

VIABILITY OF FRESH vs CULTURED ISLETS FOR TRANSPLANTS

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Pig islet work is problematic

- Pig islets are generally difficult to
 - isolate intact
 - recover during purification
 - preserve during in vitro culture
- Transplants of pig islets have been shown* both
 - not to cure STZ-diabetes in nude mice, or
 - only after ~ 2months,
 - or within days
- ? What about the role of islet viability ... —>

* Heald et al. Acta Diabetol 35: 238, 1998
Davalli et al. Diabetes 44: 104, 1995
Brandhorst et al. Exp Clin Endocrinol 103: 3, 1995

The role of the viability of isolated islets, in the outcome of culture or grafting...

- has received little attention
- We isolated islets from 12 large sows by Liberase digestion, dispersion in cold UWS, and OptiPrep-UWS* purification
- The viability of islets was studied both immediately after isolation ('fresh islets'), and after culture for 1 day or 1 week (in RPMI + pig serum, 37°C), by comparing:
 - islet recovery
 - acridine orange / propidium iodide staining (AOPI)
 - grafting ~ 750, ~1500, or ~3000 IEQs SRC in nude mice

* Working OptiPrep-UWS will be produced by Nycomed within months

ISOLATION AND CULTURE RESULTS

	FRESH ISLETS	1-DAY CULTURE	1-WEEK CULTURE
ISLET # (IEQs)	1924 \pm 346		
PURITY (%)	96 \pm 2	~ 100	~ 100
RECOVERY (%)	(100)	21 \pm 4	11 \pm 2
DIAMETER (μ m)	162 \pm 13	124 \pm 6	113 \pm 4
VIABILITY (% AO)*	83 \pm 2	87 \pm 2	90 \pm 2

* viability staining of fresh islets by AOPI does not really help in predicting recovery during culture

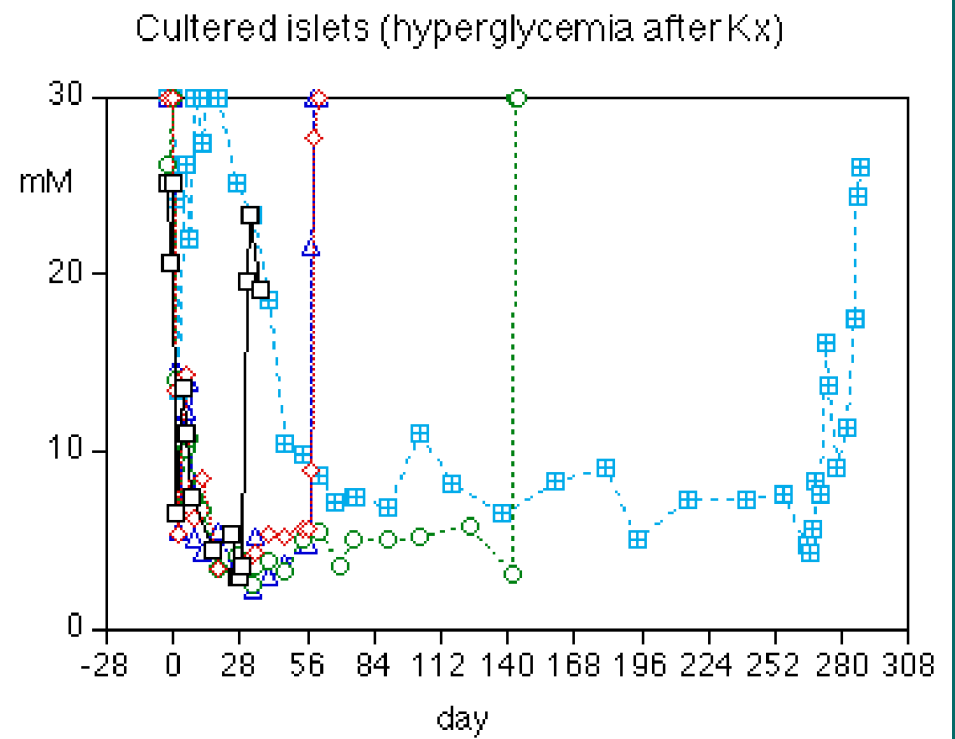
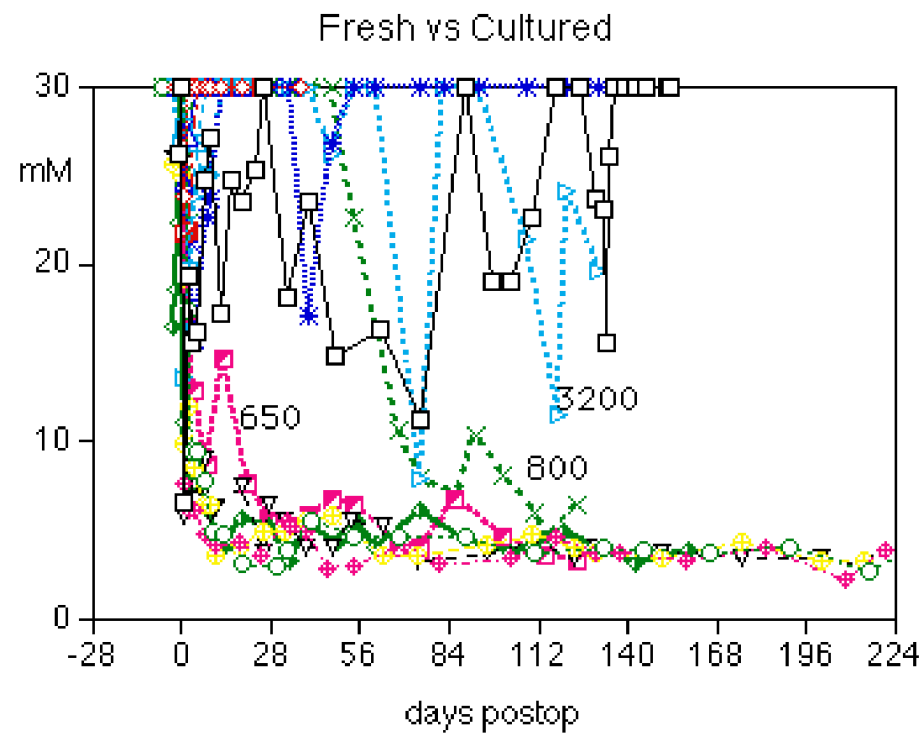
TRANSPLANT RESULTS OF FRESH vs 1 DAY OR 1 WEEK CULTURED ISLETS

CULTURE	DOSE	MICE* <i>n</i>	FAILURE		SUCCESS (<10.7 mM)		
			<i>n</i>	Glycemia	<i>n</i>	Days to normo	Glycemia mM
NO	1500	14	13**	> 24 mM	1	1	7.9
	3000	4	4**	> 25 mM			
1 DAY	750	8	3	> 25 mM	5	27 (8 - 61)	6.6 (4.2 - 10.1)
	1500	9			9	15 (1 - 53)	6.1 (4.1 - 10.6)
1 WEEK	750	4			4	56 (21 - 111)	7.4 (5.4 - 8.5)
	1500	9			9	2 (1 - 4)	5.0 (4.2 - 6.2)

* Swiss nu/nu mice received STZ 220 mg/kg at 5-7 days before Tx
normal random blood glucose in 15 animals: 5.3 - 10.7

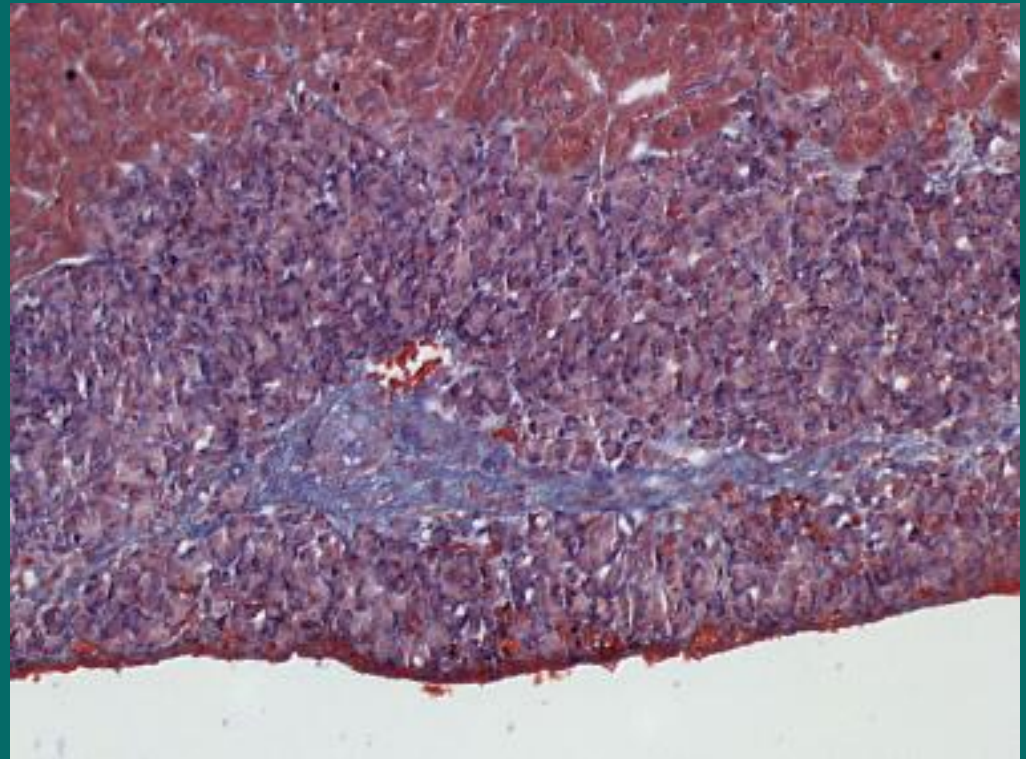
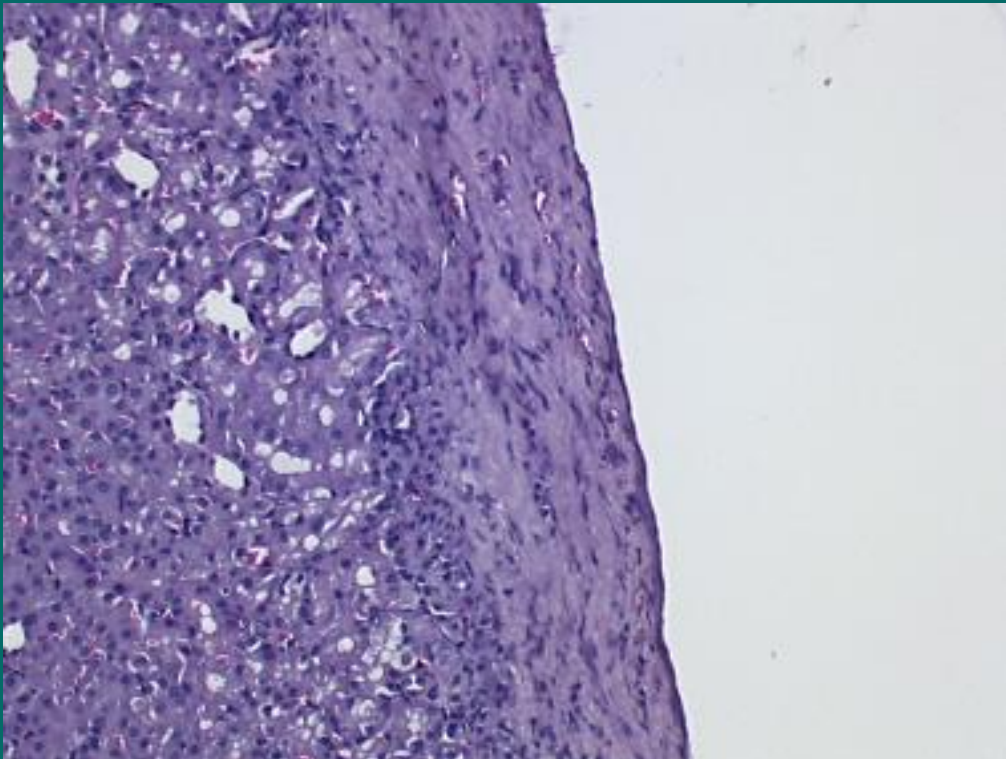
** Glycemia usually > DL of 28 mM; usually killed at ~ 3 wk for severe diabetic complications

GRAPHS



HISTOLOGY

- Substantial scarring and ~ no islets found in fresh islet recipients
- Well-preserved islets and little scarring in recipients of cultured islets



WE CONCLUDE:

- Culture of the islets before transplantation markedly improved the viability of the graft
- Disintegration of part of the fresh islets
 - may not only reduce the effective islet dose
 - but also hamper the engraftment of viable islets
 - » because of scarring, and
 - » because the cellular debris probably will attract macrophages and induce the release of harmful cytokines