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The Influence of Genetic Background on Expression of Mutations at the Diabetes Locus in the Mouse. I. C57BL/KsJ and C57BL/6J Strains

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*Two new diabetic strains, C57BL/KsJ-*db^{2J} *and C57BL/6J-*db^{2J}*, have been developed. C57BL/KsJ-*db^{2J}/db^{2J} *mice are indistinguishable from C57BL/KsJ-*db/db *mice, the original diabetes mutation. Both have severe diabetes characterized by hyperphagia, obesity, marked hyperglycemia, temporarily elevated plasma insulin concentrations, and typical degenerative changes in the islets of Langerhans. In contrast, C57BL/6J-*db^{2J}/db^{2J} *mice, although also hyperphagic and obese, have mild diabetes characterized by transitory hyperglycemia and markedly elevated plasma insulin concentrations coupled with marked hypertrophy of the islets and increased proliferative capacity of beta cells. The mild diabetes-like syndrome produced by diabetes-2J on the C57BL/6J background is similar to that produced by the obese gene* (ob) *on the same background. The islet responses, whether atrophy or hypertrophy, appear to be due to the interaction of diabetes-2J (and possibly obese) with modifiers in the genetic background rather than being peculiar to the specific mutant. The markedly different disease patterns that result when the same gene is placed on different inbred backgrounds emphasize the importance of strict genetic control in biochemical and physiological studies with these and other obesity mutants.*

INTRODUCTION

A mutant gene resembling both obese (*ob*) and diabetes (*db*) was discovered in

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1966 by Lane in an inbred strain homozygous for brown (*b*) and heterozygous for whirler (*wi*). Like *ob* and *db*, the mutation is an autosomal recessive; homozygotes accumulate excessive amounts of fat by 3–4 weeks of age and become functionally sterile as adults; heterozygotes and normals are indistinguishable except through progeny testing. To determine whether this mutant is allelic with either *ob* or *db*, mice known to be heterozygous for the new character were mated to obese heterozygotes (*ob*/+) and to diabetes heterozygotes (*db*/+). Offspring (34) of the matings to *ob*/+ were all normal, but offspring of the cross to *db*/+ included some mice indistinguishable from *db*/*db*. The new mutant was thus shown to be an allele of diabetes (*db*) and was named diabetes-2J (db^{2J}) in conformity with the rules of standardized genetic nomenclature for mice (Committee on Standardized Genetic Nomenclature for Mice, 1963).

Mice heterozygous for db^{2J} were outcrossed to hybrid ($B6CBAF_1$) and to inbred strain C57BL/6J mice. Their progeny were used to produce homozygous db^{2J}/db^{2J} mice for study. The disease in these mice did not follow the orderly progression observed repeatedly in studies of diabetics (*db*/*db*) in the strain of origin (C57BL/KsJ) (Hummel *et al.*, 1966; Coleman and Hummel, 1967; Like and Chick, 1970). As a consequence, the identity of the two alleles *db* and db^{2J} was questionable. To determine whether the differences were real or due to modifying genes in the genetic background, the new allele was established on the C57BL/KsJ and C57BL/6J backgrounds by five cross–intercross cycles. This provided diabetic mice of two new strains, C57BL/KsJ-db^{2J} and C57BL/6J-db^{2J}, for study and comparison with each other, with diabetics of strain C57BL/KsJ-*db*, and with obese (*ob*/*ob*) of strain C57BL/6J-*ob*.

MATERIALS AND METHODS

Homozygous db^{2J}/db^{2J} mice, identified at weaning by accumulations of excess fat, were observed from weaning until death. Weights and nonfasted blood sugar concentrations were recorded at frequent intervals until 6 months of age and less frequently thereafter. Plasma insulin assays were made on some of the mice at various ages, usually just before sacrifice. Sections of pancreas, liver, and kidney from many sacrificed mice were saved and processed for histological examination. Analytical and histological procedures were as previously described (Coleman and Hummel, 1967).

Mice from the C57BL/KsJ and C57BL/6J strains were used as normal controls throughout. The mutant mice were from three colonies, one heterogeneous and two inbred. The heterogeneous group consisted of 16 males and 17 females from the F_{20} and F_{22} generations of the brown whirler strain, from the F_2 and F_3 generations after a cross to strain C57BL/6J, and from

the F_2 of the cross to the hybrid B6CBAF_1 stock. Eight of the males and five of the females were homozygous also for whirler. There were 10–20 male and 10–20 female diabetic mice from each of the two inbred strains C57BL/KsJ and C57BL/6J.

All mice were housed in stainless steel pens on pine shavings with pelleted food and tap water available at all times. The pelleted food, containing 19 percent protein and 6 percent fat, is manufactured by the Emory Morse Company of Guilford, Conn.

RESULTS

Control Mice

No differences were observed between normal mice of the C57BL/KsJ and C57BL/6J inbred strains. Blood sugar concentrations ranged from 140 to 190 mg/100 ml, while plasma insulin concentrations ranged from 30 to 80 μU/ml. In general, female mice had slightly lower plasma insulin and blood sugar concentrations. The body weights of the normal control mice were variable but usually did not exceed 35 g. Islet morphology in all normal mice was similar to that previously described (Coleman and Hummel, 1967).

Heterogeneous *db*^{2J}/*db*^{2J} Mice

Heterogeneous *db*^{2J}/*db*^{2J} mice fell into two general groups with regard to development of hyperglycemia (Table I). Those mice which became hyperglycemic by 2–3 months of age and remained so thereafter fell into group I. Those which remained normoglycemic or developed a mild, transitory hyper-

Table I. Average Weights and Blood Sugar Concentrations in Noninbred *db*^{2J}/*db*^{2J} Mice at Various Ages

Group	Age (months)	Mice		Weight (g)	Blood sugar (mg/100 ml)
		Sex	No.		
I	2–3	♀	6	36	479
		♂	11	41	462
I	4–5	♀	3	44	516
		♂	8	32	535
II	2–3	♀	11	42	226
		♂	4	35	243
II	4–5	♀	10	52	206
		♂	4	44	271
II	10–17	♀	7	72	114
		♂	4	57	155

glycemia made up group II. Each group included some diabetics homozygous also for whirler; these did not differ essentially from the nonwhirler diabetics.

The disease in the six females and 11 males of group I was similar to that in C57BL/KsJ-*db*/*db* mice. Blood sugar concentrations reached 400 mg/100 ml by 3 months and did not fall below this high level thereafter. At 2–3 months, when blood sugar concentrations ranged from 340 to 700 mg/100 ml, weights ranged from 24 to 55 g, and plasma insulin values ranged from 90 to 275 μU/ml. The islets of Langerhans were not appreciably increased in number and size and contained extensively degranulated beta cells. Several mice were sacrificed, and three died before they were 15 weeks old. The three females and eight males that lived longer than $3\frac{1}{2}$ months were severely hyperglycemic. Their blood sugar concentrations ranged from 430 to 624 mg/100 ml; their weights had decreased slightly, ranging from 22 to 52 g; and their plasma insulin concentrations had also decreased, ranging from 30 to 170 μU/ml. The islets in these older diabetics showed the atrophic and degenerative changes typical of strain C57BL/KsJ-*db*/*db* mice, being small and made up of mixtures of extensively degranulated beta cells, dilated ductal structures, and acinar cells. None of this group lived beyond 8 months of age.

The disease in the 11 females and four males of group II was less severe. Blood sugar concentrations of those aged 2–3 months ranged from 146 to 366 mg/100 ml, weights ranged from 30 to 49 g, and plasma insulin concentrations ranged from 225 to 720 μU/ml. None died, and only one was sacrificed during this period. The islets in the pancreas of these diabetics were increased in number and size, and beta cells were only slightly degranulated. Up to the age of 20 weeks, there was little change either in blood sugar (range 140–313 mg/100 ml) or in plasma insulin concentrations (range 225–620 μU/ml), but the weights increased steadily (range 38–66 g). Observations on seven female and four male diabetics of group II were continued for 12 more months. During this period, all of the mice became severely hyperinsulinemic (range 338–2000 μU/ml) and increasingly obese (range 49–85 g), and many (six females and two males) became hypoglycemic or borderline normal (range 82–132 mg/100 ml). One female and two males were normoglycemic (range 154–211 mg/100 ml). The islets of all group II diabetics over 4 months showed classical signs of hyperactivity, being greatly enlarged, with dilated sinusoids and somewhat degranulated beta cells.

One male, not included in Table I, was atypical, being classified as belonging in group II at 2–4 months of age and in group I at 10 months of age.

C57BL/KsJ-db^{2J}/db^{2J} Mice

Table II and Fig. 1 show that rapid increases in weights and blood sugar concentrations occurred during the first 3–4 months in C57BL/KsJ-db^{2J}/db^{2J}

Table II. Average Weights and Blood Sugar and Insulin Concentrations in *db*^{2J}/*db*^{2J} Mice of Strains C57BL/KsJ-*db*^{2J} and C57BL/6J-*db*^{2J}

Strain	Age (months)	Mice Sex	Mice No.	Weight (g)	Blood sugar mg/100 ml	Blood sugar Range	Insulin μU/ml	Insulin No.
C57BL/KsJ-*db*^{2J}	2	♀	8	45	368	251–445	305	5
		♂	9	44	428	334–482	245	2
	4	♀	6	56	486	430–518	—	
		♂	7	41	515	518–630	—	
	6	♀	5	52	479	430–518	52	2
		♂	4	38	509	482–518	95	1
	11	♀	5	45	451	407–482	—	
		♂	3	39	472	452–482	—	
C57BL/6J-*db*^{2J}	2	♀	8	36	174	142–258	571	7
		♂	7	39	208	154–334	502	3
	4	♀	8	58	181	118–280	482	7
		♂	7	56	200	118–255	775	2
	6	♀	8	61	128	107–158	2030	4
		♂	6	62	172	110–215	1250	1
	11	♀	5	72	181	151–216	—	
		♂	4	78	157	129–183	—	

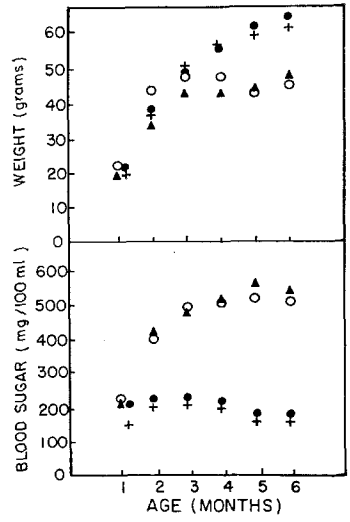

Fig. 1. Average weights and blood sugar concentrations as functions of age in mice of four strains: ▲, C57BL/KsJ-*db*/*db*; ○, C57BL/KsJ-*db*^{2J}/*db*^{2J}; +, C57BL/6J-*db*^{2J}/*db*^{2J}; ●, C57BL/6J-*ob*/*ob*. Each point represents the average value obtained from at least five mice of each sex and genotype.

mice. Thereafter, blood sugar concentrations remained high (over 400 mg/100 ml) but did not increase appreciably. Weights, especially in males, tended to decrease with age. Seventy-eight percent of males and 48 percent of females reached maximum weights by 3½ months. Exactly the same percentages of male and female C57BL/KsJ-*db*/*db* mice reached maximum weights by 3½

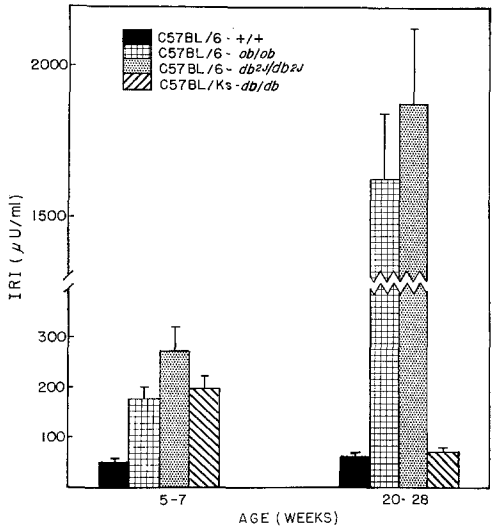

Fig. 2. Average plasma insulin concentrations in young and old diabetic and obese mutant and normal mice. IRI, immunoreactive insulin.

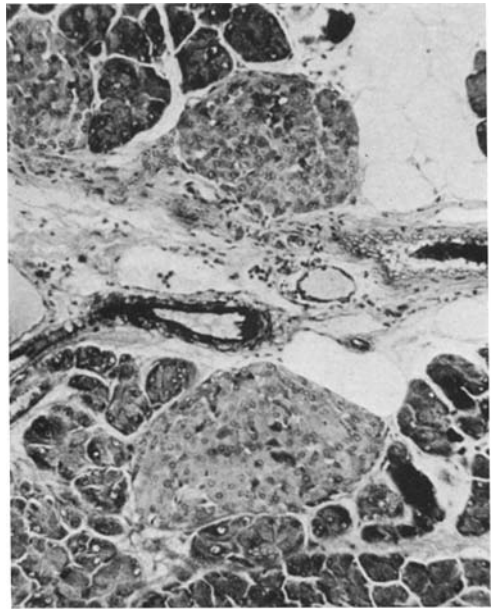

Fig. 3. Islets of a 57-day-old C57BL/KsJ-*db*^{2J}/*db*^{2J} mouse (×200; reduced 30% for reproduction). Note the marked degranulation of the beta cells.

months. The life span was shortened, many severely affected diabetics dying between 5–10 months of age. The disease in males was somewhat more severe than in females by the criteria of hyperglycemia, loss of weight, and early death. In general, the pattern of disease progression caused by the db^{2J} allele on the C57BL/KsJ background was indistinguishable from that caused by the original allele (*db*) on the same (C57BL/KsJ) background (Fig. 1).

The insulin assays (Table II and Fig. 2), although few in number, showed a pattern of high secretion during the early stage of the disease and normal secretion during later stages, as described for C57BL/KsJ-*db*/*db* mice (Coleman and Hummel, 1968). This correlates with the appearance of the islets of Langerhans, which in the young diabetics show extensive degranulation of beta cells without appreciable islet hypertrophy. Figures 3–7 are photomicrographs of representative islets of Langerhans stained with aldehyde fuchsin. Figures 3, 4, and 5 are at 200 × magnification and Figs. 6 and 7 are at 100 × magnification (all have been reduced 30% for reproduction). Figure 3 is an islet from the pancreas of a 57-day-old female with a blood sugar concentration of 348 mg/100 ml and a plasma insulin concentration of 260 μU/ml. In the older diabetic mice, the islets are typically atrophic and

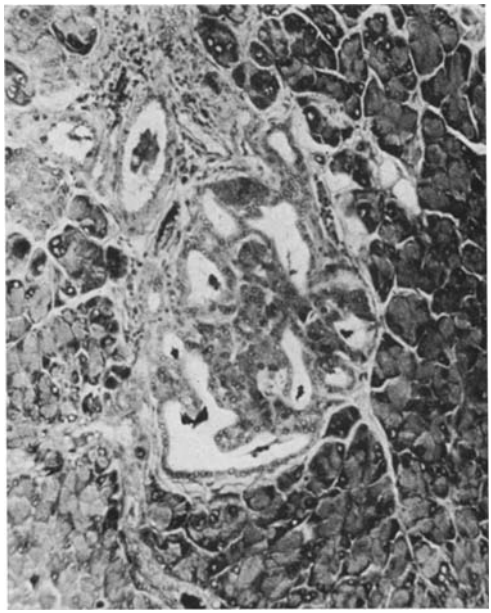

Fig. 4. Islets of a 126-day-old C57BL/KsJ-db^{2J}/db^{2J} mouse (× 200; reduced 30% for reproduction). Note the scarcity of granulated beta cells and the inclusion of acinar cells and ducts within the islet.

composed of mixtures of degranulated beta cells, epithelium-lined ducts, and acinar cells, as illustrated in Fig. 4, which is an islet from a pancreas of a 126-day-old female with a blood sugar concentration of 482 mg/100 ml and a plasma insulin concentration of 95 μU/ml.

C57BL/6J-*db*^{2J}/*db*^{2J} Mice

The results with C57BL/6J-*db*^{2J}/*db*^{2J} mice are shown in Table II and Figs. 1 and 2. Although the increase in weights during the first 4 months paralleled that of *db*^{2J}/*db*^{2J} mice on the C57BL/KsJ background, the increases in blood sugar concentrations were very slight. Very few animals attained a concentration of 300 mg/100 ml, and none remained at this level. Maximum weights were not reached in either sex by 6 months of age, and the few studied at older ages were still gaining weight when sacrificed. None of these animals died before 10 months of age, indicating that the disease is much less severe in diabetic mice of this strain. With respect to all these parameters, diabetes on the C57BL/6J background behaves in a fashion identical to that observed in obese (*ob*/*ob*) mice on the same (C57BL/6J) background (Figs. 1 and 2).

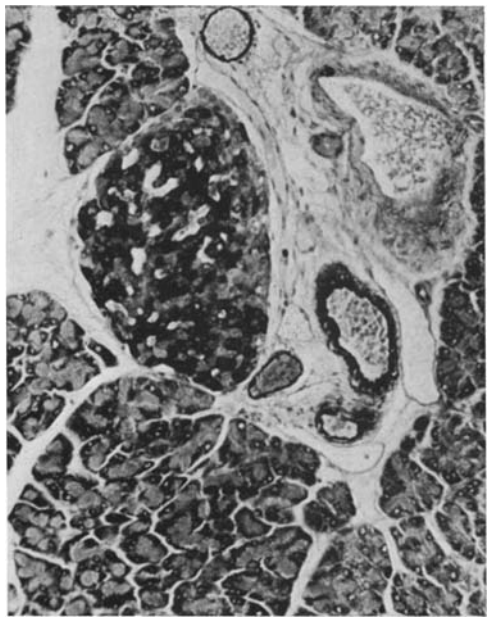

Fig. 5. Islets of a 53-day-old C57BL/6J-*db*^{2J}/*db*^{2J} mouse (×200; reduced 30% for reproduction). Note the enlargement of sinusoids and the presence of many granulated beta cells.

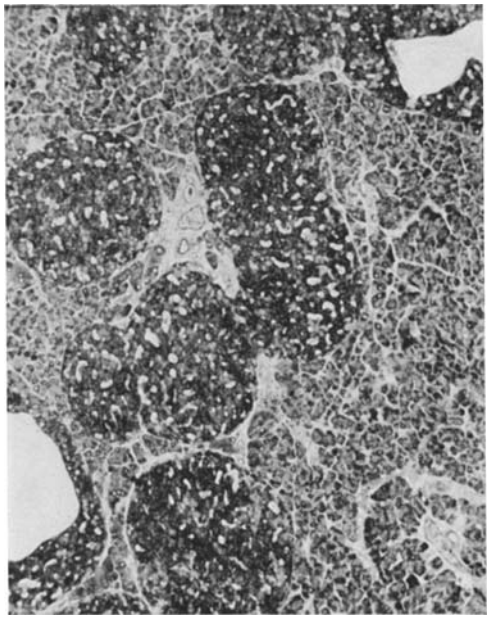

Fig. 6. Section of the pancreas of a 173-day-old C57BL/6J-*db*^{2J}/*db*^{2J} mouse (×100; reduced 30% for reproduction). Note the number and size of the islets and the presence of granulated beta cells and dilated sinusoids. Note also that the magnification is one-half that of the islet of the C57BL/KsJ-*db*^{2J}/*db*^{2J} mouse of comparable age (Fig. 4).

The plasma insulin assays showed steadily increasing insulinemia with age (Table II and Fig. 2). The islets of Langerhans reflected this state of hyperinsulinemia, being increased in number and size, with slight degranulation and dilated sinusoids indicative of hyperactivity. Figure 5 is an islet from the pancreas of a female of 53 days with a blood sugar of 169 mg/100 ml and a plasma insulin of 400 μU/ml. Islets were more numerous and much larger in older diabetics, as shown in Fig. 6, a section of pancreas from a 173-day-old male mouse with hypoglycemia (110 mg/100 ml) and hyperinsulinemia (1250 μU/ml). Note the striking similarity of the islets from this mutant to those from a C57BL/6J-*ob*/*ob* mouse (Fig. 7) with respect to number, size, and other manifestations of hyperactivity. Figure 7 is a section of pancreas from a C57BL/6J-*ob*/*ob* mouse 8 months of age with a blood sugar concentration of 171 mg/100 ml.

DISCUSSION

The disease syndrome in *db*^{2J}/*db*^{2J} mice of the C57BL/KsJ-*db*^{2J} strain is

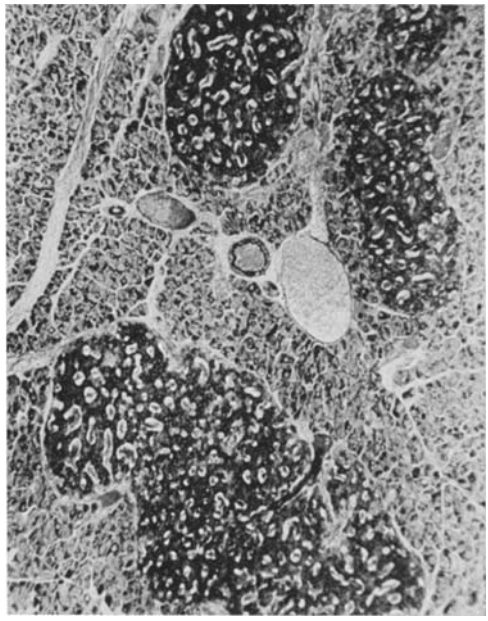

Fig. 7. Section of the pancreas of an 8-month-old C57BL/6J-*ob*/*ob* mouse (×100; reduced 30% for reproduction). Note the striking similarity to the pancreas shown in Fig. 6.

essentially identical to that of *db*/*db* mice of the C57BL/KsJ-*db* strain. If differences exist, they are insignificant and might be explained by the fact that the first discovered diabetes mutant (*db*) arose in strain C57BL/KsJ, whereas the second, db^{2J}, arose in an unrelated strain and was crossed into the C57BL/KsJ strain, a procedure that may have introduced that portion of the brown whirler stock genome closely linked to the diabetes locus. The diabetes locus is in linkage group VIII and shows 12.03 ± 0.97 percent recombination with whirler (*wi*), 8.72 ± 0.97 percent recombination with brown (*b*), and 1.40 ± 0.74 percent recombination with misty (*m*).

The differences in disease syndromes of db^{2J}/db^{2J} mice after crosses to two related strains of black mice, C57BL/KsJ and C57BL/6J, as shown in the tables and figures, are considerable. The two are alike in the early stage, both being characterized by hyperphagia, rapid weight gain, and hyperinsulinemia (Fig. 1 and Table II). In the late stage, the two differ in nearly all aspects. The C57BL/KsJ diabetics become severely hyperglycemic by 2–3 months and remain so as long as they live. At about 4 months of age, they cease gaining weight and secreting abnormal amounts of insulin, and many die before they reach 10 months of age. In contrast, the C57BL/6J diabetics are hyper-

insulinemic (Fig. 2) and continue to gain weight as long as they live. They do not develop frank diabetes, although some exhibit a mild and transitory hyperglycemia and glycosuria.

The islet morphology suggests that synthesis of insulin is halted prematurely in C57BL/KsJ diabetics, whereas insulin synthesis is enhanced in the C57BL/6J diabetics. In young C57BL/KsJ diabetics, the beta cells are extensively degranulated (Fig. 3), whereas the numerous, enlarged islets of the C57BL/6J (Fig. 5) diabetics contain well-stained beta granules, an indication of rapid replication and synthesis. The contrast in islet morphology becomes especially marked in older mice, with atrophy typical of islets in C57BL/KsJ diabetics (Fig. 4) and hypertrophy typical of islets in C57BL/6J diabetics (Fig. 6).

The similarity in disease pattern of the two unrelated obesity-producing genes, obese (*ob*) and diabetes (db^{2J}), on the C57BL/6J strain background is striking. The average weights, blood sugar concentrations, and plasma insulin concentrations of the C57BL/6J-*ob*/*ob* coincide almost exactly with those of C57BL/6J-db^{2J}/db^{2J} (Figs. 1 and 2). In both diabetic (Fig. 6) and obese (Fig. 7) mice, the islets are increased in number and size and show other characteristic signs of hyperactivity.

We are transferring the obese (*ob*) gene into the C57BL/KsJ background by the cross–intercross system and have studied eight *ob*/*ob* mice from the fourth backcross. Preliminary results suggest that they become and remain hyperglycemic and that the islets resemble those from the diabetic (*db*/*db*) mice on the C57BL/KsJ background. After the fifth backcross, the strain will be considered inbred and will provide C57BL/KsJ-*ob*/*ob* mice for detailed comparison with C57BL/6J-*ob*/*ob* mice.

It is of interest that the series of yellow alleles (A^y, A^{vy}, and A^{iy}) at the agouti locus, which also cause obesity and a diabetes-like syndrome, show the typical pattern of progressively increasing hyperinsulinemia, mild and transitory hyperglycemia, and hypertrophied islets of Langerhans when maintained on the C57BL/6J background (Hummel and Coleman, unpublished).

Our studies indicate that the interpretation of results with diabetic mice may be difficult if the genetic background is mixed or unknown. In the diabetic mice from our heterogeneous colony, we observed two classes with respect to disease syndromes. In one group (I), the hyperglycemia was uncontrolled; in the other (II), hyperglycemia was transitory if it occurred at all, and animals even became hypoglycemic in old age. It is apparent that the beta cells of mice of group I did not keep up with the demand for insulin and showed the characteristic islet atrophy of diabetics of the C57BL/KsJ strain. In contrast, beta cells of mice of group II exhibited the remarkable proliferative capacity characteristic of obesity genes on C57BL/6J background. Our results may explain the observations of Chick and Like (1970) that "DBM"

diabetic mice differ in disease syndrome and islet morphology from the C57BL/KsJ-*db*/*db* mice which they also studied. The DBM mice were produced at the Jackson Laboratory by crossing C57BL/KsJ-*db*/+ and C57BL/6J-*m*/*m* (misty coat color) mice and selecting double heterozygous offspring (+*db*/*m*+) for subsequent breeding. We can see now that the atypical features of the DBM mutants can be attributed to residual genetic factors brought into the stock with the C57BL/6J genome.

While our observations point out differences in gene action of diabetes-2J in two closely related inbred strains, they provide no clue as to the nature of the modifying factors nor as to their sites of action. It should be emphasized that the islet morphology of normal mice of the C57BL/6J and C57BL/KsJ inbred strains is identical and no abnormalities have been observed. The differences in islet morphology and consequent modifications of the diabetic syndrome result only when the alleles at the diabetes locus interact with unknown genetic factors in the C57BL/6J and C57BL/KsJ genomes. Even though the differences in gene expression are most easily observed in the islets of Langerhans (or beta cells), they may be secondary to hypophyseal or hypothalamic influences. A hypothalamic defect has been postulated as the primary cause of diabetes in homozygous *db*/*db* mice (Coleman and Hummel, 1969, 1970).

Our studies emphasize the importance of genetic control in attempts to determine causes and courses of diseases such as diabetes or obesity. The use of a noninbred stock which is an unknown genetic mix may give rise to a wide range of results which are confusing and even contradictory. The use of an inbred strain or hybrid in which the genome is uniform insures consistency of results within a narrow range.

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