Secondary Negative Effects of Isolation Enzyme (s) On Human Islets

A.N.Balamurugan

Human Islets Functional Mass Preservation

DIABETES, VOL. 51, AUGUST 2002

Preservation of Human Islet Cell Functional Mass by Anti-Oxidative Action of a Novel SOD Mimic Compound

Response of Human Islets to Isolation Stress and the Effect of Antioxidant Treatment

DIABETES, VOL. 53, OCTOBER 2004

American Journal of Transplantation 2003; 3: 1135–1142
Blackwell Munksgaard

ISSN 1600-6135

Flexible Management of Enzymatic Digestion Improves Human Islet Isolation Outcome from Sub-Optimal Donor Pancreata

American Journal of Transplantation 2005; 5: 2671–2681
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doi: 10.1111/j.1600-6143.2005.01078.x

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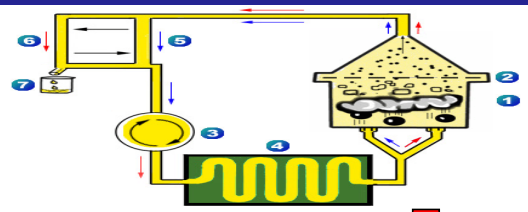
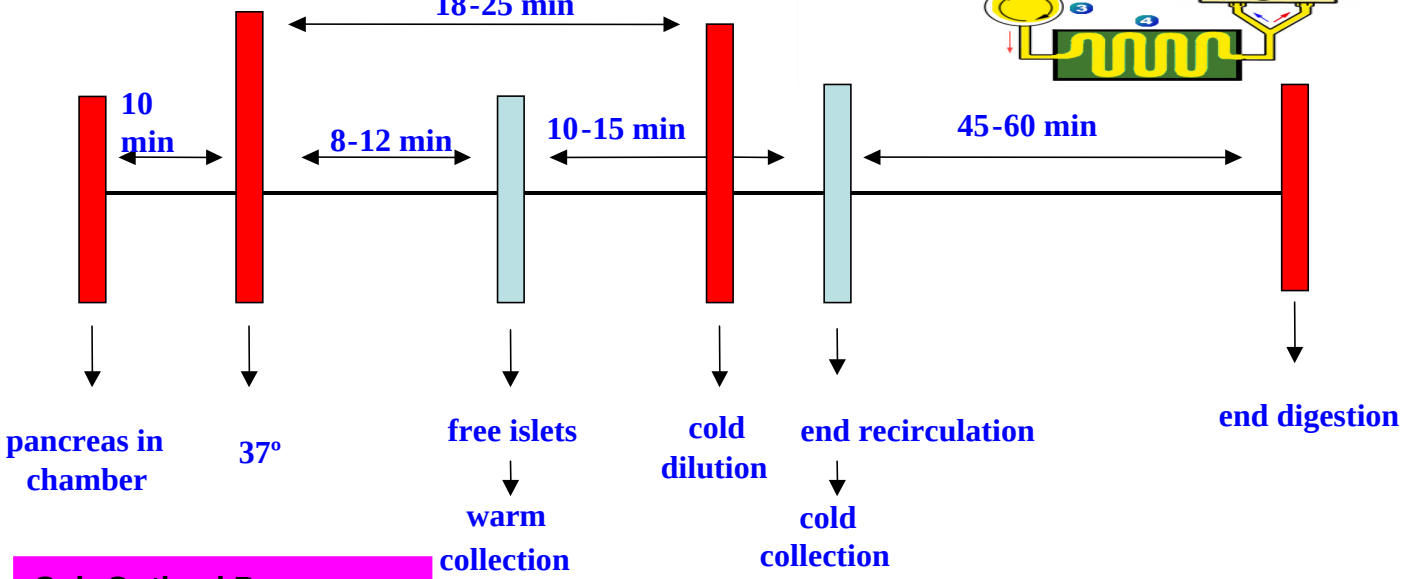
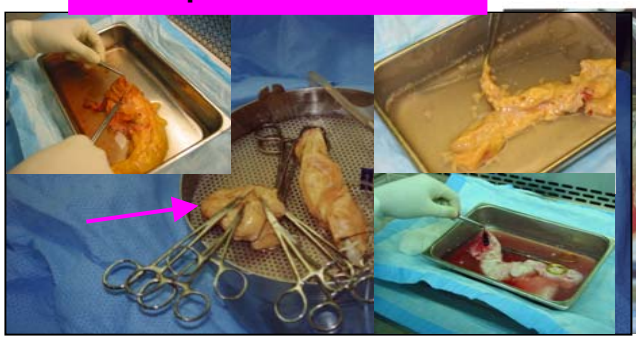
Flexible Management of Enzymatic Digestion Improves Human Islet Isolation Outcome from Sub-Optimal Donor Pancreata

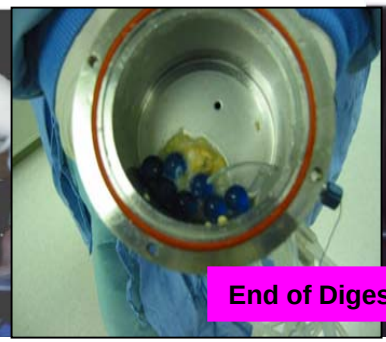




Sub-Optimal Pancreases



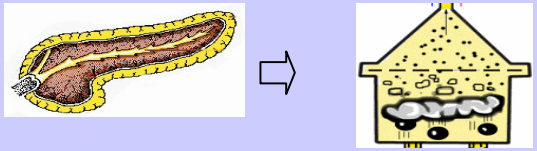
Beginning of Cold



End of Digestion

ISLET INCUBATION PROTOCOL

Organ Manipulation - Ricordi's isolation method



Start
Warm collection
T = 8-12 Minutes
Sampling

Appearance of Free Islets

Start
Cold collection
T = 25-30 Minutes

Wash Cells and Maintain at 4C

Incubate at 37C With No Washing

4C Samples

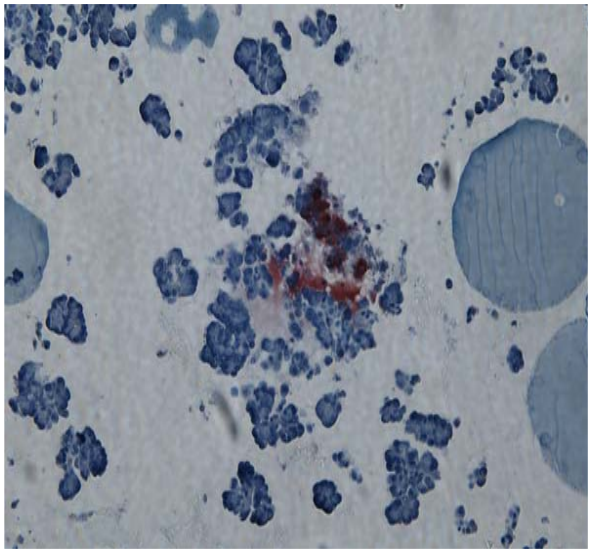
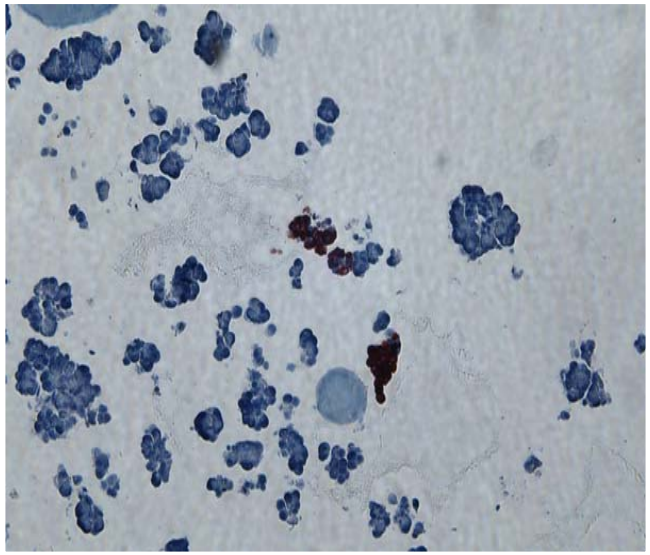
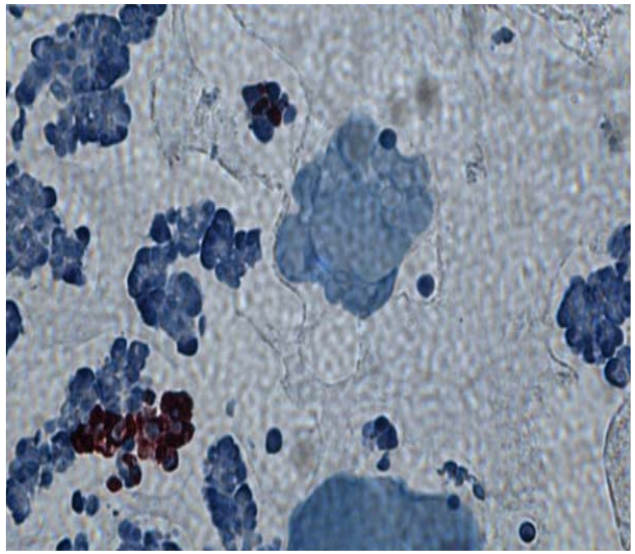
37C Samples

SAMPLES

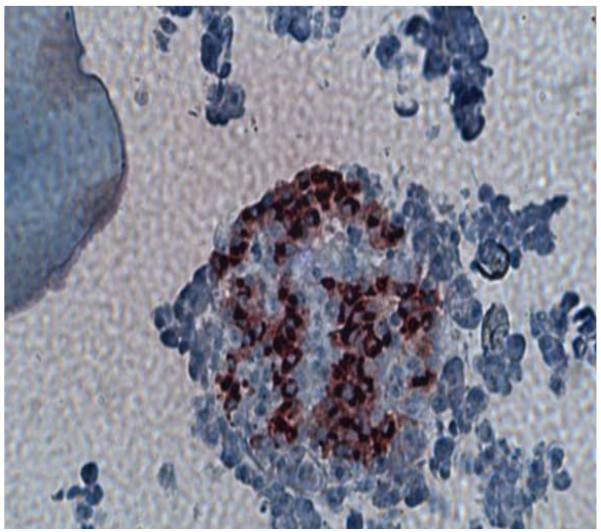
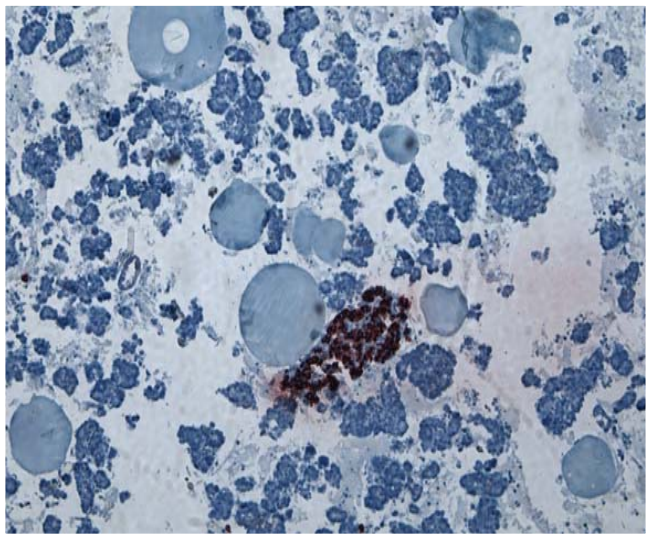
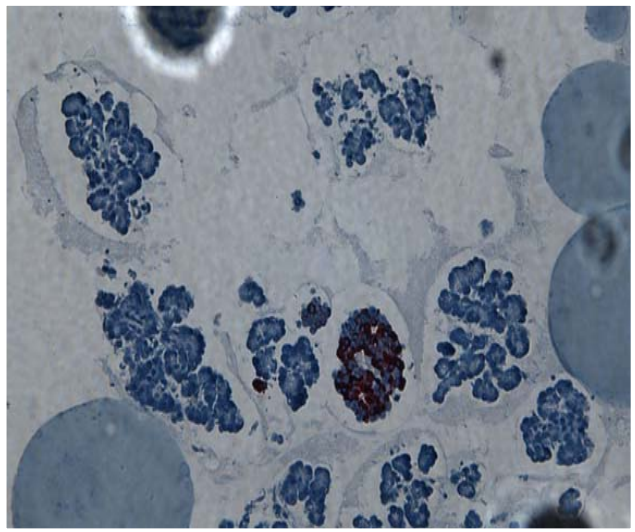
- Islet Count
- DNA Measurement
- Insulin Content
- Immunocytochemistry

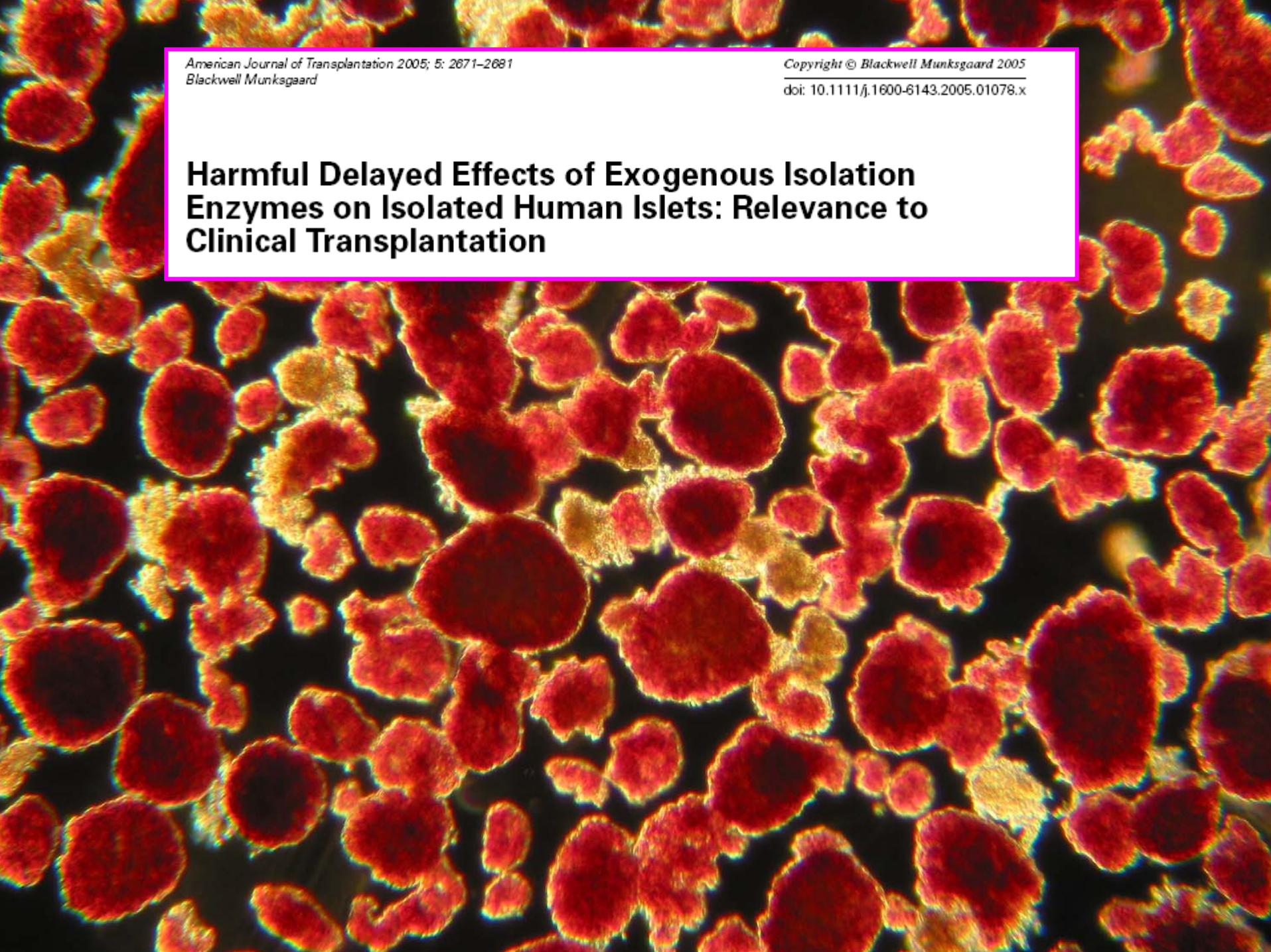
Insulin Immunostaining

Standard Collection

Early Collection



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Harmful Delayed Effects of Exogenous Isolation Enzymes on Isolated Human Islets: Relevance to Clinical Transplantation

Islet Graft Assessment in the Edmonton Protocol

Implications for Predicting Long-Term Clinical Outcome

Cale N. Street,[1] Jonathan R.T. Lakey,[1,2] A.M. James Shapiro,[1,2] Sharleen Imes,[3] Ray V. Rajotte,[1,2,4] Edmond A. Ryan,[4] James G. Lyon,[1] Tatsuya Kin,[1] Jose Avila,[1] Toshiaki Tsujimura,[1] and Gregory S. Korbutt[1,2,5]

The success of the Edmonton Protocol for islet transplantation has provided new hope in the treatment of type 1 diabetes. This study reports on the assessment of 83 human islet grafts transplanted using the Edmonton Protocol since 1999. Cellular composition, as assessed by immunohistochemistry, showed a lower islet purity

the proportion of dithizone-positive aggregates have been the standard measures used to estimate yield and purity (6,7), respectively. However, these techniques are not necessarily quantitative largely due to observer subjectivity, and more accurate methods to assess human islet grafts ar

The NEW ENGLAND JOURNAL of MEDICINE

REVIEW ARTICLE

MEDICAL PROGRESS

Islet Transplantation as a Treatment for Diabetes — A Work in Progress

R. Paul Robertson, M.D.

IN 1993 THE DIABETES CONTROL AND COMPLICATIONS TRIAL (DCCT) established the modern standard of care for the medical management of type 1 diabetes mellitus.[1] The DCCT randomly assigned 1441 patients to conventional or intensive treatment. The latter included multiple daily determinations of blood glucose levels at home by finger stick; combinations of daily injections of long-, intermediate-, l support. The clinical outcomes better in the intensively treated fter, intensive treatment became se and glycosylated hemoglobin bled patients with diabetes to at- pisodes of hypoglycemia.

Long-term Islet Graft Function?

Challenges facing islet transplantation for the treatment of type 1 diabetes mellitus

Kristina I. Rother and David M. Harlan

Islet and Autoimmunity Branch, National Institutes of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, Maryland, USA.

Islet transplantation represents a most impressive recent advance in the search for a type 1 diabetes mellitus cure. While several hundred patients have achieved at least temporary insulin independence after receiving the islet "mini-organs" (containing insulin-producing β cells), very few patients remain insulin independent beyond 4 years after transplantation. In this review, we describe historic as well as technical details about the procedure and provide insight into clinical and basic research efforts to overcome existing hurdles for this promising therapy.

Worldwide, more than 750 individuals with type 1 diabetes mellitus (T1DM) have received allogeneic islet transplants since 1974, in an effort to cure their chronic condition. Though this is still a small number (especially when compared with the estimated 1 million afflicted with T1DM and an additional 17 million with type 2 diabetes in the US, not to mention the estimated 140 million with diabetes worldwide), much has been learned, especially since the promising results of the Edmonton group were published in 2000 (1, 2). This report described 7 consecutive patients with T1DM who became insulin independent after receiving islet

standing T1DM. These studies have raised heretofore underexplored avenues for clinical investigation, which we will return to.

Brief history

In 1924, after approximately 40 years of unsuccessful attempts by various investigators to control diabetes using partial pancreas transplantation, the English surgeon Charles Pybus (1882–1975) made a statement that resonates even today: "Not much can be said about the principles of grafting, but it seems that until we are able to understand them (and I feel we do not understand them at pres-

Transplantation for Type I Diabetes

Comparison of Vascularized Whole-Organ Pancreas With Isolated Pancreatic Islets

Adam Frank, MD, Shaoping Deng, MD, Xiaolun Huang, MD, Ergun Velidedeoglu, MD, Yong-Suk Bae, BS, Chengyang Liu, MD, Peter Abt, MD, Robert Stephenson, MD, Muhammad Mohiuddin, MD, Thav Thambipillai, MD, Eileen Markmann, RN, Maral Palanjian, RN, Marty Sellers, MD, Ali Naji MD, PhD, Clyde F. Barker, MD, and James F. Markmann MD, PhD

Objective: We sought to compare the efficacy, risks, and costs of whole-organ pancreas transplantation (WOP) with the costs of isolated islet transplantation (IIT) in the treatment of patients with type I diabetes mellitus.

Summary Background Data: A striking improvement has taken place in the results of IIT with regard to attaining normoglycemia and insulin independence of type I diabetic recipients. Theoretically, this minimally invasive therapy should replace WOP because its risks and expense should be less. To date, however, no systematic comparisons of these 2 options have been reported.

Methods: We conducted a retrospective analysis of a consecutive series of WOP and IIT performed at the University of Pennsylvania between September 2001 and February 2004. We compared a variety of parameters, including patient and graft survival, degree and duration of glucose homeostasis, procedural and immunosuppressive complications, and resources utilization.

Results: Both WOP and IIT proved highly successful at establishing insulin independence in type I diabetic patients. Whole-organ pancreas recipients experienced longer lengths of stay, more readmissions, and more complications, but they exhibited a more durable state of normoglycemia with greater insulin reserves. Achieving insulin independence by IIT proved surprisingly more expensive, despite shorter initial hospital and readmission stays.

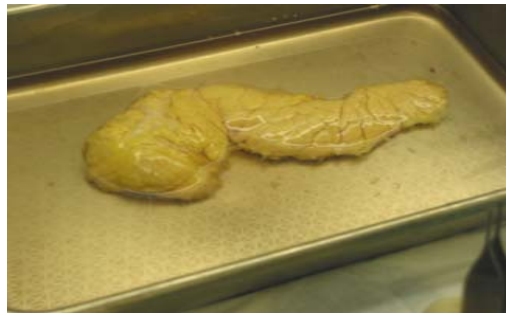
Conclusion: Despite recent improvement in the success of IIT, islets from multiple donors to gain insulin independence. Because donor pancreata that are unsuitable for WOP can often be used successfully for IIT, we suggest that as IIT evolves, it should continue to be evaluated as a complementary alternative to rather than as a replacement for the better-established method of WOP.

(*Ann Surg* 2004;240: 631–643)

Type I diabetes mellitus afflicts nearly 2 million Americans and is responsible for untold morbidity. Despite significant improvements in monitoring and administration, insulin therapy cannot fully normalize glucose homeostasis at the present time. Therefore, curative therapies for the disease have relied on replacement of the β-cell mass by transplantation. During the last 35 years, whole organ pancreas transplantation (WOP) has evolved gradually into a highly effective therapy for type I diabetic patients who are undergoing simultaneous renal transplantation.[1–5] Because the risks of severe complications of this procedure are relatively small in these patients who are already obligated to lifelong immunosuppression, the benefits of the procedure are generally ac-

Cold storage

Surgical manipulation

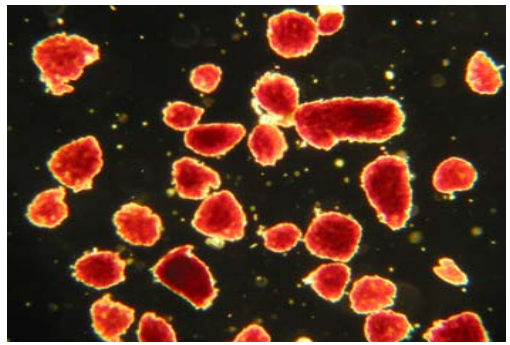


Isolation stress

Chemical stress

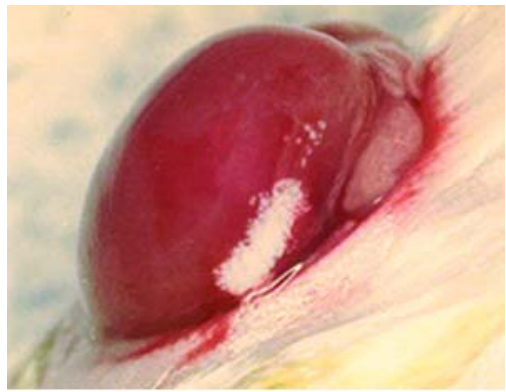
Mechanical stress

Ischemia



Non physiologic culture environment

Inadequate mass



Inflammation

Immunosuppressive drug toxicity

Rejection

Role of Isolation Enzyme?

Isolation Enzyme Collagenase

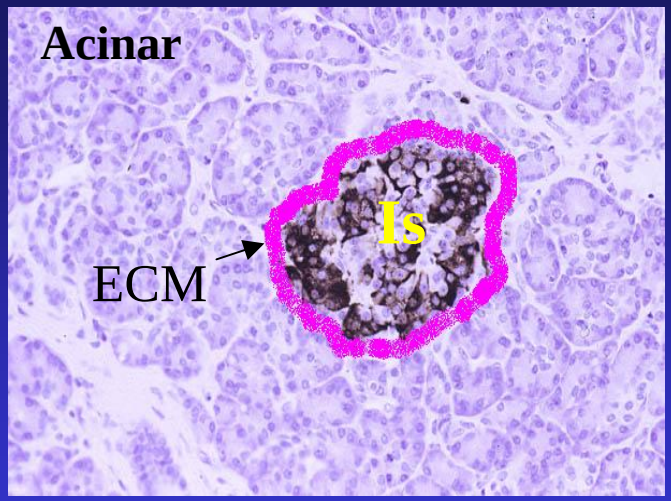
- **bacteria *"Clostridium Histolyticum"***

Sigma - crude collagenase:

- **6 different collagenases, aminopeptidase, clostripain, phospolipase-C & neutral proteases**

Roche – purified collagenase -- Liberase™

- **Collagenase Type-I, Type-II and Thermolysin**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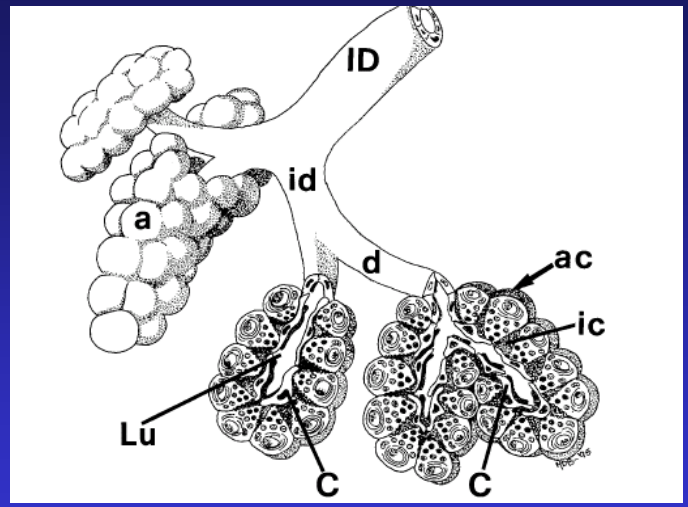
Is
ECM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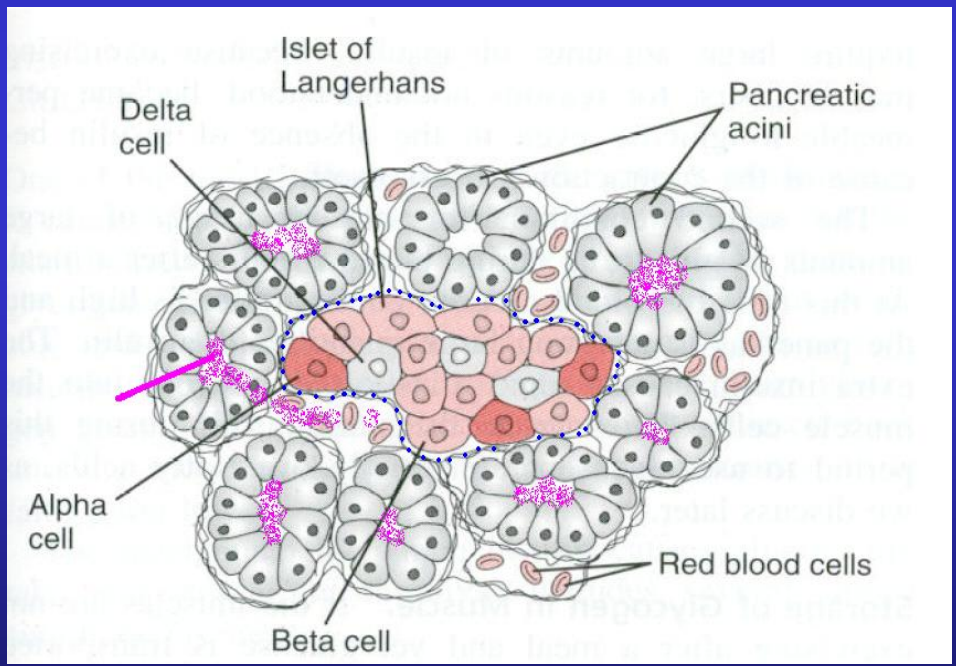
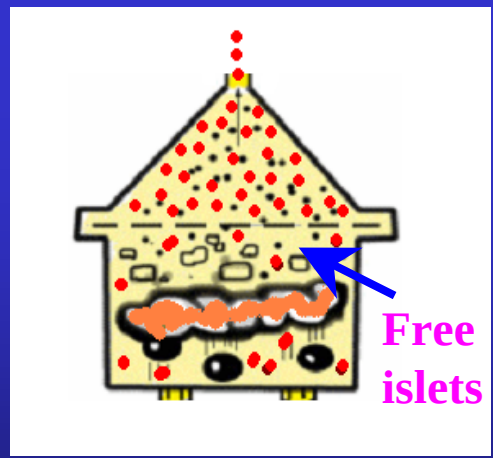

Islet of Langerhans
Delta cell
Pancreatic acini
Alpha cell
Red blood cells
Beta cell


Free islets

Experimental Design

Fluorescent Liberase-HI

- *FITC conjugated Liberase-HI (Molecular Probe)*
- *Confocal Fluorescence Microscope*
- *Immuno Electron Microscope*

Roche Liberase™ +

Insulin Secretory Capability

- *Time course exposure and culture - Basal insulin secretion*
- *Stimulated Insulin Secretion - Dynamic glucose challenge and KCL stimulation*
- *Insulin C-timer [transgenic mouse] islets - visualization of proinsulin granules*

Islet cell expressions

- *Adhesion molecules (CD 106 and CD 62p)*
- *Apoptotic and anti-apoptotic molecules (Bax, Bcl-2)*

In vivo Transplantation

- *Graft function*
- *CD 11b deposition*

Fluorescent (FITC) Conjugated Liberase-HI

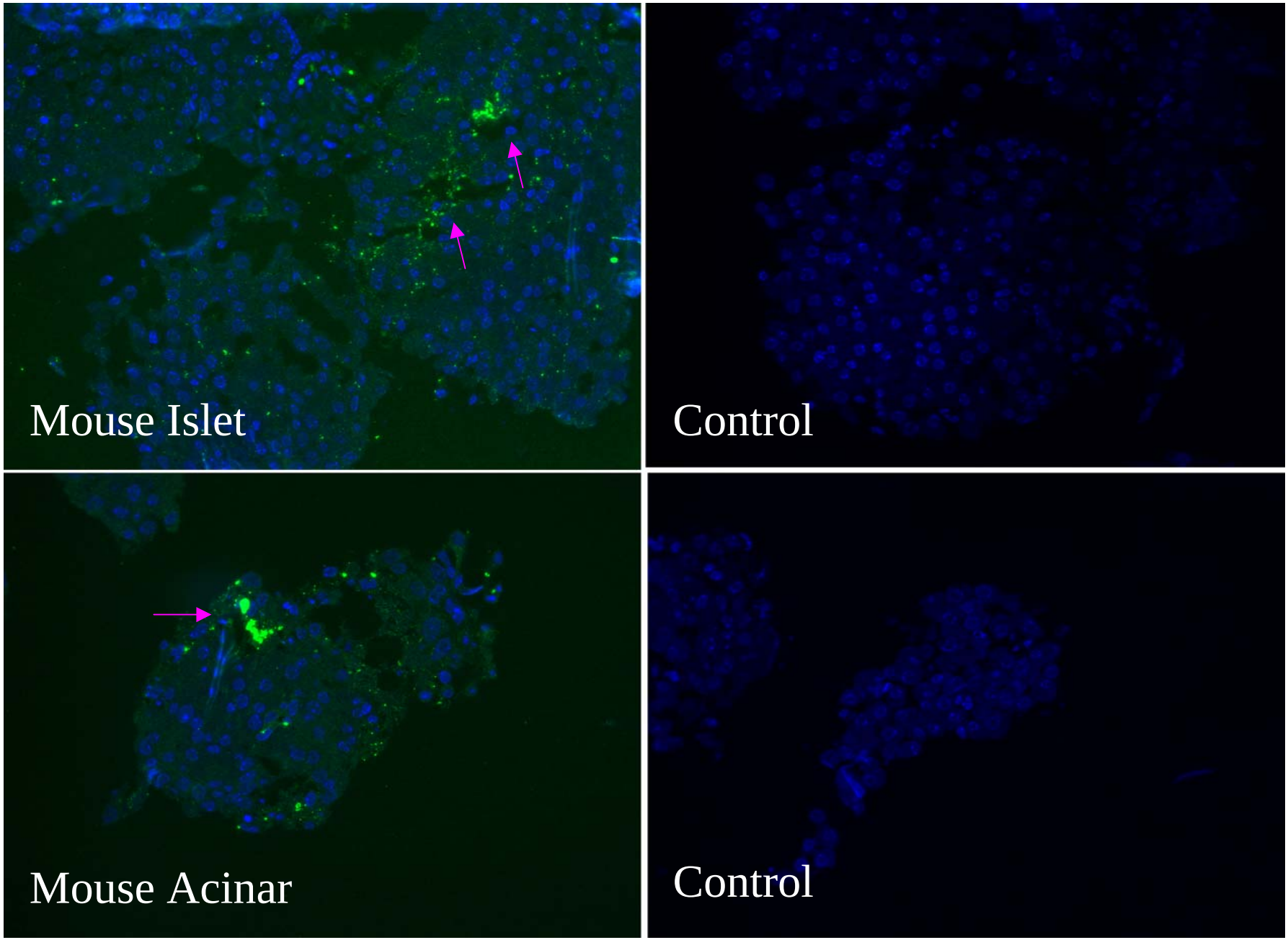
*Mouse islets -- intraductal injection of **FITC** conjugated Liberase and isolation of islets (n=4 donors)*

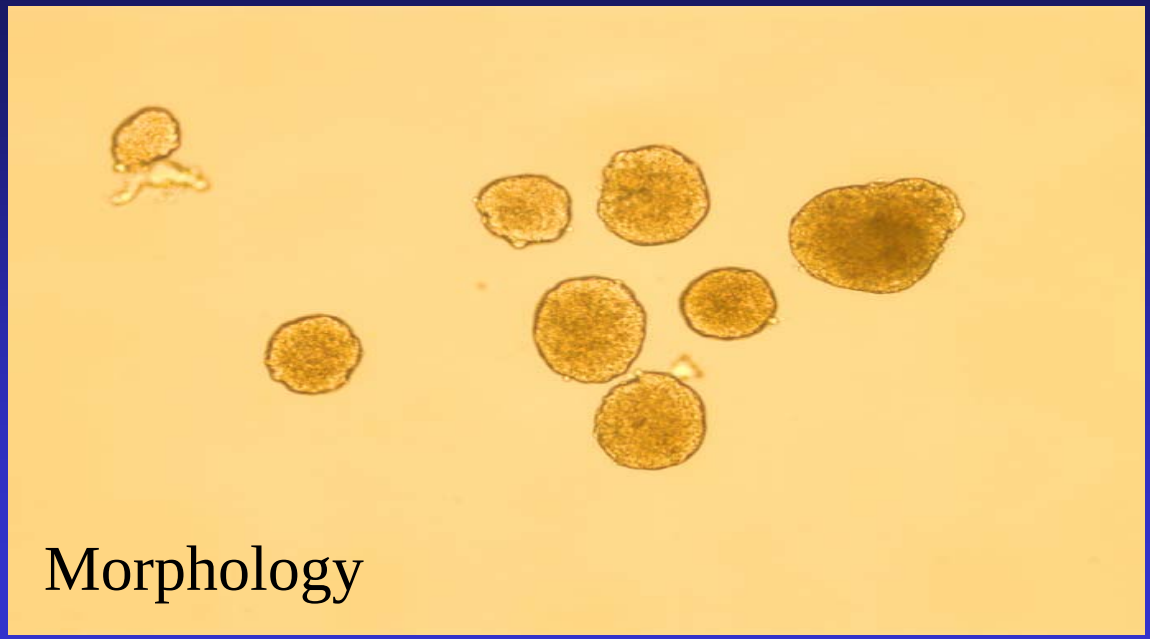
*Human islets -- one hour exposure of **FITC** conjugated Liberase in vitro (n=8 donors)*

Confocal Fluorescence Microscopic &

Immuno Electron Microscopic Examinations

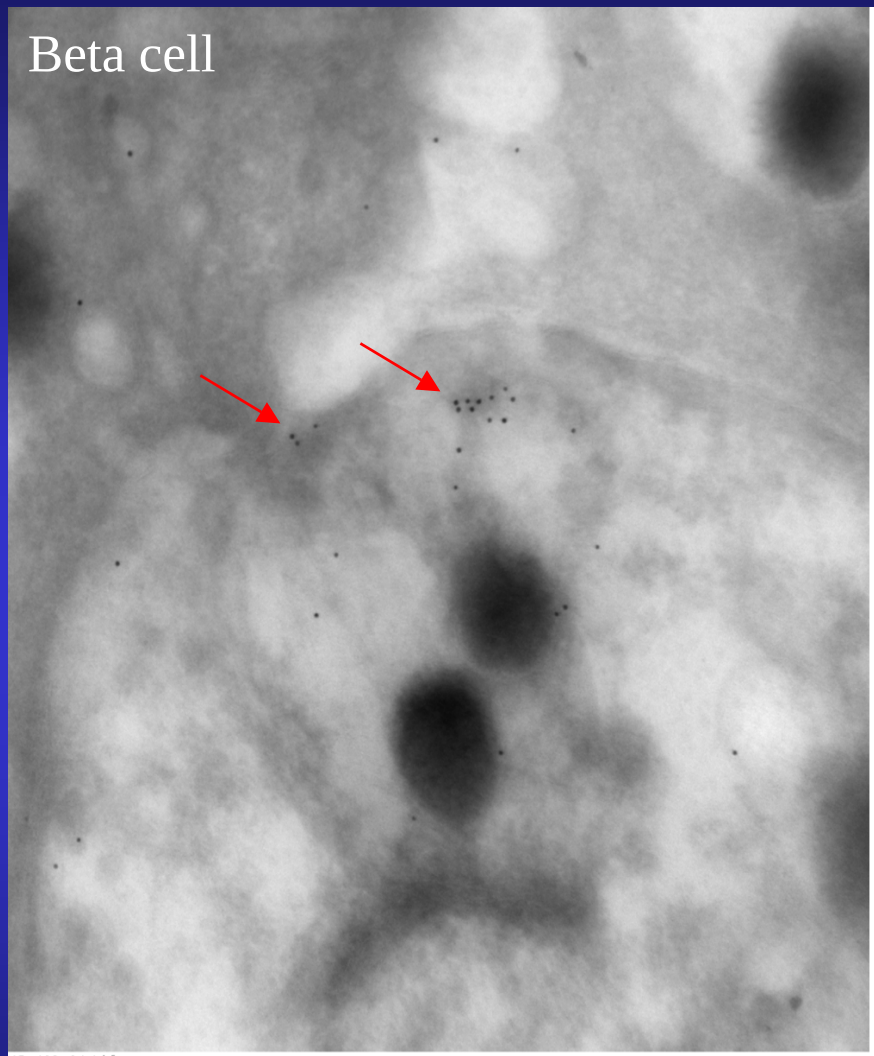
- *Fresh islets and acinar cells*
- *3 days cultured islets and acinar cells*
- *Epifluorescence double staining with insulin*


Mouse Islet
Control
Mouse Acinar
Control



Morphology

Viability ***(Calcin-AM, P.I. Stain)***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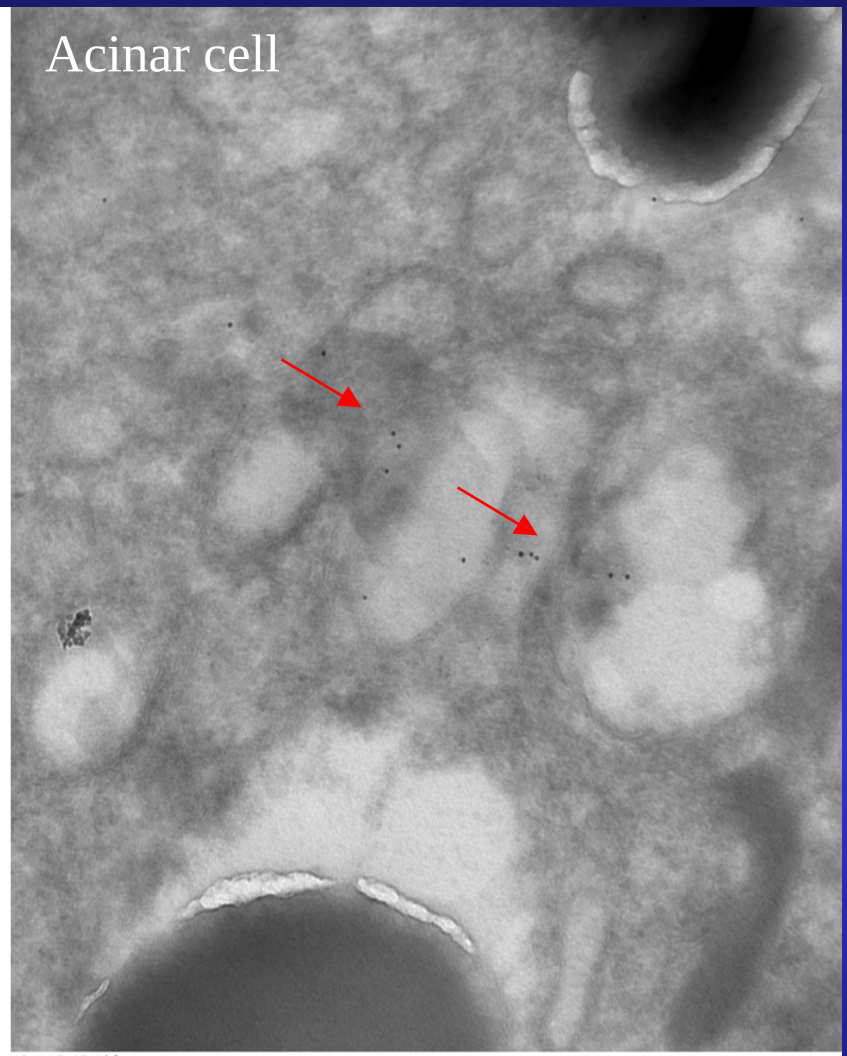
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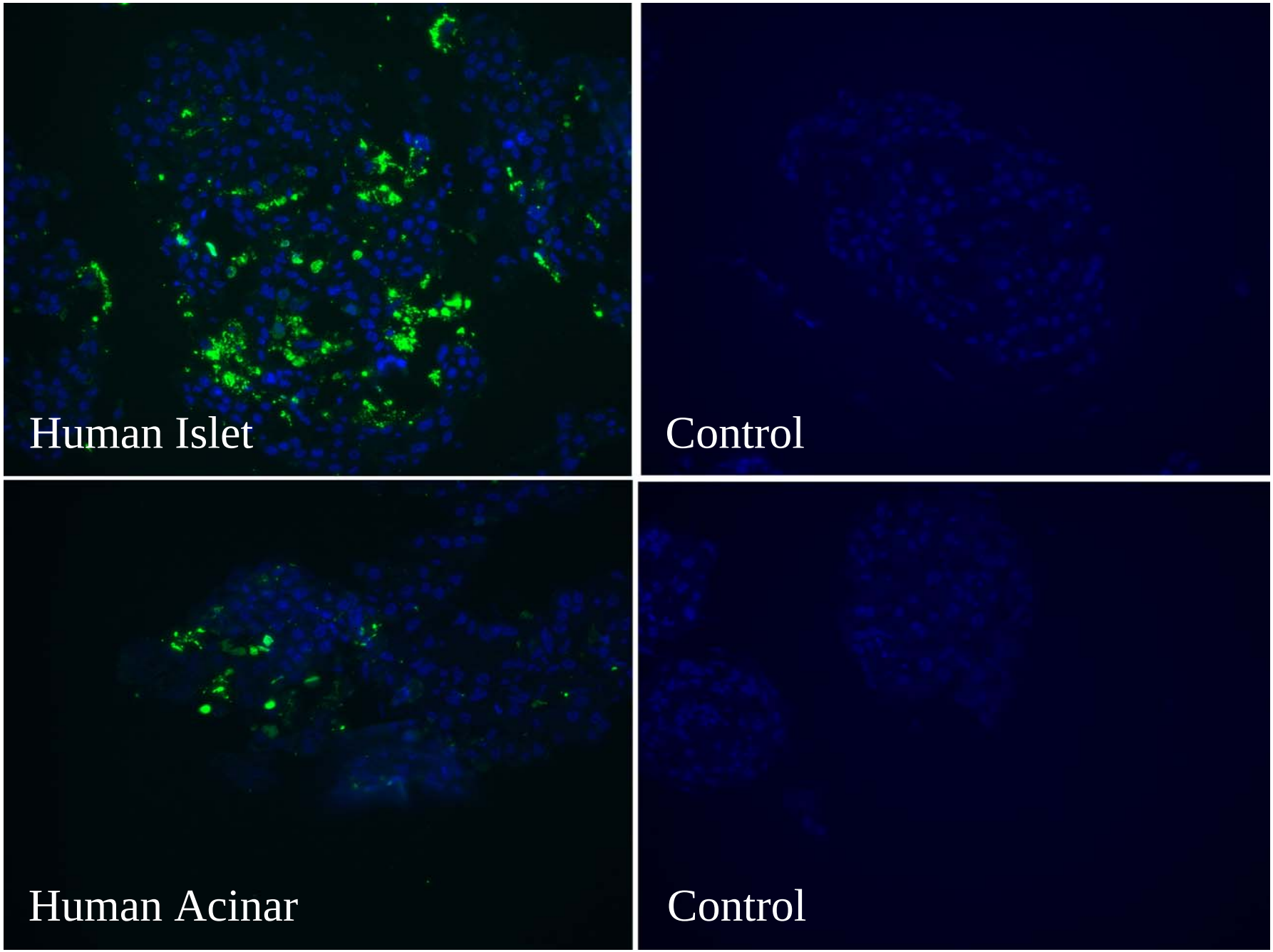
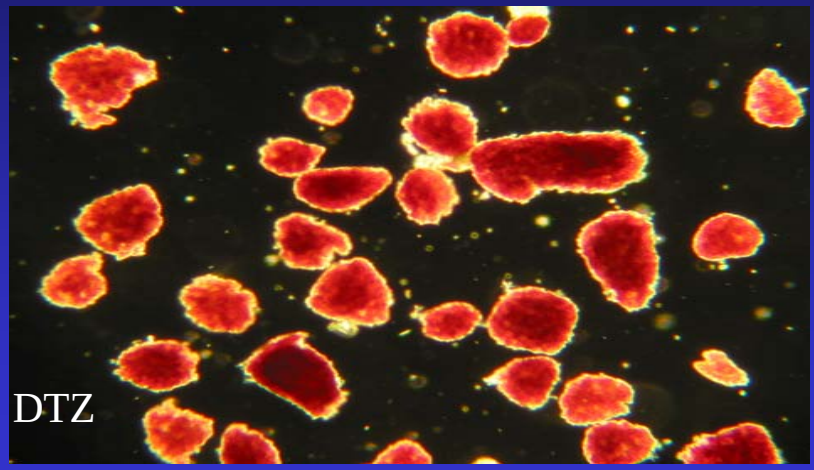

Human Islet
Control
Human Acinar
Control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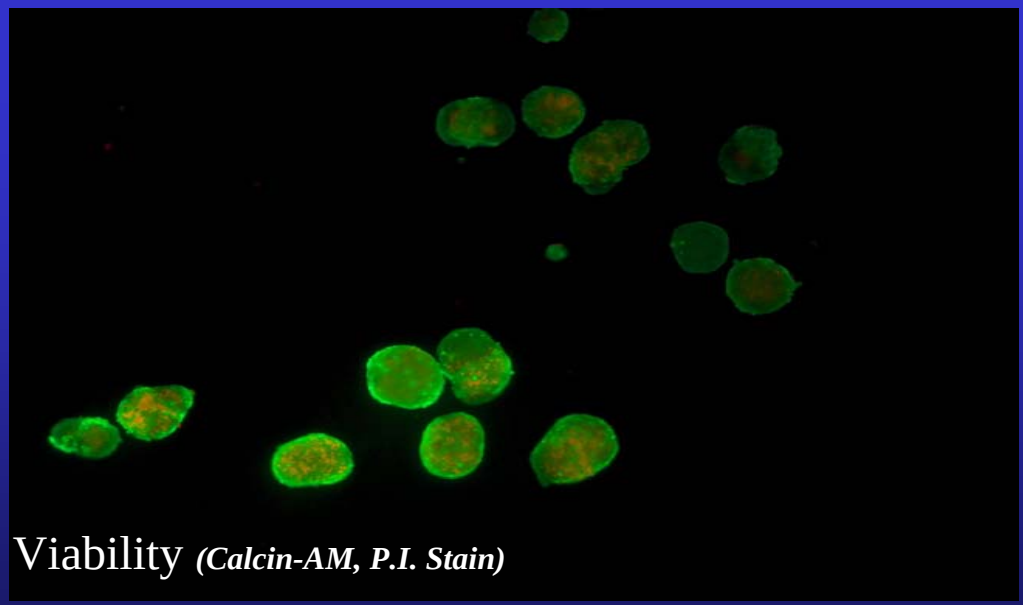
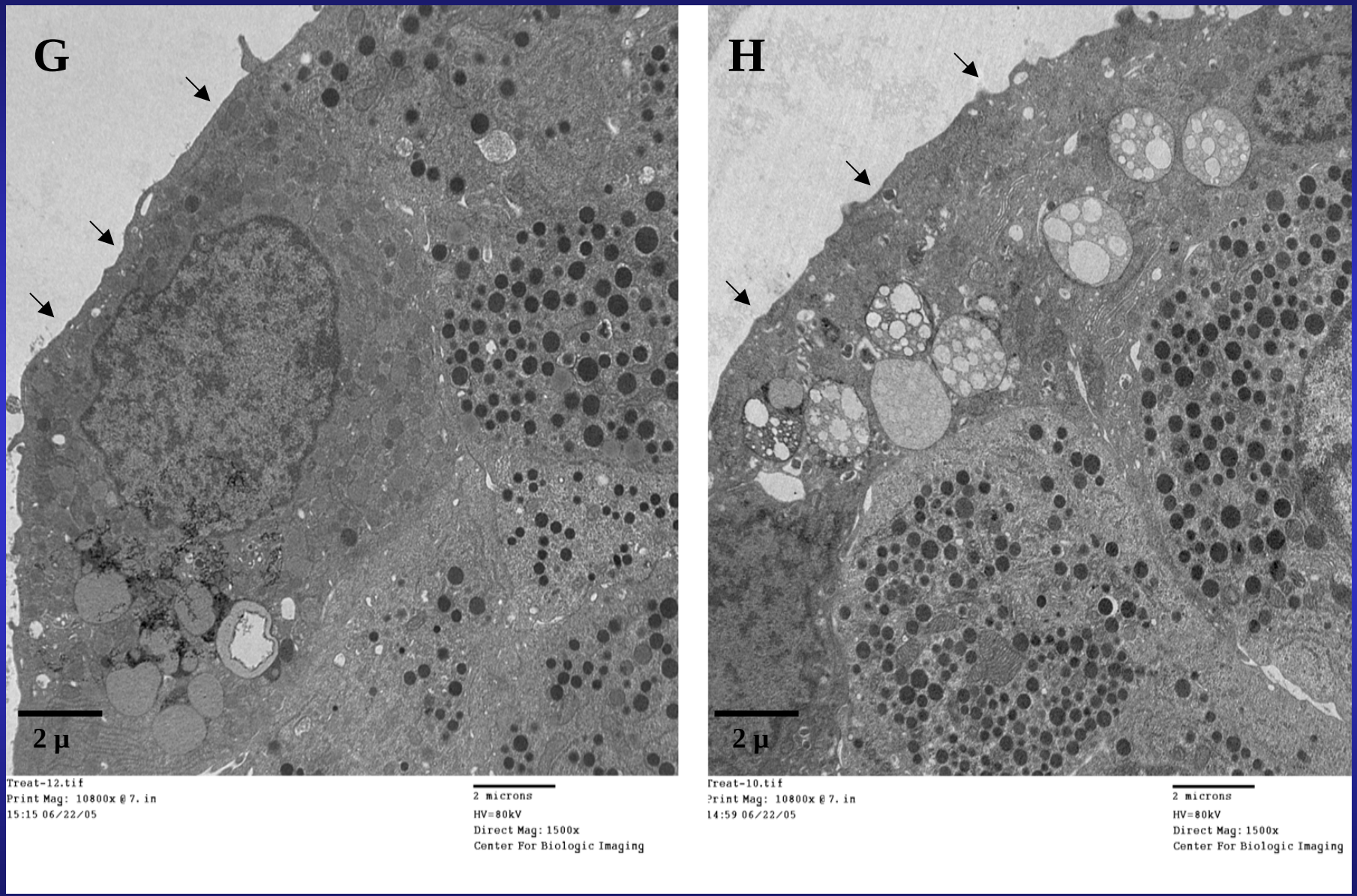
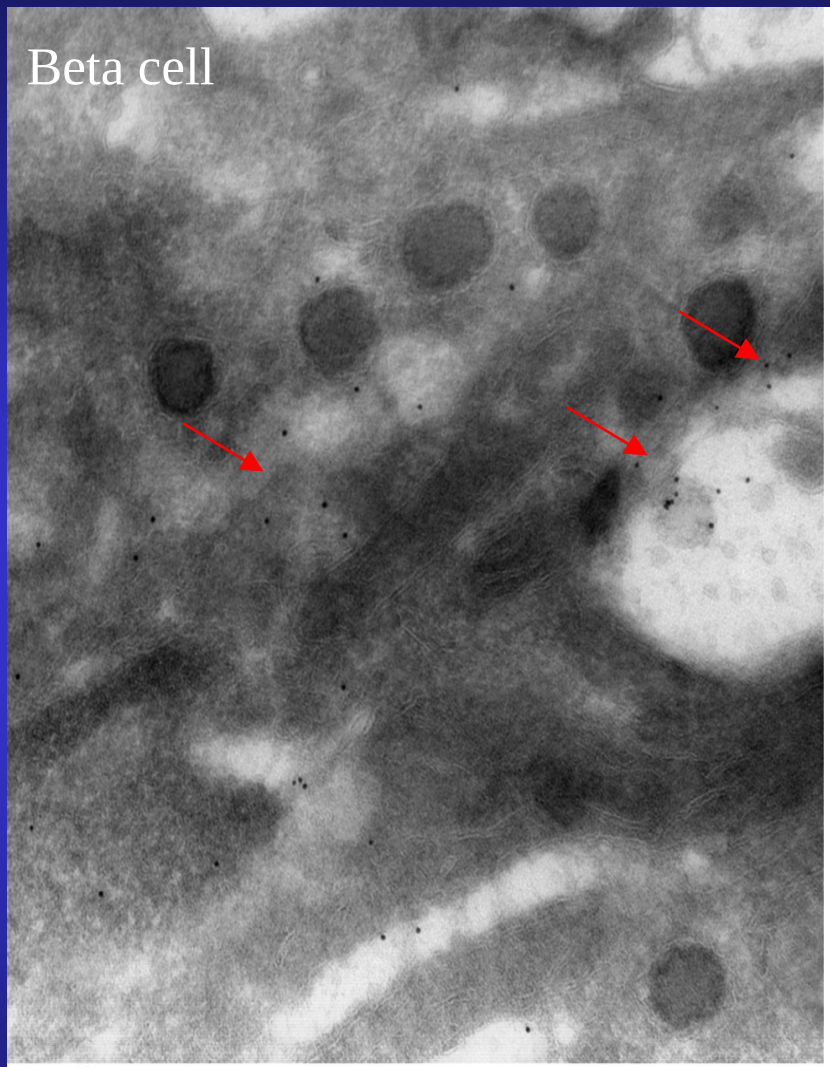

Viability *(Calcin-AM, P.I. Stain)*

Control-Hank's exposed islet

Liberase exposed islet

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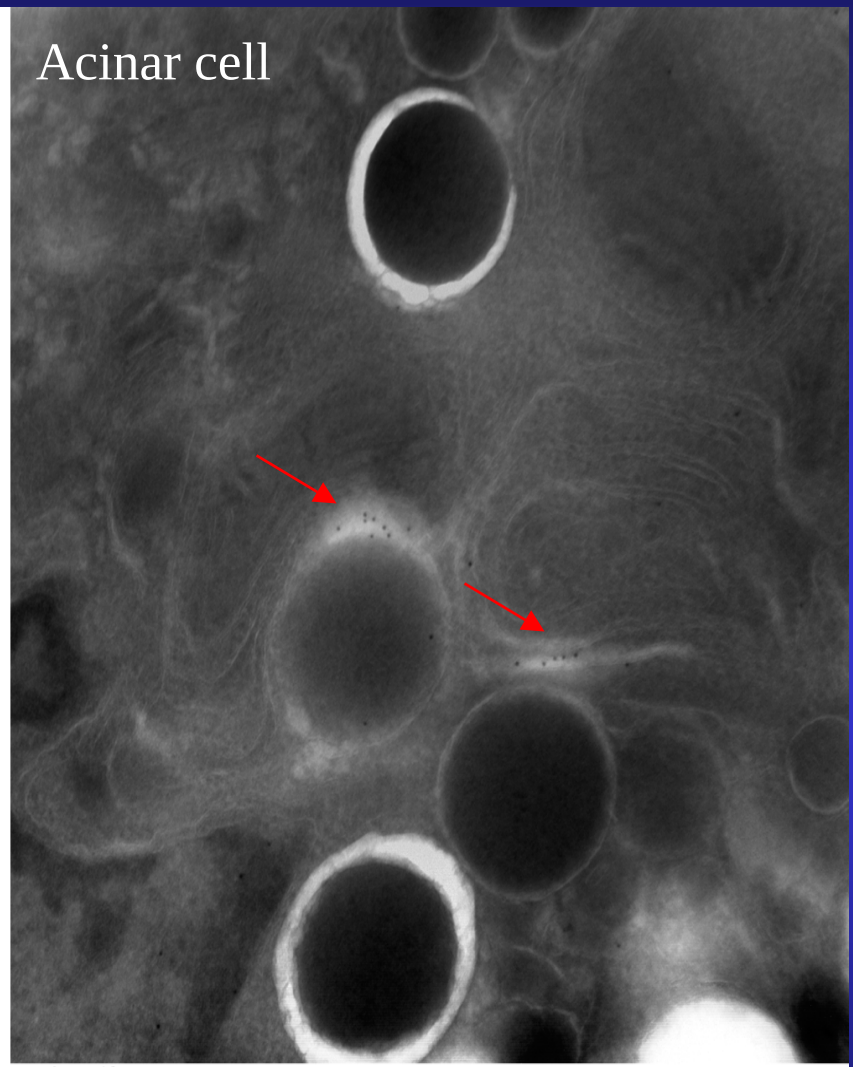
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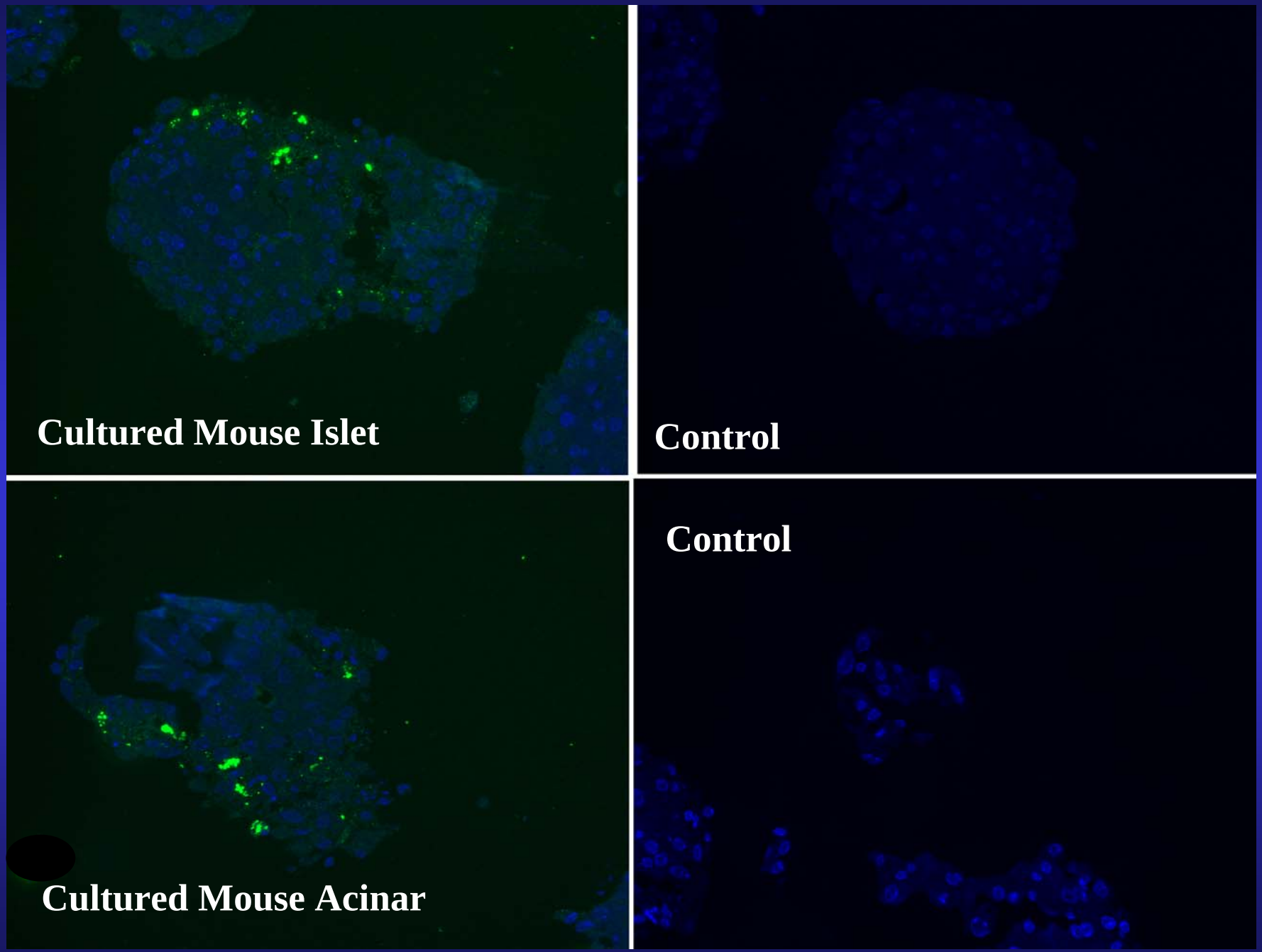
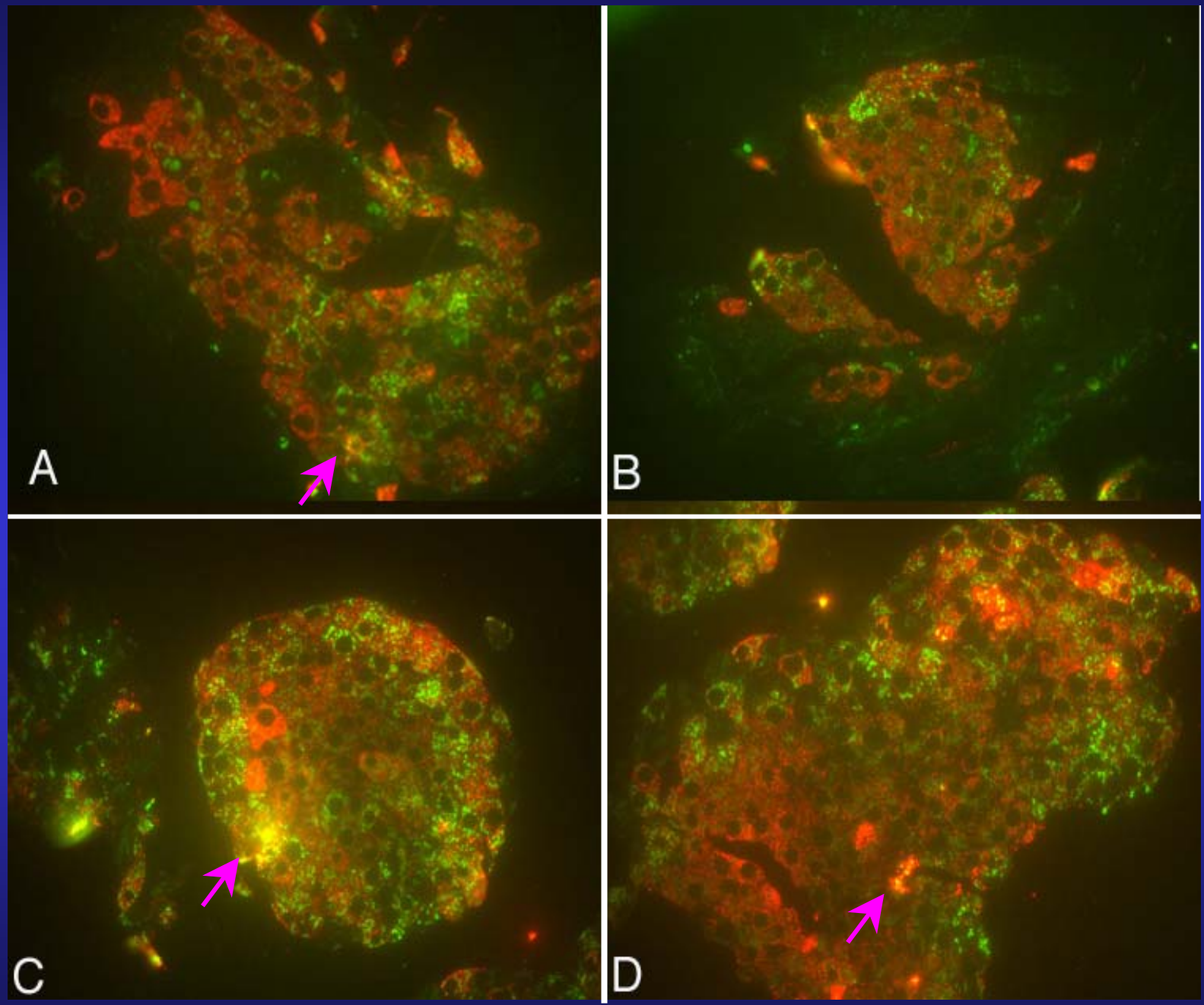

Cultured Mouse Islet
Control
Cultured Mouse Acinar
Control

Fresh

Cultured



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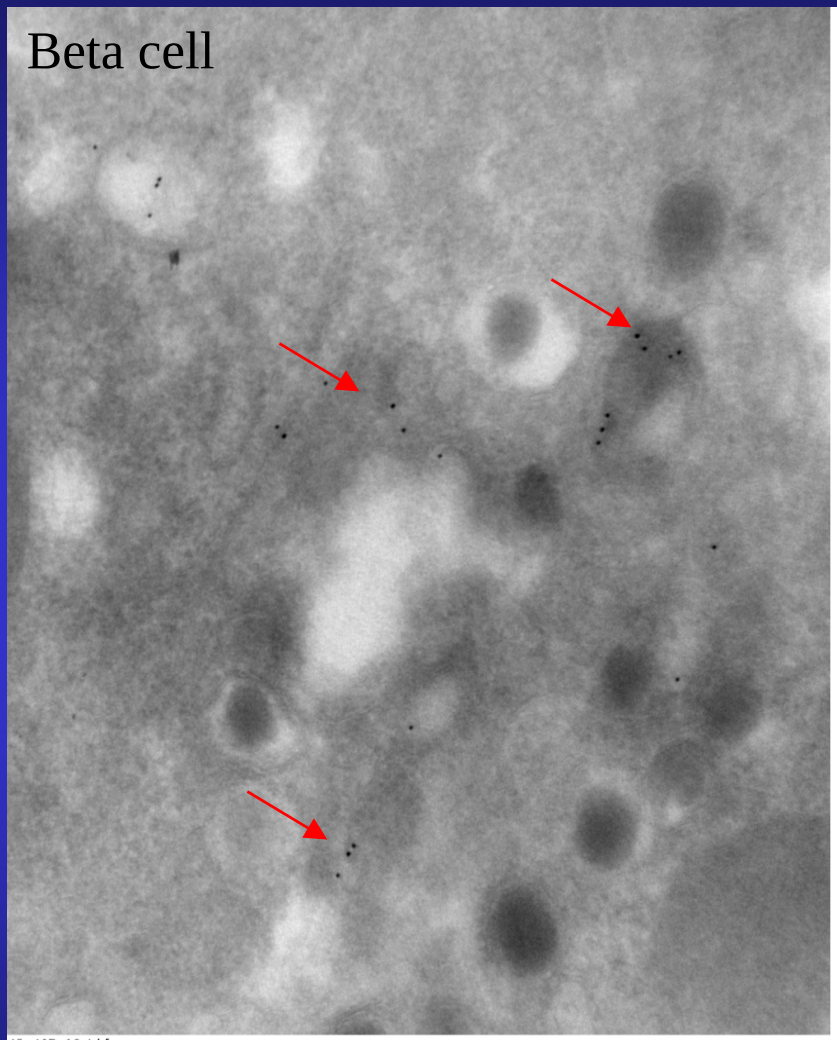

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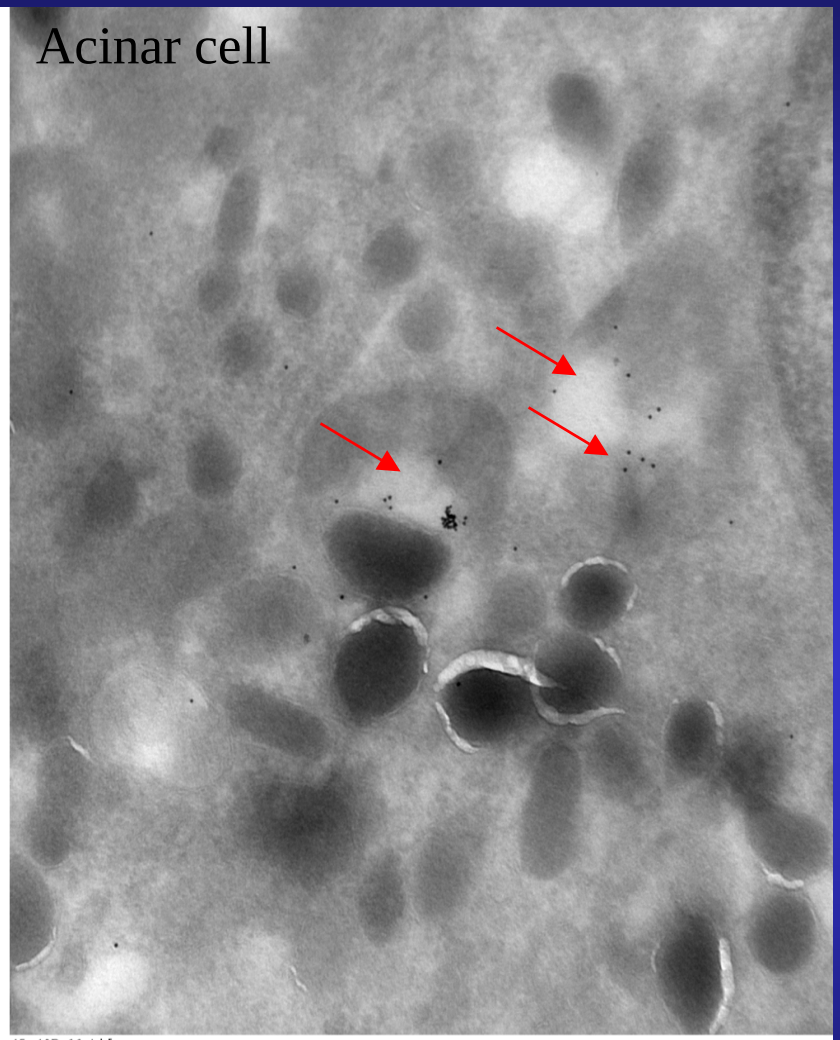
Acinar cell



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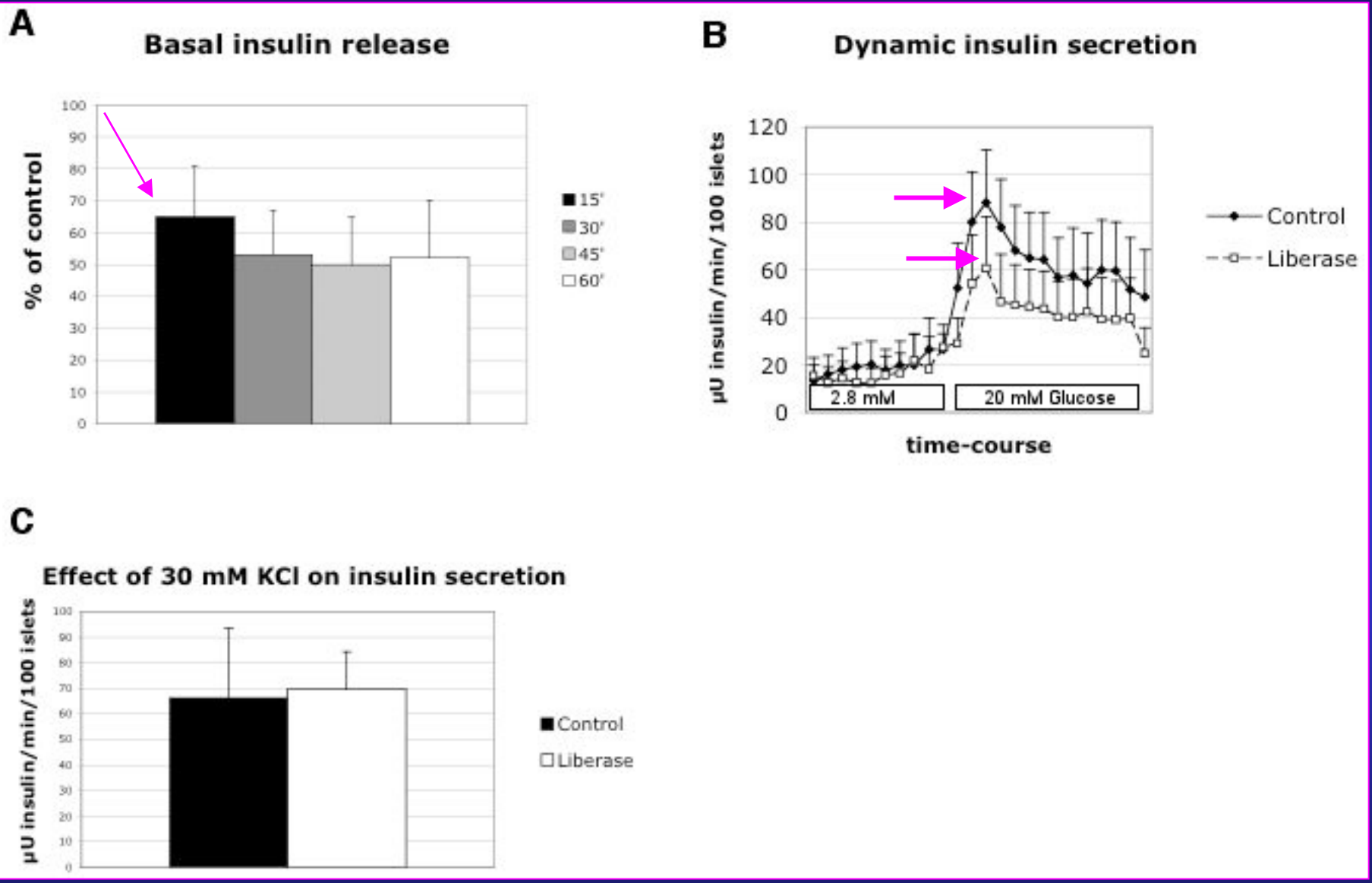
Center For Biologic Imaging

Effect of enzyme exposure on insulin secretion

- *Human islets were exposed to Liberase for 15, 30, 45 or 60 minutes and washed and cultured for 60 hours – basal insulin secretion (n=6donors)*
- *1 hour exposure of human islets to Liberase – Dynamic glucose challenge*
- *1 hour exposure – KCL stimulation (n=4 donors)*
- *Exposure of Insulin C-Timer Transgenic mouse islets – proinsulin visualization (color change)*

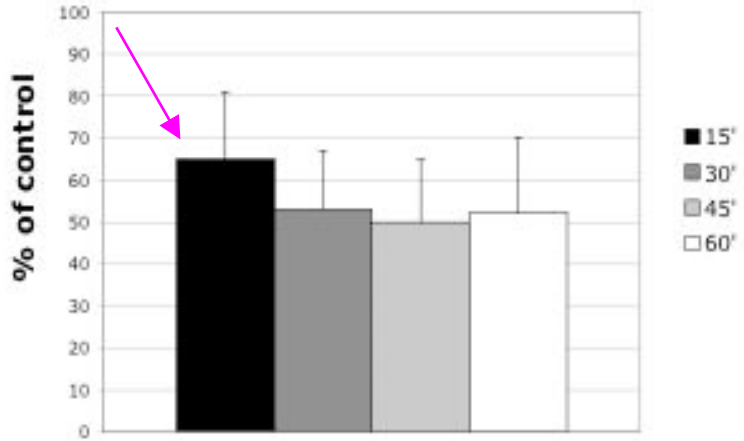
Insulin Secretory Defect



Insulin Secretory Defect

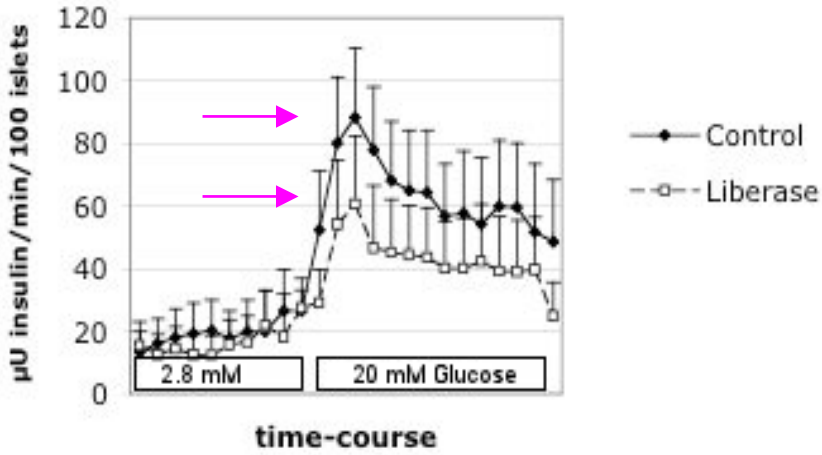
A

Basal insulin release



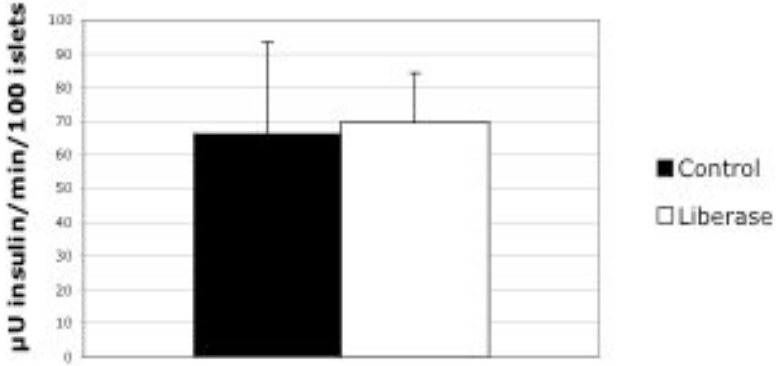
B

Dynamic insulin secretion

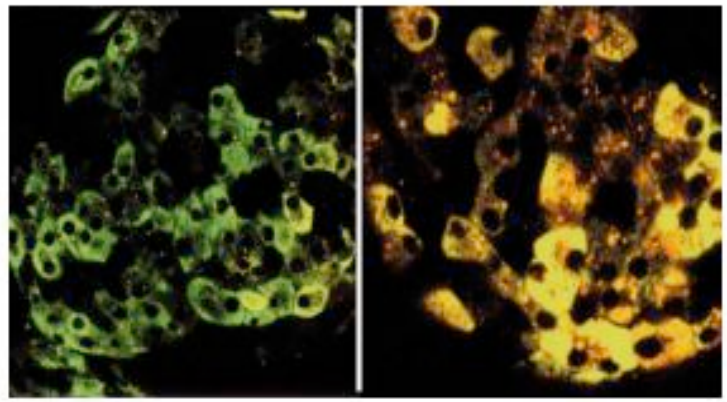


C

Effect of 30 mM KCl on insulin secretion



D Control Liberase



Insulin Retention in Islets

Effect of enzyme exposure on islet cell expressions

Human islets were exposed to Liberase for 1 hour and cultured for 60 hours (n=6 donors)

- *islet cell attachment to the dishes*

- *adhesion molecules expressions*

V-CAM-1 [CD 106], P-selectin [CD 62p]

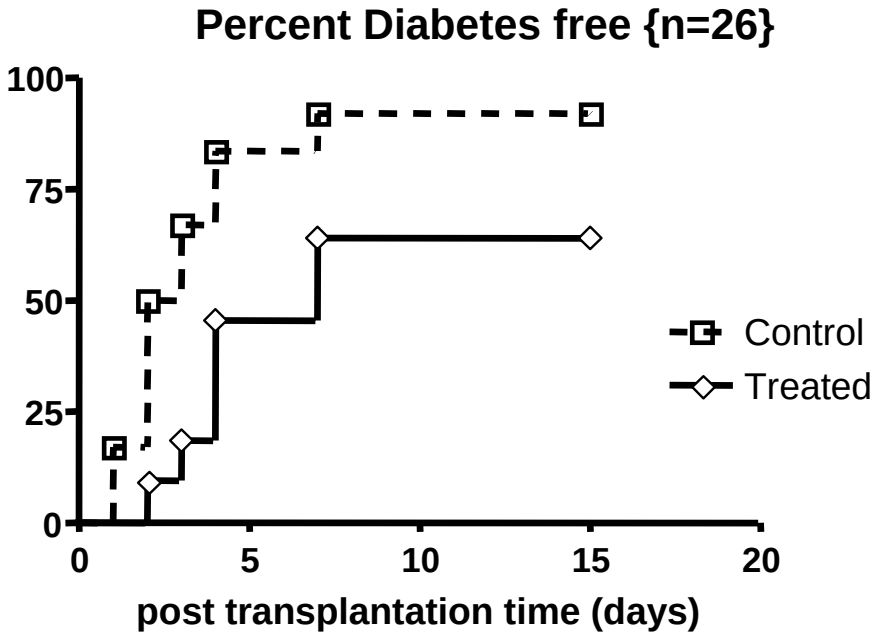
- *apoptotic and anti-apoptotic molecules (Bax, Bcl-2)*

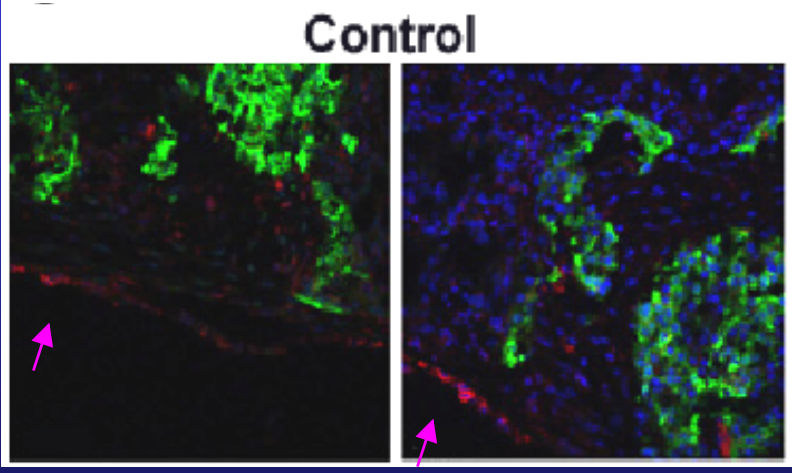
Cycloheximide (protein synthesis inhibitor) treatment – prevents cell attachment and adhesion molecule expressions

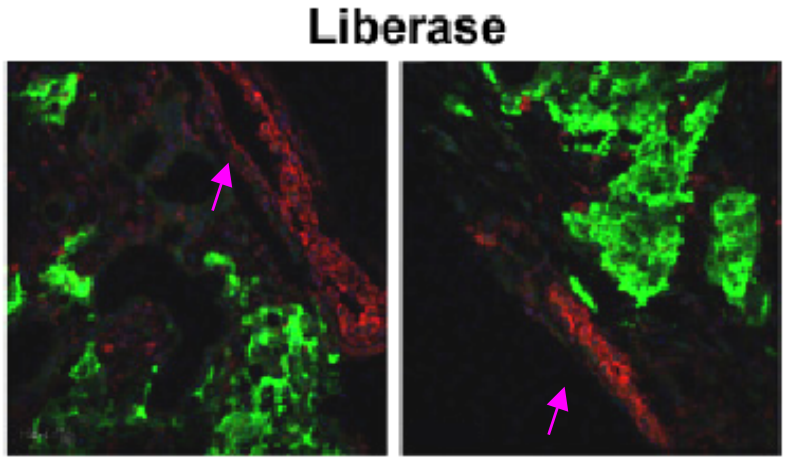
Effect of enzyme exposure on transplanted islets

Liberase exposed (1 h) Human islets were transplanted (n=26)

- *150-400 islets/graft in NODscid mice (Streptozotocin induced, renal capsule site)*
- *Graft survival and recipient survival*
- *CD11b (marker of inflammation) deposition In graft area*


Percent Diabetes free {n=26}
100
75
50
25
0
0
5
10
15
20
post transplantation time (days)
Control
Treated


Control


Liberase

Summary

- *Isolation process internalizes the enzyme particles in islet and acinar cells*
- *3 days cultured islets also contained the particles*
- *Reduction in insulin secretion correlated with time of enzyme exposure (secretory defect)*
- *Adhesion molecules were expressed and apoptotic pathways were activated in islets*
- *Enzyme exposed islets recruited intense inflammatory cells*

Conclusion

Isolated islets carry potentially unwanted isolation enzyme by-products and reducing the exposure to enzyme is crucial

Suggestions

- *Collection of islets in UW solution during isolation (collection phase) possibly prevents further activation of enzyme (further exposure)*