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Islet and Beta Cell Volumes in Diabetic Chinese Hamsters and their Non-Diabetic Siblings

ANNA-MARY CARPENTER*, G.C. GERRITSEN, W.E. DULIN, and A. LAZAROW*,

Minneapolis, Minnesota, and the Upjohn Company Kalamazoo, Michigan

Summary. Three groups of Chinese hamsters bred at the Upjohn Laboratory were studied: these included diabetic animals in which symptoms were present for 18 months, non-diabetic siblings and non-related, non-diabetic animals of the corresponding age and sex. The spontaneous diabetes is characterized by hyperglycemia, glycosuria, decreased islet volume, decreased beta cell mass, beta cell degranulation and glycogen infiltration in the islets. In the non-diabetic siblings, similar but less marked changes were noted. To our knowledge this is the first reported observation of decreased beta cell mass and beta cell degranulation occurring prior to clinical onset of spontaneous diabetes in man or animal. It suggests that the observed alteration of beta cells is a primary etiologic mechanism in the production of spontaneous diabetes in the Chinese hamster.

Volumes des îlots de Langerhans et des cellules β de hamsters chinois diabétiques et non-diabétiques de mêmes nichées.

Résumé. Nous avons étudié 3 groupes de hamsters chinois de la colonie des laboratoires Upjohn, soit (1) des animaux diabétiques ayant présenté des symptômes pendant 18 mois; (2) des animaux non-diabétiques des mêmes nichées; et (3) des animaux non-diabétiques et non-apparentés mais concordant par l'âge et le sexe. Le diabète spontané de ces animaux est caractérisé par une hyperglycémie, une glucosurie, une diminution du volume des îlots de Langerhans, une diminution du volume total des cellules β, une dégranulation des cellules β et l'infiltration en glycogène des îlots de Langerhans. Des anomalies de même type, mais moins marquées ont été observées chez les frères et soeurs des animaux diabétiques. A notre connaissance, c'est là la première description de la diminution de la masse des cellules β et de leur dégranulation avant l'apparition clinique d'un diabète spontané chez l'homme ou l'animal. Il en ressortirait que cette altération des cellules β pourrait bien être un mécanisme étiologique primaire de la pathogénèse du diabète spontané du hamster chinois.

Messungen des Inselvolumens sowie des Volumens der β-Zellen bei diabetischen und bei nichtdiabetischen Hamstern aus demselben Wurf.

Zusammenfassung. Drei Gruppen von chinesischen Hamstern aus der Upjohn-Zucht wurden untersucht. Dazu gehörten: diabetische Tiere, die seit 18 Monaten Symptome aufwiesen, nichtdiabetische Tiere aus gleichem Wurf und nichtverwandte nichtdiabetische Tiere, die in Alter und Geschlecht den anderen entsprachen. Der Spontandiabetes zeichnet sich aus durch Hyperglykämie, Glykosurie, Abnahme des Inselvolumens, Verminderung der β-Zellmasse, Degranulierung der β-Zellen und Glykogeninfiltration der Inseln. In den nichtdiabetischen Geschwistertieren wurden ähnliche aber weniger markante Veränderungen festgestellt. Unseres Wissens ist dies der erste Bericht einer Verringerung der β-Zellmasse mit Degranulierung noch ehe sich ein Spontandiabetes bei Mensch oder Tier manifestiert. Dies deutet darauf daß diese Veränderungen der β-Zellen einen primären etiologischen Faktor bei der Entstehung des Spontandiabetes beim chinesischen Hamster darstellen könnten.

Key-words: Spontaneous Diabetes, Chinese hamster, Cricetulus griseus, Islet volume, Beta cell volume, Pancreas, Beta cells, Prediabetes, Preclinical diabetes.

Material and Methods

For this preliminary report the islet and beta cell volumes were determined in a strain of Chinese Hamsters that develops spontaneous diabetes. The detailed genetic history of the various sublines, some of which have been inbred for more than ten generations at the Upjohn Company [5], has been recorded carefully. For each diabetic hamster studied there were two controls: a non-diabetic sibling as well as a non-related animal of corresponding age and sex.

The five diabetic animals were selected because they had consistent glycosuria (Tes-Tape value of 4+); this initial qualitative test for glycosuria was accomplished by expressing urine from the urethra directly onto the Tes-Tape. Quantitative urinary glucose excretion was determined using the Auto-Analyzer on five consecutive 24-hour urine samples; these collections were made after the animals were acclimatized in stainless steel metabolism cages for at least a week.

All blood samples were obtained from the orbital sinus. Glucose tolerance tests were performed on animals that were fasted overnight (16 hours). Immediately after drawing the zero-hour blood sample, the hamsters were injected intraperitoneally with a sterile 2.5% glucose solution (in physiological saline) in doses of 250 mg/kg. The animals were bled at 30, 60 and 120 minutes. Glucose concentrations were determined on blood (0.05 ml) and urine samples using the micro-method of HOFFMAN [7] as adapted to the Auto-Analyzer.

Although diabetic animals received no therapy, they were allowed food (Purina Mouse Breeder Chow) and water *ad libitum*. The animals were sacrificed by decapitation at an average age of 22 months; the average duration of diabetes was 18 months. The entire pancreas was dissected, weighed and fixed in Bouin, except for a weighed sector taken from the mid-portion which was divided into two parts: one part was immersed in cold Deane and the other in

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cold buffered osmium fixative. The Bouin-fixed tissues were embedded in paraffin and sectioned serially at 4μ. Every twentieth section was mounted and stained with our modification of GOMORI's [6] aldehyde fuchsin-ponceau (AF-P). The tissues fixed in Deane solution were processed similarly but stained for glycogen with the periodic acid Schiff (PAS) method [8].

The volume of tissue components was determined by the linear scan method using the light microscope component quantitator (LMCQ)[1] developed in our laboratory [12]. Islet volume was obtained by scanning aldehyde fuchsin-stained slides using the low power objective for a total scan distance of 500 millimeters. Volumes of the structural components within the islets were measured by scanning with an oil immersion objective. Fifty consecutive islets from each animal were quantitated using a single random pass; under these conditions the component cell volumes, as determined in 2—5 mm of scan, are accurate to $\pm 5\%$ [2].

The islet components were classified as follows: aldehyde fuchsin-positive cells (AF+), ponceau-positive cells and blood and blood vessels. The aldehyde fuchsin-positive cells (beta cells) were subdivided into several classes: in beta cells classified as 4+, the entire cytoplasm was filled with granules; in those classified as 2+, granule density was about half that of the 4+; in the 1+ classification, only a few granules were seen. Non-granular cells which had a violet-purple cast to their cytoplasm were present in the islets of both diabetic hamsters and their siblings.

Glycogen infiltration of the islet was measured using the PAS-stained tissues. Approximately sixty islets were examined in each animal; the number of islets containing PAS+ (beta) cells was tallied and expressed as a percentage of total islets. The islets, further classified in accordance with the number of PAS+ cells they contained, were arbitrarily grouped as follows: (a) more than 10 PAS-positive cells present in the islet examined (b) six to nine (c) two to five and (d) only one PAS-positive cell present.

Results

Fig. 1 illustrates the average blood sugar level and urinary glucose excretion in the three groups of animals studied. It should be noted that the siblings of diabetic hamsters, like the non-related controls, have normal blood sugar levels and they excreted only trace amounts of glucose.

Fig. 2 shows the average islet volume (expressed as percent of total pancreatic volume), whereas the average volume of the morphologic components within the islet (expressed as percent of islet volume) is shown in Fig. 3. In contrast to the normal, in which 74% of the islet volume consists of 4+ AF granulated beta cells (cf. Plate 1a, b), none of the beta cells in the diabetic hamsters have 4+ beta granulation (cf. Plate 1g, h). Less than 50% of the islet volume in the diabetic is composed of granulated beta cells and most of these contain only a few granules. The identity of the non-granulated cell with a violet cytoplasm (comprising approximately 30% of the islet volume) is uncertain.

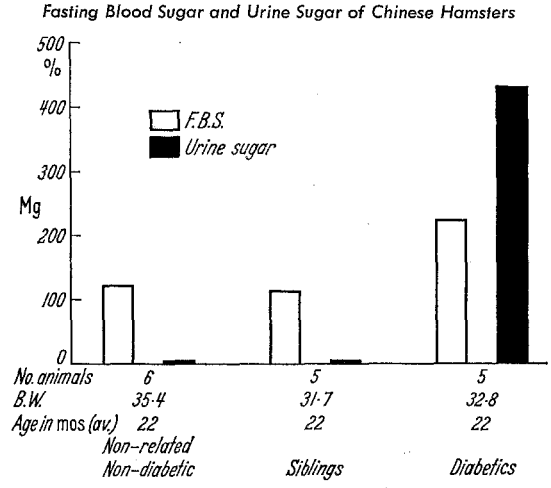

Fig. 1. Average fasting blood sugar and urine sugar levels in the three groups of Chinese Hamsters studied

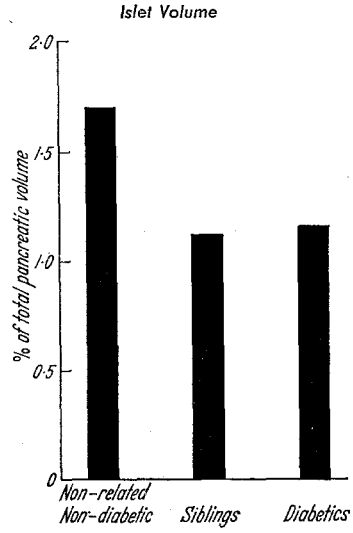

Fig. 2. Average islet volumes in the Chinese Hamster expressed as percent of total pancreatic volume

A most unexpected finding in this study was that non-diabetic siblings exhibited a reduced beta cell granulation. Despite the absence of hyperglycemia or glycosuria, less than 20% of the islet volume is composed of granulated beta cells identified as 4+; an additional 40% of the islet volume contains cells with 1+ and 2+ granulation (cf. Plate 1d, e).

Beta cell mass (expressed as percent of total pancreatic volume) was calculated by multiplying the average islet volume (as percent of pancreas) by the

[1] This was described [12] as the micrometer component quantitator.

average beta cell volume (as percent of islet). As shown in Fig. 4, the diabetic hamsters have a decreased beta cell mass. On theoretical grounds one might predict that the capacity of the pancreas to secrete insulin under conditions of maximal stimulation would be limited by the total number of beta cells present. It appears that the diabetic hamster, with 0.07% of the pancreatic volume consisting of 2 + AF granulated

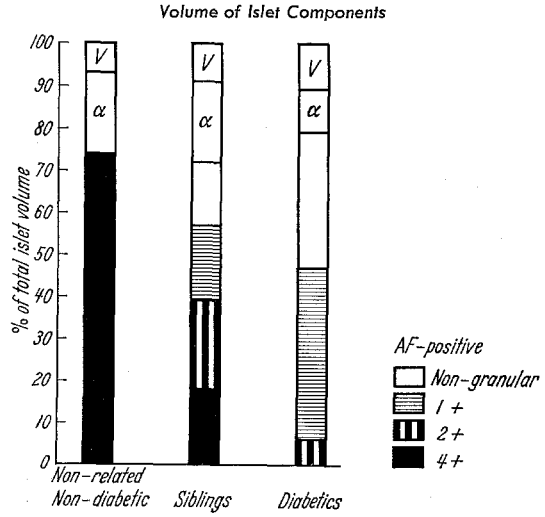

Fig. 3. Average volume of islet components determined by the linear scan method using aldehyde fuchsin-stained islets

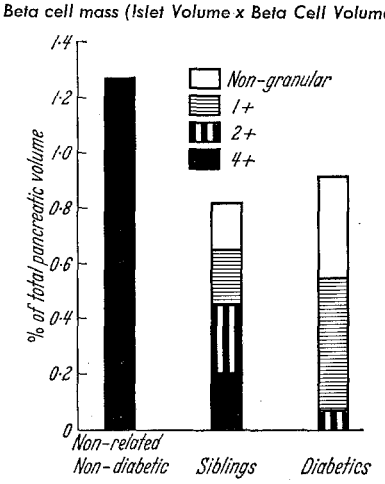

Fig. 4. Comparison of the beta cell mass in the three groups of Chinese Hamsters

beta cells and an additional 0.45% of sparsely granulated 1 + AF beta cells, does not have sufficient insulin secretory capacity to prevent symptoms. Although the non-diabetic siblings have a reduced mass of granulated beta cells — the 4 + AF, 2 + AF and 1 + AF granulated beta cells comprise 0.20, 0.24 and 0.20%, respectively, of the total pancreatic volume — this reduction is not sufficiently great to produce clinical signs of diabetes.

The number of islets containing beta cells with glycogen infiltration is shown in Fig. 5. PAS-positive cells were not found in non-diabetic, non-related controls (Plate 1, c). By contrast, 60% of the islets in the diabetic hamsters contained one or more PAS + cells, and 40% contained two to ten PAS + cells (Plate 1, i). In the non-diabetic siblings 10% of the islets showed two or more PAS + cells (Plate 1, f).

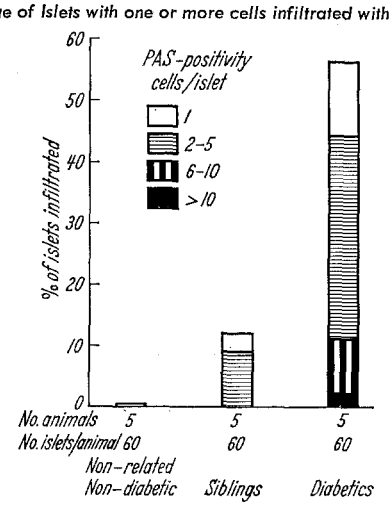

Fig. 5. Glycogen infiltration in the beta cells of the Chinese Hamster expressed as percent of islets containing one or more PAS-positive cells

Discussion

Reduced numbers of beta cells, degranulated beta cells [cf. 10] and glycogen infiltration [15] in the islet have been observed in experimental diabetic animals under various conditions. Similarly, decreased beta cell volume has been reported [16] in subdiabetic[2] rats, which have abnormal glucose tolerance but no other clinical signs of diabetes. The findings of decreased beta cell granulation and glycogen infiltration in the islet of the spontaneously diabetic hamster were expected in view of previous studies. Although it may be postulated that the observed changes in beta cells may be the primary cause of the diabetic state, it might also be argued that the beta cell changes are secondary to the hyperglycemia, which is in turn produced by extrapancreatic factors. Thus the observed degranulation and disappearance of the beta cells could be a secondary response, as is the case in metahypophyseal diabetes. Growth hormone administration produces a primary hyperglycemia [1]; the beta cell degranulation [14] and destruction are secondary to the hyperglycemia since they can be prevented by controlling the blood-sugar level with phlorizin administration [13].

Our unexpected finding that similar though less marked changes in beta cells are present in the non-diabetic siblings excludes the possibility that the beta cell changes are secondary to hyperglycemia; the siblings of the diabetic hamsters do not show

[2] Although these animals, which were given subthreshold doses of alloxan, did not have hyperglycemia or glycosuria, they did show abnormal tolerance to glucose.

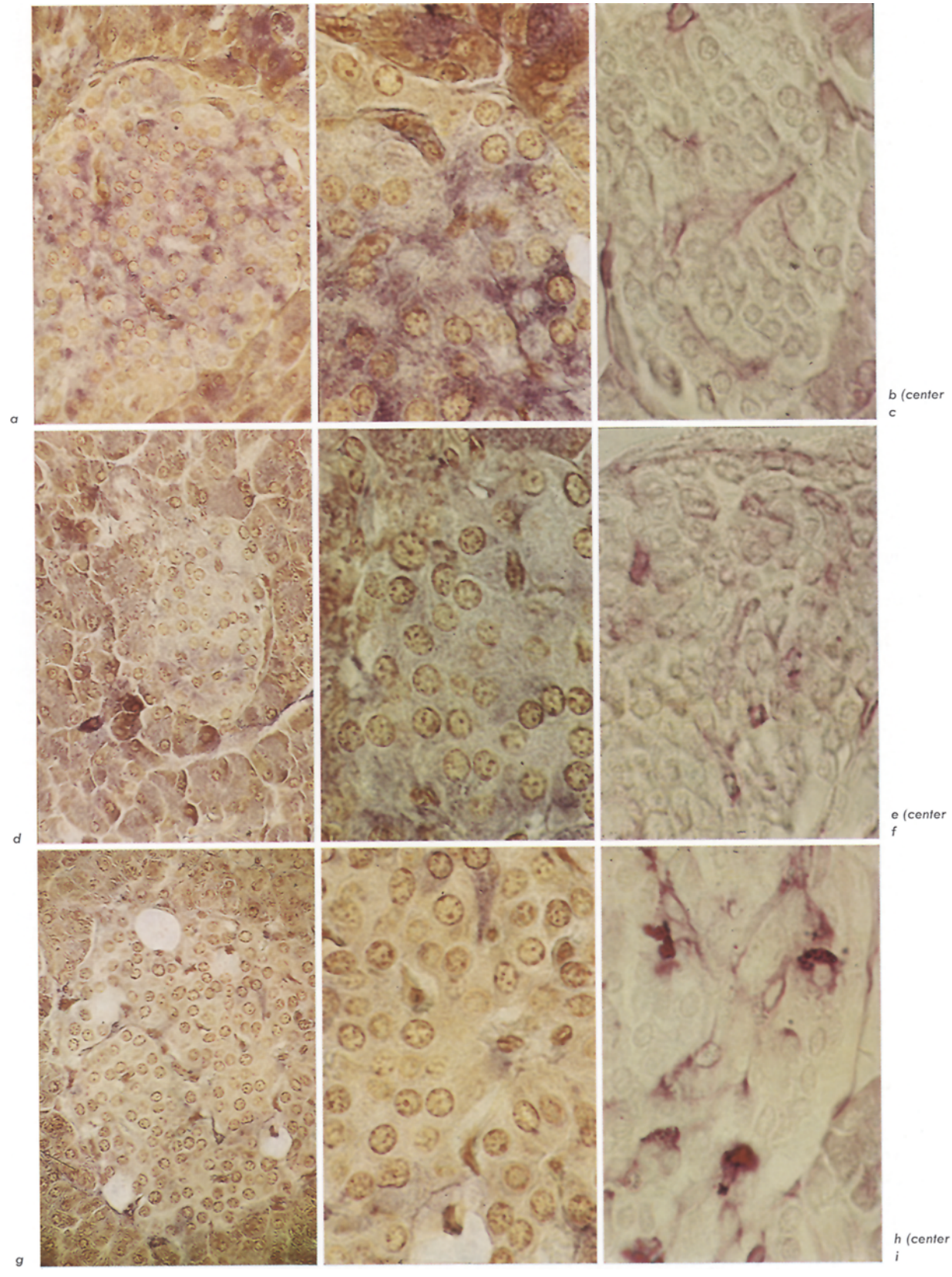

Plate 1. Top row: (a, b, c) Islets of control (non-related, non-diabetic hamster) stained with aldehyde-fuchsin showing beta cells with 4+ granulation (320× and 800×). The corresponding PAS-stained section (800×) was devoid of glycogen

Center row: (d, e, f) magnification same as above. Islets of non-diabetic sibling showing decreased numbers of AF+ cells and moderate glycogen infiltration

Lower row: (g, h, i) magnification same as above. Islets of diabetic hamster showing marked beta cell degranulation and increased amounts of glycogen in the beta cell

hyperglycemia, glycosuria or abnormal glucose tolerance. These morphologic observations suggest that genetic factors are operative at the beta cell level, and that the etiology of spontaneous diabetes in the Chinese Hamster may result from a primary alteration in the beta cell. The fact that the changes in beta cells occur initially reinforces the suggestion made earlier [10, 9] that endogenous agents cytotoxic for beta cell may produce diabetes by acting directly on the islet tissue. Alternatively, one must consider the possibility that the observed decrease in beta cells results from an imbalance between the rate of new beta cell formation and the rate of senescence of existing beta cell. If the rate of beta cell formation were decreased or the rate of beta cell destruction were accelerated, a progressive decrease in the beta cell mass would result.

We believe our finding of beta cell changes in the siblings of spontaneous diabetic Chinese Hamsters represents the first instance in which beta cell degranulation and/or destruction precedes the development of the clinical diabetic state. It should be noted that Gepts reported [4] decreased numbers of beta cells in human juvenile-onset diabetics examined one to four weeks after the acute onset of the disease. However, in this instance one cannot be certain whether the beta cell changes preceded the onset of clinical diabetes or whether they developed as a consequence of the hyperglycemia.

Our finding of glycogen infiltration in the islet cells in the non-diabetic siblings is of interest. The presence of glycogen infiltration in the beta cells is usually associated with hyperglycemia. For example, we have demonstrated [3] that the transitory production of hyperglycemia in both normal and subdiabetic rats produces an immediate glycogen infiltration, whereas lowering the blood sugar level in diabetic animals removes glycogen from the beta cells. Although it may be postulated that the glycogen level in the beta cell may influence the insulin release mechanism, no evidence has been presented thus far to support this view. Hyperglycemia brings about insulin release presumably through the action of a metabolite that appears in increased concentration as a consequence of increased glucose metabolism [11]. One might postulate that in the presence of an increased glycogen level within the beta cell, an agent that causes excessive glycogen breakdown could bring about an increased intracellular glucose concentration and a secondary release of insulin.

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Dr. A. LAZAROW
Department of Anatomy
University of Minnesota
Mineapolis, Minnesota, U.S.A.