INFLUENCE OF THE MUTATION "DIABETES" ON INSULIN RELEASE AND ISLET MORPHOLOGY IN MICE OF DIFFERENT GENETIC BACKGROUNDS

L. BOQUIST, B. HELLMAN, Å. LERNMARK, and I.-B. TÄLJEDAL

From the Departments of Pathology and Histology, University of Umeå, Umeå, Sweden

ABSTRACT

Mice, 7–8-mo old, of the C57BL/KsJ-*db* strain and homozygotic for the mutant gene *db*, exhibited marked hyperglycemia and moderately elevated serum insulin levels. Light and electron microscopy provided evidence of a slightly decreased proportion of β cells in the pancreatic islets, irregular islet architecture with intraislet ducts, and degenerative as well as hypertrophic changes in the individual β cells. As a rule, islets microdissected from these mice did not release insulin in response to glucose, theophylline, iodoacetamide, or chloromercuribenzene-*p*-sulphonic acid. The absence of secretory responses was not simply due to lack of insulin. Although the islet content of insulin was decreased in C57BL/KsJ-*db*/*db* mice, the remaining amount was severalfold larger than that released from stimulated islets of normal controls. Another mutation, db^{2J}, an allele of *db* with identical phenotypic expressions in the C57BL/KsJ strain, was studied on the genetic background C57BL/6J. In contrast to the severely diabetic C57BL/KsJ-*db*/*db* animals, the C57BL/6J-db^{2J}/db^{2J} mice were characterized by highly elevated serum insulin levels and only moderate hyperglycemia. Their endocrine pancreas was enlarged and showed an increased proportion of β cells. Like the islets of normal mice, those of C57BL/6J-db^{2J}/db^{2J} mice responded to glucose and chloromercuribenzene-*p*-sulphonic acid, the glucose-induced responses being potentiated by theophylline or iodoacetamide. C57BL/KsJ-*db*/*db* mice should provide a valuable model for studying defects in insulin secretion in relation to diabetes mellitus. Mice of the C57BL/6J strain offer a control material that may help to elucidate the dependence of the insulin secretory defect on the background genome.

INTRODUCTION

Diabetic patients exhibit a defective insulin secretion that may be significant for the etiology of the disease (Seltzer et al., 1967; Cerasi and Luft, 1967; Felig, 1971). The mechanisms of insulin release have been intensely studied in animal experiments employing a variety of in vitro models. In general, the normal physiology of the pancreatic β cells has been investigated, whereas comparatively little attention has been paid to the possible disturbances in diabetes. This is no doubt due to the fact

that the normal secretory mechanisms are so far only dimly understood. However, the lack of suitable animal models for experimental work is also obvious. Although several types of spontaneously hyperglycemic rodents are available, most of them exhibit neither morphological signs of β-cell deterioration nor an impaired secretory capacity of the islets. Poor insulin secretory responses have been observed in spiny mice (Cameron et al., 1972), sand rats (Hahn et al., 1971), and New Zealand obese mice (Larkins, 1973), but there are no self-evident controls to these animals. The Chinese hamster may develop a type of diabetes that resembles juvenile diabetes in humans (Gerritsen and Dulin, 1967; Boquist, 1969 *a*; Loge et al., 1973). However, the relative scarcity of information concerning the genetics and normal islet physiology of this species is inconvenient in experimental work.

Coleman and Hummel (1967), and Hummel et al. (1972) described "diabetes" and "diabetes-2J," recessive mutations in mice that produce diabetes in the homozygotes *db/db* or db^{2J}/db^{2J}. The manifestations of the genes *db* and db^{2J} are mutually indistinguishable but depend on the genetic background (Hummel et al., 1972). While a severe type of diabetes with β-cell necrosis occurs in the strain C57BL/KsJ, the same genes in the strain C57BL/6J produce a syndrome of mild hyperglycemia and β-cell hyperplasia. Thus the "diabetes" mutation when placed in different strains may provide valuable models for the experimental elucidation of functional β-cell abnormalities in diabetes mellitus as well as of their dependence on interactions with the background genome. We have therefore investigated the capacity for insulin release in pancreatic islets microdissected from C57BL/KsJ-*db/db* mice and C57BL/6J-db^{2J}/db^{2J} mice as well as from the normal controls C57BL/KsJ-+/+. The mice selected for this study were also characterized with respect to the concentrations of glucose and insulin in blood, the islet content of insulin, and islet morphology.

MATERIALS AND METHODS

Animals

Inbred mice were obtained from three local C57BL stocks originally established through the generous gift in 1972 of breeding couples from Dr. D. L. Coleman, Jackson Laboratories, Bar Harbor, Maine. Normal controls were of the strain C57BL/KsJ and maintained as a stock free of the *db* gene; they will be referred to as KsJ-+/+ mice. Another stock had the identical genetic background but contained the mutated *db* gene, which in the homozygous state gives rise to diabetes (Coleman and Hummel, 1967; Hummel et al., 1972); diabetic mice of this type will be designated as KsJ-*db/db* mice. The third stock had the genetic background C57BL/6J and carried the gene db^{2J}. This gene is the second mutation to "diabetes" at Jackson Laboratories, and in all investigated respects is indistinguishable from *db* (Hummel et al., 1972; Coleman, personal communication). For the sake of simplicity, diabetic homozygotes of this background will therefore be designated as 6J-*db/db*. The first mutation to "diabetes," *db*, was not available on the 6J background.

The KsJ-*db/db* mice were regularly weighed and taken for study after repeated weighings had given clear evidence of inhibited growth, or, in the majority of cases, weight reduction. Maximum body weights of 57 ± 2 g for 11 males and 59 ± 1 g for 19 females were recorded at the age of 4.5 ± 0.1 and 4.8 ± 0.2 mo, respectively (mean values ± SEM). Table I gives the sex as well as the age and weight at the time of killing for all three strains of mice. Because the morphological and chemical analyses revealed no obvious differences between the sexes, they are brought together in the following presentation and discussion of results. All mice were fed ordinary laboratory chow and water ad libitum until 1 day before killing. During the last night some animals continued to have free access to food and water, while others were given only water, as indicated in the Results section.

Serum Glucose and Serum Insulin

The mice were killed by decapitation. Mixed arterial and venous blood was collected at the neck wound, allowed to clot at 4°C, and centrifuged. Samples of serum were analyzed for glucose with the coupled hexokinase-glucose-6-phosphate dehydrogenase method (Lowry et al., 1964), and for insulin with the radioimmunoassay described below.

TABLE I

Body Weight and Age of Mice at the Time of Experimentation

Type	No. of animals	Weight	Age
		g	*mo*
Male KsJ-+/+	13	29 ± 1	7.8 ± 0.3
Female KsJ-+/+	11	25 ± 1	7.5 ± 0.3
Male KsJ-*db/db*	11	46 ± 2	7.8 ± 0.5
Female KsJ-*db/db*	19	51 ± 1	7.5 ± 0.5
Male 6J-*db/db*	7	56 ± 4	9.6 ± 1.5
Female 6J-*db/db*	14	65 ± 3	8.5 ± 1.0

Mean values ± SEM.

Insulin Release

Fresh pancreatic islets were microdissected freehand (Hellerström, 1964) in Krebs-Ringer bicarbonate buffer supplemented with 3 mM glucose and 1 mg of bovine serum albumin/ml (Sigma Chemical Co., St. Louis, Mo.; "fraction V"), equilibrated with O_2–CO_2 (95:5), and kept at room temperature. This type of medium was also used as basal medium in subsequent incubations. Preliminary incubation was carried out for 40 min at 37°C in basal medium only. The islets were then incubated for 60 min at 37°C in fresh basal medium to which were added test substances as indicated in the legends to Tables. After incubation, the islets were freeze-dried (−40°C, 0.001 mm Hg) overnight and weighed as previously described (Lernmark, 1971). Amounts of insulin released during the 60 min of incubation were determined radioimmunologically. The purity of islets microdissected from KsJ-+/+ and KsJ-*db*/*db* mice was checked by electron microscopy.

Insulin Content in Islets

In some experiments the weighed islets were extracted by sonication for 15 s (Sonifier B-12 equipped with a microtip and run at a meter reading of 50 W, Branson Sonic Power Co., Danbury, Conn.) in 0.01 M HCl supplemented with 5 mg of bovine serum albumin/ml. The extracts were then freeze-dried, dissolved in and appropriately diluted with phosphate buffer (see below), and radioimmunoassayed for insulin.

Radioimmunoassay of Insulin

For the immunoassay of insulin, insulin antibodies were obtained from Wellcome Reagents Ltd., Beckenham, England, and ^{125}I-labeled pig insulin from Farbwerke Hoechst A. G., Frankfurt am Main, Germany. The reaction between insulin and antibodies was generally carried out for 24 h at 4°C in 40 mM phosphate buffer (pH 7.4) supplemented with 0.2 mM thiomersalate, 50 mM NaCl, and 3 mg of bovine serum albumin/ml. When assaying serum samples, the concentration of albumin in the buffer was 40 mg/ml. Free and antibody-bound insulin were separated by precipitation with 81% (vol/vol) ethanol (Heding 1966), and the precipitate was washed once with 81% ethanol and analyzed for radioactivity in a Packard gamma spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). As reference we used crystalline insulin prepared from phenotypically normal mice of a local noninbred stock carrying the gene *ob*. No difference was detected between the insulins from the three inbred stocks in their reactivity with the antibodies employed (Fig. 1).

Qualitative Light and Electron Microscopy

Pieces of pancreas were fixed in Bouin's fluid and subjected to routine light microscope procedures, including embedding in paraffin. Sections 4 μm thick were stained with hematoxylin-eosin, van Gieson's stain, aldehyde fuchsin, chrome-alum hematoxylin with ponceau fuchsin as counterstain, and the silver impregnation methods of Grimelius (1968), and Hellerström and Hellman (1960).

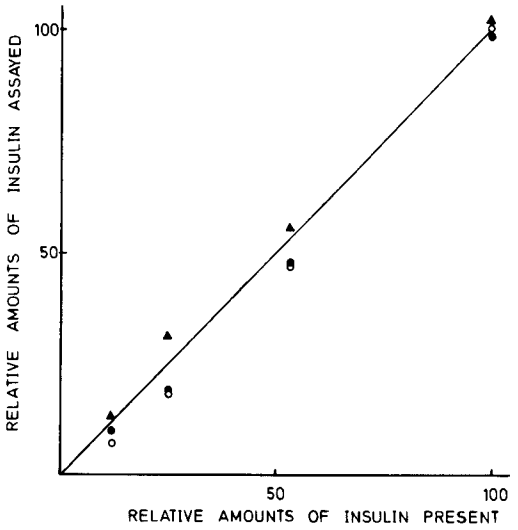


FIGURE 1 Relationship between amounts of insulin determined by radioimmunoassay and those actually present in extracts of islets from KsJ-+/+ mice (○), KsJ-*db*/*db* mice (●), and 6J-*db*/*db* mice (▲). Islets were extracted as described in the text, and the extracts were diluted to give the relative concentrations of insulin indicated on the abscissa. With reference to our routine standard of crystalline mouse insulin, 100% on the ordinate corresponds to 8.0 (KsJ-+/+), 5.2 (KsJ-*db*/*db*), and 13.3 (6J-*db*/*db*) ng of insulin per ml of extract.

Specimens for electron microscopy were fixed in 2.5% glutaraldehyde in 0.36 M Veronal acetate buffer (pH 7.4) and postfixed in 1% osmium tetroxide in the same buffer. Some specimens were directly fixed in osmium tetroxide. After rinsing and dehydration, the fixed tissue was embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in Siemens Elmiskopes 1A and 101.

Morphometry

The proportion of β cells among the endocrine parenchymal cells was estimated by differential counting on aldehyde fuchsin-stained light microscopic sections, 4 μm thick. The sections were placed on a movable stage and examined at a magnification of × 500 in the order in which they were encountered when the stage was moved in a predetermined fashion. Only cells with a visible nucleus or fragment of nucleus were counted. Corrections based on the nuclear size were not made, since it was about the same in the different islet parenchymal cells. For each animal, duplicate determinations were performed, a single determination comprising at least

TABLE II

Serum Glucose and Serum Insulin in Normal and Diabetic Mice

	KsJ-+/+	KsJ-*db/db*	6J-*db/db*
Fed animals			
Serum glucose, *mg/100 ml*	116 ± 27 (4)	690 ± 58 (9)	230 ± 38 (8)
Serum insulin, *ng/ml*	5.5 ± 1.6 (5)	11.9 ± 3.2 (7)	68.7 ± 8.9 (7)
Starved animals			
Serum glucose, *mg/100 ml*	44 ± 6 (6)	398 ± 64 (10)	142 ± 19 (4)
Serum insulin, *ng/ml*	0.2 ± 0.1 (7)	5.5 ± 1.7 (10)	22.4 ± 10.0 (5)

Mean values ± SEM for the numbers of animals stated in parentheses.

1,000 cells or 12 islet section surfaces. From the differences between duplicates, the standard deviation of a single determination (method error) was estimated as 0.5–1.1% β cells in the three strains of mice. To get an idea of the size distribution of islets, the islet section surfaces were classified with respect to their content of parenchymal cells.

The relative volumes of islet and acinar parenchyma as well as of duct epithelium were determined by point sampling in light microscopic sections. A square-ruled grid with 121 intersection points was placed in the eyepiece, and the numbers of intersections (hits) superimposed on islets, acini, and ducts were counted at a magnification of × 125. Each determination was based on at least 2,400 hits and 10 pancreatic sections.

All morphometric studies were carried out on sections from mice fasted overnight.

RESULTS

Glucose and Insulin in Serum

As shown in Table II, the KsJ-*db/db* mice had a considerable hyperglycemia and a tendency to increased concentrations of insulin in serum. One mouse differed from the others in having a serum insulin level as high as 107 ng/ml. Because this value is significantly higher than the others recorded for KsJ-*db/db* mice, it has not been included in Table II. This animal also had the lowest concentration of glucose in serum, 374 mg/100 ml, among the fed KsJ-*db/db* mice investigated. The 6J-*db/db* mice were moderately hyperglycemic with greatly elevated serum insulin levels. Starvation overnight resulted in a fall of serum glucose and insulin in both types of *db/db* mice as well as in the normal KsJ-+/+ controls. However, even after starvation the *db/db* mice had abnormally high concentrations of glucose and insulin in serum.

Qualitative Light and Electron Microscopy

The pancreatic islets of the KsJ-+/+ mice had the same appearance as those of other strains of normal mice. They were rounded or ellipsoid and contained α_1, α_2, and β cells without degenerative changes. No intraislet ducts were seen.

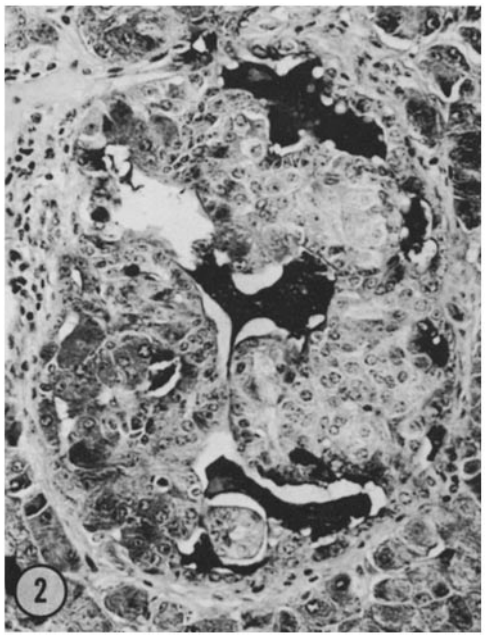


FIGURE 2 Islet of a KsJ-*db/db* mouse showing several ducts containing dark-stained fluid as well as papillary projections into the lumina. Aldehyde-fuchsin; × 80.

The appearance of the endocrine pancreas in KsJ-*db/db* mice varied considerably. Although most islets were rounded or ellipsoid, a conspicuous number were irregularly shaped. In comparison with the KsJ-+/+ mice, there was apparently a greater number of small islets in the KsJ-*db/db* mice. Small ducts lined by cuboidal epithelium were often found in KsJ-*db/db* mice at the periphery of the islets or in the islets in close connection with islet parenchymal cells (Figs. 2 and 3). Some islets were extremely rich in ducts (Fig. 2). Endocrine cells were also seen to be interposed between

the epithelial cells of ducts without any direct connection with islets (Fig. 4).

While the α_1 and α_2 cells appeared normal in the KsJ-*db/db* mice, the β cells exhibited varying degrees of degranulation, degeneration, and hypertrophy. The degenerative changes varied from slight cytoplasmic and nuclear lesions to necrosis of whole β cells. These changes included mitochondrial swelling and dissolution and disorganization of the endoplasmic reticulum and Golgi complex,

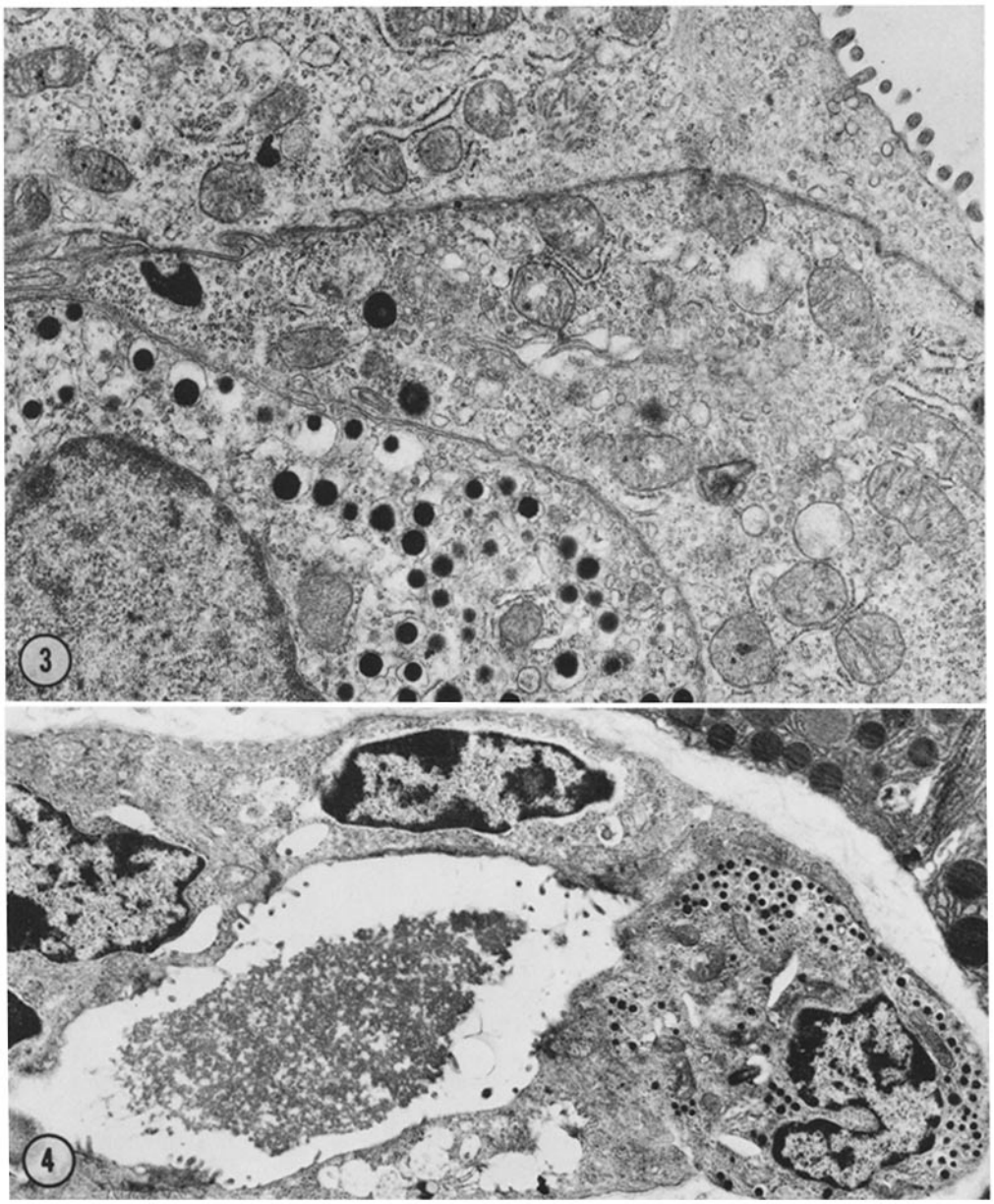


FIGURE 3 Portion of intraislet duct in a KsJ-*db/db* mouse. The duct lumen (top right) is lined by epithelial cells without secretory granules. A β cell is seen in close contact with the duct epithelium. × 9,000.

FIGURE 4 Extraislet duct in a KsJ-*db/db* mouse. One apparently endocrine cell is interposed between the ductule epithelial cells and extends to the lumen. In this endocrine cell a portion of a cilium is seen. × 6,500.

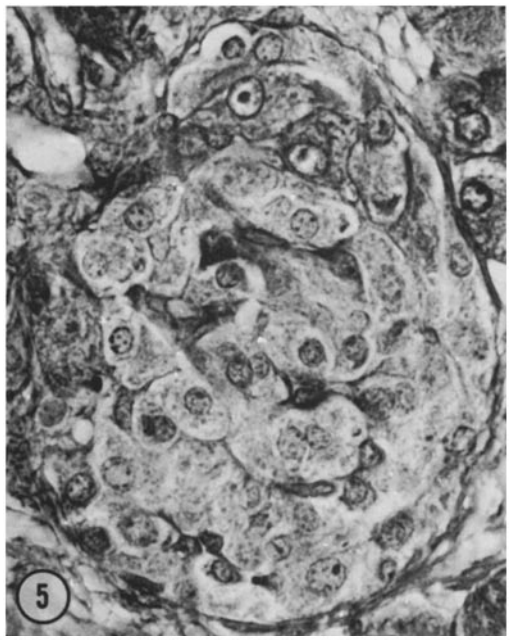


FIGURE 5 Islet of a KsJ-*db/db* mouse. Hypertrophic β cells with light-staining cytoplasm, large nuclei, and rather prominent nucleoli are seen. Chrome-alum-hematoxylin; × 120.

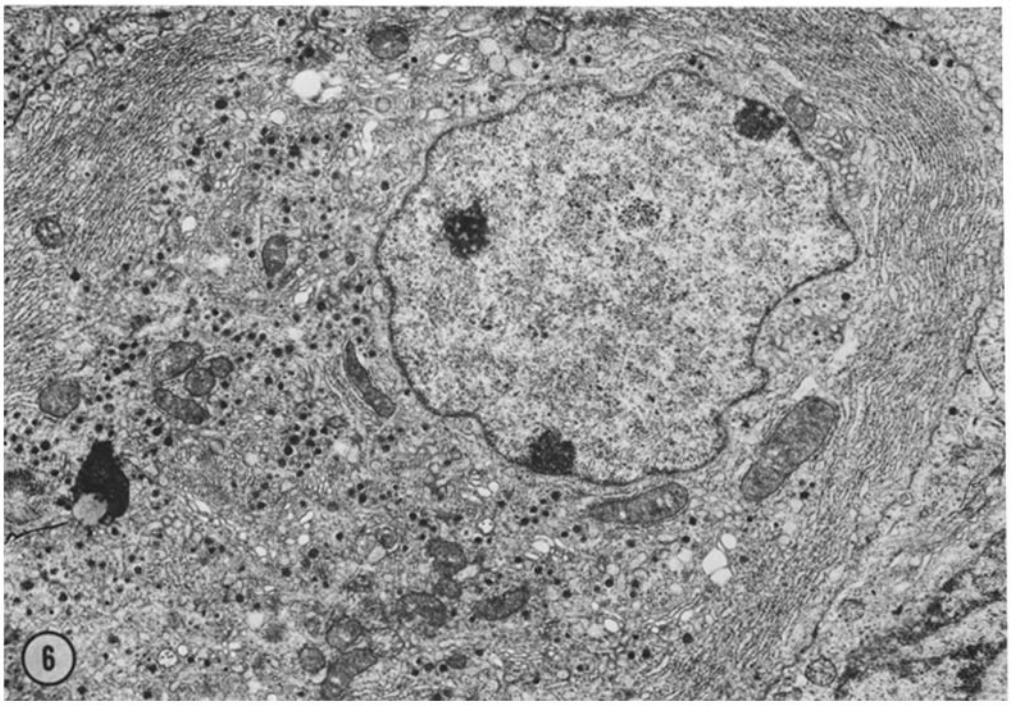
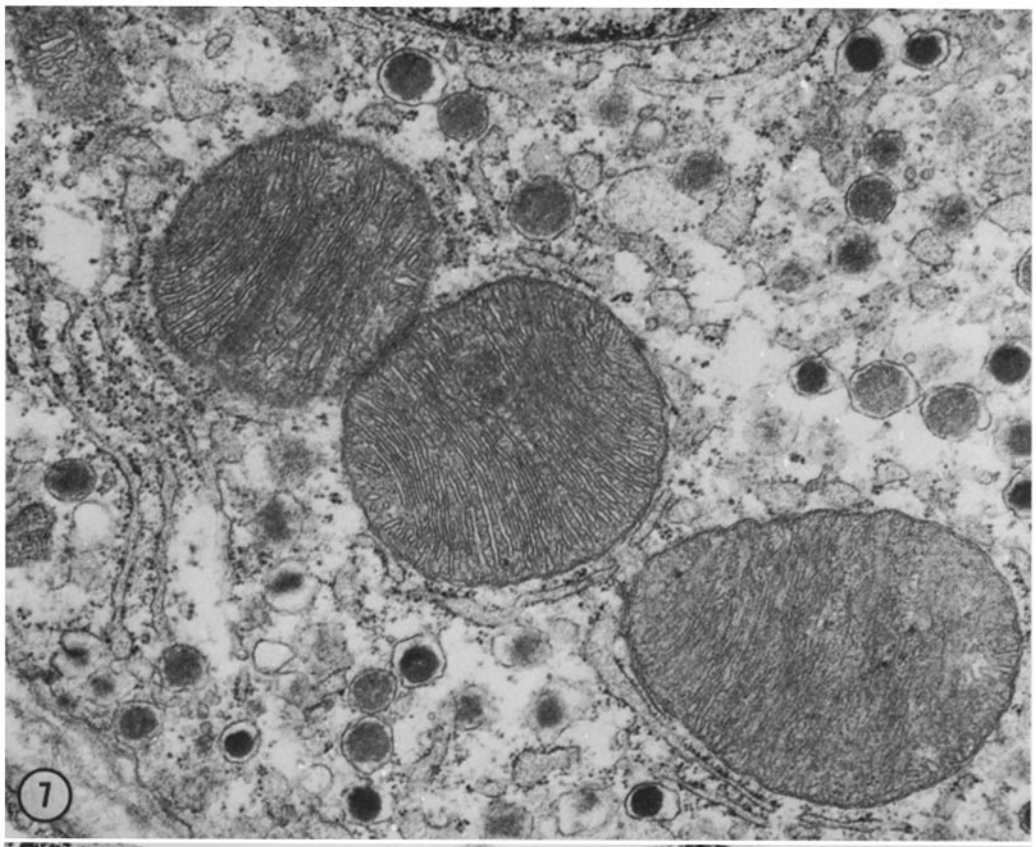


FIGURE 6 Hypertrophic β cell of a KsJ-*db/db* mouse. A prominent, lamellar endoplasmic reticulum, a well-developed Golgi complex, and a moderate number of secretory granules are seen. × 4,000.

as well as pycnosis and nuclear fragmentation. Hypertrophic β cells were characterized by large nuclei and nucleoli (Fig. 5) and by prominent Golgi complex and endoplasmic reticulum (Fig. 6); often the mitochondria were also enlarged (Fig. 7) and occasionally exhibited conspicuously elongated cristae.

In contrast to the endocrine pancreas of the KsJ



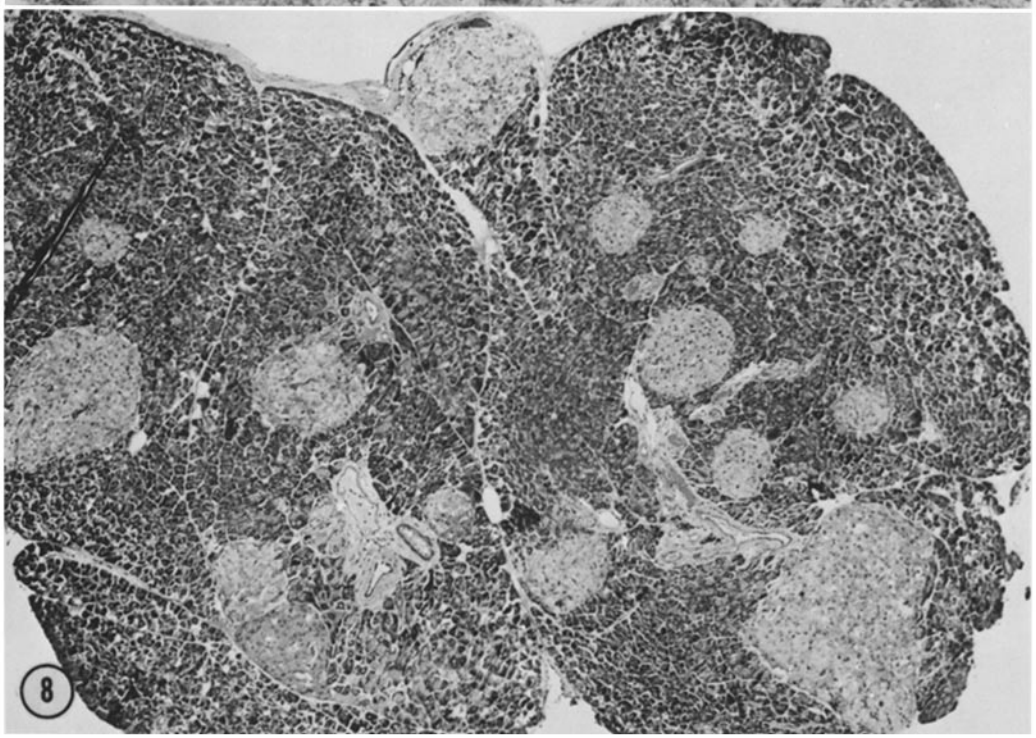


FIGURE 7 β Cell of a KsJ-*db*/*db* mouse exhibiting large, rounded, or ovoid mitochondria as well as secretory granules with varying degrees of electron density. × 15,000.

FIGURE 8 Cross section of the pancreas from a 6J-*db*/*db* mouse exhibiting numerous islets, some of which are clearly hyperplastic and one of which is localized at the periphery of the pancreas. Chrome-alum-hematoxylin; × 16.

mice, whether +/+ or *db/db*, that of the 6J-*db/db* mice was clearly hyperplastic with numerous large islets (Fig. 8). These islets were mainly composed of β cells with prominent endoplasmic reticulum and Golgi complex (Fig. 9) and varying degrees of degranulation (Fig. 10). Neither the β cells nor the α_1 and α_2 cells were degeneratively altered in the 6J-*db/db* mice. Intraislet ducts were not encountered.

There were no obvious differences between the three strains of mice with respect to the occurrence in islet cells of cilia or of cytoplasmic and nuclear rods such as have previously been described for other rodent species and strains (Boquist, 1969 *b*, 1970).

Morphometry of the Pancreas

The differential cell counts revealed that the frequency of β cells was 80.6 ± 1.2% in KsJ-+/+ mice, 72.9 ± 3.3% in KsJ-*db/db* mice, and 90.0 ± 2.4% in 6J-*db/db* mice (mean values ± SEM for 7, 11, and 5 animals, respectively). Student's *t* test as well as the Wilcoxon rank test showed the difference between KsJ-+/+ mice and 6J-*db/db* mice to be clearly significant ($P < 0.01$). The decrease of β cells in KsJ-*db/db* mice as compared with the KsJ-+/+ mice was statistically less impressive, the one-tailed Student's *t* test yielding $P < 0.05$.

Table III shows the proportions of islets, acini, and ducts in the pancreas of the three strains of mice. While there were only small and statistically nonsignificant differences between the KsJ-+/+ and KsJ-*db/db* mice, the proportion of islets was markedly increased in the 6J-*db/db* mice. Although the differences between KsJ-+/+ and KsJ-*db/db* mice were not significant, the lower mean value for islets in the KsJ-*db/db* mice is consistent with the decrease of β-cell frequency described above; both changes may signify an involution of the β cells in the KsJ-*db/db* mice. Conversely, the increase of β-cell frequency as well as of the proportion of islets gives evidence of β-cell hyperplasia in the 6J-*db/db* mice. These interpretations are supported by the size distribution of islet section surfaces presented in Table IV. While, in comparison to the KsJ-+/+ mice, the KsJ-*db/db* mice seemed to have a somewhat increased frequency of small islets, the distribution was displaced towards large islets in the 6J-*db/db* mice. In general there is a strict correlation between the total volume of the endocrine pancreas and the frequency of large islet section surfaces (Hellman et al., 1964). The data in Table IV are therefore consistent with the endocrine pancreas being somewhat reduced in the KsJ-*db/db* mice and increased in the 6J-*db/db* mice.

Release of Insulin from Microdissected Islets

Tables V and VI show that the islets microdissected from KsJ-+/+ mice and 6J-*db/db* mice released insulin in response to 20 or 40 mM glucose, or 0.1 mM chloromercuribenzene-*p*-sulphonic acid. The magnitude of the release did not differ significantly between these two strains of mice, although somewhat higher mean values were observed in the 6J-*db/db* mice. Starvation overnight had little, if any, effect. In both strains of mice, the glucose-induced secretory responses were potentiated by 0.1 mM iodoacetamide or 5 mM theophylline.

In contrast to the islets of KsJ-+/+ or 6J-*db/db* mice, those of most KsJ-*db/db* mice did not respond to glucose alone, to glucose in combination with iodoacetamide or theophylline, or to chloromercuribenzene-*p*-sulphonic acid (Tables V and VI). However, islets from the individual KsJ-*db/db* mouse which had the unusually high serum insulin level reported above did respond to glucose, as did the islets of two other KsJ-*db/db* mice whose serum insulin levels were not measured. The results obtained with islets microdissected from these three individuals are listed separately in Tables V and VI. It is noteworthy that the glucose-induced insulin release from these islets was potentiated by iodoacetamide or theophylline. The one glucose-sensitive KsJ-*db/db* mouse, whose islets were exposed to chloromercuribenzene-*p*-sulphonic acid, exhibited a clear secretory response to this stimulus as well (Table VI).

Insulin Content in Islets

After incubation for measurement of insulin release, the islets used in Table VI were extracted for determination of their insulin content. The islets from each animal were pooled before extraction, yielding eight different extracts for each strain of mice. Islets from KsJ-+/+ mice contained 191 ± 13 ng of insulin/μg dry weight (mean value ± SEM). The corresponding value for KsJ-*db/db* mice was 31 ± 6 ng/μg, and for 6J-*db/db* mice 128 ± 9 ng/μg. Student's *t* test

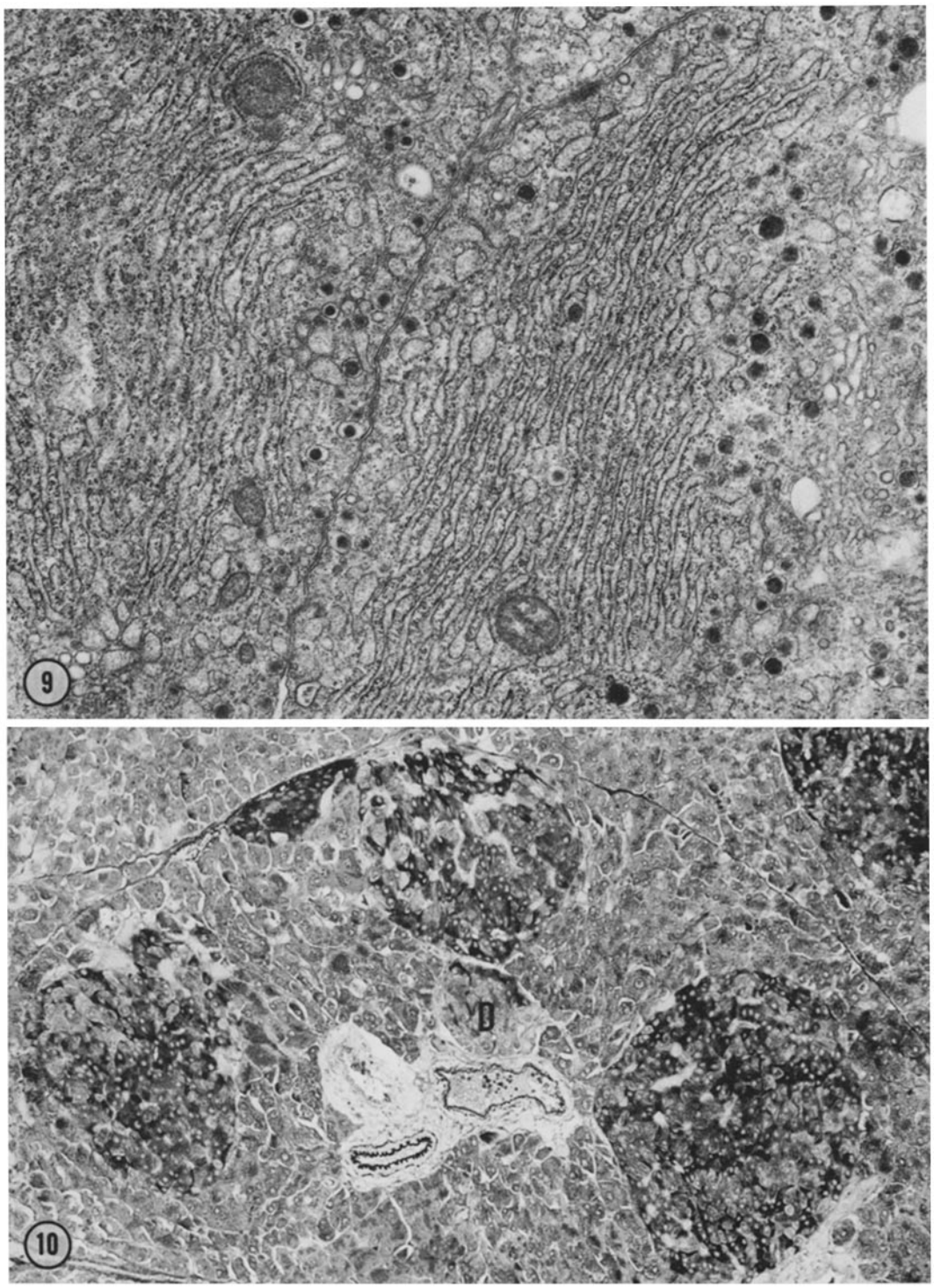


FIGURE 9 Two adjacent β cells of a 6J-*db*/*db* mouse showing prominent lamellar and rough-surfaced endoplasmic reticulum, secretory granules, and portions of Golgi complex. × 12,000.

FIGURE 10 Pancreas of a 6J-*db*/*db* mouse showing differently sized islets with varying degrees of β-cell granulation. One islet (*D*) is mainly composed of degranulated β cells. Aldehyde-fuchsin; × 40.

showed the decrease in comparison with KsJ-+/+ mice to be significant for both the KsJ-*db/db* mice ($P < 0.001$) and the 6J-*db/db* mice ($P < 0.01$).

Since the islets had been incubated before extraction, it should be noted that the highest secretory rate in Table VI (20 mM glucose + 5 mM theophylline in 6J-*db/db* mice) corresponds to only 14% of the remaining insulin content. The rates induced by 20 mM glucose correspond to about 1% of the total insulin content in KsJ-+/+ mice, 0.4% in the KsJ-*db/db* mice, and 3% in the 6J-*db/db* mice.

TABLE III

Composition of Pancreas in Normal and Diabetic Mice

Structure	Percent of total pancreas		
	KsJ-+/+	KsJ-*db/db*	6J-*db/db*
Islets	2.1 ± 0.3	1.8 ± 0.4	11.4 ± 3.1*
Acini	93.9 ± 0.8	93.4 ± 0.7	84.4 ± 3.1
Ducts	4.1 ± 0.7	4.8 ± 0.6	4.3 ± 1.0

The proportions of islets, acini, and ducts were determined by point sampling on light-microscopic pancreas sections as described in the Materials and Methods section. Results are given as the mean values ± SEM for 5 (6J-*db/db*), 7 (KsJ-+/+), and 11 (KsJ-*db/db*) animals. * $P \sim 0.01$ for comparison vs. KsJ-+/+ mice (Wilcoxon rank test).

TABLE IV

Size Distribution of Islet Section Surfaces in Normal and Diabetic Mice

Cells per islet section surface	Percent of all islet section surfaces		
	KsJ-+/+	KsJ-*db/db*	6J-*db/db*
-100	82	88	61
101-200	18	11	33
201-	0	0	6

When performing the differential cell counting (see Materials and Methods and Results sections), the islet section surfaces were also classified with respect to their absolute content of endocrine parenchymal cells. The frequencies of islet sections containing less than 100, 101-200, and more than 200 parenchymal cells are given. Results are based on 7 KsJ-+/+, 11 KsJ-*db/db*, and 5 6J-*db/db* mice, from which totals of 113, 200, and 64 islet section surfaces, respectively, were counted.

TABLE V

Effects of Glucose and Iodoacetamide on Insulin Release from Normal and Diabetic Mice

Test compounds	Rate of insulin release (ng/h per μg dry islet)		
	KsJ-+/+	KsJ-*db/db*	6J-*db/db*
Fed animals			
3 mM glucose	1.11 ± 0.16	0.42 ± 0.11 (0.95, 0.30)	0.86 ± 0.30
20 mM glucose	3.12 ± 0.80*	0.51 ± 0.19 (5.41, 5.98)	3.23 ± 1.01*
40 mM glucose	4.02 ± 1.02‡	0.62 ± 0.16 (4.48, 4.57)	5.47 ± 1.36§
20 mM glucose + 0.1 mM iodoacetamide	10.12 ± 2.30‖	0.93 ± 0.25 (11.63, 9.04)	13.14 ± 3.14¶
Starved animals			
3 mM glucose	0.76 ± 0.14	0.37 ± 0.18	1.79 ± 0.41
20 mM glucose	2.41 ± 0.49§	0.25 ± 0.08	5.21 ± 1.02
40 mM glucose	3.03 ± 0.80*	0.27 ± 0.07	5.13 ± 1.00*
20 mM glucose + 0.1 mM iodoacetamide	8.56 ± 2.61**	0.48 ± 0.13	11.60 ± 2.38‖

In each experiment, islets from one mouse were incubated in parallel in media containing glucose and iodoacetamide as indicated. Amounts of insulin released during 60 min are given as the mean values ± SEM for 5 (starved 6J-*db/db*), 7 (fed 6J-*db/db*, starved KsJ-+/+), 9 (fed KsJ-*db/db*), or 11 (starved KsJ-*db/db*) experiments. Data on two additional KsJ-*db/db* mice are given in parentheses. Statistical significances were judged from the mean ± SEM of differences between parallel incubations. Comparison vs. 3 mM glucose in same strain: * $P < 0.05$, ‡ $P < 0.02$, § $P < 0.01$. Comparison vs. 20 mM glucose in same strain: ** $P < 0.05$, ‖ $P < 0.02$, ¶ $P < 0.01$.

TABLE VI

Effects of Glucose, Theophylline, and Chloromercuribenzene-p-sulphonic Acid on Insulin Release from Normal and Diabetic Mice

Test compounds	Rate of insulin release (ng/h per μg dry islet)		
	KsJ-+/+	KsJ-*db/db*	6J-*db/db*
3 mM glucose	0.33 ± 0.15	0.13 ± 0.04 (0.30)	0.66 ± 0.21
20 mM glucose	2.09 ± 0.38*	0.12 ± 0.05 (1.49)	4.13 ± 0.98‡
20 mM glucose + 5 mM theophylline	6.80 ± 0.96§	0.29 ± 0.10 (6.60)	18.16 ± 1.22‖
3 mM glucose + 0.1 mM CMBS	6.09 ± 0.79‖	0.48 ± 0.19 (3.55)	9.21 ± 2.30*

In each experiment islets from one starved mouse were incubated in parallel in media containing glucose, theophylline, and chloromercuribenzene-*p*-sulphonic acid as indicated. Amounts of insulin released during 60 min are given as the mean values ± SEM for seven (KsJ-*db/db*) or eight (KsJ-+/+, 6J-*db/db*) experiments. Data on one additional KsJ-*db/db* mouse are given in parentheses. Statistical significances were judged from the mean ± SEM of differences between parallel incubations. Comparison vs. 3 mM glucose in same strain: ‡$P < 0.02$, *$P < 0.01$, ‖$P < 0.001$. Comparison vs. 20 mM glucose in same strain: §$P < 0.01$, ¶$P < 0.001$.

DISCUSSION

The results reported here confirm and extend previous studies showing that the mutation "diabetes" produces remarkably different effects when associated with the genetic backgrounds C57BL/KsJ and C57BL/6J (Hummel et al., 1972; Coleman and Hummel, 1973). Like *ob/ob* mice of the 6J inbred strain or of the noninbred stock kept in our laboratory (Umeå-*ob/ob;* Hellman, 1965), the 6J-*db/db* mice exhibit obesity, marked hyperinsulinemia, and considerable β-cell hyperplasia, which may reflect a compensatory hyperfunction of the endocrine pancreas in response to hyperglycemia of unknown genesis. During early life the KsJ-*db/db* mice are also characterized by obesity, marked hyperinsulinemia, and hyperglycemia, but later they develop a more severely diabetic syndrome with necrosis of β cells and decline of the serum insulin levels (Coleman and Hummel, 1967; Hummel et al., 1972). The reason for this difference between 6J-*db/db* mice and KsJ-*db/db* mice is not clear. However, studies on the interactions of both the *db* and *ob* genes with the 6J and KsJ background genomes (Coleman and Hummel, 1973) suggest that 6J mice can expand their insulin supply more or less indefinitely in response to increased demands. In contrast, the islets of KsJ mice appear to have a limited capacity and more easily succumb to the increased functional stress imposed by either the *db* or *ob* gene. This difference makes the 6J and KsJ strains appear as promising models for experimentally studying the deterioration of β-cell function in diabetes. For the present study we tried to select KsJ-*db/db* mice whose islets had started to fail to cope with functional stress. This selection was done by regular weighing of the animals, because previous studies have shown the severely diabetic phase to be associated with inhibited growth and even loss of body weight (Coleman and Hummel, 1967; Hummel et al., 1972). The morphological investigations as well as the observations of a marked hyperglycemia in conjunction with comparatively moderate hyperinsulinemia confirmed that most of the selected animals had entered into the severely diabetic phase.

Malaisse et al. (1968) reported that in comparison with normal controls, pieces of pancreas from old KsJ-*db/db* mice contained reduced amounts of insulin and released only little insulin when exposed to 30 mM glucose. These findings could reflect a decreased number of β cells, a decreased storage of insulin in each β cell, an impaired ability of the individual β cells to release stored insulin, or a combination of such factors. As shown here, the pancreas of severely diabetic KsJ-*db/db* mice contains a somewhat reduced number of β cells as well as a decreased amount of insulin per islet. Degenerative changes in the islets, including

the appearance of ducts, were observed in our material as well as in those previously described (Coleman and Hummel, 1967, 1973). However, in the great majority of mice the most striking abnormality was the complete inability of the islets to release insulin in response to glucose even at as high a concentration as 40 mM. This defect was probably not due to lack of β cells or insulin, since the reductions of these latter parameters were rather small in comparison with the total abolishment of the glucose-induced secretory response. Studies on cultured mouse islets have indicated that the insulin content can decrease by about 85% without precluding a clear-cut secretory response to glucose (Andersson and Hellerström, 1972). However, we did not measure proinsulin synthesis and conversion to insulin and do not know the exact intracellular location of the immunoreactive material. The possibility of the readily releasable pool of insulin being exhausted in these β cells should therefore be kept in mind.

The secretory defect in KsJ-*db/db* mice was not a specific insensitivity to glucose, since chloromercuribenzene-*p*-sulphonic acid also failed to evoke secretion from the islets of most KsJ-*db/db* mice. This sulfhydryl reagent is a potent insulin secretagogue with islets from the Umeå-*ob/ob* mice (Bloom et al., 1972) and, as shown here, with those from KsJ-+/+ and 6J-*db/db* mice. The marked insensitivity of the islets from KsJ-*db/db* mice to secretory stimuli in vitro is somewhat paradoxical in view of the fact that the concentration of insulin in serum was rather high and seemed to decrease upon starvation. A possible explanation could be that the isolated islets were not representative of all the β cells in the pancreas. The occurrence of functioning β cells in islets smaller than those suitable for microdissection cannot be excluded.

Among the KsJ-*db/db* mice selected for this study we encountered three individuals that differed from the majority by responding to glucose. It seems reasonable to assume that these animals represented a somewhat earlier stage in the course of diabetes with less severe damage to the β cells. The islets of these KsJ-*db/db* mice behaved like those of KsJ-+/+ mice and 6J-*db/db* mice in that the glucose-induced insulin release was potentiated by theophylline or iodoacetamide and that chloromercuribenzene-*p*-sulphonic acid elicited a significant secretory response. Previous studies have documented the potentiating effects of methylxanthines (Ashcroft et al., 1972; Hellman et al., 1973) and iodoacetamide (Hellman et al., 1973) on the islets from normal and Umeå-*ob/ob* mice.

Several factors, including ATP (Ashcroft et al., 1973), NAD (Deery and Taylor, 1973), NADP (Ammon et al., 1973), and cyclic AMP (Montague and Howell, 1973), probably must be maintained at certain concentrations in the β cells for normal insulin discharge to occur. Moreover, it has been suggested that the amount of microtubular protein plays such a critical role that a decrease in comparison with normal mice would explain the low secretory rates observed in *Acomys cahirinus* (Renold et al., 1974). Against this background, it is too early to speculate about the precise nature of the secretory defect reported here for KsJ-*db/db* mice. However, although there are numerous possible sources of this defect, elucidation of its nature and etiology do not appear to be unrealistic goals in view of the excellent control materials offered by the KsJ-+/+ and 6J-*db/db* mice.

The authors thank Dr. D. L. Coleman at Jackson Laboratories, Bar Harbor, Me, for the generous gift of normal and diabetic mice as well as for his encouraging interest and valuable advice. The assistance of Novo A/S, Copenhagen, in the preparation of crystalline mouse insulin, and the donation by Hoechst A. G., Frankfurt am Main, of [^{125}I]insulin is also gratefully acknowledged.

Financial support was given by the Swedish Medical Research Council, grants 12x-562 and 12x-718.

Received for publication 14 November 1973, and in revised form 20 February 1974.

REFERENCES

AMMON, H. P. T., and J. STEINKE. 1972. 6-Aminonicotinamide (6-AN) as a diabetogenic agent. In vitro and in vivo studies in the rat. *Diabetes.* **21**:143.

ANDERSSON, A., and C. HELLERSTRÖM. 1972. Metabolic characteristics of isolated pancreatic islets in tissue culture. *Diabetes.* **21**:(Suppl.2):546.

ASHCROFT, S. J. H., L. C. C. WEERASINGHE, J. M. BASSETT, and P. J. RANDLE. 1972. The pentose cycle and insulin release in mouse pancreatic islets. *Biochem. J.* **126**:525.

ASHCROFT, S. J. H., L. C. C. WEERASINGHE, and P. J. RANDLE. 1973. Interrelationship of islet metabolism, adenosine triphosphate content and insulin release. *Biochem. J.* **132**:223.

BLOOM, G. D., B. HELLMAN, L.-Å. IDAHL, Å. LERNMARK, J. SEHLIN, and I.-B. TÄLJEDAL. 1972. Effects of organic mercurials on mammalian pancreatic β-

cells Insulin release, glucose transport, glucose oxidation, membrane permeability, and ultrastructure. *Biochem. J.* **129**:241.

BOQUIST, L. 1969 *a*. Pancreatic islet morphology in diabetic Chinese hamsters. A light and electron microscopic study. *Acta Pathol. Microbiol. Scand.* **75**:399.

BOQUIST, L. 1969 *b*. Intranuclear rods in pancreatic islet β-cells. *J. Cell Biol.* **43**:377.

BOQUIST, L. 1970. Tubular cytoplasmic bodies in pancreatic islet B-cells of mice. *Z. Zellforsch. Mikrosk. Anat.* **106**:69.

CAMERON, D. P., W. STAUFFACHER, L. ORCI, M. AMHERDT, and A. E. RENOLD. 1972. Defective immunoreactive insulin secretion in *Acomys cahirinus*. *Diabetes*. **21**:1060.

CERASI, E., and R. LUFT. 1967. "What is inherited-What is added" hypothesis for the pathogenesis of diabetes mellitus. *Diabetes*. **16**:615.

COLEMAN, D. L., and K. P. HUMMEL. 1967. Studies with the mutation, diabetes, in the mouse. *Diabetologia*. **3**:238.

COLEMAN, D. L., and K. P. HUMMEL. 1973. The influence of genetic background on the expression of the obese (*Ob*) gene in the mouse. *Diabetologia*. **9**:287.

DEERY, D. J., and K. W. TAYLOR. 1973. Effects of azaserine and nicotinamide on insulin release and nicotinamide-adenine dinucleotide metabolism in isolated rat islets of Langerhans. *Biochem. J.* **134**:557.

FELIG, P. 1971. Pathophysiology of diabetes mellitus. *Med. Clin. North Am.* **55**:821.

GERRITSEN, G. C., and W. E. DULIN. 1967. Characterization of diabetes in the Chinese hamster. *Diabetologia*. **3**:74.

GRIMELIUS, L. 1968. A silver nitrate stain for α_2 cells in human pancreatic islets. *Acta Soc. Med. Upsal.* **73**:243.

HAHN, H. J., E. WAPPLER, and H. FIEDLER. 1971. Die Sandratte (*Psammomys obesus*): Ein an Spontandiabetes erkrankendes Tier. *Z. Versuchstierkd.* **13**:275.

HEDING, L. G. 1966. A simplified insulin radioimmunoassay method. *In* Labelled Proteins in Tracer Studies. L. Donato, G. Milhaud, and J. Sirchis, editors. Euratom, Brussels. 345–351.

HELLERSTRÖM, C. 1964. A method for the microdissection of intact pancreatic islets of mammals. *Acta Endocrinol.* **45**:122.

HELLERSTRÖM, C., and B. HELLMAN. 1960. Some aspects of silver impregnation of the islets of Langerhans in the rat. *Acta Endocrinol.* **35**:518.

HELLMAN, B. 1965. Studies in obese-hyperglycemic mice. *Ann. N. Y. Acad. Sci.* **131**:541.

HELLMAN, B., L.-Å. IDAHL, Å. LERNMARK, J. SEHLIN, and I.-B. TÄLJEDAL. 1973. Iodoacetamide-induced sensitization of the pancreatic β-cells to glucose stimulation. *Biochem. J.* **132**:775.

HELLMAN, B., B. PETERSSON, and C. HELLERSTRÖM. 1964. The growth pattern of the endocrine pancreas in mammals. *In* The Structure and Metabolism of the Pancreatic Islets. S. E. Brolin, B. Hellman, and H. Knutson, editors. Pergamon Press Ltd., Oxford. 45–60.

HUMMEL, K. P., D. L. COLEMAN, and P. W. LANE. 1972. The influence of genetic background on expression of mutations at the diabetes locus in the mouse. I. C57BL/KsJ and C57BL/6J strains. *Biochem. Genet.* **7**:1.

LARKINS, R. G. 1973. Defective insulin secretory response to glucose in the New Zealand obese mouse. Improvement with restricted diet. *Diabetes*. **22**:251.

LERNMARK, Å. 1971. Isolated mouse islets as a model for studying insulin release. *Acta Diabetol. Lat.* **8**:649.

LOGE, O., W. LOSERT, and P. JAHN. 1973. Insulinfreisetzende Wirkung von Glymidin-Natrium (Glykodiazin), Isopropylnoradrenalin, Glucagon und Theophyllin an chinesischen Streifenhamstern mit spontanem oder streptozotocininduziertem Diabetes mellitus. *Res. Exp. Med.* **160**:292.

LOWRY, O. H., J. V. PASSONNEAU, F. X. HASSELBERG, and D. W. SCHULZ. 1964. Effects of ischemia on known substrates and cofactors of the glycolytic pathway in brain. *J. Biol. Chem.* **239**:18.

MALAISSE, W. J., F. MALAISSE-LAGAE, and D. L. COLEMAN. 1968. Insulin secretion in mice with an hereditary diabetes. *Proc. Soc. Exp. Biol. Med.* **129**:65.

MONTAGUE, W., and S. L. HOWELL. 1973. The mode of action of adenosine 3′:5′-cyclic monophosphate in mammalian islets of Langerhans. *Biochem. J.* **134**:321.

RENOLD, A. E. 1974. Experimental and spontaneous diabetic syndromes in animals (a reevaluation of their usefulness for approaching the physiopathology of diabetes in man). Proceedings of the 8th Congress of the International Diabetes Federation. Excerpta Medica Foundation. In press.

SELTZER, H. S., E. W. ALLEN, A. L. HERRON, and M. T. BRENNAN. 1967. Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. *J. Clin. Invest.* **46**:323.