

Effects Of Aging On Pancreatic Islet Cell Function : An Experimental Immunohistochemical And Ultrastructural Study

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Abstract

A strong relationship between aging and diabetes mellitus has been clinically suggested, however, none of the previous published data had clearly focused on the age-related cytomorphological changes in the pancreas which are the goal of this study. Three groups of male apparently healthy rabbits have been used, ten animals each; classified as group-1 (3-5 months old); group-2 (9-12 months old) and group-3 (24-36 months old). After sacrifice, sections from the pancreas were stained by Haematoxylin and Eosin (H&E), Gomori trichromic stain & ultrastructurally to detect aging histologic changes as well as immunohistochemically to identify insulin and glucagon secreting cells using their appropriate monoclonal antibodies.

A progressive histological distortion with fibrosis and fatty changes were directly proportional to age, being mild in group-2 and severe in group-3. Morphometric studies by computerized image analysis showed that the mean number of islets was significantly higher in group-2 (8.98 ± 1.51), lowest in group-1 (5.08 ± 1.48) and intermediate in group-3 (6.37 ± 1.37). The mean diameter and square area of islets were significantly higher in group-2 compared to other groups ($P < 0.05$). The mean number of β cells per islet & their secretory granules were significantly ($P < 0.05$) higher in group-2, intermediate in group-1 and lowest in group-3. In contrast, the mean number of α cells per islet and their secretory granules were insignificantly ($P < 0.05$) higher in group-2, intermediate in group-3 and lowest in group-1. Also, the β/α ratio (β cells/ α cells) was greatest in group-2 (3.059:1), intermediate in group-1 (3.37:1), and lowest in group-3 (2.479:1).

The increased number of β cells may be due to a compensatory process to correct the hormonal feedback mechanism of insulin. The results of this work suggest that β cells are generally more vulnerable to aging, an observation which might be correlated clinically with higher incidence of diabetes in older ages.

Introduction

Elderly humans have altered cellular redox levels and suppressed regenerative responses, both of which are key events underlying the progression of chronic degenerative diseases of aging, such as atherosclerosis, Alzheimer's disease and probably diabetes mellitus. Poorly maintained cellular redox levels lead to elevated activation of nuclear transcription factors such as NF κ B (nuclear factor kappa beta) and AP-1. These factors are coordinately responsible for a huge range of extracellular signaling molecules responsible for inflammation, tissue remodeling, oncogenesis and apoptosis

progresses that orchestrate many of the degenerative processes associated with aging (Hu et al, 2000).

Aging is usually associated with decreasing glucose tolerance and more susceptibility to diabetes (De Fronzo, 1984). It was also shown that insulin secretion in rabbits decreases with age, even with calorie restriction (Reaven and Reaven 1981). The decreased ability to maintain homeostasis may be a major factor in the pathogenesis of age-induced diabetes (Meites et al., 1987). The mechanisms that are implicated in diabetogenesis could be related to functional deficiency of the

insulin producing tissue (*Draznin et al., 1985*). It is also associated with a structural damage at histological or cytological level, reflecting changes in number and distribution of both β -cells and non β -cells (*Orci, 1982*). In fact, the principal changes were described mainly in insulin-secreting cells owing to its essential role in glucose regulation and general metabolism implications (*Riccillo et al., 2004*).

The aim of this work is to investigate the different morphological aspects of the pancreatic islets in different ages, as well as the quantitative immunocytochemical & ultrastructural changes in the islet cell-populations of both α and β cells, attempting to find out the possible changes that may be associated with aging in the pancreas.

Material and Methods

Animal Groups

Animals were divided depending upon their ages, according to Doughty, 1994 & Finegood *et al.*, 1995, into 3 main groups; each consisted of ten male rabbits (with apparently healthy eyes). Group-1 were 3-5 months old; group-2 were 9-12 months old and group-3 were 24-36 months old. These age groups span the entire human age ranges as the group-1 ages correspond to 15-18 years old humans; the group-2 ages correspond to 25-50 years old humans and the group-3 ages correspond to 60-80 years old ones (*Draper et al., 1998*). Animals were housed at a temperature-controlled room where optimum nutrition and water were available. Rabbits were sacrificed and equal samples from the pancreatic tails (5mm x 5mm) were taken and processed immediately.

1) Haematoxylin and Eosin (Hx&E) Staining:

The staining was done to demonstrate the morphologic appearance and the integrity of the specimens before immunohistochemistry. Paraffin sections were cut at 6 μ m, deparaffinized in xylene, embedded in descending grades of alcohol, washed in water, then stained with haematoxylin & eosin and mounted.

2) Gomori trichromic Staining:

According to *Sheehan & Hrapchak 1987*, sections were immersed in Harris haematoxylin for 5 minutes, and washed with tap water. Then, immersed in Gomori trichrome stain for 10 minutes, followed by differentiation in 0.2% acetic acid. Sections were then dehydrated in ascending alcohol concentrations, cleared in xylene, mounted and covered.

3) Immunohistochemistry & Morphometry:

Pancreatic specimens from the animals were fixed in Bouin's solution, dehydrated and embedded in paraffin. Serial sections (6 μ m) of two different levels were processed for immunohistochemical identification of insulin and glucagon secreting cells (*Riccillo et al., 2004*). Sections were incubated with ready-to-use primary antibody against each hormone at room temperature for 1 hour (Biogenex Laboratories, San Ramon, CA, U.S.A.). After washing, the sections were immunostained by Dako EnVision System. Diaminobenzidine (DAB) was used as a chromogen. The specificity of the primary antiserum was controlled by replacing the first antiserum by phosphate buffered saline (PBS). The sections were counterstained with haematoxylin.

Measurement of cell parameters was made by the image analysis system (Media cybernetics, software Optimas v6.21). Averages of 5 images per section were analyzed. The number of islets and their mean areas (in square μ m) and diameters (in μ m) were counted for each section. The average number of α and β cells in each islet was also calculated. Comparative statistical analysis was performed by the Student T test using Microsoft Excel 2000.

4) Ultrastructural Technique:

According to *James and Leslie (2002)*, small pieces of pancreas were put in 2.5% glutaraldehyde, then in cacodylate buffer for 20 minutes & fixed in a fume cupboard with osmium tetroxide for 2 hours at 4°C. The specimens were dehydrated, cleared in propylene oxide for 30 minutes at room temperature & embedded in Beem capsule filled completely with epoxy resin

and polymerized at 60 °C for 24 hours .The capsule were then trimmed and sectioned at 1µm (semithin section) and 60 nm (ultrathin section). The sections were stained with lead citrate & uranyl acetate and examined by transmission electron microscopy.

Results

A progressive distortion in the histoarchitecture of the pancreatic parenchyma and stroma of group-3 animals (Figs.5,6) compared to group-1 animals. A marked increase in the adipose tissue was found at the expense of the functional pancreatic mass (Fig.5B). Many fragmented islets and areas of progressive interstitial fibrosis (Figs. 5A&C) were observed in group-3 animals. In addition, ductal structure was altered (Fig.5D). Thus, zones with adenomatous and cystic ductal hyperplasia were evident, showing ectasia and thinner ductal epithelium. Also, the basement membrane was strongly stained and augmented in thickness (Fig. 5D). Moreover, arteriosclerosis was observed as the main vascular alteration in aged animals.

The morphometric analysis of the pancreas showed significant differences between the three groups (P value < 0.05). The mean number of islets was significantly higher in group-2, lowest in group-1 and intermediate in group-3 (Table-1; Fig.1). The mean diameter and square area of islets were significantly higher in group-2, intermediate in group -3 and lowest in group-1 (Tables-2&3; Figs.2&3).

Immunohistochemically, the mean number of α cells per islet was higher in group-2, intermediate in group-3 and lowest in group-1 (Table 4) while the mean number of β cells per islet was higher in group-2, intermediate in group-1 and lowest in group-3 (Table 5). The β/α ratio (β cells/ α cells) was greatest in group-2 (3.059:1), intermediate in group-1 (3.37:1), and lowest in group-3 (2.479:1). (Table-5, Figs. 4&6).

Ultrastructurally, β -cells are centrally while α cells are peripherally placed in the

pancreatic islets. Also, the cytoplasmic α -cell granules are rounded & uniform in size; about 3000 \AA (300 nm), being extremely dense round body surrounded by a limiting membrane closely applied to the granule (Fig.7A). The intervening space is narrower and filled with granular materials i.e. lacking an electron – lucent periphery. The nucleus is often indented or lobulated, eccentric & less dense or hypochromic. In contrast, the secretory granules in the cytoplasm of β -cells are variable in shape & size; about 2000 \AA (200 nm), enclosed by a definite limiting membrane, having an electron dense core with a clear space devoid of any detectable density between the core and the outer limiting membrane . β -cell nucleus is single , usually spherical , fairly regular outlined , with moderate diffuse granular nucleoplasm & narrow zone of greater electron density immediately within the inner nuclear membrane. The nucleoli are well defined and being more than one (Fig. 7B).

In addition, electron microscopic examination coincided with the immunohistochemical results as the α cell number was insignificantly higher in group-2 than in group-3 which, in turn, slightly higher than those of group-1. The β -cell number was significantly higher (P< 0.05) in group-2 than in group-1 that, in turn, greater (P< 0.05) than in group-3.

Moreover, both islet cell cytoplasmic secretory granules were more numerous in group-2 animals than in any other two groups. Alpha cell granules were insignificantly lowest in group-1 and intermediate in group-3 while β -cell granules were lowest in group-3 and intermediate in group-1. Although the α cells showed no notable nuclear & organeller changes (Fig.7A) among the three studied groups, some of senescent β -cells were altered (damaged) displaying partial or complete loss of their plasma membrane with abnormal fibrous elements (filamentous material) within the cytoplasm (Fig.7C). Large vacuoles in the cytoplasm with amorphous material filling the β granular space (Fig.7D) as well as swollen mitochondria (Figs.7B&C) were also seen.

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Table-1: Comparison of the mean number of islets per section in all groups (n= 30):

Parameters	Group-1	Group-2	Group-3
Mean number of islets / section	5.078	8.980	6.370
S.D.	1.476	1.508	1.374
Gr. Comp.	Gr1:Gr2	Gr2:Gr3	Gr1:Gr3
T Value	0.000000001	0.000000283	0.000000182
P Value	< 0.05	< 0.05	< 0.05

S.D. = Standard deviation; Gr. Comp= Relative significance of groups compared according T and P values after Student T test.

Table-2: Comparison of the mean diameters of islets in all groups (n=30):

Parameters	Group-1	Group-2	Group-3
Mean diameter of islets / μm	70.613	99.683	79.415
S.D.	3.266	6.778	2.958
Gr. Comp.	Gr1:Gr2	Gr2:Gr3	Gr1:Gr3
T Value	0.000000001	0.000000023	0.000000006
P Value	< 0.05	< 0.05	< 0.05

S.D. =Standard deviation; Gr. Comp. =Relative significance of groups compared according T and P values after Student T test.

Table-3: Comparison of the mean area of islets in all groups (n =30):

Parameters	Group-1	Group-2	Group-3
Mean Islet Area in μm^2	3823.479	8124.039	4180.908
S.D.	205.361	1044.731	112.494
Gr. Comp.	Gr1:Gr2	Gr2:Gr3	Gr1:Gr3
T Value	0.000000032	0.000000162	0.000001081
P Value	< 0.05	< 0.05	< 0.05

S.D. =Standard deviation; Gr. Comp. =Relative significance of groups compared according T and P values after Student T test.

Table-4: Comparison of the mean number of α cells per islet in all groups (n = 30):

Parameters	Group-1	Group-2	Group-3
α cells number/islet	10.597	13.577	11.546
S.D.	1.367	1.282	1.023
Gr. Comp.	Gr1:Gr2	Gr2:Gr3	Gr1:Gr3
T Value	7.37513E-09	1.307E-07	4.26903E-06
P Value	NS	NS	NS

S.D. =Standard deviation; Gr. Comp. =Relative significance of groups compared according T and P values after Student T test.

Table-5: Comparison of the mean number of β cells per islet in all groups (n = 30):

Parameters	Group-1	Group-2	Group-3
β cells number/islet	35.193	41.537	28.903
S.D.	1.764	1.543	1.560
Gr. Comp.	Gr1:Gr2	Gr2:Gr3	Gr1:Gr3
P Value	< 0.05	< 0.05	< 0.05
β/α Ratio	3.37:1	3.059:1	2.479:1

S.D. = Standard deviation; *Gr. Comp.* =Relative significance of groups compared according *T* and *P* values after Student *T* test. The β/α ratio is also shown for comparison in this table.

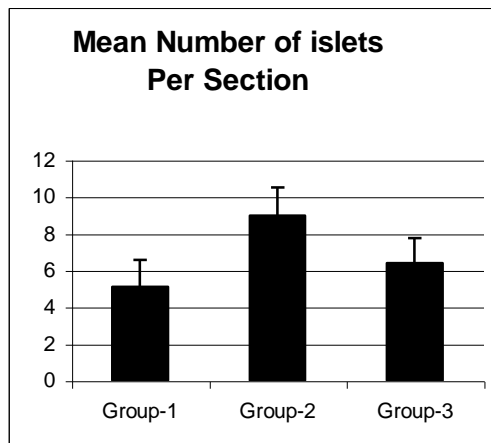


Figure-1: The histogram shows the mean number of islets per section in each group, showing their marked increase in group 2. The error bars represent the standard deviation.

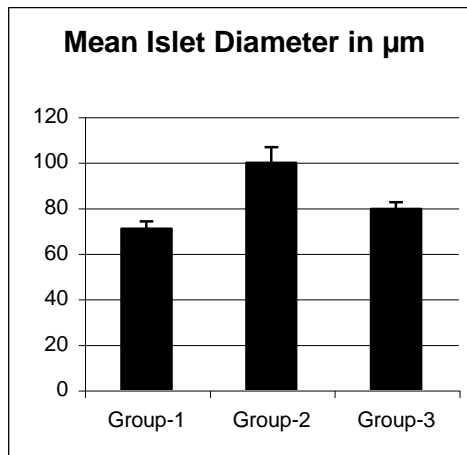


Figure-2: The histogram shows the mean islet diameter in micrometers in each group, showing their marked increase in group 2. The error bars represent the standard deviation.

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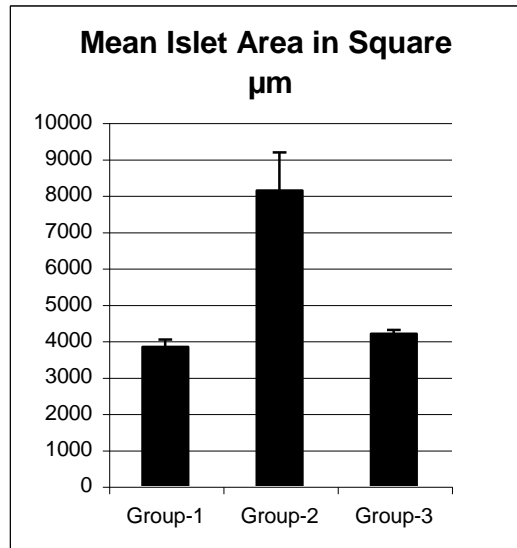


Figure-3: The histogram shows the mean area in square micrometers in each group, showing their marked increase in group 2. The error bars represent the standard deviation

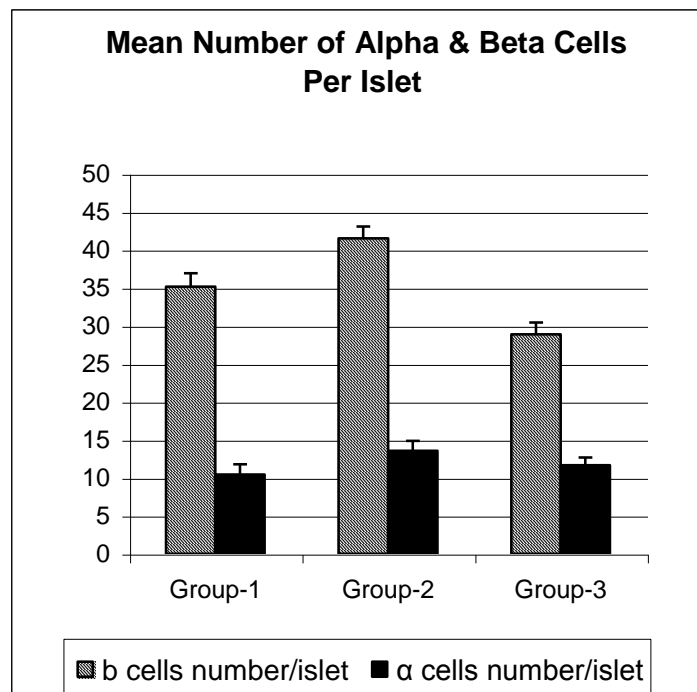


Figure-4: The histogram shows the mean number of β and α cells per islet. It demonstrates the marked predominance of β cells in group 2 which is reversed in group 3. The error bars represent the standard deviation.

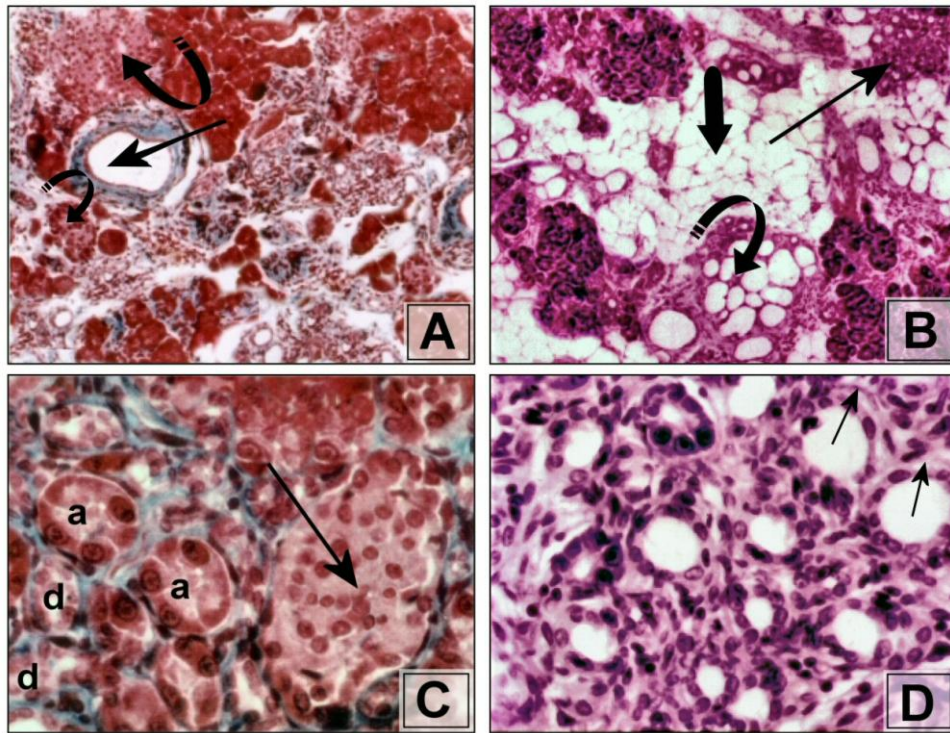


Figure-5: **A)** The pancreas from a group-3 rabbit showing isolated ductectasia with marked fibrosis (straight arrow) and the islets of different size (curved arrows);Gomori trichromic stain (X 100). **B)** The pancreas of a group-3 rabbit showing massive histological changes; the adipose tissue (thick arrow); cystic ductal hyperplasia (curved arrow) and adenomatous ductal hyperplasia (thin arrow);H&E (X 100). **C)** Pancreas from group-2 rabbit showing different pancreatic structures separated by marked fibrosis; the islets (straight arrow); acini (a) and small ducts (d);Gomori trichromic stain (X 400). **D)** Ductal hyperplasia in a group-3 rabbit also showing increased thickness of ductal basement membrane (arrow);H&E (X 400).

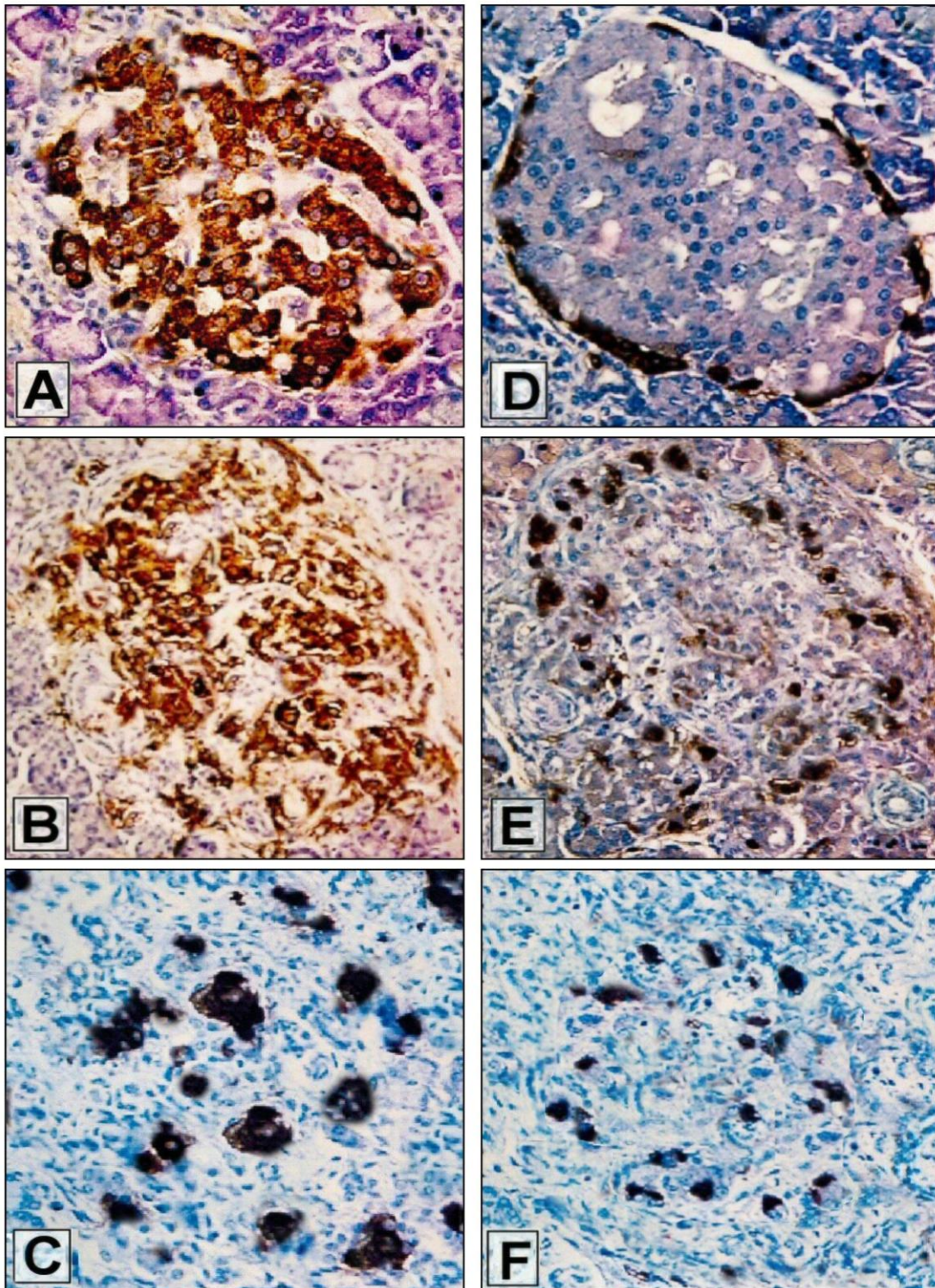


Figure-6: Immunohistochemical staining of the pancreas. Sections A , B ,C are stained with antibody against insulin while D,E,F are stained with antibody against glucagon. A and D figures represent sections from group-1 showing islets with a typical distribution of peripheral glucagon-secreting cells and central insulin-secreting cells ($\times 400$). Figures B and E represent sections from group-2, showing islets with a relatively well preserved typical distribution($\times 200$) . Fig.6-E shows an enlarged islet with newly developed blood vessels and ducts in its periphery ($\times 200$). Figures C and F represent sections from group-3 showing destroyed islets with fibrosis ($\times 400$).

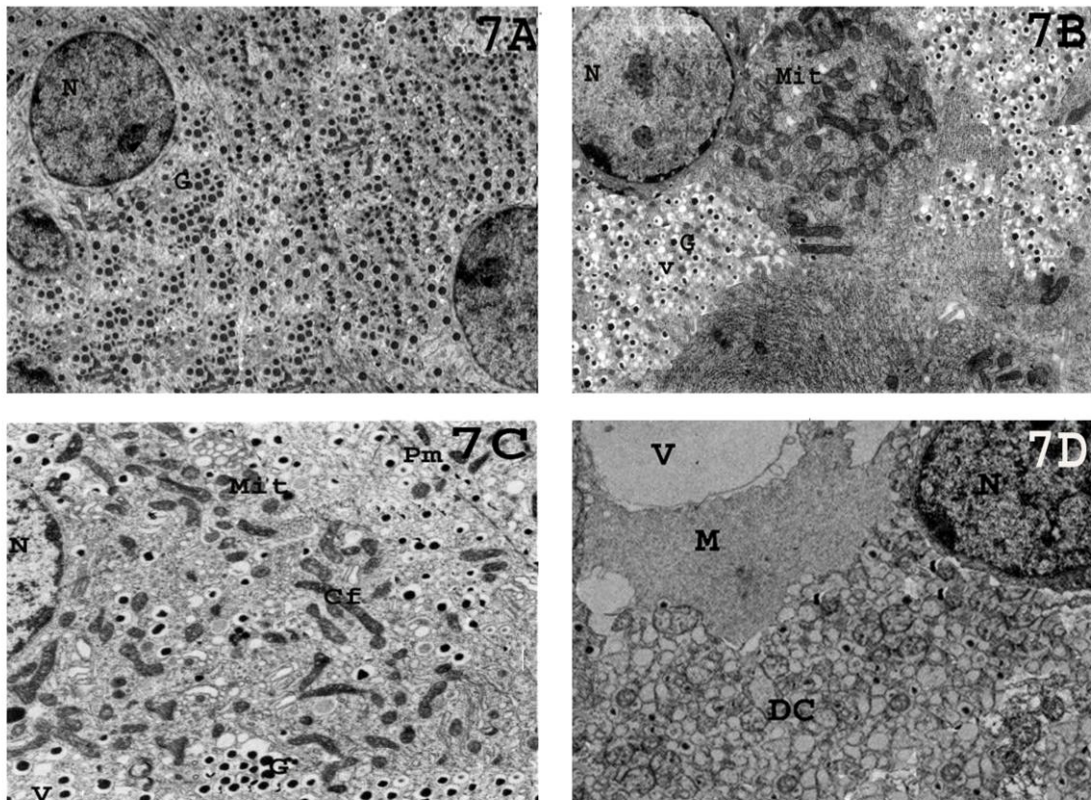


Figure-7: Electron micrographs of group-3 senescent islets. In **7A**, two adjacent alpha cells showing variable sized cytoplasmic secretory granules (G) lacking an electron lucent periphery. The nuclei (N) are irregularly outlined & having a single nucleolus. In **7B**, the damaged beta cell is extremely vacuolated (V) & contains swollen mitochondria (Mit). The cytoplasmic granules (G), in contrast, have an electron dense core surrounded by a clear zone & the nucleus (N) is fairly regular in outline with two well defined nucleoli. In **7C**, the damaged beta cell reveals partial loss of the plasma membrane (Pm) & contains collagen filaments (Cf) together with the above mentioned changes. In **7D**, the damaged beta cell (DC) contains a large vacuole (V) & amorphous material (M) filling a space formerly occupied by beta granules. (Double fixation & Double stain. X 10,000).

Discussion

The effect of aging on the endocrine system as well as the other systems represents one of the main topics in the current research. Many factors are involved in the progression of the aging process, however, it is difficult to separate those that are selectively due to aging itself, from those due to associated diseases that develop throughout the passage of time (Quay, 1995).

According to the previously published data, aging produces a disruption in the neuroendocrine system (Meites *et al.*, 1987). It was also reported that aging in the rabbit is associated with diminished pancreatic exocrine functions. However, the exact explanation of such changes is still unclear (Hollander and Dadufalza, 1984).

The present study provides a comparative analysis of the morphology of

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the pancreatic islets and the two major cells that balance the blood sugar (β and α cells) at three different ages, from both a quantitative and qualitative point of view.

It seems that the interstitial fibrosis may play an important role in the distortion of both exocrine and endocrine histology of the pancreas (*Hayflick, 1985 and Ruhe et al., 1997*). Their and our possible evidence is the presence of vascular (arteriosclerosis), ductal (ectasia as well as adenomatous and cystic hyperplasia) and insular (fragmentation and polymorphism) alterations. Furthermore, the progressive increment of the pancreatic (intraglandular) adipose tissue, highly noted in the senile rabbits, appears as a substitution of the normal exocrine pancreas, frequently associated to the pathologic processes, since aging can be considered a natural degenerative process.

Considering the fact that decreased cellular proliferation is a general hallmark of aging, *Hayflick, 1985 and Ruhe et al., 1997* proposed that the maintenance of a normal organ function during senescence may be accomplished, in part, by an increase in the percentage of cells that are responsive to stimuli, and /or by an increase in the sensitivity of such responsive cells. However, the evidence that the β -cell mass exists in a dynamic state is becoming increasingly strong. This signifies that the number of β -cells is determined by the balance of cell renewal and cell loss (*Bernard et al., 1999; Bernard and Ktorza, 2001; Bonner-Weir, 2006*).

The present morphometric analysis suggests a different behavior of the β -cell mass if compared to the α -cells coinciding with *Riccillo et al., 2004* who reported that the efficiency of beta cells to maintain euglycemia could be decreased with aging which is then compensated with an increased number in group-2, then followed by failure of appropriate growth to maintain the normal endocrine function in senile group-3 rabbits.

The appearance of these morphological changes in the endocrine pancreas of the older rabbits, reflects the effect of aging on the pancreatic structure, as an attempt to compensate a possible impairment in the insulin secretion pattern.

The β -cell behavior is generally associated with deficiency in the insulin secretion mechanism; increased glucagon secretion and/or insulin resistance (*Aizawa et al., 1994*). This may be related to the decreased glucose tolerance (*Jackson, 1990*) and the insulin metabolism alterations (*Coordt et al., 1995; Perfetti et al., 1996*), that usually appear associated with advanced ages and their possible associated diabetes (*Riccillo et al., 2004*). *Reaven and Reaven (1981)* found that while the rabbits age, there is an increasing demand placed upon the beta cell to secrete more insulin. At the same time, there is a progressive decline in beta cell capacity. The combination of these events (β cell decline plus increased total demand) may be expected to put a great deal of stress on the beta cell and this may account for their & our changes. Thus, it is possible that beta cell exhaustion occurs as the rabbits age leading to beta cell damage and death as well as connective tissue infiltration at three years of age. Also, there are, at least, some precedent documents for this notion in man (*Stanik and Marcus, 1980*). Furthermore, *Reaven and Reaven (1981)* did not know whether less insulin secretion by senescent animals was due to a decrease in beta cell capacity with aging or simply a function of the reduced content of functional beta cells in the islets resulting from cell damage and increased connective tissue infiltration. Since it had previously demonstrated that aging leads to a decrease in insulin secretion per beta cell (*Reaven et al., 1980*), they would suggest that the observed reduction in insulin secretion was due to the combination of both factors.

Glucagon-producing α cells play a crucial role during the perinatal period and possibly may play a role in type-1 diabetes. Because of their peri-islet localization near the early dendritic and macrophage cell infiltration, *Pelegri et al, 2001* investigated α -cells in the non-obese diabetic (NOD) rabbits as a model for human type I diabetes. However, *Bernard and Steven (1990)* displayed a deficiency of glucagon – stained cells in older animals explaining improved their glucose tolerance.

Other investigators have described increased islet β -cell apoptosis in NOD

rabbits from birth onward (*Trudeau et al, 2005*). The causes of these disturbances remain to be determined; they may involve genetic, maternal, and/or fetal factors. These disturbances may be related to β -cell dysfunction, resulting in increased β -cell sensitivity or even targeting of the autoimmune reaction. These observations may explain the results of our work since we found, both immunohistochemically & ultrastructurally, a significant decrease in the β -cells & their secretory granules in group-3 together with a relative insignificant decrease in α -cells & their granules in that group. These results were mimicking those displayed by *Holz et al., 1993* & *Riccillo et al., 2004*. Thus, the tendency of smaller and less numerous islets appearing in group-3, when compared to those of group-2 rabbits, could explain the outcome of previously larger islets disruption.

In conclusion, there is an increase in the size of islets and the number of β -cells as in group-2. As aging progresses, the compensatory changes may fail to maintain euglycemia which may possibly lead to the development of diabetes in group-3. Decreased insulin response to hyperglycemia may occur in the senile rabbits which, in turn, becomes associated with abnormal pancreatic changes such as severe fibrosis and decreased beta cells. In addition, since the ability of beta cell to secrete insulin declines with aging that also may render the animals significantly insulin resistant, more and more insulin secretion is demanded of the pancreas. This demand, in combination with the age – related decline in beta cell capacity, eventually leads to an injury and death of some cells. These findings, as stated by *Draper et al. (1998)*, correspond to those encountered in senile humans.

References

1. **Aizawa T, Komatsu M, Sato Y, Ishihara F, Suzuki N, Nishii N, Hashizume K and Yamada T (1994):** Insulin secretion by the pancreatic beta cell of aged animals. *Pancreas*, 9 (4): 454-9.
2. **Bernard C, Berthault MF, Saulnier C and Ktorza A (1999):** Neogenesis vs. Apoptosis as main components of pancreatic β -cell mass changes in glucose-infused normal and mildly diabetic adult rabbits. *FASEB J.*, 13: 1195-205.
3. **Bernard C and Ktorza A (2001):** Endocrine pancreas plasticity under physiological and pathological conditions. *Diabetes*, 50 (Suppl.1): s30- s35.
4. **Bernard GS and Steven PL (1990):** Age – related immunohistochemical studies of A and D cells in pancreatic islets. *J. Endocrinol.*, 228(1): 53-7
5. **Bonner-Weir S (2006):** β -cell Turnover. Its assessment and implications. *Diabetes*, 50 (Suppl.1): s20-s24.
6. **Coordt MC, Ruhe RC and McDonald RB (1995):** Aging and insulin secretion. *Proc .Soc. Exp. Biol. Med.*, 209(3): 213-22.
7. **De Fronzo RA (1984):** Glucose intolerance of aging. *Diabetes Care*; 4: 493-513.
8. **Doughty M J (1994):** The cornea and corneal epithelium in the aged rabbit. *Ophthalmology & Visual Science*, 71(12) : 809-18.
9. **Draper CE, Adeghate EA, Lawrence PA and Garner A (1998):** Age -related changes in morphology and secretory responses of male rabbit lacrimal gland. *J. Aut. Nerv. Syst.*, 69: 173- 83.
10. **Draznin B, Steinberg JP, Leitner JW and Sussman KE (1985):** The nature of insulin secretory defect in aging rabbits. *Diabetes*, 34: 1168-73.
11. **Finegood DT, Scaglia L, Bonner H (1995):** Dynamics of β -cell mass in the growing rabbit pancreas: Estimation with a simple mathematical model. *Diabetes*, 44: 249-56.
12. **Hayflick L (1985):** Theories of biological aging. *Exp. Gerontol* ,20: 145-9
13. **Hollander D and Dadufalza VD (1984):** Aging-associated pancreatic exocrine insufficiency in the unanesthetized rabbit. *Gerontology*, 30 (4): 218-22.
14. **Holz GG, Kuhlreiber WM and Habener JF (1993) :** Pancreatic beta cells are rendered glucose component by insulinotropic hormone glucagons like peptide-1 . *Nature*, 361: 362-5.
15. **Hu H, Forsey RJ, Blades TJ, Barratt MEJ, Parmar P and Powell JR (2000):** Antioxidants may contribute in the fight against aging: An in vitro model. *Mechanisms of Aging and Development* ,121: 217–30
16. **Jackson RA (1990):** Mechanism of age-related glucose tolerance. *Diabetes Care* 13: 9-19.
17. **James LH and Leslie PG (2002) :** Textbook of Histology , Introduction to Histology and Basic Histological

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- Techniques. 7 th ed., Bulter Worth, Oxford, Boston : 1-6
18. **Meites J, Goya RG and Takahashi S (1987):** Why the neuroendocrine system is important in aging process. *Exp. Gerontol* ., 22: 1-5.
 19. **Orci L (1982):** Macro- and micro-domains in the endocrine pancreas. *Diabetes* , 31: 538-65.
 20. **Pelegri C, Rosmalen JGM, Durant S, Throsby M, Alvès V, Coulaud J, Esling AA, Pléau J, Drexhage HA and Homo-Delarche F (2001):** Islet Endocrine-Cell Behavior From Birth Onward in Rabbit with the Nonobese Diabetic Genetic Background. *Molecular Medicine*, 7(5): 311-19
 21. **Perfetti R, Rafizadeh CM, Liotta AS and Egan JM (1996):** Age-dependent reduction in insulin secretion and insulin mRNA in isolated islets from rabbits. *Am. J .Physiol* , 269(6): E983-90
 22. **Quay W (1995):** Pancreatic regulation of nutrient metabolism. In: *Hormones and Aging*. P. Timiras, W. Quay, A. Vernadakis Eds. CRC Press, Boca Raton, chapter 10, pp. 169-99.
 23. **Reaven EP, Gold G and Reaven GM (1980):** Effect of age on Leucine-induced insulin secretion by the beta cell. *J.Gerontol.*,35:324-8.
 24. **Reaven EP and Reaven GN (1981):** Structure and function changes in the endocrine pancreas of aging rabbits with reference to the modulating effects of exercise and caloric restriction. *J.Clin. Invest.*, 68, 75-84.
 25. **Riccillo FL, Bracamonte MI, Console GM and Gomez CLA (2004):** Histomorphological and quantitative immunohistochemical changes in the pancreas during aging . *Biocell*, 28(2):127-34
 26. **Ruhe RC, Curry DL and McDonald RB (1997):** Altered cellular heterogeneity as a possible mechanism for the maintenance of organ function in senescent animals. *J .Gerontol .A .Biol. Sci .Med. Sci.*, 52(1): B53-58.
 27. **Sheehan DC and Hrapchak BB (1987):** Theory and practice of histotechnology . Battelle Memorial Institute, Columbus, OH, 97-101.
 28. **Stanik S and Marcus R (1980):** Insulin secretion improves following dietary control of plasma glucose in severely hyperglycemic obese patients. *J.Metabolism*, 29: 346-50.
 29. **Trudeau JD, Dutz, JP, Arany E, Hill DJ, Fieldus WE and Finegood DT (2005):** Neonatal β -cell apoptosis. A trigger for autoimmune diabetes? *Diabetes*, 49: 1-7.

تأثيرات الشيخوخة علي وظيفة خلايا جزر البنكرياس : دراسة تجريبية هستوكيميائية مناعية وتركيبية دقيقة

أحمد حسن عبد الرحمن¹ ، حسن عبد الهادي¹ ، محمد أحمد قاسم² ، حسام الدين حسين عثمان³ ، حنان سليمان عبد الحميد⁴ ، محمد الباسل⁵ ، محمد عبد الهادي مشاحيت⁶

من أقسام الباثولوجي¹ و الهستولوجي² والتشريح³ بكلية طب الأزهر بنين فرعى أسيوط والقاهرة، وقسم الباثولوجي⁴ والباطنة العامة⁵ بكلية طب القصر العيني وقسم الباطنة العامة⁶ بطب الفيوم .

تدل الأبحاث والملاحظات الإكلينيكية على وجود علاقة وثيقة بين الشيخوخة ومرض البول السكري، ولم توضح هذه الأبحاث مدى تأثير الشيخوخة على أنسجة وخلايا البنكرياس. في هذه الدراسة تم استخدام ثلاث مجموعات من ذكور الارانب تتكون كل منها من عشره، وكان عمر المجموعة الأولى من 3-5 شهور والمجموعة الثانية من 9-12 شهر والمجموعة الثالثة من 24-36 شهراً ، وبعد أخذ عينات من البنكرياس و فحصها بصبغة الهيماتوكسيلين والإيوسين وصبغة جومورى الثلاثية والهستولوجيا المناعية تم فحص الأنسجة والتعرف على الخلايا المنتجة للإنسولين والجلوكاجون باستخدام ما يظهرهما من الأجسام المضادة وحيدة الأصل وكذلك بالميكروسكوب الالكترونى للتعرف على التغييرات المصاحبه للشيخوخة في خلايا الفا وبيتا .

وقد أظهرت النتائج وجود خلل في البنية النسيجية للبنكرياس مع تليف وتغيرات دهنية بحيث تناسبت طردياً مع عمر الارانب، فقد كانت متوسطة في المجموعة الثانية وشديدة في المجموعة الثالثة، كما أظهر تحليل الصور بالحاسب الالى أن متوسط عدد الجزر في المجموعة الثانية كان أكبر من أى مجموعة أخرى (8.98 ± 1.51)، وكانت المجموعة الأولى أقلهم عدداً (5.08 ± 1.48) ومتوسطة في المجموعة الثالثة (6.37 ± 1.37).

وكان متوسط أقطار ومساحات الجزر أكبر ما يكون في المجموعة الثانية مقارنة بأى مجموعة أخرى (قيمة ب-الإحصائية > 05) . وكان متوسط عدد خلايا بيتا لكل جزيرة وحببياتها المفزره (قيمة ب-الإحصائية > 05) أكثر في المجموعة الثانية ومتوسطاً في المجموعة الأولى وأقل ما يكون في المجموعة الثالثة بينما في المقابل كان متوسط عدد خلايا الفا وحببياتها المفزره أكثر في المجموعه الثانيه وأقل ما يكون في الأولى ومتوسطا في الثالثه (ب الاحصائيه < 05) وكانت نسبة خلايا بيتا/ألفا (β/α) في المجموعة الثانية = $3.059:1$ ، وفي المجموعة الأولى = $3.37:1$ ، وفي المجموعة الثالثة = $2.479:1$.

ويتضح من هذه الدراسة زيادة خلايا بيتا في المجموعة الثانية التي من المحتمل أن تكون ناتجة عن تفاعل تعويضى لتصحيح الاستجابة الهرمونية للإنسولين، ونستخلص من هذه النتائج أن خلايا بيتا معرضة أكثر لتأثير الشيخوخة مما يرجح إرتباط مرض البول السكري مع التقدم فى السن والشيخوخة.

Effects Of Aging On Pancreatic Islet Cell Function.....