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Studies in the Diabetic Mutant Mouse: III. Physiological Factors Associated with Alterations in Beta Cell Proliferation*

W.L. Chick** and A.A. Like***

Elliott P. Joslin Research Laboratory, Departments of Medicine and Pathology, Harvard Medical School, The Peter Bent Brigham Hospital and the Diabetes Foundation, Inc., Boston, Massachusetts, USA

Summary. Beta cell replication was studied in normal (C57BL/Ks) and diabetic mutant (C57BL/Ks-*db/db*) mice following thymidine-^{3}H administration. The specific activity of DNA of isolated islets (DPM/μg islet DNA) was used as an index of proliferative activity and correlated with labeling determined by radioautography. Although thymidine-^{3}H incorporation in islets of prehyperglycemic 5 to 6 week old mutants was limited, it was significantly greater than that in normal mice. With the elevation of blood glucose values, incorporation rose sharply, reaching a maximum level above 130 mg glucose/100 ml blood. Sustained, severe hyperglycemia subsequently correlated with a decline in islet DNA synthesis. Food restriction early in the syndrome reduced hyperglycemia and resulted in low incorporation of label. Animals refed ad lib for periods of 1, 2, or 3 weeks showed significant increases in labeling, with maximal values after 1 week of refeeding. Electron microscopic radioautographs of the islets revealed labeled beta cells but no labeled alpha cells, suggesting that proliferative activity is predominantly restricted to the beta cell population.

Etudes de la mutation dbdb chez la souris. III. Facteurs physiologiques associés a des altérations de la prolifération des cellules B.

Résumé. La division des cellules β a été étudiée chez la souris normale (C57BL/Ks) et chez son mutant diabétique (C57BL/Ks-*db/db*) après administration de ^{3}H-thymidine. L'activité spécifique de l'ADN des îlots isolés (DPM/μg ADN) sert d'index de l'activité de prolifération et est corrélée avec le marquage décélé par autoradiographie. Bien que l'incorporation de ^{3}H- thymidine dans les îlots de souris *db/db* préhyperglycémiques âgées de 5 à 6 semaines soit assez faible, elle est cependant significativement plus élevée que chez la souris normale. L'augmentation des taux de glucose sanguin s'accompagne d'un accroissement très net de l'incorporation, qui est maximale pour les glycémies supérieures à 130 mg glucose %. Une hyperglycémie sévère et prolongée s'accompagne à la suite d'une diminution de la synthèse d'ADN dans les îlots. Une diminution de l'apport alimentaire au début de la manifestation du syndrome décroît l'hyperglycémie concomitante avec une incorporation faible de la substance marquée. Lorsque ces animaux peuvent de nouveau s'alimenter à volonté pendant 1, 2 ou 3 semaines, on constante une augmentation significative du marquage atteignant un taux maximum après une semaine de réalimentation. — L'examen autoradiographique combiné à la microscopie électronique des îlots démontre la présence de radioactivité dans les cellules β, mais pas dans les cellules α. Ce résultat suggère que l'activité de prolifération est essentiellement confinée aux cellules β.

Der Diabetes der dbdb-Maus. III. Beziehungen zwischen Stoffwechselveränderungen und B-Zell-Proliferation

Zusammenfassung. Mittels Messung der spezifischen Aktivität der DNA isolierter Langerhans'scher Inseln und radioautographischer Lokalisation der Radioaktivität nach Injektion von Thymidin-^{3}H wurde die Vermehrung der B-Zellen im Pankreas normaler und hereditär diabetischer (*db/db*) Mäuse untersucht. Die Inkorporation von Radioaktivität war bei 5—6 Wochen alten *db/db* Tieren signifikant höher als bei den normalen Kontrolltieren. Mit Ansteigen der Blutzuckerkonzentration nahm die Inkorporation zu und erreichte bei Blutzuckerkonzentrationen über 130 mg/100 ml ihr Maximum. Anhaltend schwere Hyperglykämie war von einem progressiven Abfall der Inkorporation von Radioaktivität in die Insel-DNA begleitet. Kalorienrestriktion in einem Frühstadium des Syndroms verminderte sowohl die Hyperglykämie als auch die Inkorporation von Radioaktivität. Nach Normalisierung der Nahrungsaufnahme zeigte sich eine deutliche Erhöhung der Inkorporation, die nach einer Woche ihr Maximum erreichte. Elektronenmikroskopische Autoradiographie ergab, daß die Radioaktivität ausschließlich in den B-Zellen lokalisiert war. Markierte A-Zellen wurden nicht beobachtet. Dies weist darauf hin, daß sich die proliferative Aktivität praktisch ausschließlich auf die B-Zellen beschränkt.

Key-words: Diabetic mutant mouse, genotype: C57-BL/Ks-*db/db*, hereditary diabetes in mice, mutation: diabetes, pancreatic islets, islets of Langerhans, beta cell replication, mitotic activity, glucose, insulin, diet, food restriction.

Knowledge of the factors controlling beta cell replication is relevant to understanding the etiologies of abnormal carbohydrate tolerance. Although beta cell proliferative activity in adult mammals appears to be quite limited, marked increases have been reported in response to a variety of stimuli including subtotal pancreatectomy [25], and the administration of agents such as alloxan [22], streptozotocin [22, 2] ACTH [4, 13], cortisone [13, 12], growth hormone [30, 24], and guinea pig antiinsulin serum [21].

Unfortunately, there have been only a limited number of studies dealing with alterations in beta cell mitotic activity in spontaneous diabetes in laboratory animals, and these have relied upon measurements of islet volumes and counting of mitotic figures [31,3], rather than the more recent technique of DNA labeling with tritiated thymidine (thymidine-^{3}H) [27].

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Since the discovery of the diabetic mutant mouse (C 57 BL/Ks-*db*/*db*) in 1965, a number of facets of the syndrome have been studied in detail [11, 7, 8]. Several of these features, including the resemblance to human diabetes, make this animal a particularly useful model for examining alterations in beta cell mitotic activity in hereditary diabetes. The present studies were therefore undertaken to examine changes in beta cell mitotic activity at different stages of the diabetic syndrome in this animal using the technique of DNA labeling with thymidine-^{3}H. Emphasis was placed on relating these changes to alterations in blood glucose concentrations, serum insulin levels, and islet morphology. In addition to studying alterations in beta cell proliferative activity during the natural course of the diabetic syndrome, the effects of dietary manipulation (food restriction and refeeding) on beta cell replication were also determined.

Materials and Methods

Animals and diet

Diabetic mutant mice from the inbred C 57 BL/Ks-*db* strain and normal control mice from the inbred C 57 BL/Ks strain were obtained from the Production Department of The Jackson Laboratory at 5 to 6 weeks of age. Animals were fed with Old Guilford mouse pellets (7.5% fat) and allowed free access to water. Two groups of mice were studied. The first (ad lib fed) was allowed free access to food at all times. In the second group (food restricted — refed) feeding was restricted to an 8-hour period on Monday, Wednesday and Friday of each week between the 7th and 11th weeks of life; these mice were sacrificed either at the end of the period of food restriction, or 1, 2 or 3 weeks following reinstitution of ad lib feeding.

Blood glucose and serum insulin

Blood samples for glucose and serum immunoreactive insulin (IRI) were obtained either by tail bleedings or at the time of sacrifice by decapitation. Glucose was measured by the ferricyanide method of Hoffman, as adapted for the Technicon autoanalyzer [10]. Insulin was measured in duplicate 50 µl aliquots of serum by the double antibody method [28, 33], using ^{125}I-pork insulin (Abbott Laboratories). Although the quantity of insulin in 50 µl of *normal* mouse serum was at the lower limits of the detectability of the assay, it was impractical to use larger amounts of serum because of the limited total blood volume of the animals. In the vast majority of *diabetic* mice, however, this quantity of serum proved more than adequate because of the higher concentrations of circulating insulin. Human insulin standards (Eli Lilly and Company) were utilized in the original assays, since the preparation of purified mouse insulin [9] was not completed until late in the study. These values were subsequently converted to mouse insulin units by simultaneously assaying both human and purified mouse insulin standards under the conditions of the original assays in order to determine the number of mouse insulin units equivalent to each point on the human insulin standard curve.

Beta cell replication

Beta cell proliferative activity was evaluated in each animal both by radioautography [17], and by determining the extent of islet DNA synthesis by means of thymidine-^{3}H labeling [5]. Mice were injected intraperitoneally with a solution of thymidine-methyl-^{3}H (Schwarz BioResearch, Inc.), specific activity 10 Ci per mmole, in 0.9% saline (1 mCi per ml) at a dose of 10 µCi per gm body weight, and sacrificed 2 h later. The majority of the pancreas, and a small segment of intestine and lung were removed, rinsed in iced Hank's solution and treated for DNA analysis as outlined below.

Radioautography. The remainder of the pancreas with a segment of duodenum was removed and fixed for conventional light microscopy and light microscopic radioautography as described in an accompanying publication in this volume [17]. In order to identify the types of islet cells which were labeled, portions of pancreas from representative animals were examined by electron microscopic radioautography [18].

DNA specific activity. Islets were isolated from the unfixed portion of pancreas by the technique of collagenase digestion [9, 15] and collected in 6×50 mm glass test tubes containing 150 µl of cold 5% trichloroacetic acid (TCA). Approximately 20 to 30 islets were isolated from each diabetic mouse pancreas and 10 to 20 islets from each normal control animal. In order to evaluate proliferative activity in the exocrine tissue, fragments of acinar pancreas free of islets were also collected in TCA. Scrapings of intestinal mucosa and small segments of lung were similarly treated. The high specific activity of the DNA extracted from the intestinal mucosa served as a check both of the absorption of the injected thymidine-^{3}H, and later steps in the analytical procedure. The lung was examined to determine the effects of food restriction and refeeding on DNA labeling in a non-insulin sensitive tissue.

Tissue lipids were extracted with cold 0.1 M alcoholic potassium acetate, followed by hot ethanol, in order to remove compounds which interfere with fluorescent DNA analysis [14]. The DNA was extracted from the lipid-free tissue residues with 60 µl of 5% TCA at 90°C for 15 min [32]. A 20 µl aliquot was removed, dried under vacuum, and assayed for DNA by reaction with 3, 5 diaminobenzoic acid dihydrochloride (Aldrich Chemical Company) [14], using purified calf thymus DNA (Sigma Chemical Company) as a standard. Fluorescence was read in an Aminco SPF-125 Fluorometer fitted with an 85 watt high pressure mercury lamp (excitation 400 mµ, emission 520 mµ).

Twenty-five microliters of the TCA extract was added to 15 ml of toluene-ethanol cocktail (14 : 1) containing PPO 0.6% and POPOP 0.01%, and counted in

a Beckman LS-133 liquid scintillation spectrometer. Count rates were converted to absolute activity (DPM) using the external standard method of quench correction.

Final results for both analyses were corrected to 60 μl (original volume of TCA extract), and the specific activity of the DNA, expressed as DPM/μg DNA, used as an index of mitotic activity.

Results

I. Ad lib Fed Diabetic and Normal Mice.

A. Blood glucose and serum insulin. Blood glucose and serum insulin values for diabetic mice ranging in age from 4 to 27 weeks are shown in Fig. 1. The progressive severity of the diabetic syndrome is apparent from these data. Although at 5 to 6 weeks of age the average blood sugar was only slightly elevated above normal, by the 7th week a more distinct elevation was apparent. There was a further increase in hyperglycemia until 15 weeks of age, when the values stabilized in the range of 400 to 500 mg glucose/100 ml blood. Occasional animals, however, manifested more marked hyperglycemia, with levels of 600 to 800 mg glucose/100 ml blood.

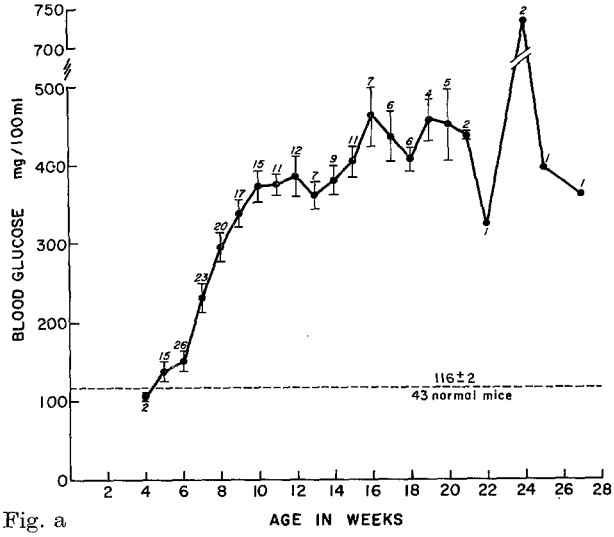

Fig. a

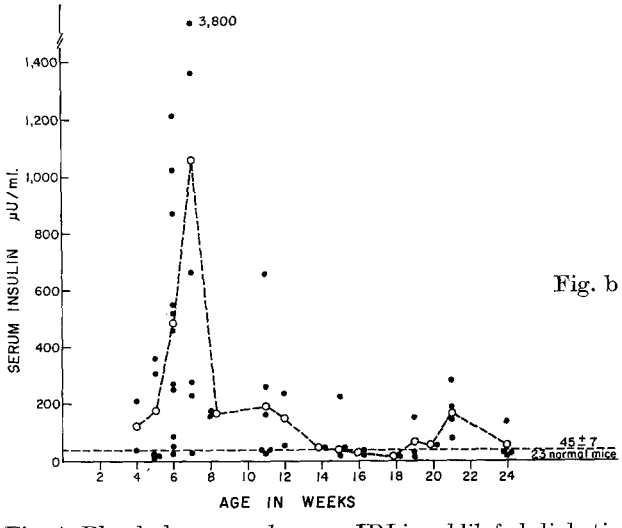

Fig. b

Fig. 1. Blood glucose and serum IRI in ad lib fed diabetic mutant mice at various ages

a. *Upper panel* — blood glucose concentrations are means ± S.E.M. The small numerals indicate the number of animals for each point.

b. *Lower panel* — each point represents the IRI concentration in a single animal; the dashed line connects the mean values.

In contrast to mean blood glucose, serum insulin levels were clearly elevated at 5 to 6 weeks of age. Hyperinsulinemia was most marked at approximately 7 weeks, beyond which there was a gradual decline. During the terminal stage of the disease, insulin levels approached those in normal mice. At this time the number of beta cells was significantly reduced, and ductal structures were seen within the islets. The beta cells which remained were characterized by marked degranulation [17].

The relationship between these changes and alterations in beta cell proliferative activity is discussed in the sections which follow.

B. Beta cell replication in ad lib fed normal mice. Thirteen control animals ranging in age from 5 to 8 weeks were used to determine the extent of beta cell replication in normal mice. The average islet DNA specific activity for the entire group was 25 ± 10 DPM/μg DNA (mean ± S.E.M.), with a range of 0—113 DPM/μg DNA. These results agreed with those of radioautography, which in most instances showed fewer than 5 labeled islet cells per random tissue section [17].

C. Beta cell replication in ad lib fed diabetic mice. Twenty-two diabetic mice ranging in age from 5 to 15 ½ weeks were examined on order to determine the extent of beta cell proliferative activity during the various stages of the diabetc syndrome, and the relationship to alterations in blood glucose, serum insulin, and acinar cell replication.

Relation of beta cell replication to blood glucose. Fig. 2 shows the relationship of thymidine-^{3}H incorporation to blood glucose concentration at sacrifice. Each point represents a single animal. The dashed lines indicate the general trends in the relationship. This appeared to be identical for male animals (closed circles) and female animals (open circles). Although there was limited incorporation below 120 mg glucose/100 ml blood, relatively small increases in blood glucose above this level were associated with a sharp rise in islet labeling, so that values as high as 800 DPM/μg islet DNA were observed above 130 mg glucose/100 ml blood. Prolonged, progressively severe hyperglycemia, however, was associated with a decline in thymidine-^{3}H incorporation, as indicated by the descending limb of the dashed line.

This same series of changes was evident on radioautography [17]. Early in the syndrome labeled beta cells were visible within each islet. With progression of the disease there was a reduction in the number of beta

cells, and the appearance of small ductal structures which surrounded and sometimes permeated the islets. The epithelial cells of these structures were occasionally labeled. Islets were not isolated from animals in the terminal stage of the syndrome because of technical difficulties resulting from the marked structural alterations which occur. The pattern of labeling on radioautography during the terminal phase revealed only occasional labeling among the collection of surviving islet cells [17].

In order to determine whether increased thymidine-^{3}H incorporation occurred prior to the onset of sustained hyperglycemia, labeling in 5 to 6 week old diabetic animals in which the blood sugar had not yet become elevated (blood glucose less than 114 mg/100 ml

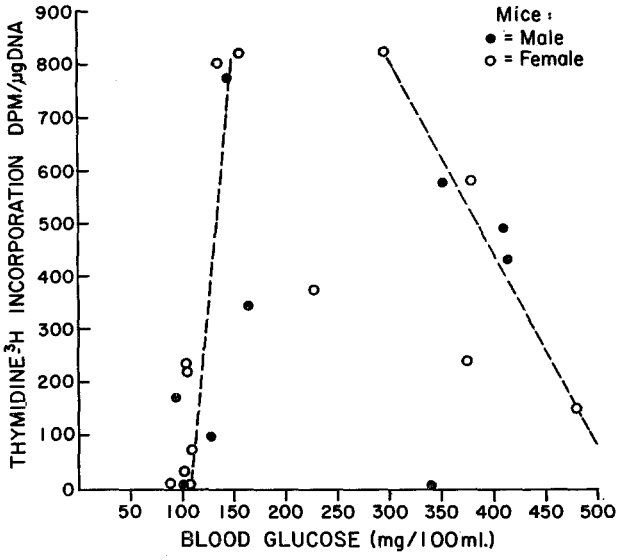

Fig. 2. Thymidine-^{3}H incorporation into islet cell DNA of ad lib fed diabetic mutants versus blood glucose concentration at sacrifice. Each point represents a single diabetic animal. The dashed lines indicate the general trends in the relationship

blood) was compared to that in age-matched normal controls (mean blood glucose 114 mg/100 ml blood). Although thymidine-^{3}H incorporation was limited during this early stage of the disease, it was still significantly greater ($P < 0.05$) than in the normals (Table 1). Although the average serum insulin in the prehyperglycemic mutants was higher than in the normals, the difference was not statistically significant at the 0.05 level. The mean blood glucose was significantly lower in the diabetics; however, this should be interpreted in light of the fact that these 8 animals were selected from a larger group of young mice on the basis of their low blood glucose values (less than 114 mg/100 ml).

Relation of beta cell replication to serum insulin. Insulin levels at sacrifice were analyzed in order to determine whether any correlation existed between beta cell functional activity, as reflected in serum insulin levels, and proliferative activity, as reflected in thymidine-^{3}H incorporation into islet cell DNA. Fig. 3 is a plot of thymidine-^{3}H incorporation versus serum insulin for mutants at various stages of the syndrome. Although a number of widely varying specific activity values occurred at identical insulin values, in general, elevations in serum IRI were associated with increased DNA synthesis, so that the coefficient of linear correlation between the two parameters approached significance at the 0.05 level ($0.1 > P > 0.05$, $N = 20$, $r = 0.3787$).

Table 1. *Incorporation of thymidine-^{3}H into islet cell DNA of prehyperglycemic diabetic mutant mice*

Animal	Average age (weeks)	Blood glucose (mg/100 ml)	Serum insulin (µU/ml)	Islet labeling (DPM/µg DNA)
Normal	6	114±2 (33) $P < 0.001$	55± 9 (16) N.S.	25±10 (13) $P < 0.05$
Diabetic (prehyperglycemic phase)	6	101±2 (8)	72±42 (7)	98±38 (8)

Values are means ± S.E.M. Numbers of mice are in parentheses.

p is the probability for the difference between the normal and the diabetic group, determined from the Student t-test.

N.S. = difference not significant ($p > 0.05$).

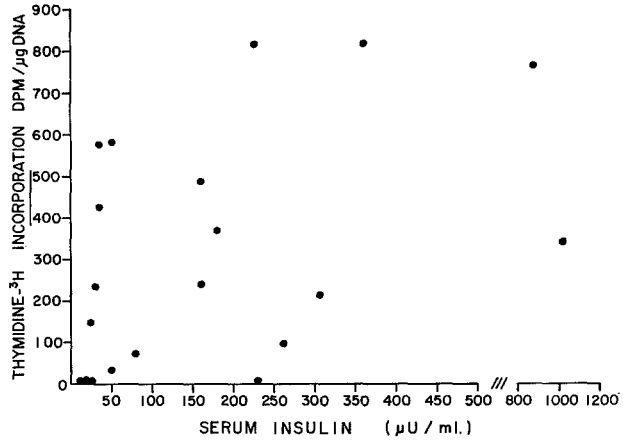

Fig. 3. Thymidine-^{3}H incorporation into islet cell DNA of ad lib fed diabetic mutants versus serum IRI concentration at sacrifice. Each point represents a single diabetic animal. The coefficient of linear correlation approaches significance at the 0.05 level ($0.1 > P > 0.05$, $r = 0.3787$)

Relation of beta cell replication to acinar cell labeling. The specific activity of the DNA isolated from the fragments of acinar pancreas was compared to the specific activity of the DNA isolated from the islets in order to determine whether any correlation existed between proliferative activity in the endocrine and exocrine portions of the pancreas. In 13 normal control mice,

acinar cell DNA synthesis was limited, with an average value of 131 ± 37 DPM/μg acinar cell DNA (mean ± S.E.M.). In general acinar cell DNA synthesis in diabetic animals was also low. Occasional animals with increased islet thymidine-^{3}H incorporation also showed significant increases in acinar cell DNA synthesis. This was not true, however, of the majority of diabetics studied, so that the linear correlation between islet and acinar cell thymidine-^{3}H incorporation was not significant at the 0.05 level ($P > 0.1$, $N = 21$, $r = 0.1967$) (Fig. 4).

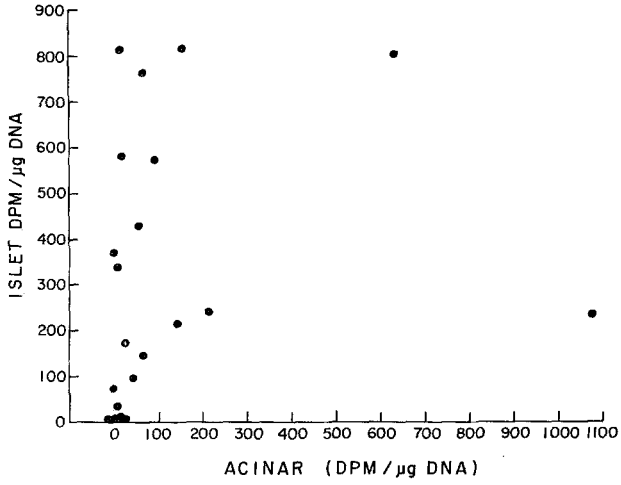

Fig. 4. Thymidine-^{3}H incorporation into islet versus acinar cell DNA of ad lib fed diabetic mutants. Each point represents a single diabetic animal. The coefficient of linear correlation is not significant at the 0.05 level ($P > 0.1$, $r = 0.1967$)

II. Food Restricted-Refed Diabetic and Normal Mice.

A. Blood glucose and serum insulin. Amelioration of hyperglycemia and hyperinsulinemia in diabetic mice placed on food restriction is apparent from Fig. 5. Blood glucose values for food restricted-refed normal mice are presented for comparison. These data should be contrasted with those already presented for age matched ad lib fed diabetics (Fig. 1). After 2 weeks of restriction, blood glucose concentrations in the diabetic group fell to levels approximating those in normal animals and remained in the range of 100 mg glucose/100 ml blood until reinstitution of ad lib feeding. With resumption of ad lib feeding, although glucose concentrations quickly rose to hyperglycemic levels, several weeks were required before values reached those attained in diabetics fed ad lib throughout life. Similarly, food restriction was associated with a marked reduction in serum insulin, with a sharp rebound following return to ad lib feeding. It should be noted that although hyperglycemia and hyperinsulinism were ameliorated by food restriction, the persistence of carbohydrate intolerance was still easily demonstrable following feeding or administration of parenteral glucose [6].

B. Beta cell replication in food restricted-refed normal mice. A group of normal mice were studied in order to ascertain the effects of food restriction and refeeding on beta cell replication. Since beta cell mitotic activity was already so limited prior to food restriction, it proved difficult to ascertain accurately whether restriction

Fig. a

Fig. b

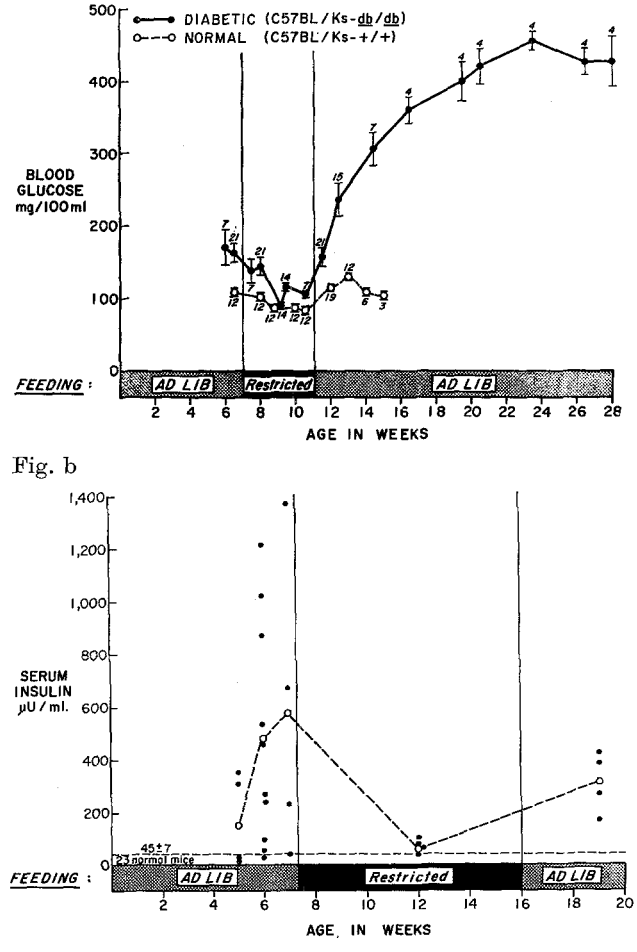

Fig. 5. Effects of food restriction and refeeding on blood glucose and serum IRI

a. *Upper panel* — blood glucose concentrations for both diabetic mutant and normal mice. Values are means ±S.E.M. The small numerals indicate the numbers of animals for each point.

b. *Lower panel* — each point represents the IRI concentration in a single *diabetic* animal. In this case, the period of food restriction extends from the 7th to 16th weeks, rather than the 7th to 11th weeks. A line is drawn connecting the mean values

resulted in a significant reduction in thymidine-^{3}H incorporation. It was apparent, however, that with return to ad lib feeding beta cell proliferative activity failed to rise above levels observed prior to the initiation of food restriction.

C. Beta cell replication in food restricted-refed diabetic mice. Although thymidine-^{3}H incorporation into islet

cell DNA of ad lib fed young mutants was significantly elevated, food restriction resulted in a marked fall, so that virtually no incorporation could be detected after 4 weeks of food restriction (Fig. 6). Similarly, thymidine-^{3}H incorporation by acinar cells was also reduced. It was apparent, however, that the effects of food restriction on DNA synthesis varied amongst different tissues. Continued incorporation of label by intestinal crypt and lung cells during the period of food restriction indicated that the method of food deprivation utilized did not necessarily result in a generalized interruption of cell replication. In contrast to findings in normal mice, resumption of ad lib feeding of diabetics was associated with a sharp rise in islet labeling, which persisted throughout the entire 3-week period of refeeding. Maximal values for thymidine-^{3}H incorporation were observed after 1 week of refeeding (Fig. 6). It is of interest that incorporation of label by acinar cells was also increased during refeeding (Fig. 7).

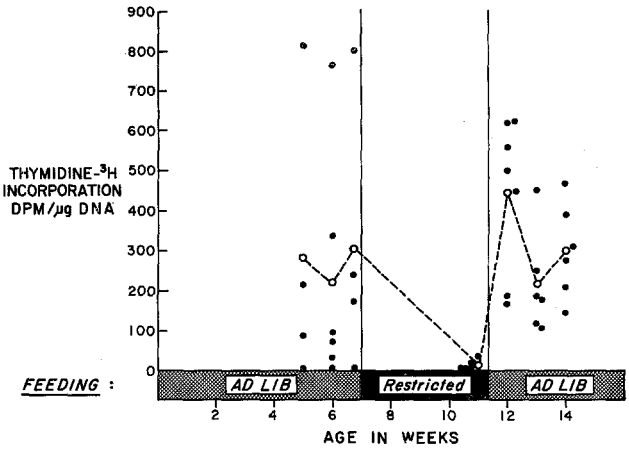

Fig. 6. Effects of food restriction and refeeding on thymidine-^{3}H incorporation into islet cell DNA of diabetic mutant mice. Each point represents a single animal. The dashed line connects the mean values

Light microscopic radioautographs of the pancreas confirmed the belief that quantitative estimates of islet cell DNA synthesis accurately reflected alterations in the frequency of islet cell labeling. A definite increase in the frequency of both islet and acinar cell labeling was readily demonstrable during refeeding. In addition, refeeding was associated with a gradual increase in islet size. The invasion of islets by ductal structures and loss of beta cells observed in mutants fed ad lib thoughout life appeared to be retarded in food restricted-refed animals. This suggested that food restriction and refeeding served to ameliorate the factors responsible for islet destruction. A representative light microscopic radioautograph of the pancreas of a food restricted-refed mutant is shown in Fig. 7. This animal had been refed for 1 week at the time of sacrifice. Blood glucose was 134 mg/100 ml, serum IRI 1140 µU/ml, and islet DNA specific activity 498 DPM/µg islet DNA. A number of labeled islet cells are visible. In addition, some of the acinar cells are labeled.

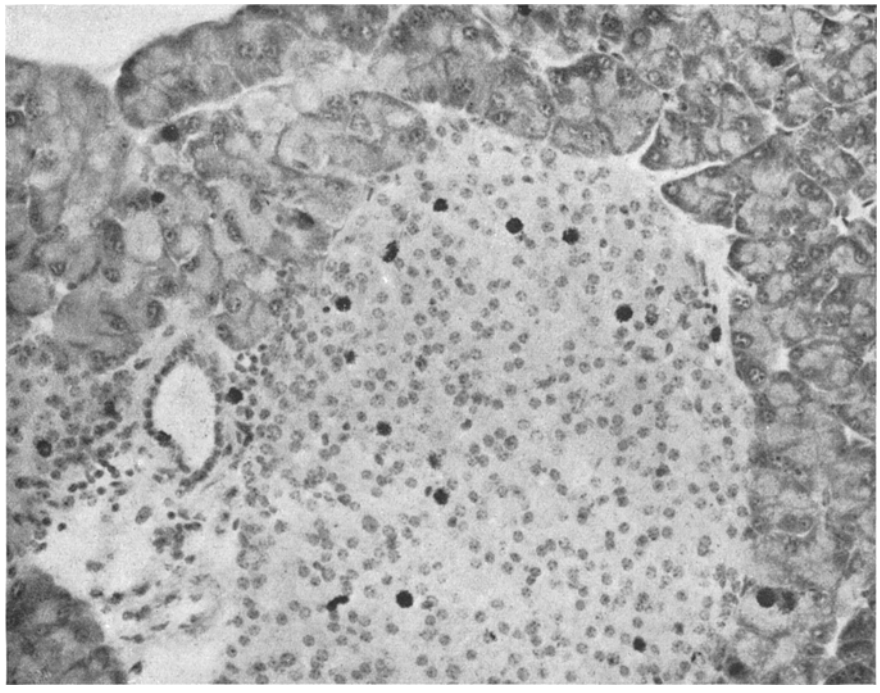

Fig. 7. Light microscopic radioautograph of the pancreas of a food restricted-refed diabetic mouse. This animal had been returned to ad lib feeding for 1 week at the time of sacrifice. Both islet and acinar cells are labeled (PAS stain 256 ×)

III. Electron Microscopic Radioautography.

Electron microscopic radioautography was performed in order to identify the types of labeled cells

within the islets of ad lib fed and food restricted-refed mutants. Results indicated that proliferative activity was primarily confined to the beta cell population, since alpha cell labeling was absent or too infrequent to be documented by this technique and capillary endothelial cell labeling was rare. Fig. 8 is an example of a labeled beta cell from a 6 ½ week old mutant. The blood glucose concentration at sacrifice was 136mg/100 ml. The electron dense silver grains in the photographic emulsion overlying the tritium labeled nuclear DNA are clearly visible. Characteristic beta cell granules are present in the cytoplasm.

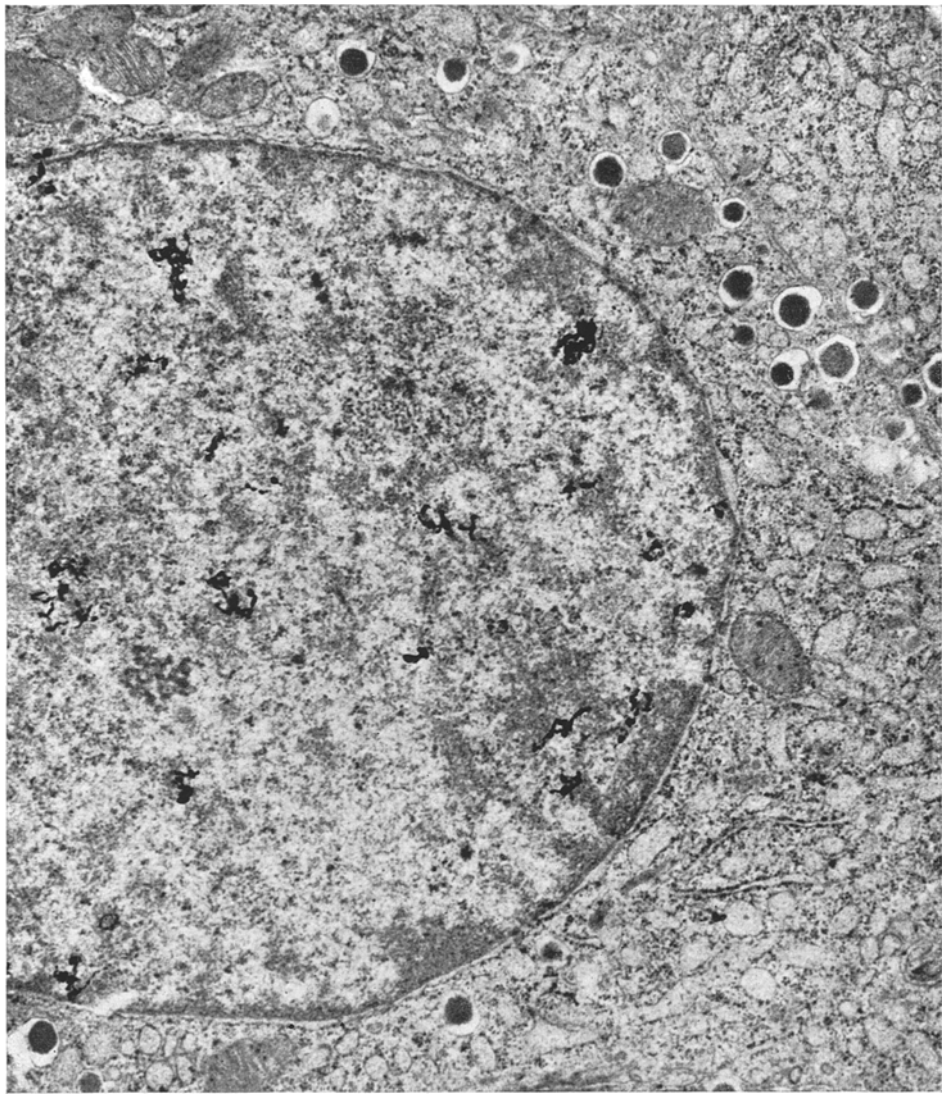

Fig. 8. Electron microscopic radioautograph of a beta cell from a diabetic mouse. Electron dense silver grains are present overlying the tritium labeled nuclear DNA. Characteristic beta cell granules are present in the cytoplasm (Approx. 14.250 ×)

Discussion

Compensatory hyperplasia of the beta cell population is an obvious possible mechanism for increasing insulin secretion in response to a diabetogenic stress. It is of interest, however, that while some authors have reported failure of hyperplasia to occur in cases of hereditary diabetes in man others have reported stimulation of beta cell replication under these same circumstances [16]. It is conceivable that such discrepancies have resulted, at least in part, from the fact that most studies of beta cell replication have relied upon the relatively insensitive methods of either counting mitotic figures or determining total islet volumes by planimetric methods without definitively identifying the cell types involved. The technique of DNA labeling with thymidine-^{3}H avoids these shortcomings. In particular, the method of direct quantitation of tritiated islet DNA extracts offers the advantages both of rapidity and of the ability to sample relatively large numbers

of cells, while avoiding the problems associated with counting large numbers of nuclei in random tissue sections. When used in conjunction with radioautography, the quantitative data can be correlated with information regarding the types of labeled cells, and their relative frequency and distribution.

Our findings of limited but consistently demonstrable beta cell mitotic activity in the normal adult C 57 BL/Ks mouse are in agreement with those of Logothetopoulos and co-workers, who reported 2 to 8 labeled nuclei per 1000 cells in mice of the C 57 BL/6J strain [21]. It is attractive to hypothesize that this low level of mitotic activity is essential for maintenance of an appropriately sized beta cell population under normal conditions of "wear and tear".

It is apparent, however, that mitotic activity is increased several fold in response to a variety of stimuli. The question remains as to the mechanisms by which such stimuli affect beta cell proliferative activity. In the case of the diabetic mutant mouse, it appears that early in the syndrome there is a significant rise in beta cell mitotic activity which begins prior to the onset of sustained hyperglycemia. It therefore seems doubtful that hyperglycemia *per se* is the sole signal for regulating beta cell proliferative activity in adult animals. Although in most instances previously reported [25, 22, 2, 4, 13, 12, 30, 24, 21] expansion of the beta cell population indeed is associated with rises in blood glucose levels, this may be a parallel rather than a causative factor.

It is of interest that sustained, progressively severe hyperglycemia in the diabetic mutant mouse is associated with an eventual decline in beta cell mitotic activity and a decrease in the number of beta cells [17], rather than continued beta cell proliferation. It therefore appears that in the diabetic mutant, although beta cell mitotic activity may increase several fold, the overall capacity for proliferation is limited, so that in the late stages of the syndrome the imbalance between beta cell regeneration and beta cell degeneration now favors the latter process, resulting in an overall reduction in the number of cells.

With regard to the possibility that alterations in the rate of insulin synthesis and/or secretion provide a signal through which various stimuli affect beta cell mitotic activity, there have been reports, although controversial, of increased beta cell proliferation produced by oral hypoglycemic agents known to increase insulin secretion [23]. In the present experiments, the linear correlation between beta cell functional activity, as reflected in serum IRI levels, and proliferative activity, as reflected in islet DNA synthesis, approached significance at the 0.05 level. Increases in proliferative activity therefore appear to occur in association with increased beta cell functional activity.

The marked decrease in beta cell mitotic activity in the food restricted mutant suggests that diet plays an essential role in modulating the size of the beta cell population. It is of interest that curtailment of islet cell proliferation has been described in the food restricted rat [1], and the C 57 BL/6J-*ob* mouse [31]. Although the marked rise in proliferative activity which occurs in mutants returned to ad lib feeding is associated with elevations in both blood glucose and serum insulin concentrations, again, it is unclear as to whether these are parallel or causative factors. Failure to observe a similar rise in beta cell mitotic activity in food restricted normal mice returned to ad lib feeding indicates that the process of food restriction and refeeding *per se* does not invariably evoke such an increase. It is conceivable that the abnormally food intake in mutants returned to ad lib feeding provides the signal responsible for the changes in proliferative activity which occur.

One may speculate that the rise in beta cell proliferative activity which occurs during the return to ad lib feeding is responsible, at least in part, for retarding the progression of the diabetic syndrome in food restricted-refed mutants. Other instances of amelioration of diabetes associated with increased beta cell proliferation have been described in the partially pancreatectomized rat [26] and C 3 Hf × I F_1 mouse [19].

With regard to beta cell degeneration in the diabetic mutant mouse, the factors responsible for this process are not entirely clear [18]. It is not, however, associated with glycogen infiltration, as described in other animals with experimental spontaneous diabetes [20].

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William L. Chick, M. D.
Elliott P. Joslin Research Laboratory
170 Pilgrim Road
Boston, Massachusetts 02215, USA