obtained by alkaline treatment of liver protein from rats administered AAF or *N*-hydroxy-AAF (*15*). Thus, although esters of *N*-hydroxy-AAF such as *N*-acetoxy-AAF have not yet been identified as metabolites, some reactive compound of this type (for example, *N*-acyloxy-AAF or the *N*-phosphate or *N*-sulfate of AAF) must be formed in vivo. The reactivity of *N*-acetoxy-AAF towards proteins and nucleic acids and the carcinogenicity of this ester for the subcutaneous tissue of the rat (*3*) suggest that metabolic esters of *N*-hydroxy-AAF may react with one or more critical cellular macromolecules to induce neoplasia.

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Diabetes, a New Mutation in the Mouse

Abstract. *Diabetes* (db), *which occurred in an inbred strain of mouse, is inherited as a unit autosomal recessive and is characterized by a metabolic disturbance resembling diabetes mellitus in man. Abnormal deposition of fat at 3 to 4 weeks of age is followed shortly by hyperglycemia, polyuria, and glycosuria. Accompanying morphological changes in the islets of Langerhans suggest neogenesis to compensate for insulin depletion.*

The mutation diabetes, which occurred in an inbred mouse strain (C57BL/Ks) at Jackson Laboratory, is characterized by a metabolic disturbance resembling diabetes mellitus in man. The diabetic mutant is similar to the obese mutant (*1*) in appearance but exhibits a severe disease syndrome with onset at an early age and a shortened life-span. Diabetes (*db*), like obese (*ob*), is inherited as a unit autosomal recessive with complete penetrance. Homozygotes are fat, hyperglycemic, and nonfertile; heterozygotes cannot be distinguished morphologically or physiologically from normal. Our preliminary observations on the inheritance, onset, and course of the diabetic condition and changes in the islets of Langerhans in a limited number of these mutants is reported. Other studies, including the chemistry, endocrinology, pathology, and genetics of the diabetes mutant, are in progress and will be reported in detail elsewhere.

Matings between mice heterozygous for diabetes and obese ($+db \times +ob$) resulted in 21 offspring, none of which exhibited the parental type of fat deposition. Therefore it was concluded that diabetes was not an allele of obese. Fifty-seven (30 percent) of 191 young born to $+db$ parents were of the diabetes (fat) phenotype and 13 (46 percent) of 28 offspring of $++$ by $+db$ parents were shown by progeny tests to be heterozygous for diabetes. Although *dbdb* females do not breed, their ovaries function normally when transplanted into a normal environment. Ten (43 percent) of 23 offspring resulting from crosses between females having *dbdb* ovaries and $+db$ males were of the diabetes phenotype. None of the above ratios is different from that expected of a unit recessive.

One distinguishing feature of the diabetes homozygote, deposition of fat in axillary and inguinal regions, is first noticeable at 3 to 4 weeks of age and, although not correlated with body weight, is unmistakable. Some homozygotes are small and plump and others are large and plump, the range in weights shown in Table 1 being considerable at all ages. Table 1 compares body weights, at weekly intervals, of diabetic (*dbdb*) and normal ($+db$ or $++$) mice and shows that in the former there is a rapid increase during the first few weeks with a plateau reached at about 10 weeks. One exceptional female attained a maximum weight of 62 g, whereas most females have not exceeded 55 g. This is in contrast to obese mutants (*obob*), in which the maximum weight attained is about 120 g. Not shown by figures in the table is the decrease in weight that occurs as the diabetics begin to succumb to the disease. This deterioration has occurred usually between 3 and 6 months of age, although two females survived 9 and 12 months. Obese usually survive about 15 months.

To determine the age of onset and degree of hyperglycemia, nonfasting blood sugar was determined weekly at the same time of day on groups of diabetic and normal mice of the same ages. For the assay, blood was withdrawn from the orbital sinus into a 50-μl pipette, deproteinized in 5 ml of tungstic acid reagent, and assayed for total reducing sugars by the micromethod of Folin and Malmros (*2*).

Results of the assays are given in Table 2. The blood sugar of control mice did not exceed 200 mg/100 ml of blood at any age, whereas all diabetics had concentrations of at least 200 mg/100 ml by 8 weeks of age, and all reached or exceeded a level of 300 mg/100 ml by 10 weeks of age. Although 28 percent of diabetics had blood sugar concentrations of 200 mg/100 ml when 3 to 4 weeks old, others did not reach this level until they were 8 weeks old. Our records suggest that the latter were more often females than males, and also that males tended to reach blood sugar concentrations of 500 mg/100 ml at earlier ages

Table 1. Body weight of diabetic (*dbdb*) and normal (+ + and + *db*) mice. Sexes are combined.

Age (weeks)	*dbdb*			+ + and + *db*		
	No. of mice	Weight (g)		No. of mice	Weight (g)	
		Av.	Range		Av.	Range
2– 3	6	6.7	7– 10	7	7.6	7– 9
3– 4	12	14.8	9– 20	21	11.3	9– 13
4– 5	25	19.8	10– 27	16	16.9	15– 21
5– 6	20	25.4	14– 34	19	18.5	15– 23
6– 7	16	28.3	18– 39	17	19.8	16– 28
7– 8	12	32.4	21– 42	17	21.9	19– 28
8– 9	10	34.5	31– 41			
9–10	11	37.9	31– 44			
10–11	11	39.4	32– 45			
11–12	7	38.1	32– 46			
12–13	5	44.4	34– 51			
13–22	11	45.3	32– 62	5	27.3	20– 36
23–54	5	45.9	37– 61	26	28.9	20– 38

Table 2. Blood sugar concentrations of diabetic (*dbdb*) and normal (+ + and + *db*) mice. Sexes are combined.

Age (weeks)	*dbdb*			+ + and + *db*		
	No. of mice	Blood sugar (mg/100 ml)		No. of mice	Blood sugar (mg/100 ml)	
		Av.	Range		Av.	Range
2– 3	3	95.3	85–108	3	92.3	88–101
3– 4	7	187.3	135–268	16	159.7	144–190
4– 5	23	208.5	147–374	8	162.4	132–196
5– 6	12	246.2	144–500	7	171.8	152–188
6– 7	16	322.7	153–576	14	157.5	134–199
7– 8	13	347.0	147–530			
8– 9	12	380.1	221–550			
9–10	11	461.8	226–576			
10–11	11	413.9	304–570			
11–12	8	466.0	390–550			
12–13	6	458.0	367–547			
13–22	9	548.4	446–675	6	154.0	145–159
23–54	4	563.2	494–682	12	158.5	135–188

than females. Studies on more mice are necessary to confirm these points. The possibility that the *dbdb* mice with later onset of hyperglycemia live longer is being investigated. Hyperglycemia in *obob* mice is irregular in occurrence, age of onset, and concentration attained.

At blood sugar concentrations of 250 to 300 mg/100 ml, *dbdb* mice have other symptoms, among which are: polyuria, polydipsia, polyphagia, and glycosuria. Amounts of urine up to 1 ml/hr have been measured, and, during the short periods (20 to 22 hours) in metabolism cages tolerated by the diabetics, the urine output often exceeded the water intake. Although sugar was detected (by Ames "Clinistix" and "Clinitest") in urine of diabetic mice when the blood sugar reached 250 to 300 mg/100 ml and usually exceeded 2 percent at blood sugar concentrations of 350 mg/100 ml, urine specific gravity was not greater than that of normal mice. Total solids, measured on the American Optical TS meter, made up 8 to 17 percent of the urine volume in diabetics, a ratio not different from that in urines of ++ and +*db* mice nor of those of many inbred strains that we have measured.

Seven diabetics (4 males and 3 females) were fasted for 24 hours with resultant losses in weight of 3 to 9 percent and lowering of blood sugar of 41 to 69 percent. They all rapidly regained weight and blood sugar levels after the free availability of food was restored. Attempts to control weight by restriction of food, as has been successful with obese, failed. The diabetics were unable to survive on one or two 3-g pellets per day and they failed to lose weight or become less hyperglycemic on three a day. Diabetics with well-established hyperglycemia were unable to withstand the stress of being in metabolism cages over a period of several days, even though they were supplied with adequate amounts of food and water. This and other stressful situations appeared to bring on metabolic deterioration, signs of which were sluggishness, ketonuria (detected by Ames "Ketostix"), decrease in body temperature, and the presence of traces of blood in urine and feces. Autopsies of mice in the terminal stages of diabetes often showed evidence of bleeding into stomach, intestine, and other internal organs.

Organs and tissues of diabetics, killed at 3 to 5 months of age, showed no gross histological lesions except in the islets of Langerhans. These were strikingly abnormal; were not always clearly demarcated from pancreatic acinar cells; and consisted of mixtures of acinar cells, islet cells with few, if any, beta granules, and dilated pancreatic ducts. The cystlike pancreatic ducts were lined with cuboidal epithelium in which there appeared to be transitions to islet and pancreatic acinar cells, which suggests that neogenesis was in process. Islets of normal mice of this age showed no such process, and the islets of younger, less severely affected diabetics contained fewer dilated ducts and more beta cell granules. In the islets of 3- to 4-week-old diabetes homozygotes, there appeared to be a decreased number of beta granules, compared with the number in normal mice of the same age, but no neogenesis, which suggests that this process is a compensatory mechanism initiated as the disorder becomes severe and not a developmental or congenital defect. The islets of diabetes mice are quite different from those of obese which are hyperplastic with enlarged sinusoids and have many beta granules.

The ovaries of the diabetes mutant, like those of the obese, contain no follicles beyond the antrum stage and remain small; testes appear to be normal. Kidneys, adrenals, retinas, thyroids, lungs, and hearts of *dbdb* have been examined and no gross pathology was noted.

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