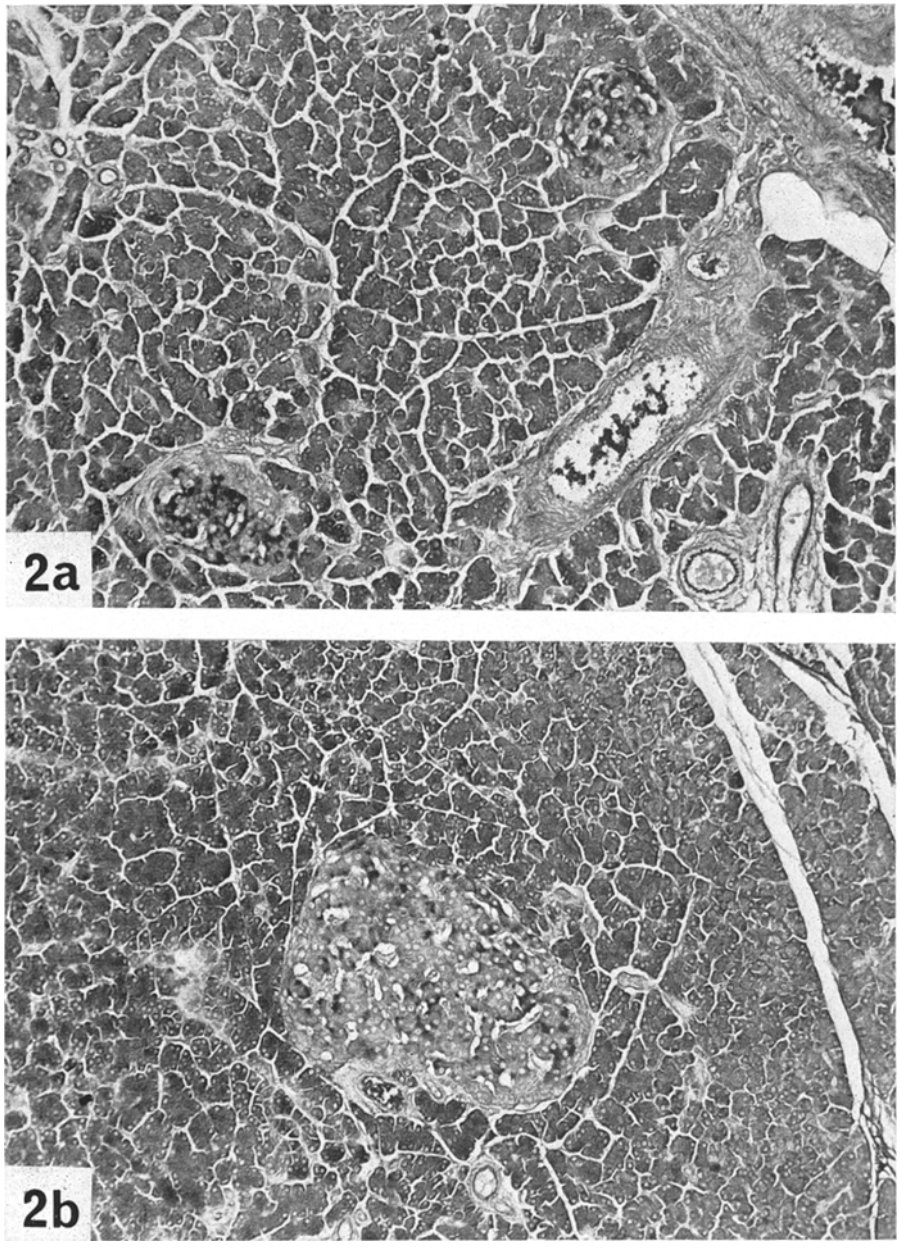

Fig. 2. Pancreatic islets from lean and fatty rats at 8 weeks of age. Degranulation of B cells is remarkable in fatty rats. Gomori's aldehyde-fuchsin stain. a) lean, b) fatty. 125 ×

In 52 weeks old fatty rats, whose plasma IRI level was decreased, pancreatic islets were still hypertrophied but B cells were filled with granules. An increase of fibrous tissue was remarkable in these islets (Fig. 9).

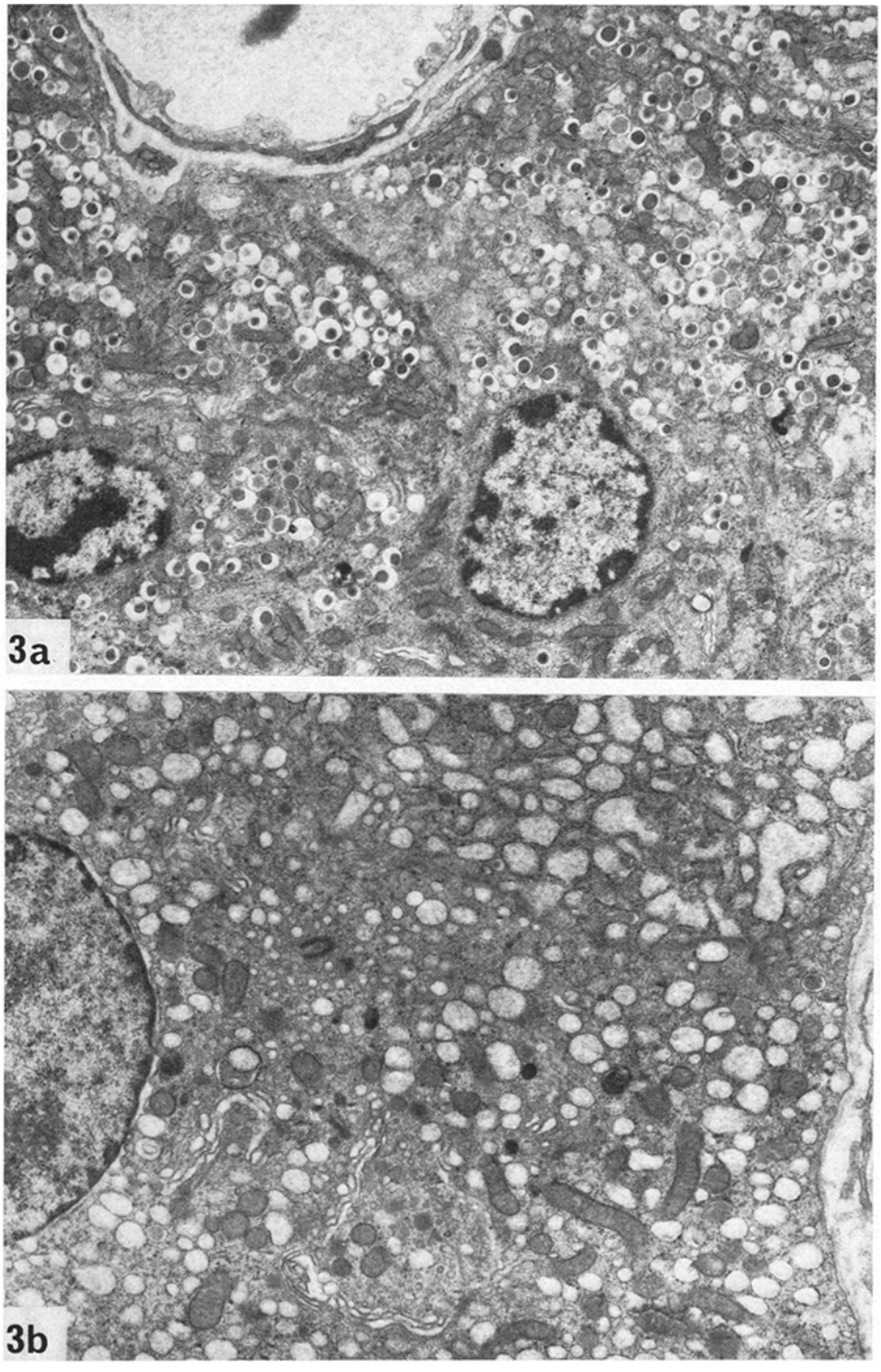

Fig. 3. Electron micrographs of B cells of pancreatic islets from lean and fatty rats at 8 weeks of age. The B cells of lean rat are well granulated. In fatty rats, the secretory granules of B cells are diminished and Golgi complex and rough endoplasmic reticulum are well developed. a) lean : 9200×, b) fatty : 12700×

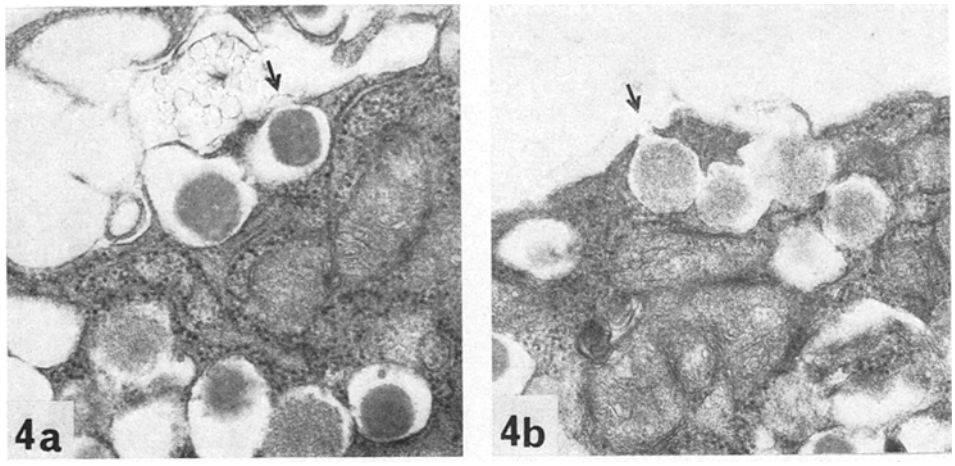

Fig. 4. Portions of B cells showing emiocytosis. Both electron dense and pale granules are liberated into subcapillary space (Arrows). 41000×

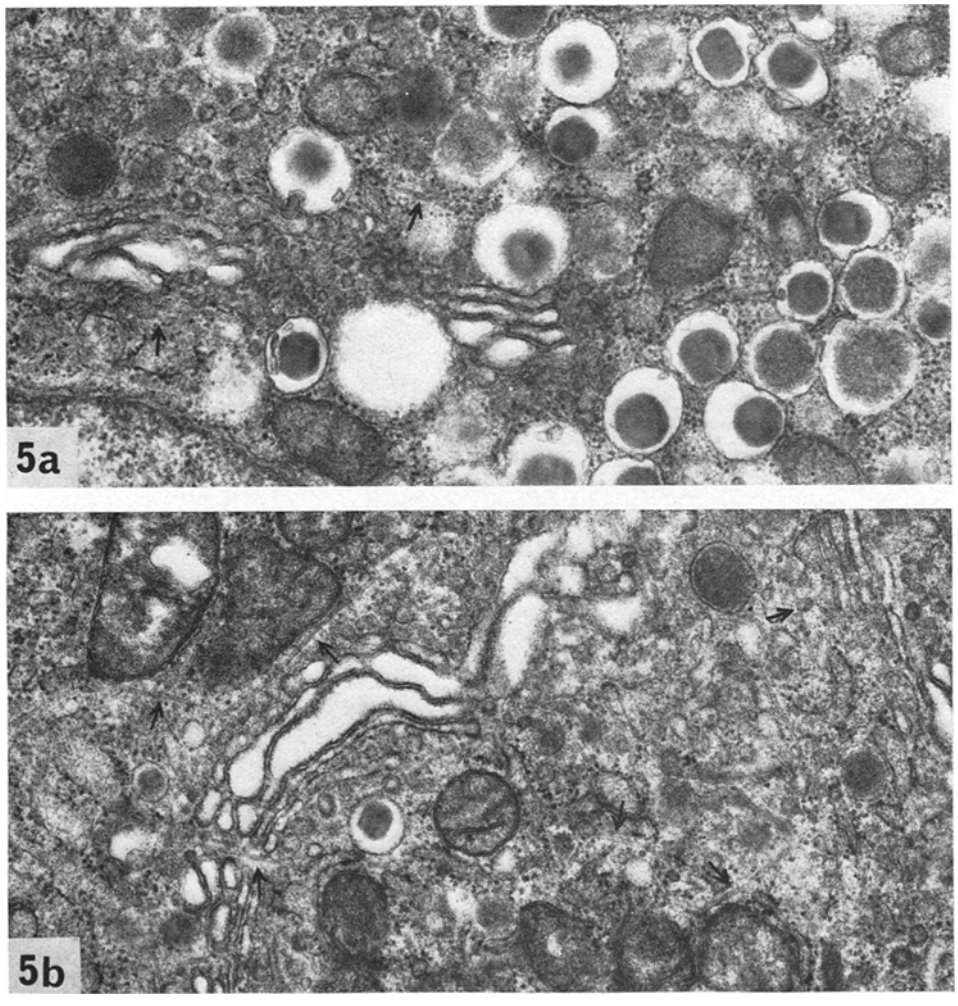

Fig. 5. Electron micrographs of B cells from lean and fatty rats at 8 weeks of age. These pictures are obtained from the tissue fixed in glutaraldehyde at room temperature. Profiles of microtubules are more prominent in fatty than in lean rat (Arrows). 36000×

Deposition of glycogen in B cells could not be detected by light or electron microscopy in the pancreas from all of the fatty rats used in the present studies. In control rats, there was no structural alteration of pancreatic islets, except a mild increase of fibrous tissue in islets from 52 week old rats.

Discussion

Close association of obesity and hyperinsulinaemia suggests the presence of active synthesis and secretion of insulin in humans and animals [1, 18, 22]. Many studies on animals with spontaneous diabetes and obesity have given clear cytological evidence for pancreatic islet hyperfunction, which appeared to explain hyperinsulinaemia [12, 16, 18, 22].

Stern *et al.* observed a positive correlation between an elevation of the plasma insulin level and enlargement of adipocytes in fatty rats [19]. Zucker and Antoniades [27] also confirmed an increase in body fat accompanying elevation of the plasma insulin level with increasing age.

The causal relationship between obesity and hyperinsulinism is very complicated in genetically obese and/or diabetic animals [6, 7, 24]. The case of fatty rats is not exceptional. York *et al.* confirmed advanced insensitivity to insulin in fatty as compared to hypothalamic-lesioned obese rats [24]. This finding suggests that a primary defect of fatty rats is insensitivity to insulin, which results in obesity through hyperinsulinism. On the other hand, it was shown that the plasma insulin level of fatty rats could hardly be reduced by reduction of the carbohydrate content of the diet

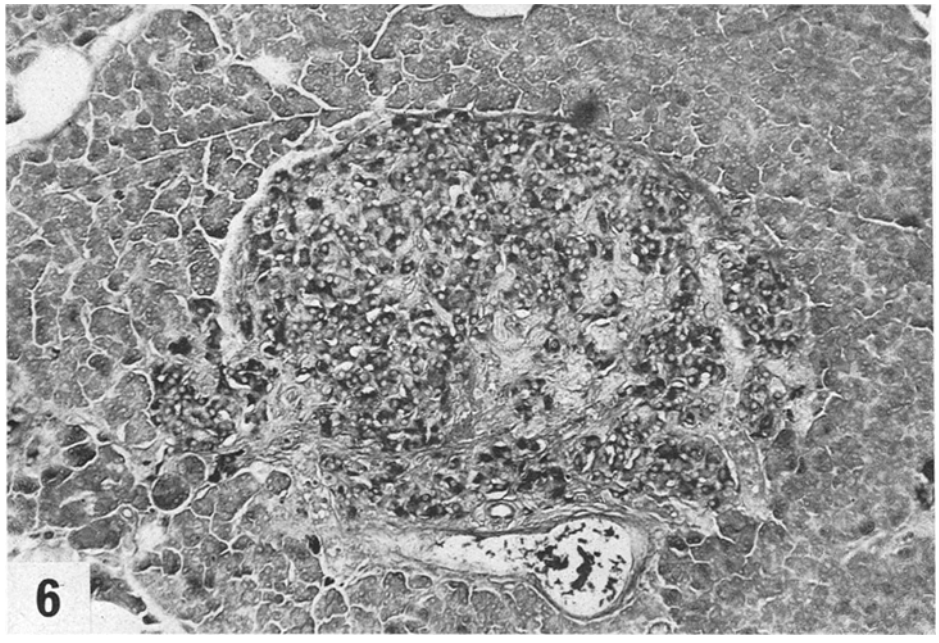

Fig. 6. Light micrograph of pancreatic islet from 24 weeks old fatty rat. Hypertrophy of islets and degranulation of B cells are prominent. Proliferation of fibrous tissue is also remarkable. Aldehyde-fuchsin stain. 125×

The present study confirms the same kind of changes in the pancreas from fatty rats, such as hypertrophy of islets and highly developed endoplasmic reticulum and Golgi complex, which suggested active insulin synthesis in the B cells. Furthermore, emiocytosis, microtubular formation and increased ratio of pale granules in B cells were observed as cytological evidence of active insulin secretion [2, 9, 15, 21].

The transformations of extrainsular cells into B cells were observed in fatty rats as well as in diabetic mice [16, 18]. These, together with marked hypertrophy of islets, suggest a compensatory adaptation of the pancreas to increased demand of insulin by peripheral tissue, associated with advancing obesity. [27] or restriction of food intake [24]. This suggests that B cell function of fatty rats is quite different from that of normal ones.

The absence of glycogen deposits in degranulated B cells of fatty rats is cytological evidence to exclude the onset of a transient hyperglycaemia in fatty rats, because Carpenter and Lazarow confirmed that artificial transient hyperglycaemia produced glycogen deposits in rat B cells [3]. In fact, we observed glycogen deposits in degranulated B cells from intermittently hyperglycaemic yellow KK mice [18]. It is likely that insulin secretion of fatty animals is highly stimulated even by normoglycaemia. Furthermore, fasting was found to be more effective on the insulin level than on

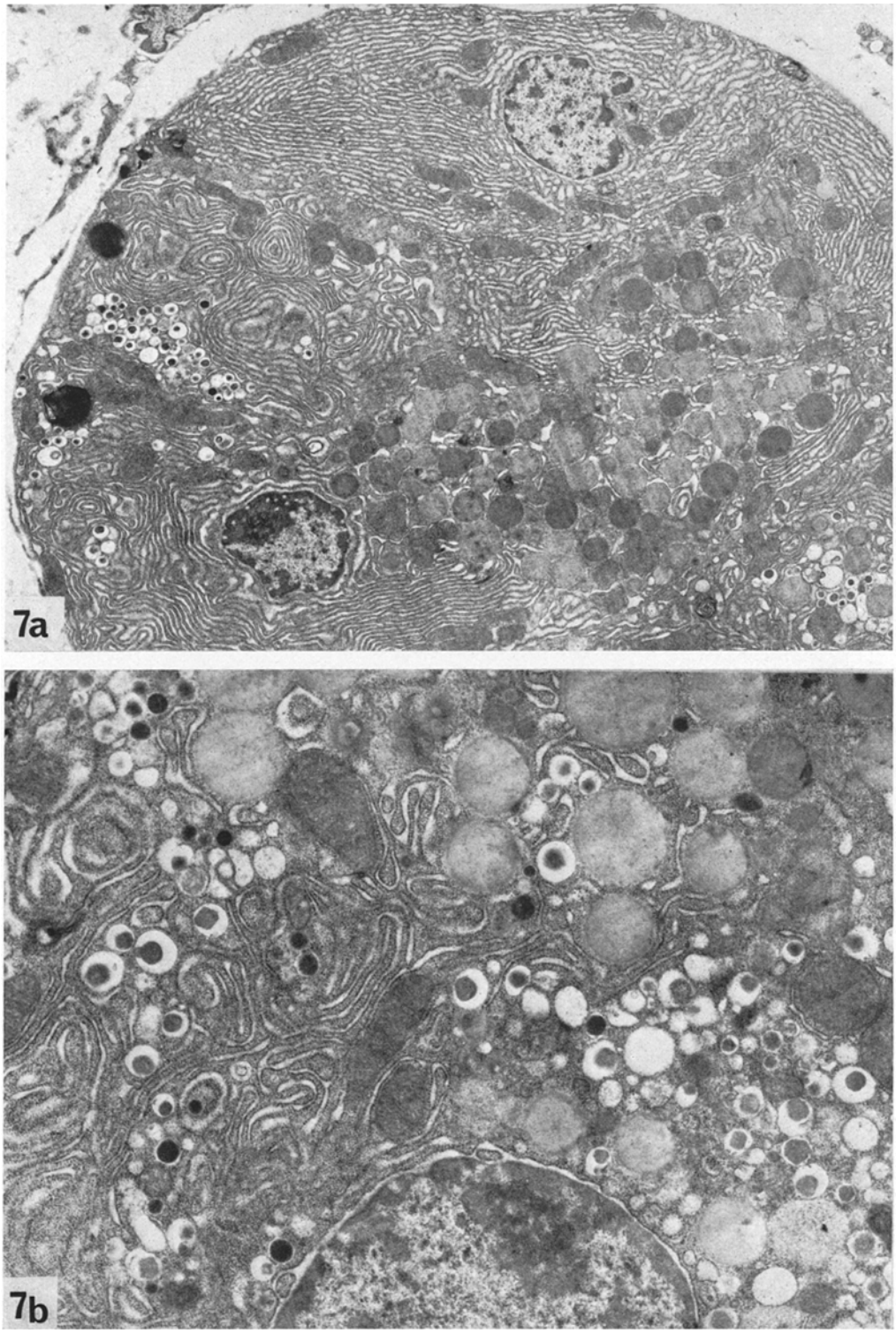

Fig. 7. Mixed cells in pancreas from 24 weeks old fatty rat. a) Cells contained B and zymogen granules. 5500×. b) A mixed cell contained A, B and zymogen granules in cytoplasm. 15000×

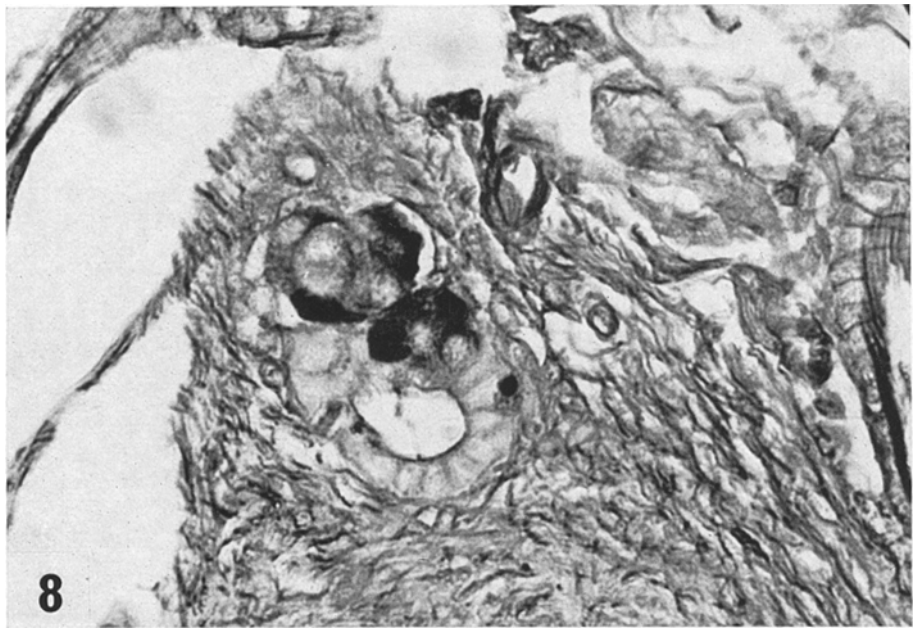

Fig. 8. Ductus B cells in pancreas from 24 weeks old fatty rat. Aldehyde-fuchsin stain. 460×

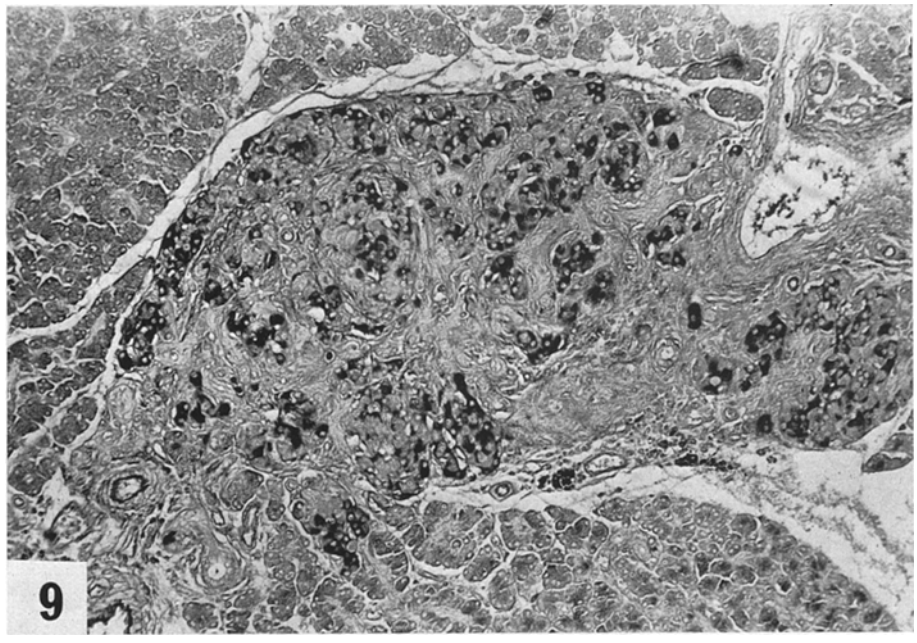

Fig. 9. Pancreatic islet from 52 weeks old fatty rat. Extensive proliferation of fibrous tissue is remarkable. Aldehyde-fuchsin stain. 125×

the glucose level in the fatty rats. This phenomenon was also observed by Zucker and Antoniades [27]. It also seems that insulin secretion of these rats depends mostly on stimulants produced by feeding substances other than glucose. Therefore, the primary defect due to *fa* gene is still obscure.

In one year old fatty rats, there were observed reduction of the plasma insulin level and degenerated B cells, manifested by fibroplasia. The regression of obese syndromes in old age is not specific for these animals, because hyperinsulinaemia and hypertrophy of adipocytes disappeared in old ob/ob and yellow KK

mice [5, 18]. At the present time, there is no plausible explanation for this phenomenon with genetically obese animals.

Acknowledgement. We are gratefull to Dr. T.F. Zucker for a generous gift of fatty rats. Our thanks are also due to Mrs K. Shimakawa, E. Ishikawa, S. Taketomi and Miss M. Fukuwatari in our laboratory for routine supply of the specific pathogen free rats and usefull technical assistance in the experiments. We wish to thank Dr. K. Shimamoto for his encouragement.

References

1. Bagdade, J.D., Bierman, E.L., Porte, D.Jr.: Influence of obesity on the relationship between insulin and triglyceride levels in endogenous hypertriglyceridemia. Diabetes **20**, 664–672 (1971)
2. Bencosme, S.A., Tsutsumi, V., Martin, J.M., Åkerblom, H.K.: Ultrastructural changes in B cells of pancreatic islets from rats with elevated levels of circulating growth hormone secreted by MtT-W15 tumor. Diabetes **20**, 15–26 (1971)
3. Carpenter, A.M., Lazarow, A.: Effects of hyper- and hypoglycemia on beta cell degranulation and glycogen infiltration in normal, subdiabetic and diabetic rats. Diabetes **16**, 493–501 (1967)
4. Gomori, G.: Aldehyde-fuchsin: new stain for elastic tissue. Amer. J. clin. Path. **20**, 665 (1950)
5. Herberg, L., Major, E., Hennigs, U., Grünneklee, D., Freytag, G., Gries, F.A.: Differences in the development of the obese-hyperglycemic syndrome in obob and NZO mice. Diabetologia **6**, 292–299 (1970)
6. Iwatsuka, H., Shino, A., Suzuoki, Z.: General survey of diabetic features of yellow KK mice. Endocr. jap. **17**, 23–35 (1970)
7. Iwatsuka, H., Shino, A.: Studies on diabetogenic action of obesity in mice.: Congenital insulin resistance of KK mice. Endocr. jap. **17**, 535–540 (1970)
8. Krebs, H.A., Dierks, C., Gascoyne, T.: Carbohydrate synthesis from lactate in pigeon-liver homogenates. Biochem. J. **93**, 112–121 (1964)
9. Lacy, P.E.: Beta cell secretion — from the standpoint of a pathologist. Diabetes **19**, 895–905 (1970)
10. Lemonnier, D.: Hyperinsulinism in genetically obese rats. Horm. Metab. Res. **3**, 287–288 (1971)
11. Like, A.A., Jones. E,E.: Studies on experimental diabetes in the wellesley hybrid mouse. Diabetologia **3**, 179–187 (1967)
12. Like, A.A., Miki, E.: Diabetic syndrome in sand rats. IV. Morphologic changes in islet tissue. Diabetologia **3**, 143–166 (1967)
13. Like, A.A., Chick, W.L.: Studies in the diabetic mutant mouse.: II. Electron microscopy of pancreatic islets. Diabetologia **6**, 216–242 (1970)
14. Luft, J.H.: Improvements in epoxy resin embedding methods. J. biophys. biochem. Cytol. **9**, 409–414 (1961)
15. Malaisse, W.J., Malaisse-Lagae, F., Walker, M.O., Lacy, P.E.: The stimulus-secretion coupling of glucose-induced insulin release V. The participation of a microtubular-microfilamentous system. Diabetes **20**, 257–265 (1971)
16. Pictet, R., Orci, L., Gonet, A.E., Rouiller, C., Renold, A.E.: Ultrastructural studies of the hyperplastic islets of Langerhans of spiny mice (Acomys Cahirinus) before and during the development of hyperglycemia. Diabetologia **3**, 188–211 (1967)
17. Reynolds, E.S.: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. **17**, 208–212 (1963)
18. Shino, A., Iwatsuka, H.: Morphological observations on pancreatic islets of spontaneous diabetic mice, "yellow KK". Endocr. jap. **17**, 459–476 (1970)
19. Stern, J., Johnson, P.R., Greenwood, M.R.C., Zucker, L.M., Hirsch, J.: Insulin resistance and pancreatic insulin release in the genetically obese Zucker rat. Proc. Soc. exp. Biol. (N.Y.) **139**, 66–69 (1972)
20. Van Handel, E., Zilversmit, D.B.: Micromethod for the direct determination of serum triglycerides. J. Lab. clin. Med. **50**, 152–157 (1957)
21. Williamson, J.R., Lacy, P.E., Grisham, J.W.: Ultrastructural changes in islets of the rat produced by tolbutamide. Diabetes **10**, 460–469 (1961)
22. Wrenshall, G.A., Andrus, S.B., Mayer, J.: High levels of pancreatic insulin coexistent with hyperplasia and degranulation of beta cells in mice with the hereditary obese-hyperglycemic syndrome. Endocrinology **56**, 335–340 (1955)
23. Yalow, R.S., Berson, S.A.: Immunoassay of endogenous plasma insulin in man. J. clin. Invest. **39**, 1157–1175 (1960)
24. York, D.A., Steinke, J., Bray, G.A.: Hyperinsulinemia and insulin resistance in genetically obese rats. Metabolism **21**, 277–284 (1972)
25. Zucker, L.M., Zucker, T.F.: Fatty, a new mutation in the rat. J. Hered. **52**, 275–278 (1961)
26. Zucker, L.M.: Hereditary obesity in the rat associated with hyperlipidemia. Ann. N. Y. Acad. Sci. **131**, 447–458 (1965)
27. Zucker, L.M., Antoniades, H.N.: Insulin and obesity in the Zucker genetically obese rat "Fatty". Endocrinology **90**, 1320–1330 (1972)

Dr. A. Shino
Biological Research Laboratories
Central Research Division
Takeda Chemical Industries Co.
Osaka (532)
Japan