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ORIGINAL ARTICLE

An Immunocytochemical Profile of the Endocrine Pancreas Using an Occlusive Duct Ligation Model

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ABSTRACT

Context
Ligation of the pancreatic duct, distally to its confluence into the bile duct has been shown to induce endocrine tissue regeneration. The surplus endocrine tissue formed is presumed to be able to replace pathologically and/or experimentally compromised tissue.

Objective
This is a quantitative study on the histology of duct ligated pancreas employing immunocytochemistry and computerised morphometry.

Interventions
Pancreatic duct ligation was performed on 25 groups of six normal Sprague-Dawley rats. Experimental animals were sacrificed at 12-hour intervals from day one to ten post-duct ligation and every 24 hours thereafter to day 14, the pancreas removed, fixed and processed. Six consecutive 3-6 micron serial sections were cut on a rotary hand microtome, floated onto 3-aminopropyl-trimethoxysilan coated slides and alternatively immunocytochemically stained for insulin, glucagon, pancreatic polypeptide and somatostatin.

Results
Pancreas transformation between days ½ and 3½ was characterised by acinar deletion and the appearance of immunoreactive cells for the primary endocrine hormones. Transdifferentiation of existing endocrine tissue saw islet insulin core cells replaced by pancreatic polypeptide- and somatostatin positive cells, glucagon deletion and random appearance of all endocrine cell types within the inter-islet interstitium by day 3½. Days 4 to 14 were characterised by cellular migration and islet reconstruction.

Conclusions
To date our laboratory has investigated transplantation of foetal tissue beneath the renal capsule in syngeneic, isogeneic and allogeneic normal and diabetic rats. As pancreatic duct ligation induces the development of surplus endocrine tissue our next step would be to investigate the use of ligated pancreas as a replacement for foetal tissue.

INTRODUCTION

The scientific community recognises the potential significance of inducing pancreas endocrine cell regeneration in the treatment of diabetes mellitus [1]. Study groups, using a variety of experimental techniques, have found evidence which suggests that it might be possible to stimulate change in the pancreas thereby potentially inducing an increase in insulin secreting tissue [2, 3, 4, 5, 6, 7, 8, 9, 10].

Controversy however surrounds the precise cell developmental sequence during normal embryonic development and after experimental intervention. Also in contention is whether the endocrine proliferation, shown to occur after experimental intervention in laboratory rats, follows the same developmental sequence as that of the embryo [11, 12].

Partial (90%) pancreatectomy [13], cellophane wrapping [7], beta-cell destruction by streptozotocin [14] and pancreatic duct ligation (PDL) [4, 15, 16, 17, 18] have all been shown to induce noticeable changes in both the exocrine and endocrine pancreas. PDL has also been shown to induce endocrine proliferation in rodents on a much larger scale (2.54±1.49%), than any of the other techniques mentioned (partial pancreatectomy - endocrine replacement only [19]; Streptozotocin - re-establishing normoglycaemia [20]; cellophane wrapping: 2.4±0.9% [21]). Questions in need of answers include issues surrounding the nature, sustainability and viability of experimentally induced regenerated endocrine tissue, especially in a clinical setting. A question that has as yet not been answered is whether PDL was able to, and to what extent, induce normoglycaemia in a diabetic model.

Cells with multiple phenotypes have been reported in both embryonic [22, 23, 24, 25] and experimentally manipulated [3, 12] and/or endocrine character co-expressing either amylase, cytokeratins [23, 26]. Cells even sometimes differentiate into pancreatic hepatocytes [27].

Studies to date [4, 17, 28, 29] on PDL induced cellular change have mostly focused on the acinar and ductular compartments.

PDL was first reported by Edstrom and Falkmer [30]. Chrom-hematoxylin staining was used. They found a number of mainly quantitative changes in the alpha- and beta-cell compartments. This was however before the advent of immunocytochemical techniques as we know them today. Hence the limitations in their study. Subsequent work has been published on PDL induced endocrine cell lineage development but it has been sporadic.

The purpose of this study was to follow the changes shown to occur in the endocrine compartments of the pancreas after experimental manipulation [31], using a PDL rat model, a wider range of immunocytochemical (ICC) immunolabelling and computerised morphometry. PDL induces endocrine proliferation. No consensus has been reached as to whether it follows the same cellular sequence as the embryonic development of the pancreas [8, 15, 18].

MATERIALS AND METHODS

Animals

One hundred and ninety-eight normal adult Sprague-Dawley rats (body weight greater or equal to 300 g) were studied. One hundred and ninety-two animals were randomly grouped into 24 groups of eight animals each: numbers 1-6 in each of the 24 groups were experimental animals while the remaining two (7 and 8) were sham operated controls. The normal control group consisted of six animals. Identical PDL procedures were performed on six members of each of 24 groups according to methods previously described by Edstrom and Falkmer [30]. The remaining two animals in each group acted as sham-operated controls, and only underwent midline laparotomy. Anaesthesia was maintained with 0.1 mL 10 mg/mL Ketamine Hydrochloride (Ketalar, Warner Park-Davis Research Laboratories, Retreat, Western Cape, South Africa).

Pancreatic Duct Ligation

Anaesthetised rats were prepared for surgery by shaving the abdominal surface and thereafter cleaning it with Betadine antiseptic solution containing providone-iodine at 10 mg/mL (Adcock Ingram Pharmaceuticals, Industria, Johannesburg South Africa). A short (2 cm) mid-line laparotomy incision extending from the xiphisternum was utilised to gain access to the abdominal cavity. After exposing and drawing out the pancreas, it was prised away from the surrounding tissue with blunt forceps. A single stitch of resorbable suture material (5/0 Dexon polycaprolate coated braided polyglycolic acid, Davis and Geck, Cyanamid, Hampshire, UK), was carefully placed at a point immediately distal to the confluence of the splenic and common bile ducts (Figure 1). This ligated the distal aspect of the duct affecting the tail portion of the pancreas, which accounted for between 50 and 60 percent of the entire organ.

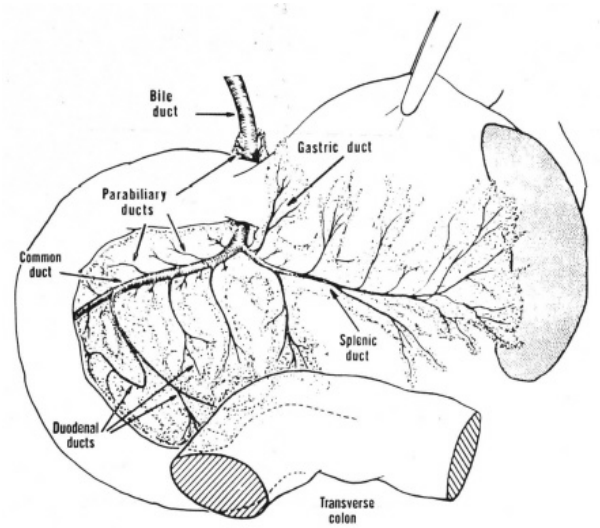

Figure 1. Arrangement of bile and pancreatic ducts in the laboratory rat. Arrow indicates point of ligation.

After ligation, the abdomen was closed in layers using chromic and silk. Post-operatively the animals were housed in standard laboratory cages in a thermally controlled environment with free access to standard rat chow (Epol, Midrand, South Africa) and drinking water. A suitable analgesic was administered every six hours under the supervision of a qualified vet.

Harvesting of Pancreas

Every 12 hours from days one to ten, every 24 hours from day 11 to 14, and on day 30, one group of duct ligated animals was anaesthetised and prepared for surgery, as described above, in order to harvest the pancreas for histological assessment. The whole pancreas was isolated, excised together with a small piece of duodenum and spleen and cut in half longitudinally through the middle line. The exposed surfaces (longitudinal sections) were placed face down on a piece of cardboard and fixed in Bouin's solution. The tissue was transferred to the base of a labelled cassette, processed in an automated tissue processor and embedded in histological embedding wax (Paramat, BDH Laboratory Supplies, Poole, England). After a number of Haematoxylin and Eosine (H&E) stained trials to assess tissue composition, the tissue blocks were trimmed to remove excess pancreas tissue, leaving only ligated tissue and a one to two millimetre strip of normal unligated tissue for comparison and further assessment.

Immunocytochemistry

Two series of seven consecutive 3-6 µm sections from each animal 200 µm and apart as described by Rosenberg and Duguid [1] were consecutively stained for H&E, insulin (1/200 dilution), glucagon (1/200 dilution), somatostatin (ST) (1/200 dilution) and pancreatic polypeptide (PP) (1/2000 dilution) using the Avidin-Biotin ABC Technique (DAKO LSAB 2 kit, Dako Corporation, Carpinteria, California, USA) method described by [32] (Refer also to Table 1).

Table 1. Details of anti-sera used for immunocytochemistry.

Immunogen	Raised in	Supplier	Lot No.	Dilution	Specificity
Porcine glucagon	Rabbit	Dakopatts	052	1/200	X-reacts with many mammalian species.
Synthetic C-peptide of insulin	Rabbit	Incstar	881 6019	1/200	X-reacts with human or primate tissue.
Synthetic cyclic (1-14) somatostatin	Rabbit	Dakopatts	072	1/200	X-reacts with many mammalian species.
Synthetic C-terminal hexapeptide of pancreatic polypeptide	Rabbit	Bioproducts	001	1/2000	X-reacts with PP but not with PYY or NPY or any known gastrointestinal hormone.
Synthetic peptide neuropeptide tyrosine (NPY)	Rabbit	Bioproducts	3RG2	1/2000	X-reacts with 1-33 NPY and <0.01% PYY.
Porcine PYY peptide	Rabbit	Biogenesis	940 520A	1/1000	X-reacts with PYY but not with NPY or PP.
MIB-5, mouse IgG1, Kappa	Mouse	Dianova	Dia 5055	1/100	Reacts with Ki-67 equivalent in primates, monkeys, cow, goat, sheep, pig, dog, rabbit and rat.

Tec Vectastain ABC substrate was prepared by mixing together equal volumes of 0.02% hydrogen peroxide, made in distilled water from a 30% stock, and 0.1% (1 mg/mL) diaminobenzidine (DAB) tetrahydrochloride (Sigma Fast, Sigma Chemical Company, Steinheim, Germany) made in 0.1 M Tris buffer, pH 7.2. The hydrogen peroxide solution should be freshly prepared from concentrated stock. Many peroxidase substrates are unstable in the presence of hydrogen peroxide or when exposed to light. For best results, the peroxidase substrate is made just prior to use.

Paraffin sections were stained by firstly deparaffinising and hydrating tissue sections through xylene and graded alcohol series then rinsed for five minutes in distilled water. The sections were incubated in 0.3% H_2O_2 in methanol for 30 minutes to quench endogenous peroxidase activity. The sections were then washed in buffer for 20 minutes and then incubated for a further 20 minutes with diluted normal serum from the species in which the secondary antibody was made and the excess serum blotted from the sections.

Sections were then incubated for 30 minutes with primary antiserum diluted in buffer and then washed for 10 minutes in buffer, after which they were incubated for 30 minutes with diluted biotinylated antibody solution and then washed for 10 minutes in buffer. A 30 to 60 minute incubation with Vectastain ABC was then required before another wash of 10 minutes in buffer. Sections were incubated for two to seven minutes in peroxidase substrate solution, washed for 5 minutes in tap water, counter-stained with toluene, cleared and mounted.

Antibody Specificity Controls

Method Controls

Method controls consisted of omission of each stage, in turn of the labelling procedure.

Specificity Controls

Specificity controls included adsorption of the primary anti-sera with excess homologous or heterologous antigen (10–100 µg per mL diluted anti-serum) for 24 hours before immunolabelling. In the case of anti-sera to peptides sharing amino acid sequences, cross reactivity of anti-sera was analysed by dot blotting and the results compare with tissue labelling, bearing in mind the different availability of epitopes between native antigen and antigen fixed in the tissue section.

Antisera Controls

Drops of 2 µL (1 µg/µL) of antigens sharing amino acid sequences (PP, NPY, and PYY) were placed on nitro-cellulose strips and allowed to dry at room temperature. After the strips were fixed in Bouin's fluid for 1 h at RT, the dot blots were stained using the Enhanced Chemiluminescence (ECL) kit (Amersham Pharmacia, Buckinghamshire, UK) [33]. The strips were incubated with unadsorbed anti-sera to assess cross reactivity and with anti-sera adsorbed with antigen combinations to try and reduce or eliminate cross reactivity. After incubation with the detection reagents, the strips were exposed to autoradiography film to detect immunolabelling and assess antibody specificity.

Morphometry

Immunocytochemically stained consecutive serial sections of post-PDL pancreas were morphometrically assessed according to previously described methods [11, 31, 34] using a computerised system which consisted of a Pulnix TMC-6 CCD video camera on a standard Zeiss light microscope coupled with a DT 3153 MACH Series Frame Grabber graphics card in a 486 PC. A HL Image ++ (image plus-plus) Windows 95 compatible software package (Western Vision Software, Salt Lake City, UT, USA) was used to capture and process microscopic images. Data was exported to MS Excel (Microsoft Inc, Johannesburg, South Africa) and processed through the Statistica package (Statsoft Inc, Tulsa, Oklahoma, USA).

We were not able to predict the results of the PDL. Retrospectively however, it was possible to categorise three broad phases of morphological change post duct ligation using changes in insulin content as a standard. The first, designated phase 1, occurred from the time of ligation to total acinar deletion (days 1 to 3) and was characterised by no noticeable change to islets. The second phase (days 3½

to 7½) was evidenced by a sudden increase (day 3½) in the percentage of insulin ICC positive tissue together with the appearance of spheroid structures (ducts?). The former gradually tapered off to day 7½. This was followed by a steady increase in the percentage of insulin ICC positive tissue from day eight onwards, culminating in a peak on day 12 (phase3: from day 8 to the end).

Ten randomly selected tissue areas, totalling at least 10^5 μm^2 from each specimen, where possible, were assessed. The immunoreactive areas for each were totalled and expressed as a percentage of the total area measured. Two further randomly selected areas for each slide were photographed for comparison and manually measured for control purposes and to determine islet size and frequency. Data obtained from ligated and sham operated controls animals were compared with values from published literature.

ETHICS

This study was approved by the ethics committee of the University of Stellenbosch and has rigorously adhered to the ethical NIH guidelines for the care and use of laboratory animals.

STATISTICAL ANALYSIS

Data is reported as mean±SEM and was statistically evaluated using the Student's t-test for independent samples. Differences between experimental results were considered significant if the two-tailed P was less than 0.05.

RESULTS

The observed trends in morphological change evoked in the exocrine and endocrine compartments of the pancreas by duct ligation, at different time intervals for all experimental groups (n=6), are summarised in Table 2.

Table 2. Composite tabulated summary of all events occurring in each of the compartments of duct ligated pancreatic tissue from day one through 14 and day 30. (This and the following table should be viewed side-by-side).

Day	Acinar compartment	Existing islets	Emerging islets	Ducts
1	Normal	Normal	None	None
1½	Oedema evident	Normal	None	None
2	Inter-acinar PP+	I, G and ST+ core G+ mantle	None	None
2½	Inter-acinar PP+ and ST+	I, ST and PP+ core G+ mantle	None	None
3	Inter-acinar PP+ and ST+	I, ST and PP+ core G+ mantle	None	Ligated tissue NPY+
3½	Acinar deletion completed	I, ST and PP+ core G+ mantle	I+	Proliferation NPY/PYY+ I, G, ST, PP+
4		I+ core G/PP+ mantle	I+	NPY/PYY+ I, G, ST, PP+
4½		I+ core G/PP+ mantle	I+	NPY/PYY+ I, G,
5½		I+ core G/PP+ mantle	I+ core G/PP/ST+ mantle	NPY/PYY+ I
6½		I+ core G/PP+ mantle	I+ core G/PP/ST+ mantle I+ core	NPY+
7½		I+ core G/PP+ mantle (No ST!)	G/PP/ST+ mantle	NPY+
8		I+ core G+ mantle	I+ core G/PP/ST+ mantle	NPY+
9	Limited acinar reappearance	I+ core G+ mantle	I+ core G/PP/ST+ mantle	NPY+
14		I+ core G+ mantle	I+ core G/PP/ST+ mantle	NYP+
30	None	Indistinguishable from new I+ core thin G+ mantle	Indistinguishable from new I+ core thin G+ mantle	None

Table 2 (continue). Composite tabulated summary of all events occurring in each of the compartments of duct ligated pancreatic tissue from day one through 14 and day 30. (This and the previous table should be viewed side-by-side).

Day	Insulin	Glucagon	Pancreatic polypeptide	Somatostatin
1	Existing islet core	Islet mantle	Islet mantle	Islet mantle
1½	Existing islet core	Islet mantle	Islet mantle	Islet mantle
2	Islet core[1]	Islet mantle	Intra-acinar	Islet mantle
2½	Islet core[1]	Broken mantle	Intra-islet PP and ST	Intra-islet PP and ST
3	Islet core[1]	Broken mantle	Intra-islet PP and ST	Intra-islet PP and ST
3½	Scattered	Scattered	Scattered	Scattered
4	Reconverging into islets	Scattered and re-converging into islets	Scattered and re-converging into islets	Scattered and re-converging into islets
4½	Reconverging into islets		Islet mantle	Mantle and inter-islet
5½	Reconverging into islets	Mantle	Mantle	Mantle and inter-islet
6½	Islet core Islet clusters	Mantle	Mantle	Mantle and inter-islet
7½	Islet core	Mantle	Mantle	Mantle and inter-islet
8	Islet core	Mantle	Mantle	Mantle and inter-islet
9	Islet core	Mantle	Mantle	Mantle and inter-islet
14	Islet core	Mantle	Mantle	Mantle and inter-islet
30	Indistinguishable from new I+ core thin G+ mantle	Mantle	None	None

[1] in existing islets

Acinar Compartment

Near total acinar deletion was achieved by day three in four of the six animals, and total deletion by day 3½ (Figures 2 and 3) post duct ligation in all but one animal. Acinar deletion was accompanied by the replacement of the space, previously occupied by acinar tissue, with circular/spheroid structures of varying size (10-40 µm in diameter) and frequency (3-6 per 1000 μm^2) in all experimental groups.

Endocrine Compartment

Three types of islet-like structures were found (two from within the space previously occupied by acinar tissue) to gradually emerge from day 3½. The first consisted of a clearly defined, usually uniform insulin core, ranging from between 100 and 200 µm in diameter, surrounded by a mantle, of varying thickness, of glucagon cells. These were presumed to be pre-existing islets. The second type consisted of either an insulin or glucagon mass (but mostly insulin), devoid of any mantle, usually less than 100 µm in diameter, clustered together and associated with duct-like structures. Such were presumed to be new and/or developing islets and were described by naming them emerging islets. Finally there were group of very large (>200 µm in diameter), usually irregular islet-like areas of mainly insulin immunoreactivity devoid of either PP or ST with a very thin, broken glucagon positive mantle, which formed toward the end of the series. Beyond day 14 all islet-like structures consisted of either an insulin mass only, or a regular to irregular, medium (150-200 µm diameter) to large (>200 µm diameter) insulin mass with a thin glucagon perimeter. The latter were presumed to be amalgamated islets resulting from the consolidation of insulin positive tissue from pre-existing and emerging islets as suggested by Rosenberg [19].

Initial endocrine activity was characterised by the increase in ST (day 2½: 1.95±0.24%) and PP (day 2 and 2½: 1.20±0.09% and 1.50±0.09%, respectively) represented as peaks in Figure 4, which coincided with that of glucagon. The hormone levels in the ligated tissue fluctuated over the fourteen-day period of the main part of the study (Figure 4). Sections in ligated tissue at 30 days were devoid of both PP and ST. The total endocrine area of each of the respective hormones expressed as a percentage of the total area surveyed, generally tended to follow a cyclic pattern. Glucagon, ST and PP could be seen to be in reverse series to insulin (Figure 4).

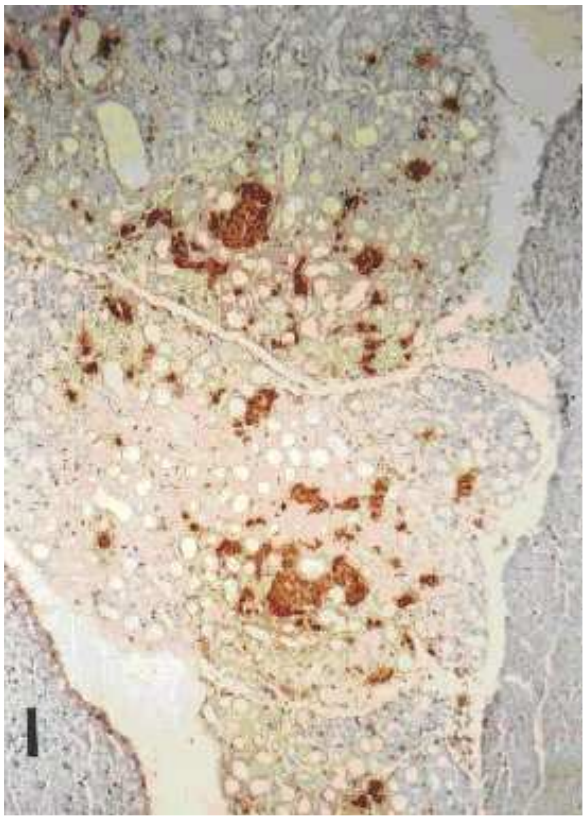

Figure 2. Day 3½ post PDL pancreatic tissue. ICC stained for insulin. x100. Bar = 100 µm.

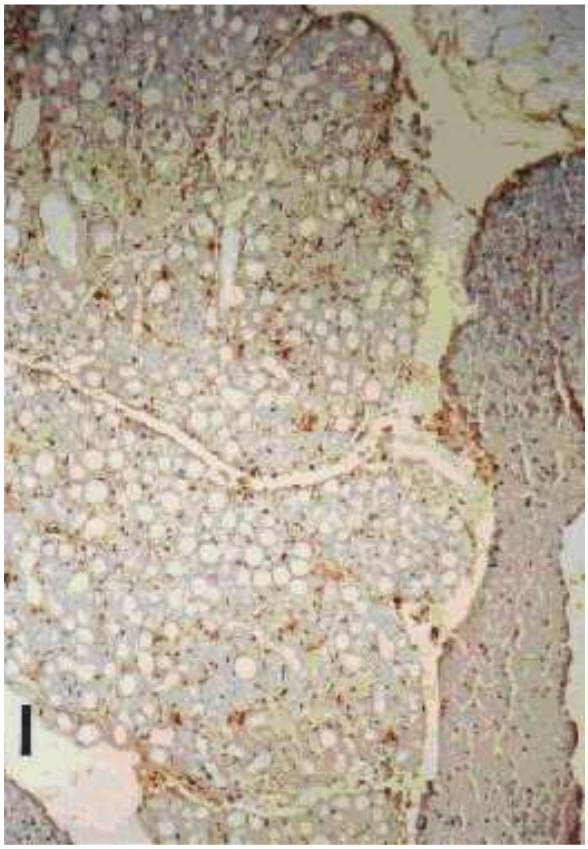

Figure 3. Day 3½ post PDL pancreatic tissue. ICC stained for glucagon. x100. Bar = 100 µm.

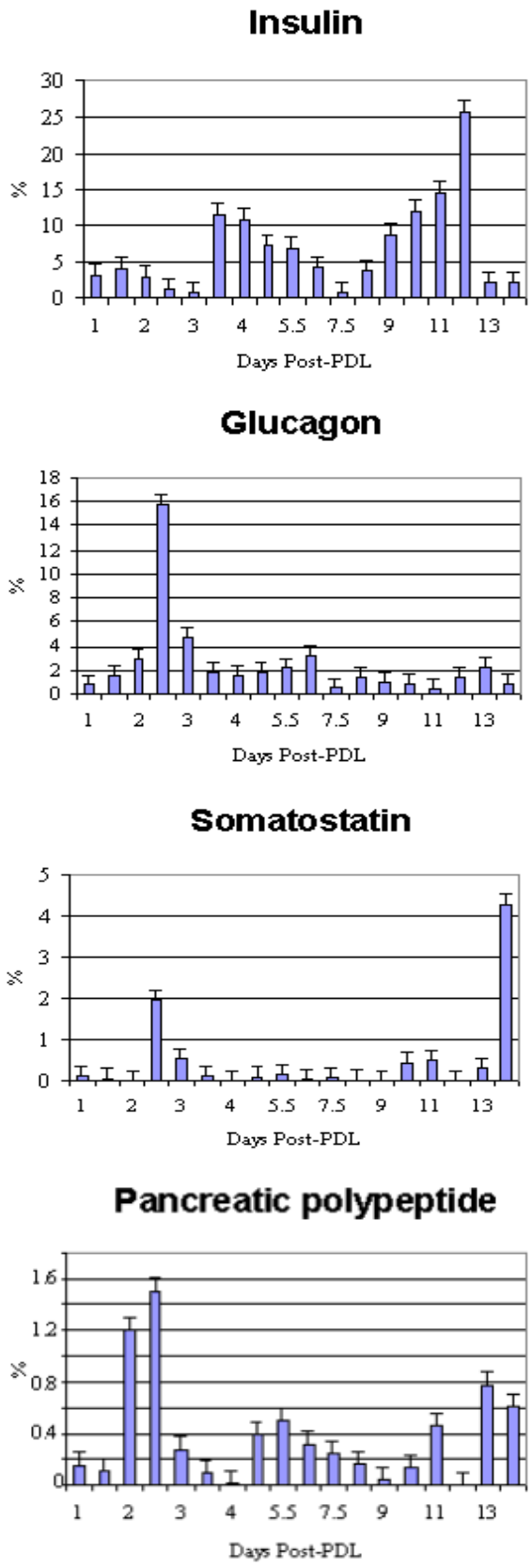

Figure 4. Composite graph of percentage endocrine area over duration of the study for insulin, glucagon, somatostatin, and pancreatic polypeptide.

Individual Hormone Profiles

Insulin

Histology - Islets remained unaffected during the first three days post-ligation. Changes in core composition occurred as PP and ST could be seen to take up residence in the islet core. Glucagon, PP and ST deletion from the mantle and an increase in small (diameter <100 μm) and large (diameter >500 μm) islets were observed (Figure 2).

Morphometry - The overall mean increase in cells ICC positive for insulin, between day one and 14, occurred from a pre-ligation percentage insulin positive area of 1.04±0.21% for normal pancreas tissue to 6.77±1.51% ($P<0.05$). The morphometric assessment of the total insulin content, expressed as a percentage of the total tissue area measured for each series, reveals a biphasic insulin response as illustrated in Figure 4. Significant differences were found to be present between internationally published (1.04±0.05%) [35], and accepted normal values (normal in this study 1.04±0.21%), and experimental animals (3.06±1.35%) ($P<0.01$) as well as between sham operated controls (1.3±0.35%) and experimental animals with regard to insulin positive tissue area ($P<0.01$).

The total percentage insulin area during days one to three (phase 1) post-PDL show a slight rise at day 1½ (from day 1: 3.06±1.51% to 4.08±1.51%) after which it declined to day three (day 2: 2.88±0.11%; day 2½: 1.09±0.5%; day 3: 0.65±0.3%). Significant differences were found between the results for normal and days 1, 1½, 2, 2½ and 3 respectively ($P<0.05$). Results however indicate an increase in the number of insulin positive areas less than 30 μm in diameter from zero on day one to 79/mm^2 by day 1½, even although the general insulin trend shows a decline. Between day 3½ and 7½ (phase 2) and initial increase in the peak for insulin immunoreactivity followed by a gradual decline in both the percentage of insulin positive tissue and the number of areas less than 30 μm^2. From day 8 until the termination of the experiment (phase 3), insulin percentage area increased to 25.8±5.3% (day 13) followed by an inexplicable value (2.1±0.18%) on day 14.

Glucagon

Histology - Insulin accounted for 2.4±0.12% of the total pancreas tissue area studied in control animals while glucagon accounted for 0.78±0.03%. An insulin:glucagon ratio of 3:1 was found in the sham operated control group animals. The percentage of glucagon tissue in the pancreas in the experimental group averaged 3.25±0.9% ($P<0.05$), with peaks at days 2½ (15.76 ±2.45%), 6½ (3.30 ±0.42%) and 13 (2.29 ±0.37%) representing virtually equidistant time intervals, 4½ and 5½ days respectively (Figure 4.). Days 1, 2½ and 5½ conformed closely to the structural arrangement found in the glucagon immunoreactivity for unligated and control animals. Glucagon formed a virtually continuous two to three cell layer around the perimeter of an islet. In endocrine tissue, which can be considered to be "emerging" islets, glucagon did not form a uniform continuous band of cells around an islet. At day 3½ glucagon immunoreactivity cells were in consort with insulin immunoreactive cells but mostly as independent individual cells or cell clusters (Figure 3). At 30 days this glucagon mantle was exceptionally thin and lacked uniformity.

Morphometry - Although the percentage glucagon immunoreactive area only had one significant peak (day 2½: 8608 μm^2). The glucagon peak (15.76±2.45%) preceded the insulin peak by 24 hours and appeared to be a single occurrence.

Somatostatin

Histology - In the pancreatic tissue of unligated and sham operated controls, ST was restricted chiefly to the perimeter of islets where they could be found with varying frequency as single cells. Some evidence of ST-positive cells, in apparent isolation, was present in amongst acinar cells of these groups. ST positive tissue accounted for a very small percentage (0.1%) of the ICC stained tissue in the sections studied with no significant difference noticed between normal/experimental ($P>0.05$) and

control/experimental groups (P>0.05). Values for ST between normal and control groups only differed by 0.01%. Very rarely could ST-positive cells be found amongst the cells making up the insulin core of normal islets. In ligated tissue ST immunoreactivity presence in "emerging" islets is absent but is present in clusters of 2 to 5 cells within the insulin core of pre-existing islets. Morphometric quantification of the average amount of ST and PP throughout the whole series indicates significantly more of the latter (4918 vs. 3476 μm^2). This observation is confirmed by the percentages exhibited in the graphs for ST and PP in Figure 4.

Morphometry - Phase one was characterised by a peak of ST immunoreactivity at day 2½, coinciding with those of glucagon and PP. Phase 2 shows no increase in ST while phase 3 shows that an increase occurs over the three day period (days 9½-13) with day 14 more that doubling that of day 2½.

Pancreatic polypeptide

Histology - Control animals possessed virtually equal numbers of PP immunoreactivity cells as normal animals (0.12±0.02% vs. 0.10±0.04%; P>0.05). A significant difference was found in the percentage area of PP immunoreactivity between control and experimental animals (0.38±0.09%; P<0.05). Although the apparent norm for the position of PP immunoreactivity cells in islet context follows a fixed pattern (i.e. are they restricted to the glucagon mantle together with ST), significant numbers of PP immunoreactivity cells were present in areas of acinar transformation and ductular formation as early as day 2½ post duct ligation. These cells were in no fixed pattern and were in association with cells exhibiting insulin and glucagon immunoreactivity.

Morphometry - Three significant areas of PP immunoreactive activity could be found. These were between days 2½ and 3½, 6½ and 7½ and 11-14. Phase one (day 1-3) is in synchrony with glucagon and ST but not with insulin. After ligation PP was found in between islet insulin core cells, as isolated pockets of PP immunoreactivity cells in association with acini and around ducts and areas of endocrine proliferation.

DISCUSSION

After careful consideration of the histological (Figures 2 and 3) and morphometric (Figure 4) results, especially those for insulin, it became evident that the post-PDL events found to take place in this study could be grouped into three categories. The first, covering days zero to three, was designated phase one. Although the phase was characterised by acinar deletion [16, 30, 35], it occurred simultaneously with a significant increase in insulin activity [30]. Phase two, spanned days 3½ to 7 and was characterised by a continued increase in extra islet insulin immunoreactivity. Phase three, spanning days 7½ to 14 appeared to involve islet reconstitution probably due to cell migration as suggested by Rosenberg [19]. Previously recorded post ligation events from studies over fourteen days [15] and of longer duration (nine months) [30] grouped results into chronological moieties (1-7 days, 2-6 weeks, 3-9 months) without apparent consideration to its relationship to cellular and morphometric changes taking place. Correlation of the chronology to the histology of the post ligation cellular change in this study is therefore a unique finding nor previously described.

The results therefore show that the change reported to occur in pancreas morphology after ligation, is not a consistently reported finding. The extent and span for the various events differ somewhat. Most reports reflect some events as less dramatic, occurring over a longer time-period.

Exocrine Compartment Remodelling

Acinar deletion

The acinar compartment remodelling that precedes endocrine neoformation and has been shown to consist of acinar deletion and increase in the number of ducts [23]. The post duct ligation structural changes reported to take place over a seven day period or longer in a number of reports, include oedema and inflammation in the interstitial compartment; lobular necrosis, shrinkage, loss of zymogen

granules (Edstrom and Falkmer [30]; and apoptosis [4, 16, 28, 35] in the acinar compartment rather than necrosis as proposed by Gukovskaya [17]. In addition, there was an increase in budding in the ductular compartment [23, 25] and the formation of ovoid and/or kidney shaped islet bodies [28, 29, 30] in the endocrine compartment as observed especially on day 3½ in this study. Pre-existing islets were described as remaining unchanged [30] although it would appear that no real distinction can be made between the pre-existing and new islets.

The most significant contrast in this study was the general lack of inflammation in ligated tissue lending credence to the notion that rather transdifferentiation is involved in acinar deletion as suggested in reports [4, 35].

Ductular Compartment Remodelling

Whatever their origin, no attempt was made to categorise them except to take note of their increased frequency compared to incidence in reports from similar studies and to speculate on their origin based on the absence of mitotic activity supported by BrdU studies. This led us to speculate on the origin of these so-called ductular complexes being from the transdifferentiation of acinar cells rather than the replication of duct epithelial cells. This finding is in contrast to BrdU and CK20 studies [15, 23].

Endocrine Compartment Remodelling

Endocrine neoformation, described by Edstrom and Falkmer [30], lacked precise morphological categorisation due to the antiquated techniques employed. They made no distinction between pre-existing and newly formed islets and showed dramatically less endocrine tissue (1%) than the 10.5±0.81% found present during the course of this study. Subsequent studies [3, 16, 36] have reported the development of new endocrine tissue distributed haphazardly throughout the space previously occupied by acinar parenchyma from duct epithelial tissue.

Although PP has been suggested as a likely candidate [6, 7] for the onset of endocrine proliferation, as demonstrated by the inter-acinar and intra-islet PP appearance in this and similar studies [6, 7] this is contested by a number of groups on both embryonic and experimentally induced lineage development which propose NPY [31] or PYY [24, 25, 26] as the most likely precursor to this process. The difference of opinion is said to involve possible cross-reactivity of antibodies raised against PP, NPY and PYY [34]. The finding of significant quantities of PP-positive cells (0.38±0.09%) compared to published values for normal tissue ($P<0.05$) and sham operated control animals ($P<0.01$) is a finding not previously described. The early PP presence is however overshadowed by the appearance large areas of glucagon immunoreactivity (2.5±0.8%) immediately prior to a significant increase in tissue positive for insulin (Figure 4). Explaining the origin of such large quantities of endocrine cells has proved to be problematic. The suggestion has been made that both acinar and endocrine cells are capable of transdifferentiation. This sentiment is supported by reports from various authors which cite findings demonstrating the presence of cells within the pancreas with intermediary characteristics [22, 24, 36].

Cell Proliferation Marker

A positive result from the post embedding marker MIB5 has remained illusive. This would seem to suggest that the morphometric changes seen to occur during this study are not due to cell proliferation but rather differentiation as suggested by Rosenberg [19]. This is in contrast to a number of similar studies [23, 37, 38] which found widespread evidence of cell proliferation during the remodelling process in experimentally manipulated pancreatic tissue. This discrepancy is noted and no explanation is forthcoming

CONCLUSION

The results suggest that PDL firstly, induces the development of endocrine tissue distributed haphazardly throughout the space previously occupied by acinar parenchyma. Secondly, that the appearance of insulin is preceded by the appearance of PP, glucagon and ST by 24-hours. Thirdly, that a still to be

determined proportion of the newly formed endocrine tissue associates with existing islets, resulting in a number of very large irregular islets.
In conclusion, the study confirms that PDL in a rat model gives rise to a significant increase in the quantity of endocrine tissue in the pancreas in the short term. Possible avenues for further exploration include the in vitro culture of pancreas tissue from duct ligated animals, or the transplantation of such tissue beneath the kidney capsule as is done in foetal rat pancreas transplantation studies described by du Toit *et al* [39].

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Key words Cell Differentiation; Islets of Langerhans; Ligation; Pancreas

Abbreviations ICC: immunocytochemical; PDL: pancreatic duct ligation; ST: somatostatin

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