Diabetologia 11, 431–438 (1975)

Function of the Pancreatic B-Cell during the Development of Hyperglycaemia in Mice Homozygous for the Mutations "Diabetes" (*db*) and "Misty" (*m*)

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Received: September 3, 1974, and in revised form: June 20, 1975

Summary. The function of the pancreatic B-cell was studied in relation to the development of the diabetic syndrome in a new variety of the diabetic mutant mouse, which was produced at The Jackson Laboratory, Bar Harbor, Maine, U.S.A. by outcrossing of a C57BL/KsJ-*db* stock with C57BL/6J mice. The expression of the *db*-gene in the resulting strain was evaluated by measurements of the body weights and the concentrations of serum glucose and serum insulin at different ages of the animals. In the diabetic mice the body weights increased rapidly between 5 and 25 weeks of age to a weight twice that of the lean controls. During the same time hyperglycaemia and hyperinsulinaemia occurred, the maximal serum glucose and insulin values being observed between 17 and 25 weeks of age. Later on the serum glucose and serum insulin concentrations gradually decreased.

Islets were isolated with collagenase from animals 5, 10 or 20 weeks old, and studied with respect to insulin content, glucose oxidation and the secretion and synthesis of insulin. The results were compared with data from control experiments with islets isolated from non-diabetic littermates. No major differences were found between islets from diabetic and control mice with regard to the glucose oxidation rate, whereas an exaggerated insulin response to glucose was observed in islets from 5 weeks old diabetic mice. In the 20 weeks old diabetic animals there was a significantly decreased islet insulin content and a considerably lowered insulin biosynthesis.

Key words: Experimental diabetes, isolated pancreatic islets, insulin content, glucose oxidation, insulin secretion, insulin biosynthesis.

In 1965 a new autosomal recessive hereditary trait for diabetes (*db*) was discovered in the C57BL/KsJ mouse strain at The Jackson Laboratory, Bar Harbor, Maine, U.S.A. [1]. As in many other rodents with syndromes characterized by inappropriate hyperglycaemia [2] the homozygotes *db/db*, in addition to increased blood glucose values, displayed a moderate hyperinsulinaemia and pronounced obesity in the early phase of the disease. In contrast to the obese-hyperglycaemic mouse (C57BL/6J-*ob/ob*) the capacity for islet cell regeneration seemed, however, to be low in this new mutant [3]. After an initial period of increased mitotic activity, the B-cells began to show marked degenerative changes at about the age of 10 weeks [4, 5]. At the same time the serum insulin concentration fell and the diabetic state became aggravated. Because of the progressive islet cell degeneration the syndrome has been considered to be a suitable experimental model for the B-cell degeneration observed in human juvenile diabetes mellitus [6, 7].

The present study was performed with the specific aim of relating the function of the pancreatic B-cell with the development of the diabetic syndrome in the *db*-mouse. The experimental animals belonged to a local colony of "*dbm*" mice (see below). Since this particular variety of the *db*-mouse has not previously been studied with regard to development of diabetes the different phases of the syndrome were evaluated in some detail.

Materials and Methods

Animals

Mice of both sexes homozygous for the *db*-gene and their non-diabetic littermates were used, the latter serving as controls.

Four breeding pairs were obtained from The Jackson Laboratory, Bar Harbor, Maine, U.S.A., in 1971 and a colony has since been bred in our department using a random breeding system. At The Jackson Laboratory the C57BL/KsJ-*db* stock was outcrossed with C57BL/6-*m* mice; the latter being characterized by a misty coat colour. The resulting hybrids were back-crossed four times to C57BL/Ks mice and although not inbred would be expected to have much of the C57BL/Ks genome (Dr. Douglas L. Coleman, personal communication). Since the loci for *m* and *db* are closely linked on the same chromosome the coupling cross *dbm*/+ + will give 25% mice with a misty coat colour, of which about 98% will display

inappropriate hyperglycaemia and obesity. This means that homozygous mice (*dbm/dbm*) could be identified already at 8–10 days of age although the metabolic abnormalities did not become manifest until about the age of 4–5 weeks.

Unless otherwise stated the animals had free access to tap water and pelleted food (Anticimex 213, Anticimex AB, Sollentuna, Sweden) with the following composition in weight per cent (except for trace elements and vitamins): crude protein 26.5, crude fat 10.5, nitrogen free extractable substances 41, fiber 4.7, ash 9, water content 7.3. The caloric value was 3.2 kcal/g. The litters were weaned at 3 weeks of age.

Determination of Serum Glucose and Insulin Concentrations

The development of the diabetic syndrome was followed by measurements of serum glucose and insulin concentrations at different ages. Blood samples of about 200 µl each were taken from the orbital venous plexus.

The blood glucose concentrations were determined in duplicate 5 µl samples of serum using a glucose oxidase method [8].

The immunoreactive serum insulin concentration was measured in duplicate by the radioimmunoassay of Heding [9] using an antibovine insulin serum from guineapigs (Miles Laboratories, Inc., Kankakee, Ill., U.S.A.) and ^{125}I-insulin (bovine) from The Radiochemical Centre, Amersham, England. Crystalline mouse insulin, kindly prepared by Novo A/S, Copenhagen, Denmark (biological activity 23 µU/ng) was used as a standard.

Tests for the presence of glucose and ketone bodies in the urine were performed with the use of conventional test tapes (Clinistix and Ketostix, Ames Co., Stoke Poges, Slough, Bucks, England).

Body Dry Weight

In order to get a crude measure of the body fat content, the dry body weight was determined [10] by drying the weighed bodies at +65° C to constant weight (7 days). The dry weights were expressed as per cent of the initial wet weights.

Isolation of Islets

In all 183 controls and 153 diabetic mice, 5, 10 and 20 weeks old were used for the *in vitro* studies of isolated islets. The three different groups roughly corresponded to three developmental stages of the diabetic syndrome previously described by Like and Chick [4]: early hyperglycaemia (5 weeks), established hyperglycaemia (10 weeks) and maximal hyperglycaemia (20 weeks). After being fasted overnight the animals were killed by neck elongation and the pancreas was then immediately excised. Blood samples for determination of serum glucose and insulin concentrations were taken from the different groups of fasted animals. Pancreatic islets were isolated by a collagenase method mainly as described by Howell & Taylor [11]. After a subcapsular injection of Hanks' balanced salt solution (glucose concentration 2.2 mM) the gland was minced and shaken by hand in a plastic tube containing 2 ml of Hanks' solution with 10–12 mg of crude collagenase (Worthington Biochemical Corp., Freehold, N.J., U.S.A.). The digest was frequently controlled by inspection under a stereomicroscope and the enzymatic process was interrupted by dilution with Hank's solution. The digested material was allowed to sediment and after three subsequent washings at room temperature the material was suspended in a plastic Petri dish. Islets were isolated using a braking pipette [12] and were washed in Hanks' solution before commencement of the incubations.

Islet Insulin Content

Islet insulin content was measured in animals fasted overnight and 1–3 mice were used in each experiment. One to four groups of about 50 islets each were homogenized in 50 µl redistilled water and 20 µl of homogenate was removed for duplicate determinations of the protein content [13]. The remaining 30 µl of the homogenates were extracted in 170 µl acid ethanol [1.5 ml of 12M HCl in 100 ml 75% (v/v) ethanol] overnight at +4° C, centrifuged and analyzed for insulin content as described above. The mean value for the homogenates in each experiment was used in the statistical calculations. Eight to eleven experiments were performed in each of the three groups of *dbm*-mice and lean mice.

Islet Glucose Oxidation

Islets were incubated at +37° C in 100 µl of Krebs-Ringer phosphate buffer [14] with (U-^{14}C)D-glucose (The Radiochemical Centre, Amersham, England). The glucose concentration was 3.3 or 16.7 mM and the specific activity was 1 mCi/mmole. The incubation system was as described by Keen *et al.* [15]. After a 90 min incubation period glucose oxidation was arrested by addition of 100 µl 0.05 mM antimycin A (Sigma Chemical Co., St. Louis, Mo., U.S.A.) dissolved in 70% ethanol. $^{14}CO_2$ was then liberated by a subse-

quent injection of 100 µl 0.40 M sodium phosphate buffer, pH 6.0, and trapped during a second 2-hour incubation in 0.25 ml hyamine (1 M solution in methanol) (Packard Instrument Co., Inc., Downers Grove, Ill., U.S.A.). Radioactivity was then counted in a liquid scintillation spectrometer (Packard Model 3380) using a toluene based scintillator [toluene 1000 ml, 2,5-diphenyloxazole 5 g, 1,4-bis(4-methyl-5-phenyloxazol-2-yl) benzene 0.05 g]. Counting efficiency was 93% and the recovery of $^{14}CO_2$ was 87%. Blank values were obtained with samples of islet-free incubation medium, which was processed in the same way.

After the incubation procedures islets were recovered, quickly washed in redistilled water, dried at +70° C for 10 min and weighed on a ultramicrobalance (Mettler UM7, Mettler Instrumente AG, Switzerland). The oxidative rates were then expressed on a dry islet weight basis.

Insulin Release

Insulin release was determined at both a low (3.3 mM) and a high (16.7 mM) glucose concentration. The incubation medium was a bicarbonate buffered solution [16] with albumin, 2 mg/ml, and the gas phase was 95% O_2/5% CO_2. After a 20 min preincubation period at the low glucose concentration, samples with 5–10 islets each were incubated for 30 min in 250 µl medium with either the low or the high glucose concentration. Samples of the medium were frozen and stored at −20° C until assayed for immunoreactive insulin. Islet dry weights were determined as described above.

Insulin Biosynthesis

Incorporation of ^{3}H-leucine into proinsulin and insulin was determined by immunoprecipitation [17, 18] using an antiserum (Miles Laboratories, Inc., Kankakee, Ill., U.S.A.) reacting with both proinsulin and insulin and coupled to a solid phase (see below). Islets were isolated and divided into groups of approximately equal size with 10 islets in each. Four groups were incubated in 100 µl of a bicarbonate buffered medium [16] with albumin, 2 mg/ml (w/v) and added amino acids [19] [except that 10 µCi L-(4,5-^{3}H)leucine (specific activity 51 Ci/mmole; The Radiochemical Centre, Amersham, England) was substituted for non-radioactive leucine]. The glucose concentration was adjusted to either 3.3 or 16.7 mM. After gassing with 95% O_2/5% CO_2 samples were incubated for 120 min. The islets were then washed twice in cold Hanks' solution with 10 mM nonradioactive leucine and sonicated in 200 µl redistilled water. After dilution with phosphate buffer (0.04 M sodium phosphate buffer, pH 7.4; 0.1 NaCl; albumin 2 mg/ml) duplicate samples were incubated for 2 hrs with the antiinsulin serum which had been coupled to Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) [20, 21]. Incubations were performed in 1 ml of the sodium phosphate buffer with albumin and carried out at room temperature under continuous vertical rotation to keep the Sepharose beads in suspension. The amount of antiserum added in order to precipitate the proinsulin and the insulin in the islet homogenates was evaluated in two separate sets of experiments. The dose of antibodies needed to completely precipitate a tracer dose of ^{125}I-insulin (bovine) added to an islet homogenate was titrated and three times this dose was subsequently used in the routine experiments. This dose of Sepharose-linked antiserum was sufficient to adsorb in addition to all insulin about 90% of the proinsulin in the islet homogenates as indicated by the corresponding reduction of radioactive proinsulin in the supernatant when this was recovered and subjected to gel chromatography (Sephadex G-50 Fine, Pharmacia Fine Chemicals, Uppsala, Sweden).

Non-specific binding of labelled proteins and ^{3}H-leucine to the Sepharose-anti-insulin serum complex was checked by simultaneous incubations of samples with normal guinea-pig serum linked to Sepharose. After centrifugation and washing three times with the phosphate buffer (without albumin) the Sepharose beads were dissolved in Soluene 350 (Packard Instrument Co., Downers Grove, Ill., U.S.A.) and radioactivity determined after addition of the toluene based scintillator. Counting efficiency was 33% and the non-specific binding was subtracted as blank [18, 21]. The non-specific binding was about 50% and 20% with homogenates of islets incubated at 3.3 mM and 16.7 mM glucose respectively. Total islet protein synthesis was estimated by precipitation of proteins with 5% (w/v) trichloracetic acid. The precipitates were washed twice in 5% trichloracetic acid, dissolved in Soluene 350 and radioactivity measured as described above.

In order to express on a dry weight basis the radioactive labelling of islet proteins 1–3 groups of isolated islets in each experiment were dried and weighed on a Cahn Model 4100 Electrobalance (Cahn/Ventron Corp., Paramount, Ca., U.S.A.) with a sensitivity of 0.1 µg.

Results

Figs. 1–3 show the body weight, serum glucose and insulin concentration at various ages of the lean and

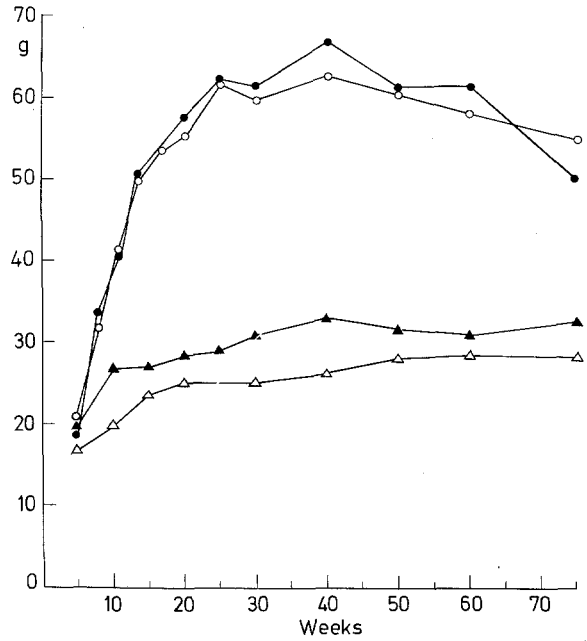

Fig. 1. Body weight of diabetic mice (males •, females o) and control mice (males ▲, females △) at different ages. Each point represents the mean of 5–33 measurements. The total number of observations was 120–198 in each animal group

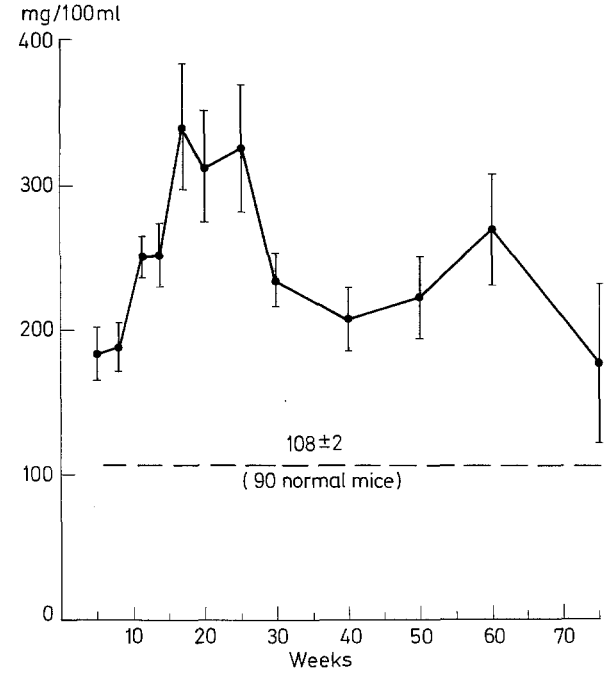

Fig. 2. Serum glucose concentrations in non-fasted diabetic mice at various ages. Each point represents the mean ± S.E.M. of 8–33 animals. There were no age differences in the control animals and the pooled mean and S.E.M. of 90 controls is also shown

diabetic mice. The maximal weight (60–65 g) of the diabetic mice was reached at about 40 weeks of age followed by a slight decrease in older animals (Fig. 1). In the diabetic group there was no obvious difference

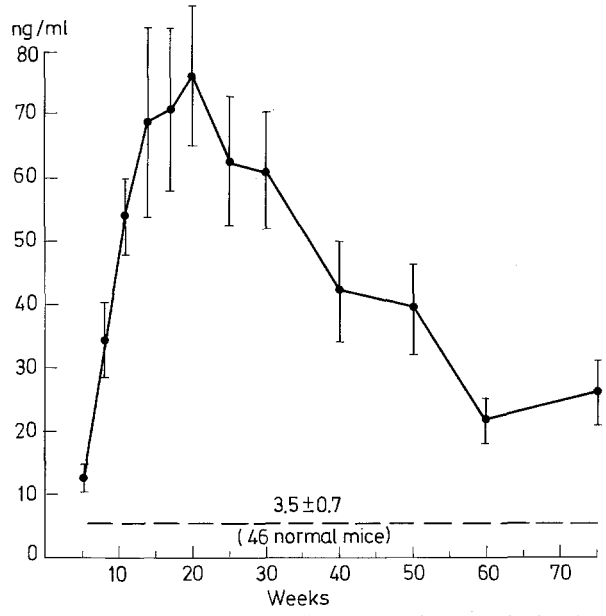

Fig. 3. Serum insulin concentrations in non-fasted diabetic mice at various ages. Serum immunoreactive insulin was measured in 155 diabetic mice. Each point represents the mean ± S.E.M. of 5–23 observations. There were no age differences in the control animals and the pooled mean and S.E.M. of 46 controls is also shown

Table 1. *The dry body weight expressed as per cent of the wet body weight. Each group consisted of 10 animals. Means ± S.E.M.*

	Diabetic mice			Control mice		
Age in weeks	5	10	20	5	10	20
	52 ± 1	69 ± 1	78 ± 1	33 ± 1	36 ± 2	37 ± 1

in weight between males and females whereas in the non-diabetic group the males were heavier.

The increasing proportion of body fat in the diabetic mice manifested itself as a relatively high dry body weight in relation to the wet body weight. In the diabetic mice dry weight was not less than 78% of the wet weight as compared to 37% in the controls.

The blood glucose concentrations increased to a peak value at 17–25 weeks (300–350 mg/100 ml) which coincided with a marked peak in the serum insulin concentrations (70–80 ng/ml) (Figs. 2 and 3). Both the serum glucose and the serum insulin values tended to decrease in the oldest animals. Glucosuria was frequent whereas ketonuria was never observed.

An attempt was made to correlate the early phases of the diabetic syndrome with islet function and metabolism by a comparative study of isolated islets from diabetic and normal animals. For this purpose isolated islets were obtained from normal and diabetic mice at 5, 10 and 20 weeks of age and studied with regard to insulin content, glucose oxidation, insulin

Table 2. *Characteristics of fasted experimental animals. Mean ± S.E.M. of serum glucose (20 animals) and serum insulin (10 animals) in each group*

	Diabetic mice			Control mice		
Age in weeks	5	10	20	5	10	20
Serum glucose mg/100 ml	92 ± 8	138 ± 12	258 ± 31	62 ± 5	61 ± 5	65 ± 6
Serum insulin ng/ml	6.2 ± 1.2	16.2 ± 4.6	23.4 ± 3.0	2.7 ± 0.4	4.0 ± 0.5	3.4 ± 0.5

Table 3. *The insulin content (ng/μg protein) in isolated islets from diabetic and control mice. The values are given as means ± S.E.M. with the number of observations within parentheses*

	Diabetic mice			Control mice		
Age in weeks	5	10	20	5	10	20
	238 ± 16 (8)	224 ± 23 (11)	125 ± 28 (9)	271 ± 23 (8)	289 ± 27 (8)	303 ± 43 (9)

Table 4. *Insulin released (ng/30 min/μg dry islet wt.) The values are given as means ± S.E.M. with the number of observations given within parentheses*

	Diabetic mice			Control mice		
Age in weeks	5	10	20	5	10	20
Glucose conc.						
3.3 mM	2.4 ± 0.6 (18)	1.8 ± 0.3 (20)	1.8 ± 0.2 (23)	2.0 ± 0.4 (22)	2.2 ± 0.3 (22)	2.1 ± 0.2 (19)
16.7 mM	13.4 ± 1.5 (21)	4.8 ± 0.5 (22)	4.7 ± 0.7 (27)	5.9 ± 1.1 (30)	4.1 ± 0.6 (23)	6.6 ± 1.2 (22)

release and insulin biosynthesis. Islets were isolated from mice that had been fasted overnight, and the diabetic state of these animals was evaluated by measurements of their serum glucose and insulin concentrations. As seen in Table 2 the fasted *dbm*-mice showed persistant hyperglycaemia and hyperinsulinaemia although not as pronounced as in the non-fasted state.

A comparison of the islet insulin content in the three groups of diabetic animals showed a decrease by about 50% from 5 to 20 weeks. At the latter age the islet insulin content of diabetic animals was only 40% of that in the controls (Table 3).

The islet glucose oxidation was similar in diabetic and control mice and no statistically significant differences were found between the three age groups (Fig. 4). In all groups there was a strong stimulation of the glucose oxidation when the glucose concentration of the medium was increased.

The insulin release from the isolated islets was also increased ($P < 0.005$) in all groups investigated when the glucose concentration was raised from 3.3 to 16.7 mM. The youngest diabetic mice displayed a hyperstimulatory response (Table 4) ($P < 0.001$ in comparison with the other groups). There was no difference between the three control groups.

As can be seen in Fig. 5 glucose also stimulated leucine incorporation into proinsulin-insulin of isolated islets from the control mice at both 5, 10 and 20 weeks of age ($P < 0.001$). A corresponding stimulation was noted in the diabetic mice at 5 and 10 weeks of age, whereas in 20 weeks old diabetic mice any glucose stimulation was doubtful ($P > 0.10$). In comparison with the controls there was a significant elevation ($P < 0.05$) of incorporation of ^{3}H-leucine into proinsulin-insulin at a low glucose concentration in the 5 weeks old diabetic mice whereas no such difference was seen at 10 and 20 weeks ($P > 0.05$). Fig. 6 shows the incorporation of radioactivity into proinsulin-insulin expressed as per cent of total trichloracetic acid precipitable islet radioactivity from the same groups of mice as shown in Fig. 6. In the controls 25–30 per cent of the total radioactivity was recovered in the proinsulin-insulin fraction at all ages after 2

hours incubation in the high glucose concentration. By contrast, in the diabetic mice the corresponding incorporation was 36–40 per cent at 5 and 10 weeks and then decreased to only 10 per cent at 20 weeks of age ($P < 0.001$ compared with control mice of the same age). Similarly there was a sharp decrease of the percentage incorporation at a low glucose concentration in the 20 weeks old diabetic animals. The diminished synthetic capacity in the 20 weeks old diabetic mice seemed to affect only proinsulin-insulin formation, since the total trichloracetic acid precipitable radioactivity was not depressed.

Discussion

A comparison of the present results with those of Coleman and Hummel [3] and Like and Chick [4] shows that the diabetic manifestations in our *dbm*-mice differ in several respects from those observed in the original C57BL/KsJ-*db* strain. Thus, at comparable ages there were lower blood sugar concentrations and no signs of ketosis in the *dbm* variety. Furthermore, the serum insulin values appeared higher in the latter animals and the hyperinsulinemia was of a longer duration. In agreement with these observations most diabetic animals of the present colony were still alive at the age of 18 months whereas Like and Chick [4] observed a life span of only 5–7 months in the C57BL/KsJ-*db* mouse.

A likely explanation for the shorter life span in the KsJ-*db* mouse as compared to the *dbm*-mouse is the observation in the former mice of sharply decreasing plasma insulin concentrations associated with islet degeneration at the age of about 10–20 weeks [4]. Morphological signs of islet degeneration were not observed in the *dbm*-mice of this age; degenerative changes of the islets do not appear in fact until the age of about 12 months [22]. Several explanations for this phenotypic difference may be put forward. Thus, a slight change in the original genetic background introduced by outcrossing C57BL/KsJ-*db* mice with C57BL/6J-*m* could weaken the tendency for islet degeneration, perhaps by increasing the capacity for mitotic division of the islet cells. A high regenerative capacity for the islet cells is a characteristic of the 6J-*db* mice, which in turn seems to give rise to a higher concentration of circulating insulin [23]. Corresponding observations have been made with the *ob*-gene on the 6J and KsJ background respectively [24]. Therefore it seems likely that in our *dbm*-mice there is still some of the C57BL/6J genome present. An alternative explanation for the differences between the two varieties of diabetic mice would be differences in the composition of their diets. According to Chick

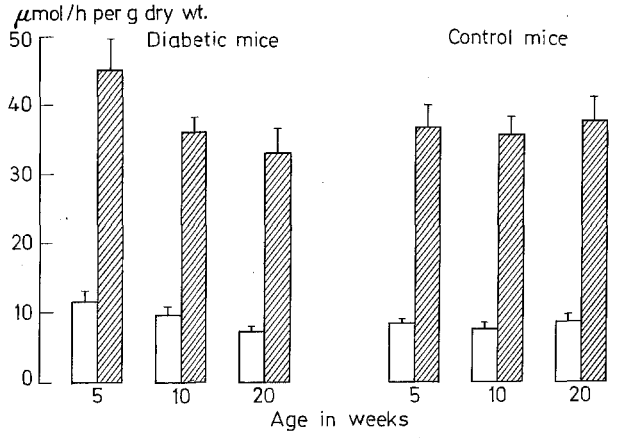

Fig. 4. The oxidation of ^{14}C-glucose to $^{14}CO_2$ as determined at 3.3 mM (open bars) or 16.7 mM (filled bars) of glucose. Each bar shows the mean ± S.E.M. of 15–23 observations

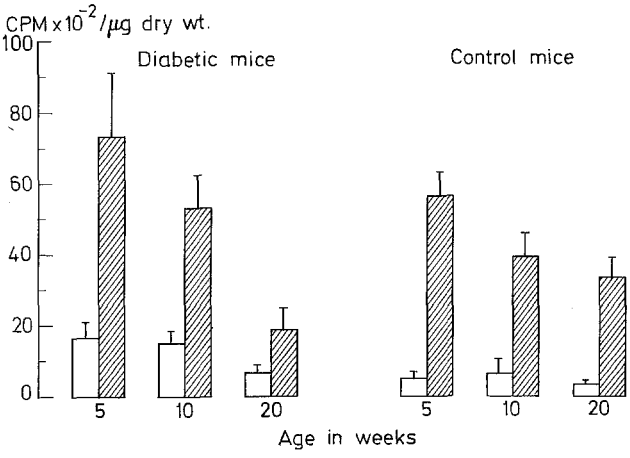

Fig. 5. Incorporation of radioactive leucine into the islet proinsulin/insulin fraction. An increase of the glucose concentration from 3.3 mM (open bars) to 16.7 mM (filled bars) stimulated the incorporation significantly ($P < 0.01$) in all groups except for the 20 weeks old diabetic mice ($P > 0.10$). Each bar represents the mean ± S.E.M. of 5–7 experiments

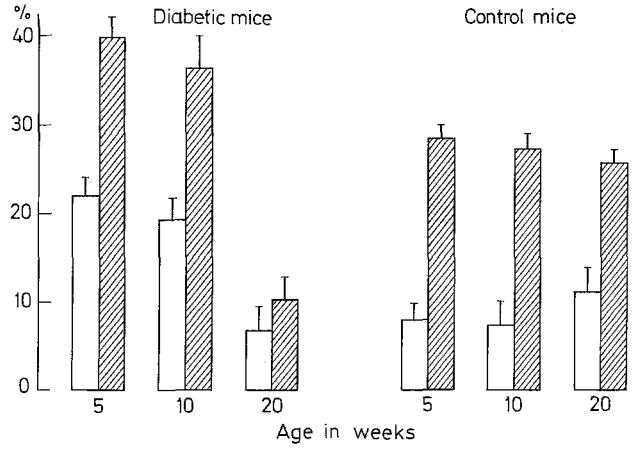

Fig. 6. The incorporation of ^{3}H-leucine into islet proinsulin-insulin in Fig. 5 expressed as per cent of the incorporation into the trichloracetic acid precipitable protein fraction of the islet homogenates. Means ± S.E.M.

and Like [24] different dietary compositions can indeed influence the insulin levels and the B-cell replication considerably in these animals. The authors found that a diet rich in proteins stimulated the incorporation of ^{3}H-thymidine into islet DNA more than a carbohydrate rich diet. It should be noted that the commercial laboratory chow used in our study is well supplied with proteins (27.5%).

Despite the absence of structural signs of islet degeneration it is obvious from the present results that the progressive development of hyperglycaemia between the ages 5–20 weeks in the *dbm*-mice was associated with several functional changes in the B-cells. It is worthy of note in this context that the *in vitro* metabolic studies were performed with islets isolated from animals starved overnight, but that this short fasting period did not abolish the diabetic state. The islet insulin content became considerably smaller as hyperglycaemia worsened. Nevertheless, the islets continued to respond to a glucose challenge with increased insulin release and glucose oxidation at all ages investigated. By comparison, Cameron *et al.* [26] found a stimulatory effect of glucose on insulin secretion *in vivo* in another variety of KsJ-*db* mice outcrossed with 6J-*m* mice [27]. Both these results and the present ones differ from *in vitro* observations on microdissected islets from 7–8 months old KsJ-*db* mice by Boquist *et al.* [28] suggesting that the islets of the KsJ-*db* mouse have a deficient insulin response to glucose. The lack of such a defect of the B-cell in the *dbm*-mouse in the present study could possibly explain why these animals do not develop a fulminating diabetes. There was in fact a hyperstimulatory insulin response to glucose in the 5 weeks old *dbm*-mice.

The observation of an apparent deficiency of the proinsulin-insulin biosynthesis in the 20 weeks old *dbm*-mice deserves special attention. Before discussing the biological significance of this observation some possible methodological errors should be considered.

Firstly, because of the reduced islet insulin content in islets from 20 weeks old *dbm*-mice a relatively high specific radioactivity of the proinsulin-insulin pool should be present after incubation with ^{3}H-leucine in comparison with control mouse islets. Secretion of insulin with a relatively high specific activity from the islets of 20 weeks old *dbm*-mice during incubations for the insulin biosynthesis experiments should thus lead to a reduction of the radioactivity incorporated into the islets, producing an erroneous impression of a low synthetic rate. However, separate experiments showed that only a few per cent of the newly synthesized insulin appeared in the medium during the 2 hours incubation period. This finding is in accordance with previous results obtained with slices of rabbit pancreas and with rat islets [29, 30].

Another methodological point concerns possible differences in the islet cellular composition which might affect the results when the insulin synthesis is calculated on an islet dry weight basis. Separate morphometric analyses of the cellular composition of the islets in fact suggested some increase in the proportion of B-cells in the *dbm*-mice at both 10 and 20 weeks of age. This indicates that the apparently reduced biosynthesis of insulin as calculated on a dry weight basis did not simply reflect a reduction of the B-cell number. It is therefore suggested that the present figures reflect a true reduction of insulin biosynthesis in the individual B-cell of the 20 weeks old *dbm*-mice.

There seems so far to be no previous report indicating a deficient insulin biosynthesis in either spontaneously diabetic animals or in man. In fact, Poffenbarger *et al.* [31] failed to observe any abnormalities of the insulin biosynthesis in isolated islets of the KsJ-*db* mouse and Berne [21] found a normal or even increased incorporation of ^{3}H-labelled leucine into the insulins of the *ob/ob*-mouse of the Uppsala colony. In the latter animal the synthetic rate was found to be still high at 12 months of age. The apparent disagreement between the present results and those of Poffenbarger *et al.*[31] may be explained in several ways. Firstly, the different genetic backgrounds of the two varieties of the *db*-mouse may produce different pathological expressions of the *db*-gene at the level of the islet cells. Secondly, the latter authors put their animals on a restricted food intake for several weeks before killing, which might have delayed or prevented the development of an otherwise occurring functional degeneration of the B-cells. In line with this notion, the animals presently used were about one month older than those of Poffenbarger *et al.* [31], and, as amply demonstrated in the present study, the age factor is of decisive importance for the manifestation of the various symptoms.

In seeking an explanation for the diminished insulin formation in the 20-week-old *dbm*-mice there are several points which deserve consideration. A gradual derangement of the islet glucose metabolism is probably of no etiological significance because of the intact glucose oxidation of the islets and the persisting glucose stimulation of the insulin release. One possible explanation could be a minor functional change at the ribosomal level. In view of the long-standing functional load on the B-cell this could possibly reflect a progressive exhaustion phenomenon which develops into a more general disturbance of the protein synthesis and ultimately into death of the B-cell.

The apparently contradictory observations of a maximal circulating insulin concentration coinciding

with a decreased rate of insulin biosynthesis may reflect either a decreased elimination of insulin from the blood or an enhanced production of insulin by an increased total B-cell mass, or a combination of these factors. Although in the *dbm*-mouse there are so far no measurements on the rate of insulin elimination from the circulation, corresponding observations in another mouse strain with hereditary diabetes indicated no reduction of the clearance rate [32]. On the other hand the morphological analyses suggested an increased B-cell mass in the *dbm*-mouse [22]. In further support of this the mean dry weight of isolated islets was more than three times higher in the *dbm*-mice as compared to their lean controls. Altogether these data make it reasonable to suggest that the decreased rate of insulin biosynthesis is compensated for by an enlarged B-cell mass.

Acknowledgements. I am indebted to Associate Professor Claes Hellerström for valuable advice and to Mrs Parri Wentzel and Mrs Ing-Britt Hallgren for skilful technical work. Financial support was received from The Medical Faculty of the University of Uppsala, The Swedish Diabetes Association and The Swedish Medical Research Council.

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