Effect of Age on Glucose-Stimulated Insulin Release by the β-Cell of the Rat

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ABSTRACT To assess the effect of age on β-cell insulin release, collagenase-isolated islets of Langerhans were obtained from rats aged 2–18 mo and incubated with increasing concentrations of glucose. Similar islets were analyzed for insulin content or subjected to morphometric measurements to identify both the number of β-cells and the volume of β-granules per islet. In parallel studies, the islet content of intact pancreata was also determined. The results showed that β-cell number increased from 2,300 to 5,000 cells as rats aged from 2 to 18 mo and islet insulin content doubled. However, glucose-stimulated insulin release decreased progressively with age, and this was especially striking when considered in terms of the increase in number of β-cells/islet; e.g., mean (±SEM) insulin secretion (nanounits per minute per β-cell) of islets incubated with 450 mg/dl of glucose was 1.3 (±0.2), 1.0 (±0.1), 0.4 (±0.05), and 0.3 (±0.01), respectively for 2-, 6-, 12-, and 18-mo-old rats. Thus, insulin secretion per β-cell was decreased, despite increased stores of insulin per cell. These findings demonstrate that the aging process leads to a profound defect in glucose-stimulated insulin release from the β-cell. Whether this is a global secretory defect, or solely a failure of the β-cell to respond to glucose, remains to be defined.

INTRODUCTION

Controversy still exists as to the effect of age on β-cell function. The majority of studies of both man and rat in which plasma insulin levels have been measured suggest that the insulin response to a glucose challenge does not deteriorate with age (1–13). However, this does not necessarily mean that B-cell function is normal. The plasma insulin concentration at any given time after glucose stimulation is a function of a number of complex homeostatic interactions, only one of which is the intrinsic ability of the β-cell to respond to glucose. Thus, factors such as the activity of intestinal insulin secretagogues, prevailing plasma glucose concentration, insulin turnover rates, total β-cell mass, etc., can significantly affect plasma insulin concentrations, and make it extremely difficult to define the effect of age on the insulin secretory capacity of the β-cell. The complex nature of this problem has been clearly stated by Andres and Tobin (14). These authors approached the dilemma by using a "glucose clamp" technique in which the insulin secretory response is measured after plasma glucose concentration is "clamped" at a fixed plateau by varying glucose infusion rates. They concluded that β-cell sensitivity to glucose diminishes with age, but that the response to a maximal glucose challenge is unaltered. Although this approach is preferable to simple measuring plasma insulin concentrations after a glucose challenge, it is not free of problems. Its validity is based upon at least three assumptions: (*a*) plasma insulin response is a direct function of co-existing plasma glucose concentration; (*b*) total β-cell mass does not change with age; (*c*) insulin turnover rate is essentially the same in all individuals. Unfortunately, none of these issues has been well studied, and there are experimental observations which cast doubt on the validity of the first two premises (15, 16). In light of these considerations it is obvious that the question as to the effect of age on β-cell function is not a simple one, and that the data currently available will not suffice for an answer.

In an effort to deal with this issue we have approached it in a different fashion. On the one hand we have studied glucose-stimulated insulin release by isolated islets, permitting us to quantify insulin secretion in a system which is free of many of the confounding variables described above. In parallel studies we have used stereological techniques to determine islet and β-cell size, and the ratio of β-cells

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per total islet tissue. From these latter measurements we can determine the number of β-cells per islet, and by the use of this combined functional and structural approach, we can relate insulin secretion to a single (average) β-cell. In this present report we have used these techniques to define the effect of age on glucose-stimulated insulin release by the individual β-cell.

METHODS

Animals

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.) with documented birthdates were used for all experiments. Young rats were allowed to mature from 2 to 6 mo of age before being studied, whereas older rats were used at 12 mo or allowed to age to 18 mo. Major emphasis has been placed on comparisons between 2- and 12-mo old rats, but various measurements were made also on rats 6 or 18 mo of age. Rats were fed standard rat chow (Wayne Lab Blox, Allied Mills, Inc., Chicago, Ill.) ad lib. and maintained on a 12-h light-dark (6 a.m./6 p.m.) cycle. Food was removed at 8:30 a.m., and experiments begun at 12:30 p.m.

Pancreas

Dry Weight

In order to visualize and remove the entire pancreas, the organ was first inflated via the bile duct with distilled water. Subsequently the distended organ was removed from each animal, lyophilized for several days and weighed.

Morphometric Measurements

In these studies, pancreas inflation was carried out with Bouins' fixative. The entire pancreas was then removed, cut into two or three pieces (without regard to region), submerged in Bouins' fixative for an additional 24 h, processed, and then embedded as a single block in paraffin. The total pancreas of each animal was serially sectioned at 5 μm, and every 100th tissue section (or 500 μm interval) was stained with hematoxylin and eosin. Four sections (separated from each other by at least 500 μm) were chosen for analysis from each embedded block by a random number system. Using an eyepiece ocular grid it was possible to obtain values (by point-counting stereological methods [17] for the percent of islet, acinar, and connective tissue per unit volume pancreas. With these same procedures it was possible also to obtain values for the area of individual islets (from 200 to 300 islets were viewed from each pancreas). These figures, when corrected by the Giger-Riedwyl equation (17), were used for calculations of average islet diameter.

Isolated Islets

Preparation

Pancreatic islets were isolated by a modification of the method of Lacy and Kostianovsky (18). For each preparation, three 2-mo-old, or two older rats were anesthetized with 5 mg/100 g Surital, i.p. (Parke, Davis & Company, Detroit, Mich.) and their pancreata inflated via the bile duct with Hanks' salt solution. The distended pancreata were minced and incubated with collagenase (CLS-IV, Worthington Biochemical Corp., Freehold, N. J.; 6 mg/ml packed volume of pancreas) with vigorous shaking.[1] After several washes, the sediment was examined under a dissecting microscope and individual islets were transferred to vials in a randomized fashion[2] for various chemical, functional, or morphological studies. For some experiments, islets were separated from the digested acinar tissue by Ficoll density gradient centrifugation at 22°C (19). Islets isolated by these methods continue to secrete insulin (in response to 300 mg/dl glucose) without a decline in rate over three consecutive 30-min intervals.

Dry Weight

Triplicate vials, each containing 80 randomly selected islets, were obtained after Ficoll separation. The islets were collected on preweighed filters (0.45 μm pore, Millipore Corp., Bedford, Mass.), washed, and lyophilized. Subsequently the filters were weighed (M5/SA microbalance, Mettler Instrument Corp., Hightstown, N. J.) and the weight of individual islets calculated.

Morphometric Measurements

Determination of β-cell number. The number of β-cells per "average" islet was calculated from the following formula:

$$\beta\text{-cell number per islet} = \frac{(\text{islet volume}) \times \left(\frac{\beta\text{-cells}}{\text{total islet cells}}\right)}{(\beta\text{-cell volume})}. \quad (1)$$

Islet volume. 25 islets from each preparation used for determination of insulin secretion were viewed in a dissecting microscope under ×30 magnification. The X and Y diameter of each islet was measured with the use of an eyepiece ocular, and a mean diameter calculated. Measurements were made using blindfold techniques: i.e., the individual measuring the islets did not know the age of the animals from which the islets were obtained. From these data, islet cell volume was obtained from the formula $(4\pi r^3)/3$ (r = mean islet radius), based on the assumption that islets are essentially spherical in shape (20).

Ratio of β-cells to total islet cells. These estimates were obtained from low magnification electron micrographs. Islets were fixed overnight in 2% glutaraldehyde (prepared in 0.1 M

[1] The exact incubation time varied somewhat with the batch of collagenase used; in general, tissue from 2-mo-old rats was digested at 37°C for slightly less time (6 min) than tissue from older rats (7 min).

[2] To avoid bias in selection of islets, the following procedure was rigidly adhered to: all islets, regardless of size, which were freed from the acinar tissue mass were transferred to a second Petri dish. This dish of "clean" islets was swirled and all islets which settled within the field of the objective lens were collected in a plastic-tipped pipette and transferred 5–10 islets at a time to 10 vials. This procedure was repeated until each vial contained 25 islets. Thus, for each experiment, 250 islets were picked and these islets were dispensed evenly into 10 vials. The vials themselves were rotated daily so that the position of a vial in the line-up could not influence the experimental results.

cacodylate buffer, pH 7.2, 22°C, 400 mosmol), subsequently dehydrated, and embedded in Epon-araldite plastic. Thin sections (400–600 Å) from one block per islet preparation were placed on copper bar grids (Ted Pella, Inc., Tustin, Calif.), and all islet tissue found within a randomly selected area[3] was photographed at ×2,700 and enlarged to a final magnification of ×8,000. Using these photographs, β-cells were identified, and their volume density (as compared to the volume density of total islet tissue) determined by standard point-counting stereologic techniques (17). These data provide an estimate of the ratio of β-cells to total islet cells.

β-cell volume. β-cell volume was calculated from measurements of β-cell diameter and use of the following formula (21):

$$\beta\text{-cell diameter} = \sqrt{\frac{(\text{nuclear diameter})^2 \times (\text{nuclear + cytoplasmic volume density})}{(\text{nuclear volume density})}}. \quad (2)$$

Volume density of β-cell nucleus and cytoplasm was determined by standard point-counting stereological methods (17). Nuclear diameter was estimated by placing a transparent grid containing concentric circles of various known diameters over photographs of β-cells, and each nuclear profile was measured with a best-fit circle. Since these measurements of diameter were obtained from random slices through cells, each value was subsequently corrected to obtain a true diameter by use of the Giger and Riedwyl transform equation (17). β-Cell volume was then calculated from β-cell diameter as described by the formula, $(4\pi r^3)/3$.

DETERMINATION OF β-GRANULE VOLUME PER ISLET. The content of mature β-granules per islet was estimated from the following formula:

$$\beta\text{-granule volume/islet} = (\beta\text{-cell volume}) \times (\text{percent } \beta\text{-granules per } \beta\text{-cell}) \times (\text{number } \beta\text{-cells/islet}). \quad (3)$$

β-cell volume. This estimate was obtained from Eq. 2.

Percent β-granules per β-cell. β-cells are recognized by their characteristic "mature" granules (membrane-bound structures containing both an electron-dense center and a prominent halo (Fig. 1A and B [22]). In these studies, the percent of the cell occupied by mature β-granules was estimated by standard point-counting stereological (volume density) techniques applied to electron micrographs (×16,000) of randomly selected β-cells. A profile of a representative β-cell appears in Fig. 1A. Distinguishing features between "mature" and "immature" β-granules can be seen in Fig. 1B.

Number of β-cells per islet. This estimate was obtained from Eq. 1.

INSULIN MEASUREMENTS

DETERMINATION OF INSULIN CONTENT OF ISOLATED ISLETS. Total islet insulin was extracted from duplicate groups of 100 islets by the procedures of Davoren (23). A trace amount of ^{125}I-insulin was added to each extract. The extract was adjusted to pH 8.3, the precipitate removed by centrifugation and the supernate brought to pH 5.3. Addition of absolute alcohol and anhydrous ether resulted in a precipitate, which was assessed both for immunoreactive insulin (24) and for preparative yield. The preparative yield was estimated by determining the percentage of trichloroacetic acid-precipitable ^{125}I-insulin that was carried through the procedures.

DETERMINATION OF GLUCOSE-STIMULATED INSULIN RELEASE BY ISOLATED ISLETS. Duplicate vials, containing 25 islets each, were incubated at 37°C in Krebs-Ringer bicarbonate buffer containing dialyzed albumin (3 mg/ml) and 50 mg/dl glucose in an atmosphere of 95% O_2-5% CO_2. After 30 min, the islets were washed twice with warm (37°C) Krebs-Ringer bicarbonate containing albumin and various concentrations of glucose. The vials were then incubated for an additional 60 min under comparable conditions. After this, the medium was separated from the islets by filtration, and kept frozen at −25°C until assayed for immunoreactive insulin (24). Comparisons were made between islets isolated by digestion and washing with those separated by means of Ficoll density gradient centrifugation. Since no differences in insulin secretion were noted, the former method was used for the routine preparation of islets for measurement of insulin secretion.

RESULTS

Effect of age on body weight, pancreatic weight, and pancreatic composition. Table I indicates that rat body weight increases rapidly from 2 to 6 mo of age, but appears to plateau at this point. No substantial changes in weight seem to occur from 6 to 18 mo of age. Pancreatic dry weight also increases with age, and the results in Table I indicate that the ratio of pancreatic to body weight is essentially identical in 2- and 12-mo-old rats. On the other hand, the composition of the pancreas does change with age. The data in Table I indicate that the proportion of the pancreas occupied by islet tissue increases by 30% as rats age from 2 to 12 mo of age ($P < 0.05$). This age-related increase in islet tissue mass per volume pancreas is associated with a relative increase in connective tissue volume and a decrease in acinar tissue volume. Measurements of sections of individual islets from these tissues indicate that the corrected (17) average diameter of islets from 2- and 12-mo-old rats is 237 and 268 μm, respectively. When these direct measurements of islet diameter are used to calculate islet volume $[4\pi r^3]/3$, it can be seen that the islets of 12-mo-old rats are 47% larger than the islets of 2-mo-old rats. Therefore, the 30% increase in islet mass estimated per volume pancreas is more than accounted for by the increase in islet size. Since the weight of the average pancreas of the 12-mo-old rat is also twice that of the 2-mo-old

[3] Although the number of islet cells viewed by this random selection procedure varied, in general ≅120 cells (between 90 and 140) were examined per block.

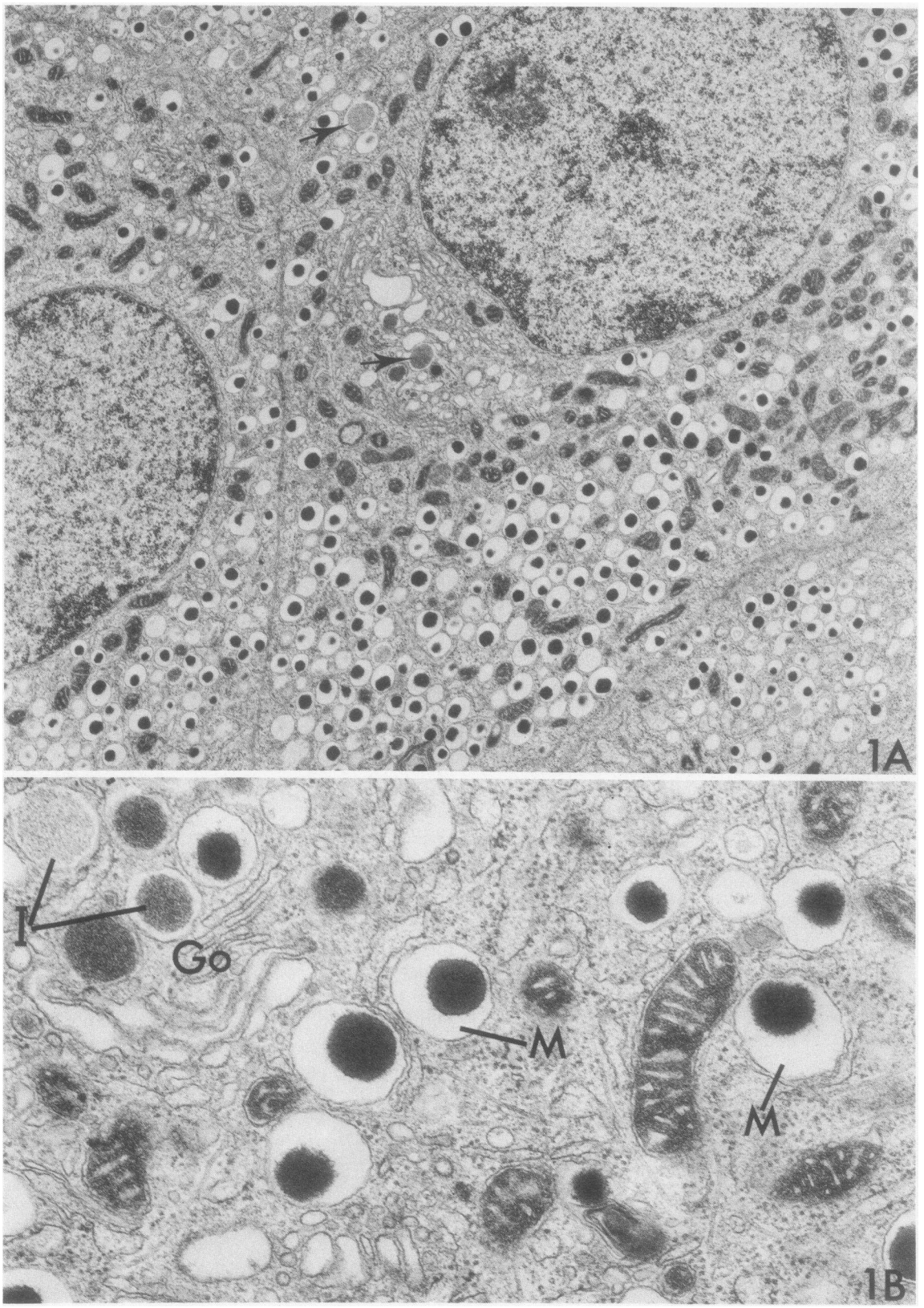
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TABLE I

Effect of Age on Mean (±SEM) Body Weight, Pancreatic Weight, and Pancreatic Composition

Age	Body weight	Pancreatic dry weight	Pancreatic composition		
			Islet tissue	Connective tissue	Acinar tissue
mo	*g*	*g*	*% pancreatic volume*		
2	220±11 (12)	0.24±0.02 (12)	1.0±0.1 (6)	6.0±0.8 (6)	93.0±1.0 (6)
6	528±12 (8)*	—	—	—	—
12	560±17 (12)*	0.52±0.03 (12)*	1.3±0.1 (6)‡	7.7±0.7 (6)‡	91.0±1.0 (6)‡
18	553±15 (12)*	—	—	—	—

The numbers in parentheses indicate the number of animals.

* $P < 0.01$ as compared to values for 2-mo-old rats.

‡ $P < 0.05$ as compared to values for 2-mo-old rats.

rat, it appears that the total islet tissue mass present in the 12-mo-old rat will far exceed that found in the younger rat. Thus, there is an increase in the proportion of islet tissue per volume pancreas, plus an absolute doubling of pancreatic size.

Effect of age on β-cell number. Table II indicates that when islets of rats of different ages are separated by collagenase and measured directly, the average islet volume increases progressively with age. For example, the mean islet volume of 12-mo-old rats is 47% greater in volume than islets obtained from 2-mo-old rats. This observation is further defined in the histogram shown in Fig. 2, which indicates that the range of islet diameter is comparable in 2- and 12-mo-old rats, but that the pancreas of a 12-mo-old rat contains relatively more of the larger islets. These morphometric observations regarding islet size are supported by differences in the dry weight of islets from young and old rats: thus, the mean (±SEM) islet dry weight of 2-mo-old rats ($n = 10$) is 3.36±0.10 μg, as compared to 4.08±0.25 μg for an equal number of 12-mo-old rats ($P < 0.01$).

In contrast to islet volume, the data in Table II indicate that the volume of individual β-cells does not increase as rats grow from 2 to 12 mo of age. Furthermore, the results in Table II demonstrate that the percentage of β-cells per islet does not increase with age. If islet volume increases with age, and there is no change in either the size of the individual β-cell, or the percentage of β-cells per islet, there must be an increase in number of β-cells per islet as a function of age.[4] Utilizing these data, and Eq. 1, it was possible to estimate the average number of β-cells present in islets obtained from 2- and 12-mo-old rats. These calculations appear in Table II, and indicate that islets from 2-mo-old rats contain ≅2,330 β-cells. In contrast, islets from 12-mo-old rats contain 3,410 β-cells, representing a 46% increase. Determinations of β-cell volume, and percentage of β-cells per islet, were not made in 6- and 18-mo-old rats. However, as seen in Table II, the values for these two variables are comparable in 2- and 12-mo-old rats. If one assumes that this is also true of 6- and 18-mo-old rats, it is possible to generate estimates of β-cell number for these animals also by using the mean of the values for these variables found in the 2- and 12-mo-old rats. These estimates appear in Table II, and indicate that increasing age leads to a progressive increase in the number of β-cells per islet.[5]

Effect of age on islet insulin content. Although the

[4] Likewise, there must be an increase in other islet cells as a function of age. However, in these studies no further distinction was made between glucagon, somatostatin, pancreatic polypeptide, and connective tissue cells, and it is not known whether the percentage of any of these cell types is altered as animals age.

[5] Although we have considered the islets to be spheres, some are clearly spheroids with a short and long axis. If islets from rats were considered "prolate spheroids" with a volume of $(4\pi ab^2)/3$ (where a = half the long axis and b = half the short axis) their volumes would be 18±2% (2-mo-old rats) and 16±2% (12-mo-old rats) less than their respective sphere volumes; if islets were "oblate spheroids" with a volume of $(4\pi a^2 b)/3$, their volume would be 15±2% (2-mo-old rats) and 13±2% (12-mo-old rats) more than their respective sphere volumes. Although the absolute number of β-cells per islet would be altered by these changes in islet volume, relative differences in β-cell number between islets of young and old rats would remain the same.

FIGURE 1 Portion of β-cell of isolated islet from 2-mo-old rat. (A) Low magnification (×12,000) view showing numerous mature β-granules (spherical structures with electron dense centers and characteristic halos). Immature granules are marked by arrows. (B) Higher magnification (×48,000) view showing details of β-cell structure: Golgi (Go), mature granules (M), immature granules (I).

TABLE II
Effect of Age on β-Cell Number (Mean±SEM)

Age	Islet volume	β-cell volume	β-cells/total islet cells	β-cell number/islet‡
mo	$\times 10^6 \mu m^3$	$\times 10^3 \mu m^3$	%	
2	8.7±0.8 (10)	3.2±0.16 (14)	86±2 (14)	2,330
6	10.3±1.0 (10)	—	—	2,760
12	12.8±1.2 (10)*	3.3±0.16 (12)	88±2 (12)	3,410
18	18.9±3.2 (10)*	—	—	5,060

The numbers in parentheses indicate the number of islet preparations on which analyses were conducted.
* $P < 0.01$ as compared to values for 2-mo-old rats.
‡ β-cell number/islet = (islet volume) × (β-cells)/(total islet cells)/(β-cell volume).

β-cell volume of young and old rats may be similar, the results of the ultrastructural measurement seen in Table III indicate that the β-cells of islets from old rats contain 40% more mature β-granules than do β-cells of young rats; i.e., β-granules represent 10.3 and 7.3% of β-cell volume in 12- and 2-mo-old rats, respectively. Utilizing these figures in Eq. 3, it appears that islets from old rats contain approximately twice the volume of mature β-granules of islets of young rats. Because β-granules presumably contain insulin, then islets from 12-mo-old rats should contain approximately twice the immunoreactive insulin of islets of young rats. This was confirmed by direct measurement, i.e., the mean (±SEM) insulin content of isolated islets was 2,100±300 and 1,150±150 μU/islet, respectively for nine preparations each of 12- and 2-mo-old rats ($P < 0.01$). Thus, islets from old rats not only contain more β-cells than islets from young rats (Table II), but they contain more presumptive insulin-containing granules and more immunoreactive insulin as well.

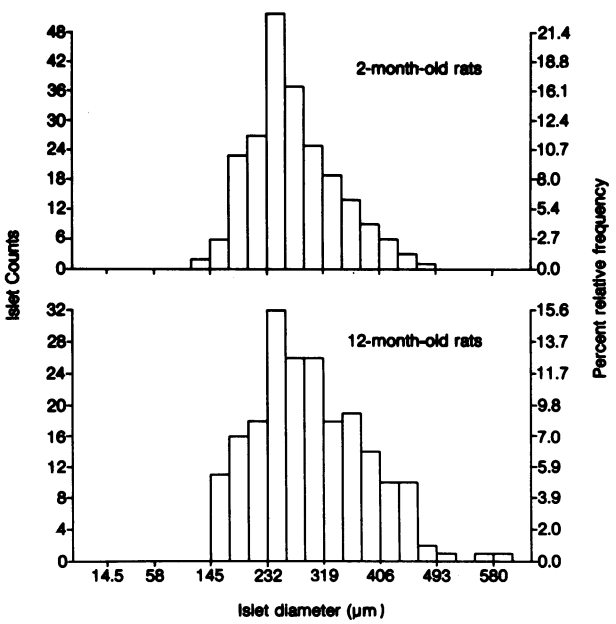

FIGURE 2 Histogram showing size distribution of islets isolated from 2- and 12-mo-old rats. Islet diameter is a mean of X and Y diameters of intact islets measured with a dissection microscope.

Effect of age on glucose-stimulated insulin release. Given the results to this point, one might anticipate that insulin secretion per islet would increase progressively with age. However, this is not the case. In Fig. 3, insulin secretion rates for islets incubated at different glucose concentrations are shown for 2-, 6,- 12-, and 18-mo-old rats. With a glucose concentration of 50 mg/dl, essentially no insulin secretion is detected: at increasing glucose concentrations, islets from 2-mo-old rats show a linear insulin response reaching a maximum rate at a glucose concentration of 300 mg/dl. Insulin secretion from islets of 6-mo-old rats is somewhat lower ($P < 0.01$) than 2-mo-old rats at 300 mg/dl glucose. However, this difference is diminished at 450 mg/dl glucose, and the insulin response at this concentration is not statistically different from that of the 2-mo-old rats. In contrast, islets from older (12 and 18 mo old) rats show a dramatically reduced insulin secretory response at every glucose concentration studied.

From the data in Fig. 3 it appears that insulin release from islets of old animals may not reach maximal levels at a glucose concentration of 450 mg/dl. To study this more thoroughly, we carried out additional experiments on islets from 2- and 12-mo-old rats at glucose concentrations ranging from 50 to 900 mg/dl. These data appear in Fig. 4, and indicate that the maximal rate of glucose-stimulated insulin release for islets of 12-mo-old rats does, in fact, occur between 300 and 400 mg/dl, and is ≅50% lower than that of islets from 2-mo-old rats. The glucose concentration at which half maximal secretion occurs is 200 and 210 mg/dl, respectively, for islets of 2- and 12-mo-old rats.

The age-related decrease in insulin secretory rates noted for islets is even more dramatic when expressed per individual β-cell. Thus, when glucose-stimulated insulin secretion (data from Fig. 3) is calculated per

TABLE III
Effect of Age on the Volume of β-Granules per Islet

Age	β-cell volume‡	Number β-cells/islet‡	β-Granules/β-cell	β-Granule volume/islet§
mo	$\times 10^3 \mu m^3$		%	$\times 10^6 \mu m^3$
2	3.2	2,330	7.3±1.0 (8)	0.54
12	3.3	3,410	10.3±1.1 (8)*	1.16

The numbers in parentheses indicate the number of islet preparations on which analyses were conducted.
* $P < 0.01$ as compared to 2-mo-old rats.
‡ These values (from Table II) are repeated here since they are necessary for calculation of β-granule volume/islet.§
§ β-Granule volume/islet = (β-cell volume) × (β-granules/β-cell) × (number β-cells/islet).

β-cell (based on the estimate of β-cell number per average islet), there appears to be a stepwise decrease in β-cell insulin secretion as a function of age (Fig. 5). As a result, at 450 mg/dl glucose, the average β-cell from an 18-mo-old rat secretes insulin at only one-fourth the rate of an average β-cell from a 2-mo-old rat.

DISCUSSION

The results of our studies of the intact pancreas have indicated that the weight of this organ increases with age, and that the incremental growth is proportional to the degree of weight gain. However, the increase in total islet mass that occurs with age is not simply a function of an increase in number of islets, since our morphometric measurements of the intact pancreas have indicated that there is also an increase in islet size (25). Thus, age results in a change in both pancreatic weight and composition. Parenthetically, it is worth noting that islets isolated by collagenase reflect this age-related increase in size, and, as such, provide confidence that the population of islets used in the in vitro studies resembles that which exists in vivo.

Information gained from more detailed morphometric analyses of isolated islets from different aged rats demonstrate that aging also leads to an increase in both the average number of β-cells per islet (Table II) and the volume of mature β-granules per β-cell (Table III). As a result, the islet content of mature β-granules essentially doubles as rats grow from 2 to 12 mo of age, and this figure approximates very closely the difference in total immunoreactive insulin content per islet between 2- and 12-mo-old rats.

However, despite this large increase in stored insulin, the data in Figs. 3 and 4 clearly indicate that glucose-stimulated insulin release from isolated islets decrease as rats age. Furthermore, the results depicted in Fig. 4 suggest that this age-related defect in insulin secretion is due primarily to a loss of maximal insulin secretory capacity without any significant change in the sensitivity of the islet to glucose stimulation. On the other hand, one could argue that the decrease in glucose-stimulated insulin release is an artifact of inadequate

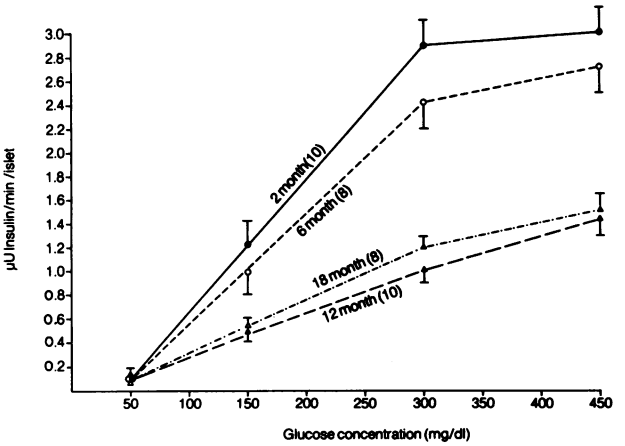

FIGURE 3 Glucose-stimulated insulin response of isolated islets from 2-, 6-, 12-, and 18-mo-old rats. The figures in parentheses represent the number of studies conducted for each age group. Each point represents the mean (±SEM) secretory rate of islets which have been isolated from each age group of rats and incubated at different glucose concentrations.

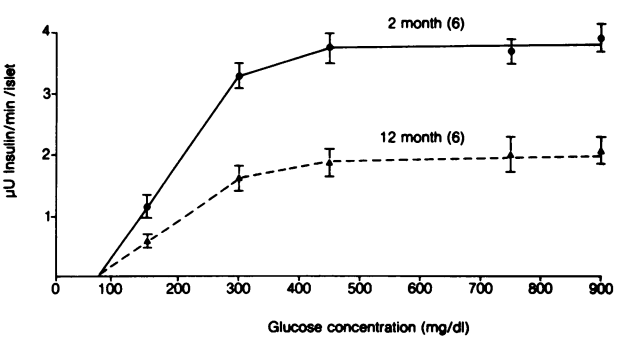

FIGURE 4 Insulin response of isolated islets from 2- and 12-mo-old rats to an extended range of glucose concentrations. Experimental details are the same as in Fig. 3.

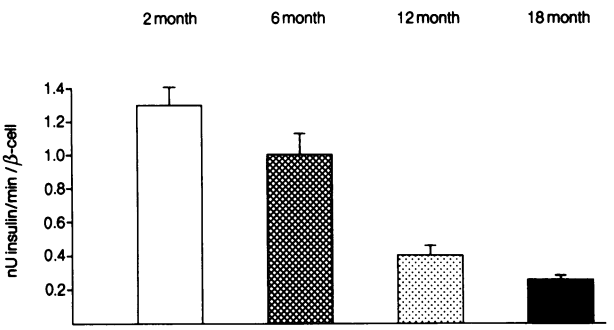

FIGURE 5 Insulin secretory response calculated per β-cell from islets isolated from 2-, 6-, 12-, and 18-mo-old rats. Values are based on insulin secretion measured per islet (at a glucose concentration of 450 mg/dl, Fig. 3) and on the number of β-cells estimated to be present per average islet obtained from rats of different ages (Table II).

diffusion of glucose into the interstices of the larger islets. If this were true, there should be a progressive decrease in the rate of insulin secretion from isolated islets as rats age from 2 to 18 mo. That this is not the case can be seen from the data in Table II and Fig. 3; i.e. islets from 18-mo-old rats are larger than islets from 12-mo-old rats (Table II), yet insulin secretion is approximately equal (Fig. 3). A similar statement can be made concerning the islet volume and insulin secretion of isolated islets from 2- and 6-mo-old rats. Thus, it seems reasonable to conclude that age leads to a decrease in glucose-stimulated insulin release, and this is not an artifact of an age-related increase in islet volume.

The quantitative nature of this defect in glucose-stimulated insulin release is best appreciated when the insulin secretory data are expressed on the basis of the insulin response of a single β-cell as illustrated in Fig. 5. These estimates vividly document the impaired ability of the β-cell to secrete insulin in response to glucose as a function of age. That this defect in β-cell insulin response is a progressive phenomenon associated with age (rather than with the growth or adiposity of the rats), is also apparent from inspection of Fig. 5. For example, although 6-mo-old rats cannot be considered "old," they are as large and have sustained the same growth as the 12- and 18-mo-old rats. However, in spite of the fact that weight gain has plateaued at 6 mo, each additional 6-mo increment of age is associated with a proportionate fall in the β-cell's secretory response to glucose.

Finally, given the magnitude of the observed defect in the insulin secretory response of the β-cell that occurs with age, it is necessary to ask how the aging animal can maintain near normal plasma glucose and insulin levels (1–14). Our studies were not aimed at answering this question, but the morphological measurements that were made provide the framework for a reasonable hypothesis. Thus, Hellman (26) and Remacle et al. (25) have previously shown that age results in an increase in the islet tissue mass of rats and our own results support these observations. Furthermore, our data indicate that the age-related increase in relative islet volume is due to an absolute increase in number of β-cells. The documentation of a decrease in the insulin secretory response of the β-cell in association with an increase in β-cell mass suggests a relationship between these phenomena. At the present time we feel that the simplest hypothesis to explain these events is that the aging process is associated with a loss of the β-cell's response to glucose (and possibly other secretagogues). As a result, less insulin is secreted from each β-cell in response to a glucose challenge, and glucose tolerance in general tends to deteriorate. In an attempt to maintain glucose tolerance, there is a compensatory increase in pancreatic β-cell mass. The degree to which glucose tolerance deteriorates in a given individual may be a combined function of how successfully the pancreas has increased its β-cell mass (and thus its ability to secrete biologically effective insulin) and the presence of other, as yet unspecified systemic factors which may either augment the insulin response (gastrointestinal hormones?) and(or) lead to a loss of normal insulin sensitivity.

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