

Introduction

In this study, a baseline stereological evaluation of untreated nonhuman primate pancreas was conducted to determine β -cell volume and α -cells and β -cells counts. Data collected from this study could subsequently provide valuable information for diabetes research and other pharmaceutical applications. This stereological approach demonstrates an initial representation of the quantification of cells in the pancreas and the variation of α -cell and the β -cell quantities and volume.

Methods

Pancreatic tissues from selected paraffin blocks from 4 control nonhuman primates were processed into 2 pairs of consecutive thin (2-3 μ m) microscope slides. Each consecutive slide set were stained with glucagon (for α -cell determination) or insulin (for β -cell determination) using standard immunohistochemical (IHC) methods. The IHC-stained nonhuman primate pancreases tissue slides were scanned on an Aperio ScanScope[®] XT scanner at 40X magnification and images hosted on a dedicated and secure Flagship Biosciences server.

The stained and scanned images were analyzed using the Visiopharm NEWCast stereology software.

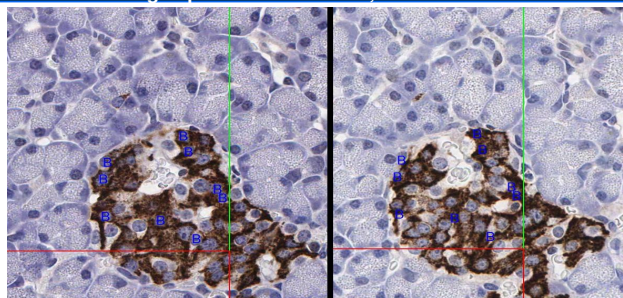


Figure 2: Determination of cell hits. Figure 2A (top row) demonstrates the top right corner of a counting frame, showing the "look up" section on the left, and the "reference section" on the right. Markers are "A" for reference tissue hits, and "B" for β -cell hits. Figure 2B (bottom row) illustrates volume grid overlaid on tissue image; showing half-circle "A" marker for reference tissue, and "B" for β -cell hits.

Estimation of β -cell volume. Prior to analysis, the sections were cut into consecutive sections 7 units apart (2-3 μ m). A zero-dimensional point grid probe was randomly translated across the tissue image in an 8x6 pattern for cell volume, and a 2x2 group pattern for reference volume. The tissue images were then sampled, with approximately 200 sampling views per slide. In conducting the analysis, a 'count' was made for every cell hit, and reference volume hit. With the determined number of β -cell hits, the Cavalieri equation was used to calculate approximate β -cell volume (Table 1).

Estimation of β -cell and α -cell count. For estimation of cell counts, the physical disector principle was used with a 3-dimensional counting probe for each consecutive section. The tissue section images were precisely aligned and the regions of interest were sampled at a constant percentage, and a counting frame added to each sampling view. The consecutive tissue section images were viewed next to each other, left being the "Reference section" and the right the "Look-up section". Target cells that were present in the "Reference section", and absent in the "Look-up section" were counted. After the tissue images had been sampled, numerical density was calculated and multiplied by the pancreas reference volume to yield an estimated numerical count of cells (Tables 2 and 3).

Results

Table 1: β -cell Volume

Animal	β -cell	Pancreas	N	% Volume
1	1,835	7,461	8,618,803 μ m ³	7.20%
2	1,234	5,119	4,220,477 μ m ³	7.00%
3	657	2,463	3,657,861 μ m ³	4.20%
4	1,589	5,389	712323 μ m ³	8.40%

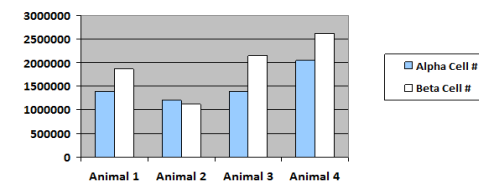
Table 2: α -cell Count

Animal	α -cell	Pancreas	N
1	230	424	1,384,067
2	198	427	1,204,257
3	173	302	1,388,538
4	202	200	2,040,134

Table 3: β -cell Count

Animal	β -cell	Pancreas	N
1	517	315	1,865,998
2	228	81	1,120,080
3	294	82	2,140,050
4	338	77	2,620,091

Stereological Analysis and Comparison of Primate Beta and Alpha Cell Count



In the estimation of β -cell volume, a mean volume of 6.7%, and a range of 4.2%-8.2% per pancreas was established. In the calculation of α -cell count, a mean of 1,504,249, and a range of 1,204,257 - 2,040,134; and for β -cell count, a mean of 1,936,555 with a range of 1,120,080 – 2,620,091 were determined.

Advantages in Stereology

This study provides a baseline determination for α -cell and β -cell count quantity and β -cell volume in the primate pancreas. In the determination of whole organ quantitative data such as volume, surface area, length, and number, stereology has proven to be a reliable method.

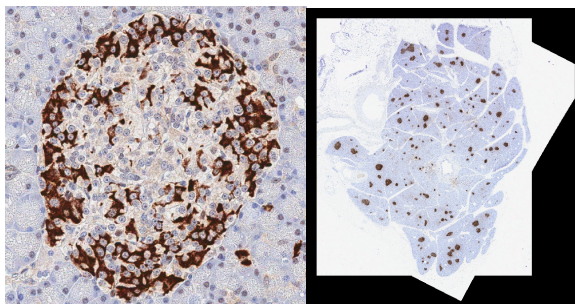


Figure 1: Figure 1A (left) 40X image of a Islet of Langerhans, containing IHC staining for insulin (β -cells). Figure 1B (right) demonstrates two consecutive images of pancreas tissue being overlaid and precisely aligned in the preparation for β -cell counting.