AURIGENE
Accelerating Discovery

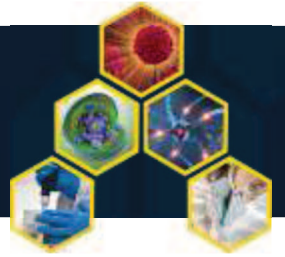
Pancreas in Preclinical Toxicology

Fifth Conference of
Society of Toxicologic Pathology – India

CONTINUING EDUCATION IN TOXICOLOGIC PATHOLOGY
-ENDOCRINE AND GASTROINTESTINAL SYSTEM

October 31 - November 2, 2014

Introduction



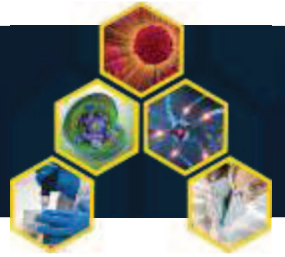
- Only organ in the body with both exocrine and endocrine parts intermixed in parenchyma
  - Islet-Acinar axis
- Finger of the liver- Talmud (200BC-200AD), Cushion of Stomach (Vesalius)

  Wirsung-pancreatic ducts (1642), de Graaf-secretions (1664), Digestive actions- 1834-1844

  Enzymes-Trypsin, amylase, lipase- 1879, enterokinase- 1889, Histology-Langerhans-1869

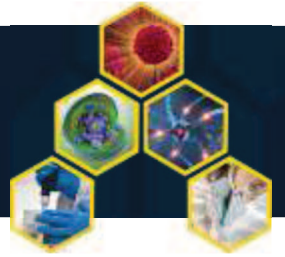
  Disease first reported only in 19th century, >500 drugs affecting pancreas

Introduction



- Silent and specialized organ
  - Not involved in absorption of ingested xenobiotics
  - Not involved in detoxification
  - Synthesize and secrete variety of specific proteins
- Low frequency of xenobiotic induced toxicity
  - When toxicity occurs, major impact on health
- Toxicity is more common where exposure to environmental pollutants, contaminants and alcohol is high
- Organ is pale with grossly visible lobules and ducts but not islets, except in prominent peri-insular holos
- Compact in hamster, primates, dogs and humans with comparable anatomy, less compact in rodents, diffuse in rabbits

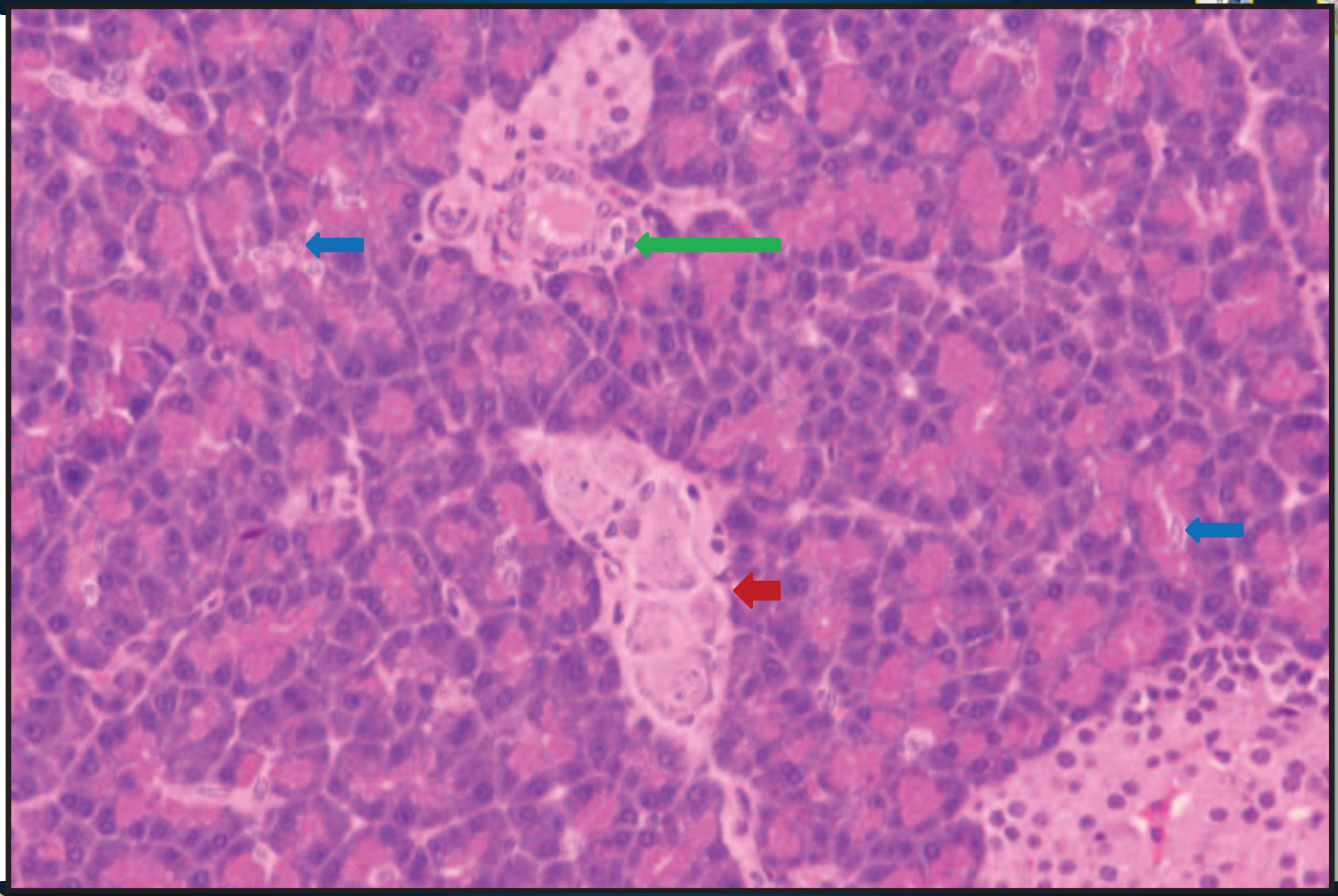
Exocrine Pancreas



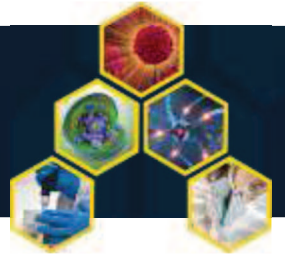
- ~90% - consists of acinar cells and ductal cells, centroacinar cells
- Acinar cells, pyramid shaped, convoluted, often interconnecting, tubuloacinar network in lobules, cyclic changes in morphology
- Basophilic cytoplasm around nucleus due to RER, apical granular eosinophilia due to zymogen granules - digestive enzymes
- Pancreatic stellate cells –play major role in disease and cancer
- Centroacinar cells- interface b/n acinar cells & intercalated duct, due to their tonofibrillary architectures, act as a valve regulating the amount of secreted zymogen granules
- Intercalated duct continues as Intralobular ducts - basement membrane of extracellular matrix

Front Physiol. 2012; 3: 344.

Pancreas Histology

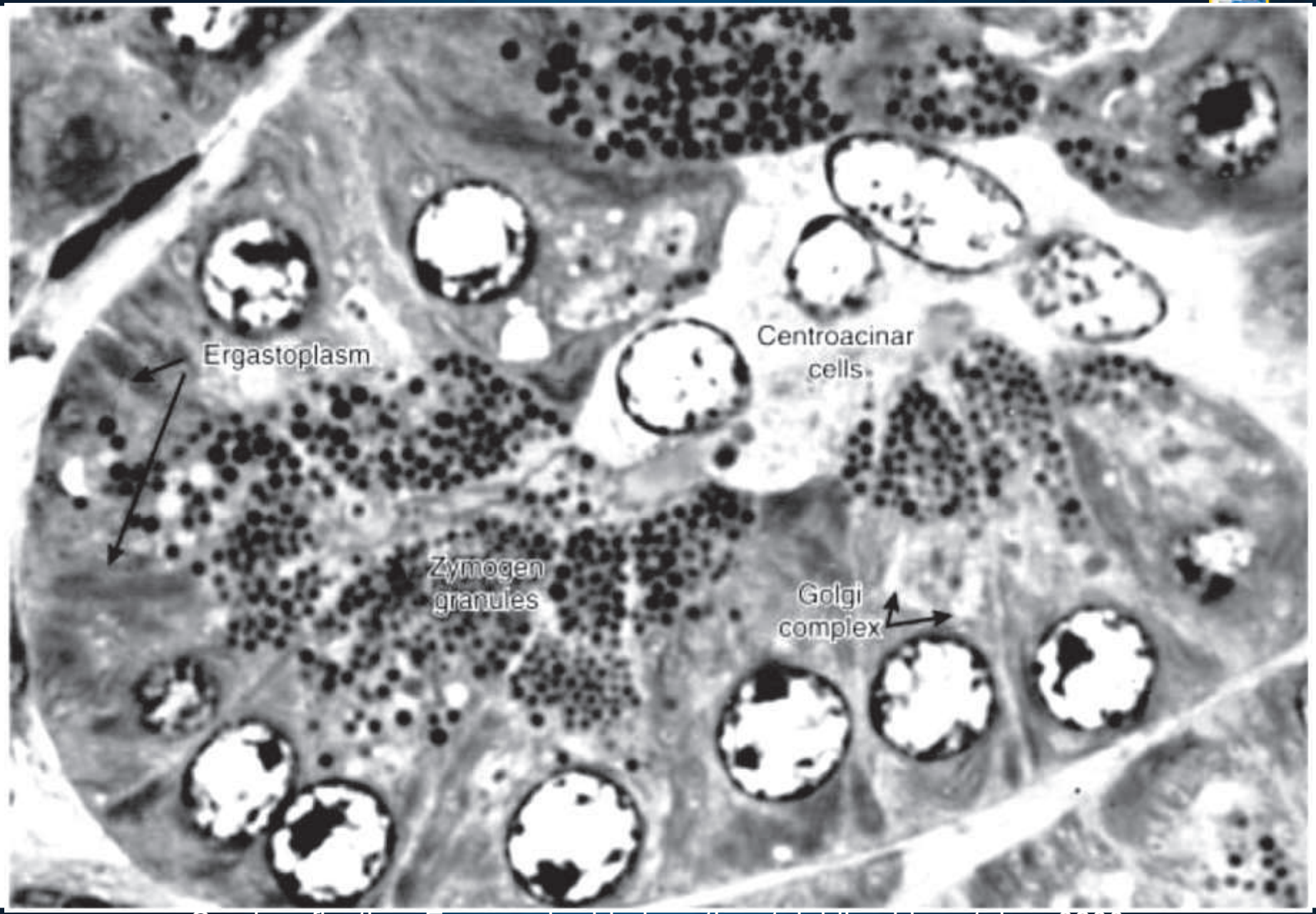


Exocrine Pancreas



- Ductal epithelium- cuboidal or low columnar
- Interlobular ducts - collagenous layer
- Exocrine pancreatic secretion is tightly regulated by neuroendocrine system
- Species specific differences in physiology – pathobiology of pancreatic disease in animal models – relevance to human disease
- Rats and mice- gastric lobe, duodenal head and tail
- Peri-insular and tele-insular regions, greater in mice than rats
- Acinal and ductal cells can divide in experimental animals- regeneration can occur

Electron Microscopy



Osmium fixation. Epon-embedded section, toluidine blue stain; ×3200.
(From Bloom W, Fawcett DWA: Textbook of Histology, 11th ed. Philadelphia, WB Saunders, 1986)

Species differences in distribution of drug metabolizing enzymes in pancreas

TABLE 1.—The distribution of CYPs and GSTs in the ductal cells of the syrian hamster (SGH), nude mouse (NM), rat, rabbit, guinea pig, dog, monkey, and human.

CYP	SGH	NM	Rat	Rabbit	Pig	Dog	Monkey	Human
1A1	+	–	+	+	–	++	+	+
1A2	+	–	–	–	–	–	–	+
2B6	+	++	+	–	–	–	+	–/+[a]
2C8,9,19	+	++	+	–	–	–	+++	++[b]
2D1	+	+	+	++	+	+	+	++
2E1	–	+	+	+	–	–	–	+[c]
3A1	+	++	++	++	+	++	++	++
3A2	+	+	+	+	++	+	++	+
3A4	+	++	–	–	+	–	–	++
GST-π	++	+	+	–	+	++	+++	++
GST-α	+	–	++	–	+	++	++	++
GST-μ*	++	++	+	++	+	++	+++	++*,[d]

Staining intensities: –, none; +, weak; ++ moderate; +++ strong.

TABLE 2.—The distribution of CYPs and GSTs in the acinar cells of the syrian hamster (SGH), nude mouse (NM), rat, rabbit, guinea pig, dog, monkey, and human.

CYP	SGH	NM	Rat	Rabbit	Pig	Dog	Monkey	Human
1A1	++	–	–	+	–	++	+	+
1A2	+	–	++	–	–	+++	+	+
2B6	+	++	–	–	+	++	++	–/+[a]
2C8,9,19	++	++	–	–	++	+	++	++[b]
2D1	++	+++	–	–	+	+	+	++[c]
2E1	–	+	+	+	–	–	–	–/+[d]
3A1	++	++	+	++	+	++	++	+
3A2	++	+	+	++	++	++	–	+
3A4	+	+	+	+	++	–	++	+
GST-π	–	–	–	–	–	–	–	–
GST-α	–	–	–	–	++	–	+	++[e]
GST-μ*	–	–	–	–	–	–	–	+*

Staining intensities: –, none; +, weak; ++ moderate; +++ strong.

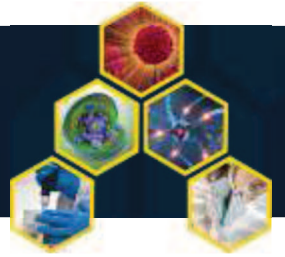
TABLE 3.—The distribution of CYPs and GSTs in the islet cells of the syrian hamster (SGH), nude mouse (NM), rat, rabbit, guinea pig, dog, monkey, and human.

CYP	SGH	NM	Rat	Rabbit	Pig	Dog	Monkey	Human
1A1	++	+	++	+++[a]	+++	+	++	+/+++[b]
1A2	+++	+++[a]	+[a]	+++	–	–	–	+
2B6	+++[a]	+++	+++[a]	++[a]	+++[b]	–	+++[c]	–/+++[b,d]
2C8,9,19	+	+++	+++[a]	–	+++[b]	–	+	–/+++[b,e]
2D1	+	++	++	+	+	+	+	+++[f]
2E1	+++	++	+++	–	–	–	–	–/+[h]
3A1	+++[a]	++	+++	++	–	++[b]	+++	++[b]
3A2	+	+++[a]	+	+	+	+	+	++
3A4	+	++	+++	–	–	–	+	–/+++[k]
GST-π	+++	++[a]	+	+	–	++	++	–/+++[m]
GST-α	+++[a]	+	–	–	–	–	–	–/+[n]
GST-μ*	++	+	+	+++[a]	+[b]	–	++	++*

Staining intensities: –, none; +, weak; ++ moderate; +++ strong.

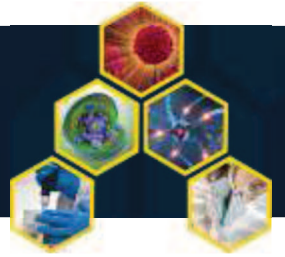
- Most enzymes expressed in human, hamster, mouse, and monkey.
- Several isozymes are lacking in rats, pigs, rabbits, and dogs
- Islet cells of all 8 species express more enzymes than ductal and acinar cells

Pancreatitis



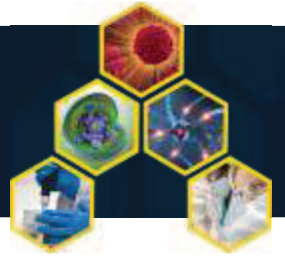
- Types of pancreatitis
  - Toxic – Alcohol(2), pesticides, xenobiotics
  - Obstructive- gallstones(1), tumors, worms, congenital defects
  - Metabolic- hyperlipidemia, hyperkalemia, acidosis
  - Infectious- parasitic, viral, bacterial
  - Genetic- cystic fibrosis transmembrane conductance regulator, pancreatic secretary trypsin inhibitor 1, serine protease inhibitor Kazal-type 1, cationic trypsinogen 1
- exocrine pancreas has great reserve capacity. No clinical evidence of exocrine pancreatic insufficiency - up to 90% destruction
- Sorafenib, mesalazine, azathioprine, simvastatin – definite pancreatitis inducing drugs; alcohol, corticosteroids, estrogens, opiates.
- dasatinib, nilotinib, and bosutinib- up to 70% increase in serum amylase activity and/or lipase activity with no frank pancreatitis

Animal models of Pancreatitis



- Invasive Models of Pancreatitis
- Noninvasive Models of Pancreatitis
  - caerulein-induced pancreatitis
    - abnormal secretory vesicle maturation & formation of large cytoplasmic vacuoles
    - acinar cell injury with both autophagy and apoptosis observed as end points (and occasionally necrosis)
    - cholecystokinin antagonists effective, proglumide and benzotript
  - muscuranic receptor agonist- carbachol, OP- fenthion & diazonin
    - augmenting the normal pancreatic flow and increase the intrapancreatic ductal pressure

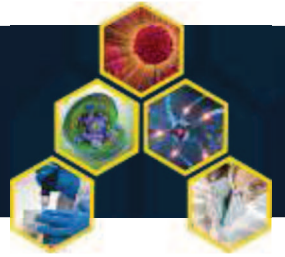
Animal models of Pancreatitis



- Alcohol-induced Pancreatitis
  - diets rich in unsaturated fat, with gradually ascending ethanol administration regime - reproducible, chronic alcohol-induced pancreatitis
- Choline deficient diet containing 0.5% DL-ethionine

- Genetic Animal Models of Pancreatitis
  - CFTR Deficient Tg Mouse, SPINK3 KO Mouse, PRSS1 overexpressing Transgenic Mouse Model
- Amylase, Lipase and pancreas-enriched miRs-216a and -375, promise as novel biomarkers for pancreatic injury

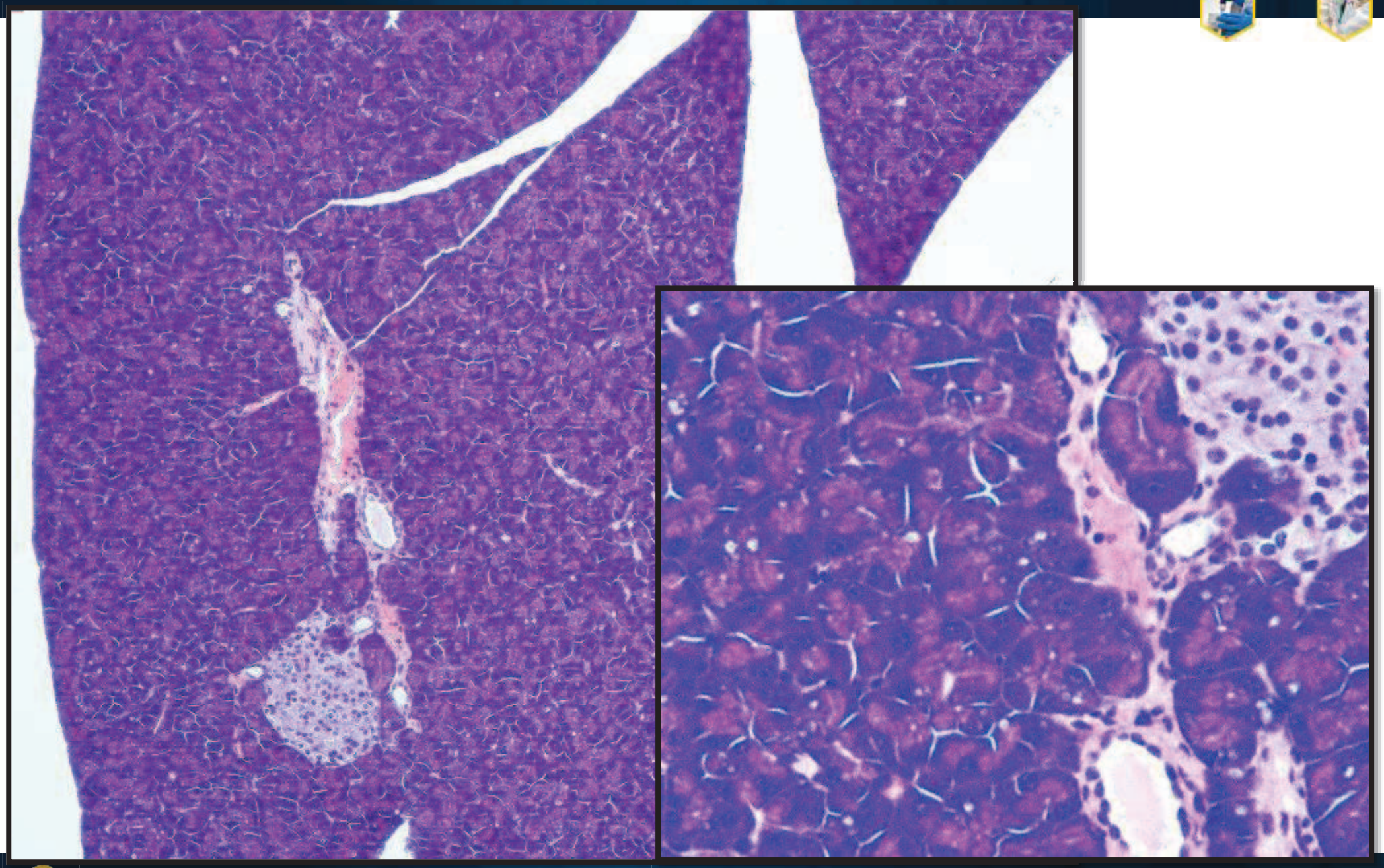
Exocrine Pancreas



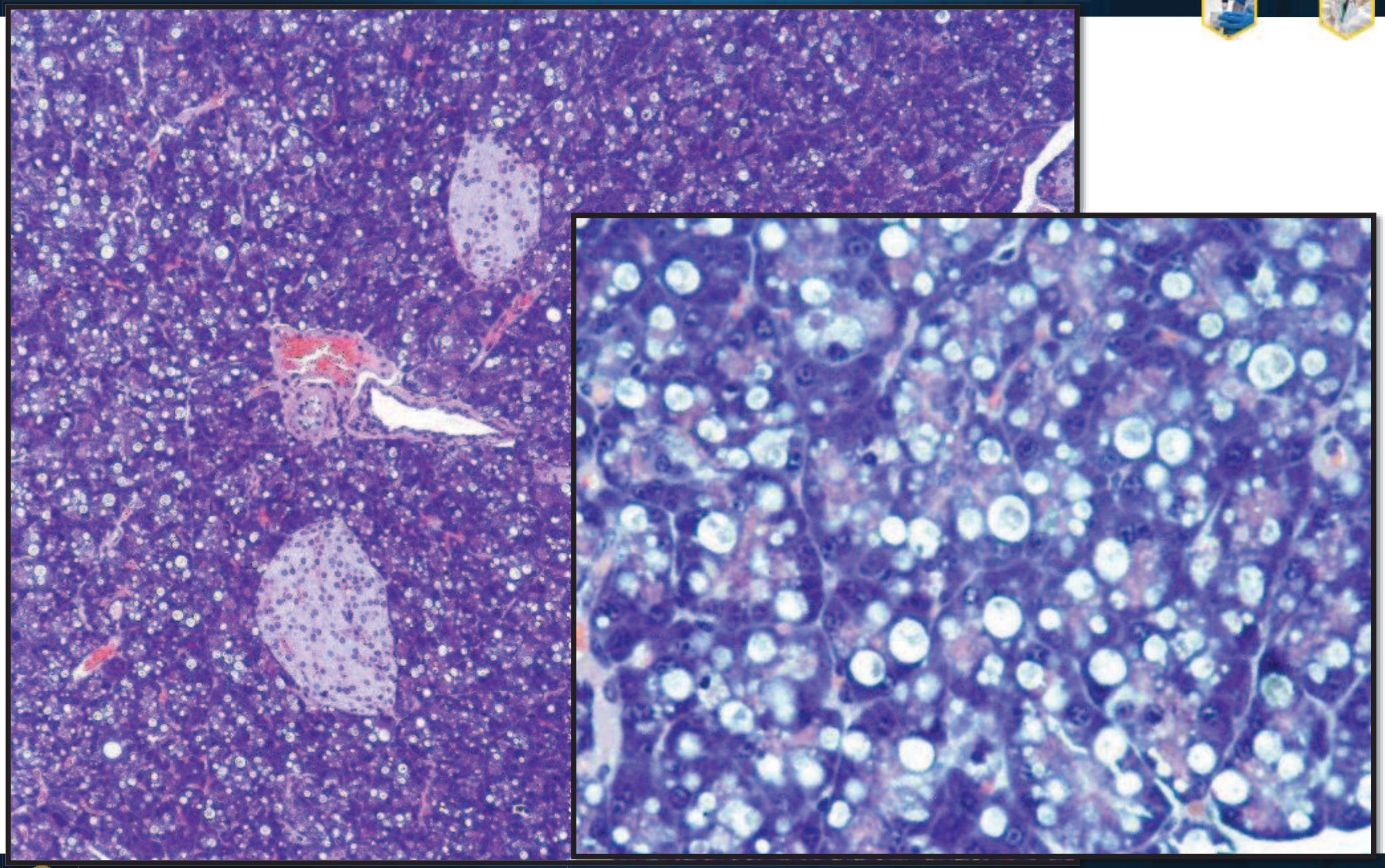
INHAND nomenclature and diagnostic critiera

- a. Congenital Lesions
  - Ectopic tissue
- b. Cellular Degeneration, Injury and Death
  - Vacuolation, acinar cell
  - Fatty infiltrate
  - Eosinophilic globules
  - Autophagic vacuoles, acinar cell
  - Single cell necrosis/apoptosis
  - Necrosis
  - Zymogen granules decreased, acinar cell
  - Atrophy, acinar cell
  - Mineralization
  - Amyloid
  - Pigment
- c. Inflammatory Lesions
  - Infiltrate
  - Inflammation
- d. Vascular Lesions
  - Inflammation, vessel
  - Hemorrhage
- e. Miscellaneous Lesions
  - Hypertrophy, acinar cell
  - Halos, peri-insular
  - Focus, basophilic
  - Metaplasia, hepatocytic
  - Metaplasia, ductular
  - Ectasia, duct
  - Edema
  - Fibrosis
- f. Non-neoplastic Proliferative Lesions
  - Hyperplasia, acinar cell
  - Hyperplasia, ductal cell
- g. Neoplasms
  - Adenoma, acinar cell
  - Adenoma, ductal cell
  - Adenocarcinoma, acinar cell
  - Adenocarcinoma, ductal cell

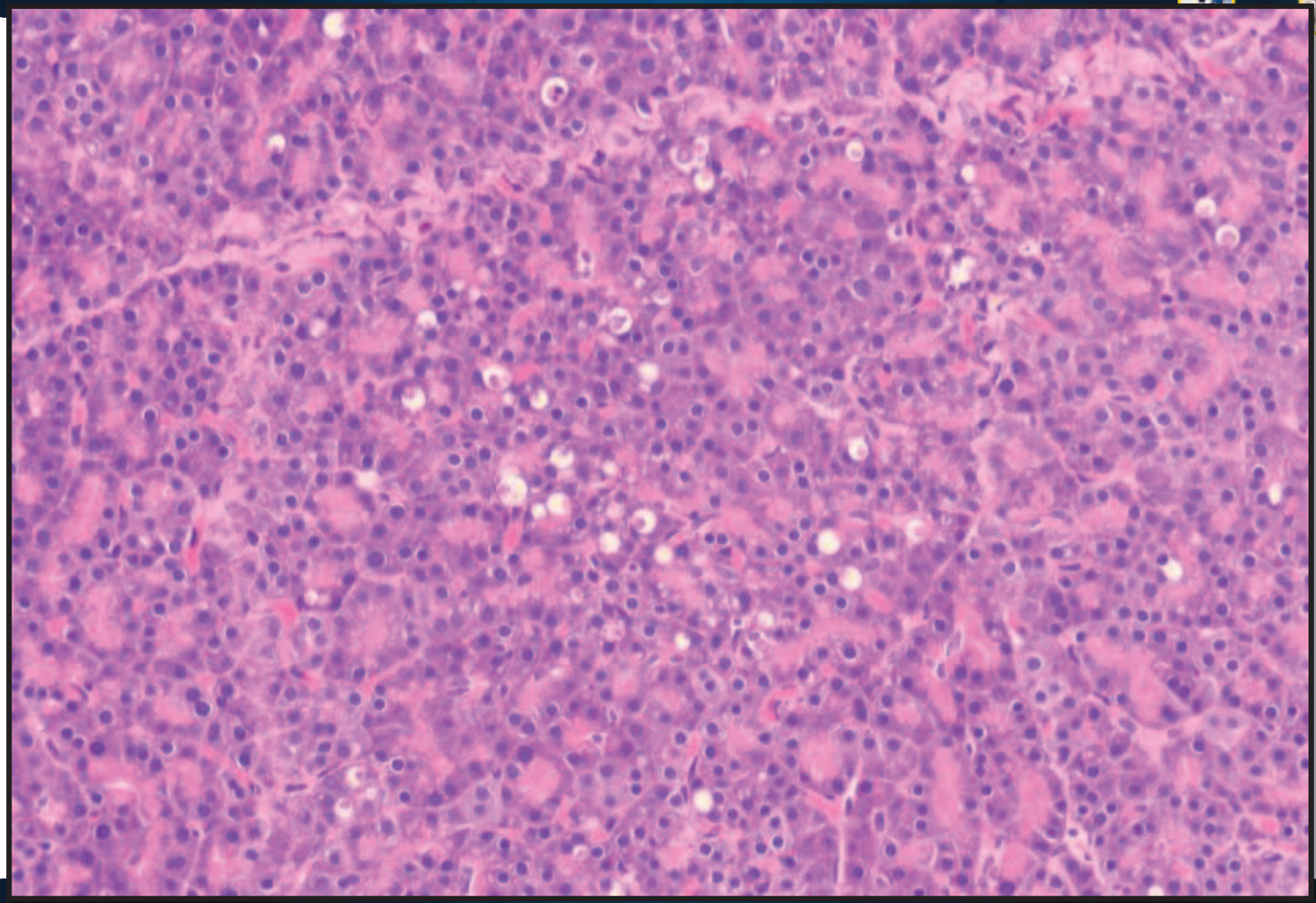
VACUOLATION, ACINAR CELL



VACUOLATION, ACINAR CELL

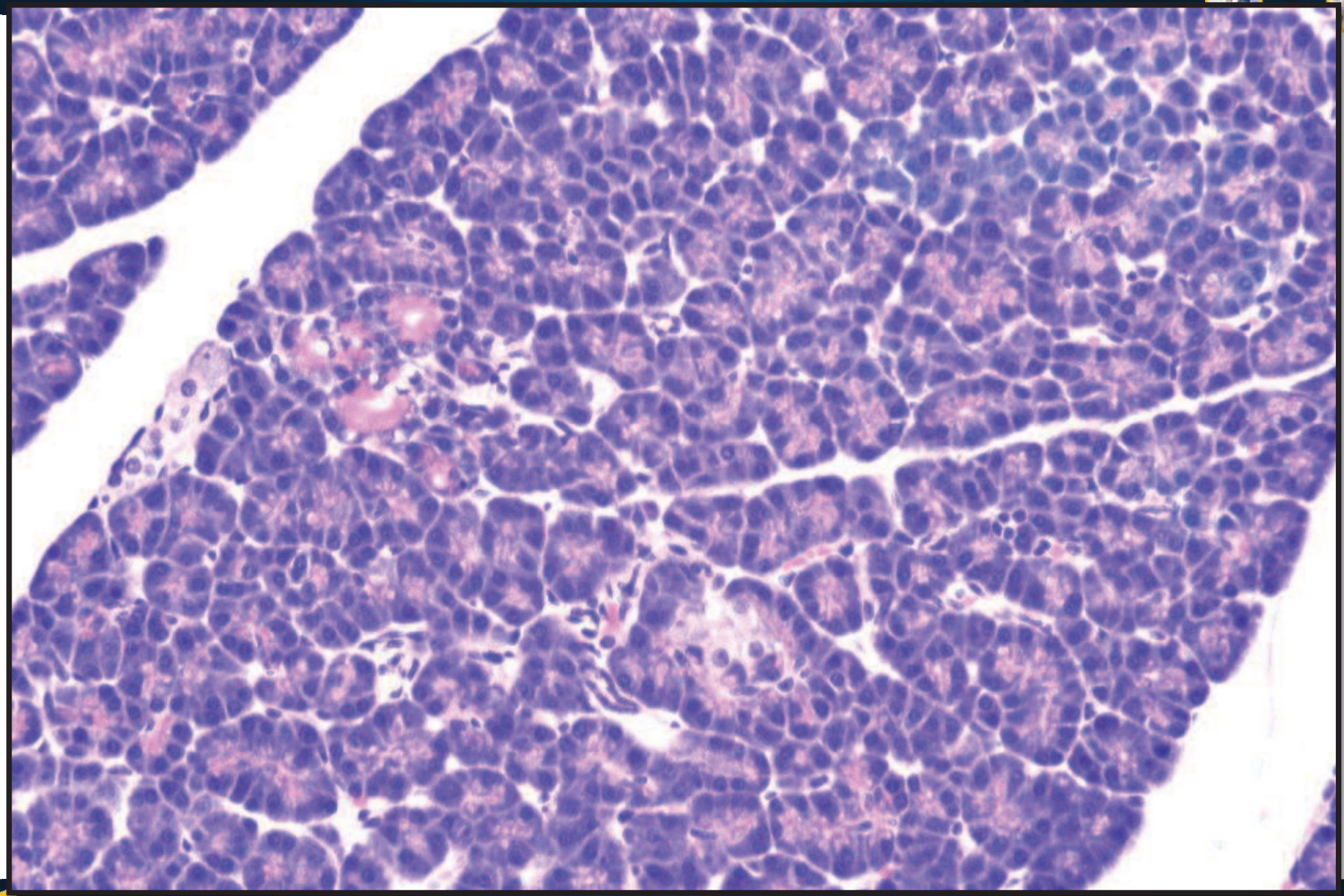


AUTOPHAGIC VACUOLES, ACINAR CELL



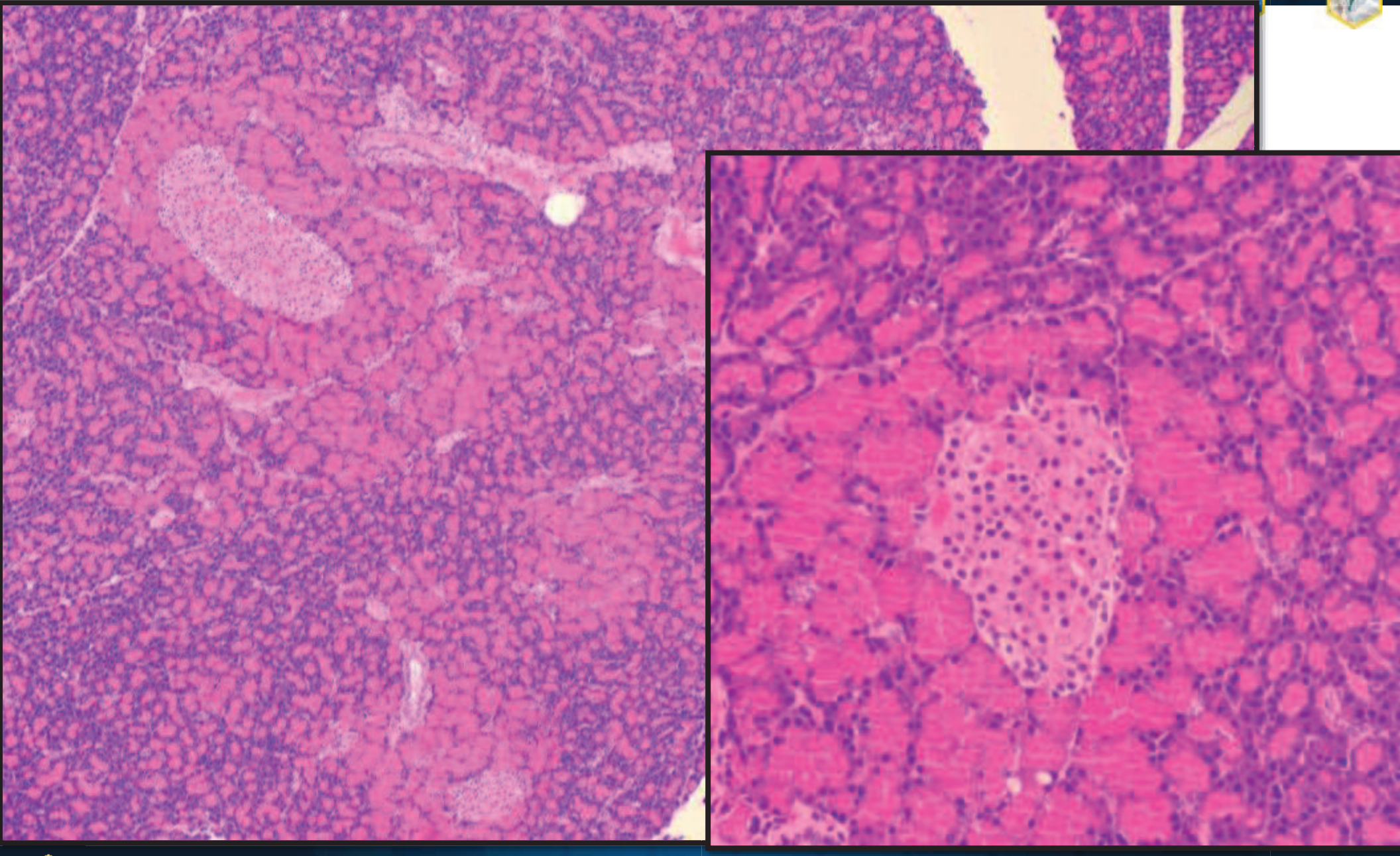
DD- Acinar cell vacuolation, apoptosis

ZYMOGEN GRANULES DECREASED, ACINAR CELL

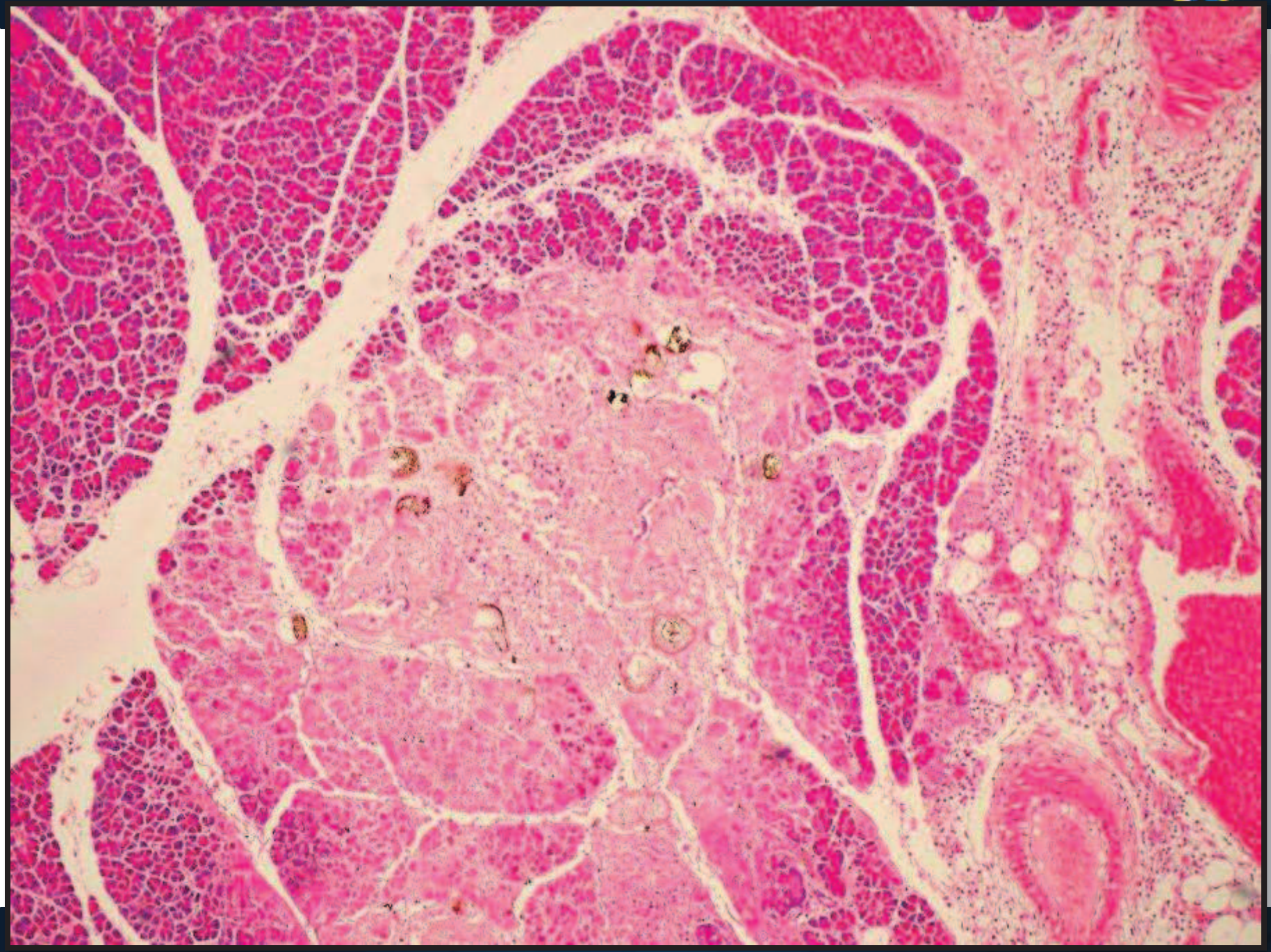


DD- Acinar cell atrophy, Basophilic cell focus

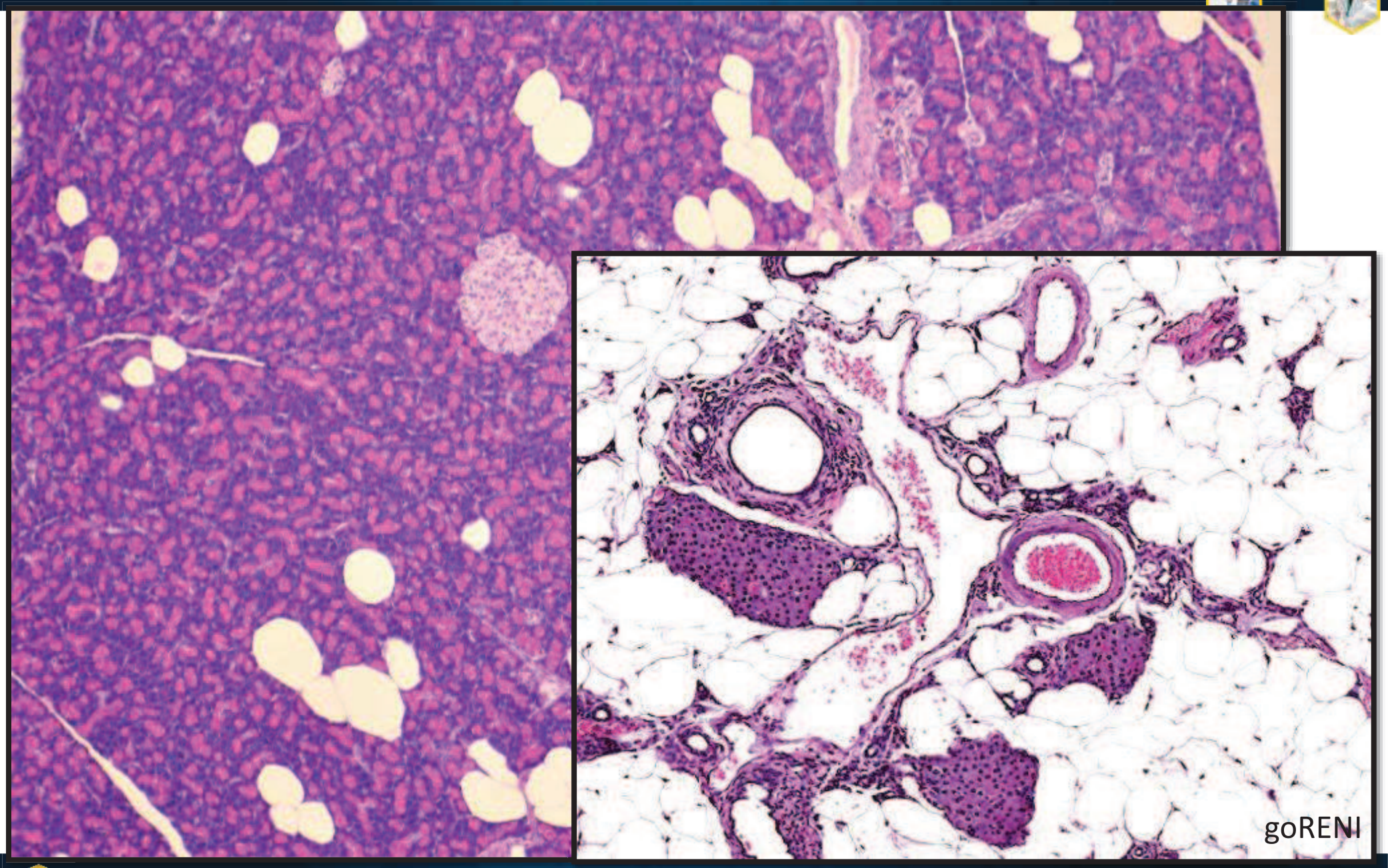
HALOS, PERI-INSULAR



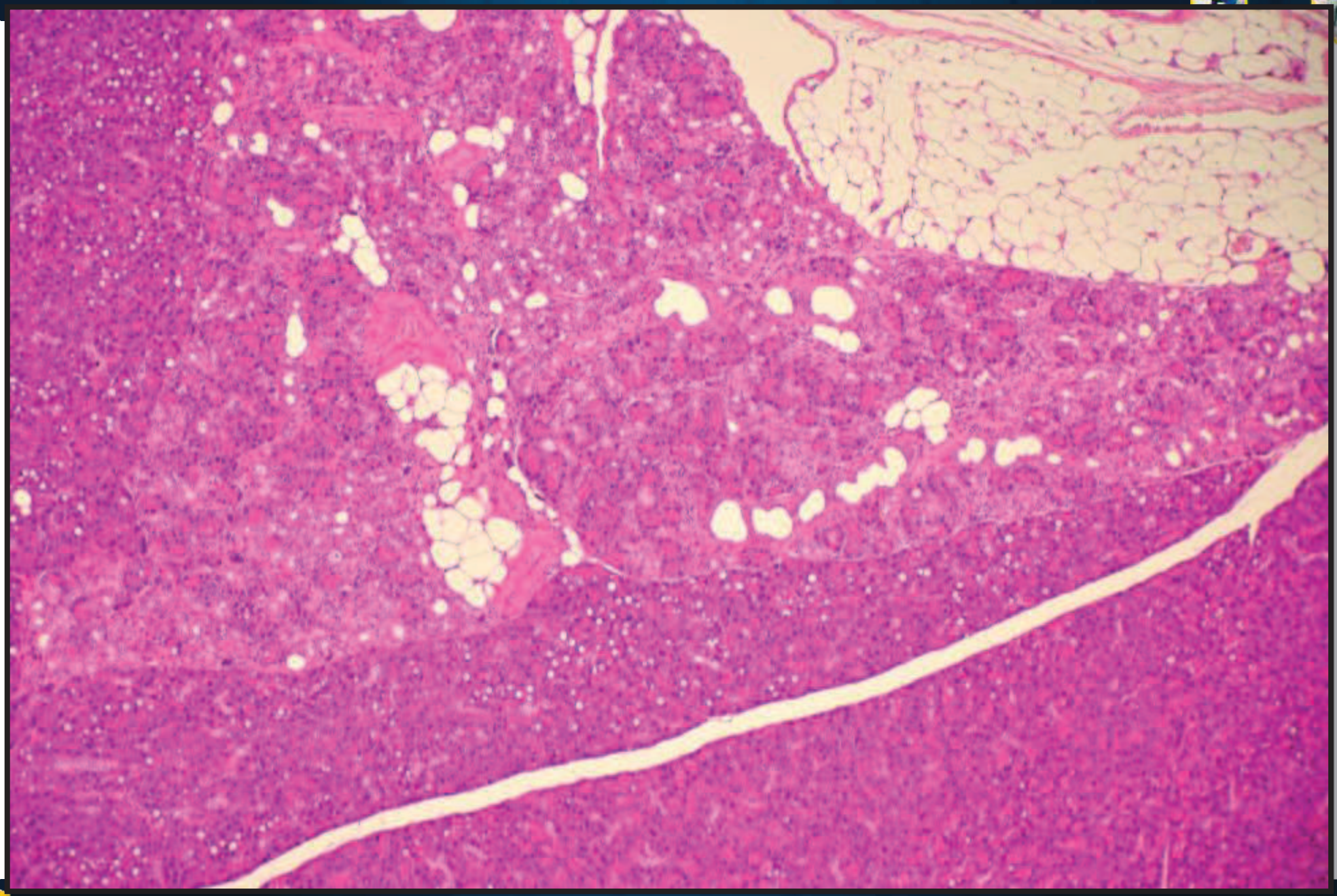
NECROSIS



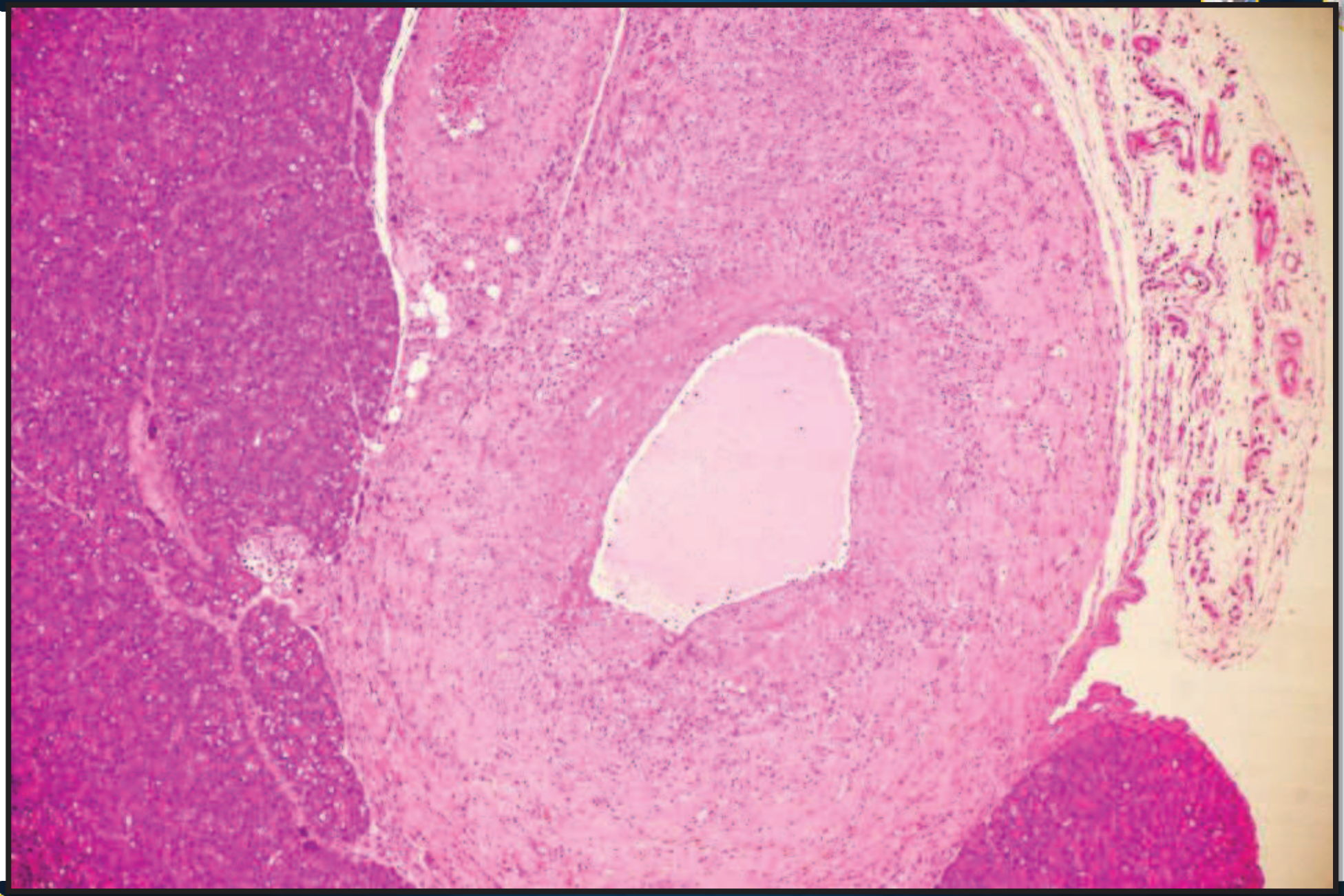
FATTY INFILTRATE



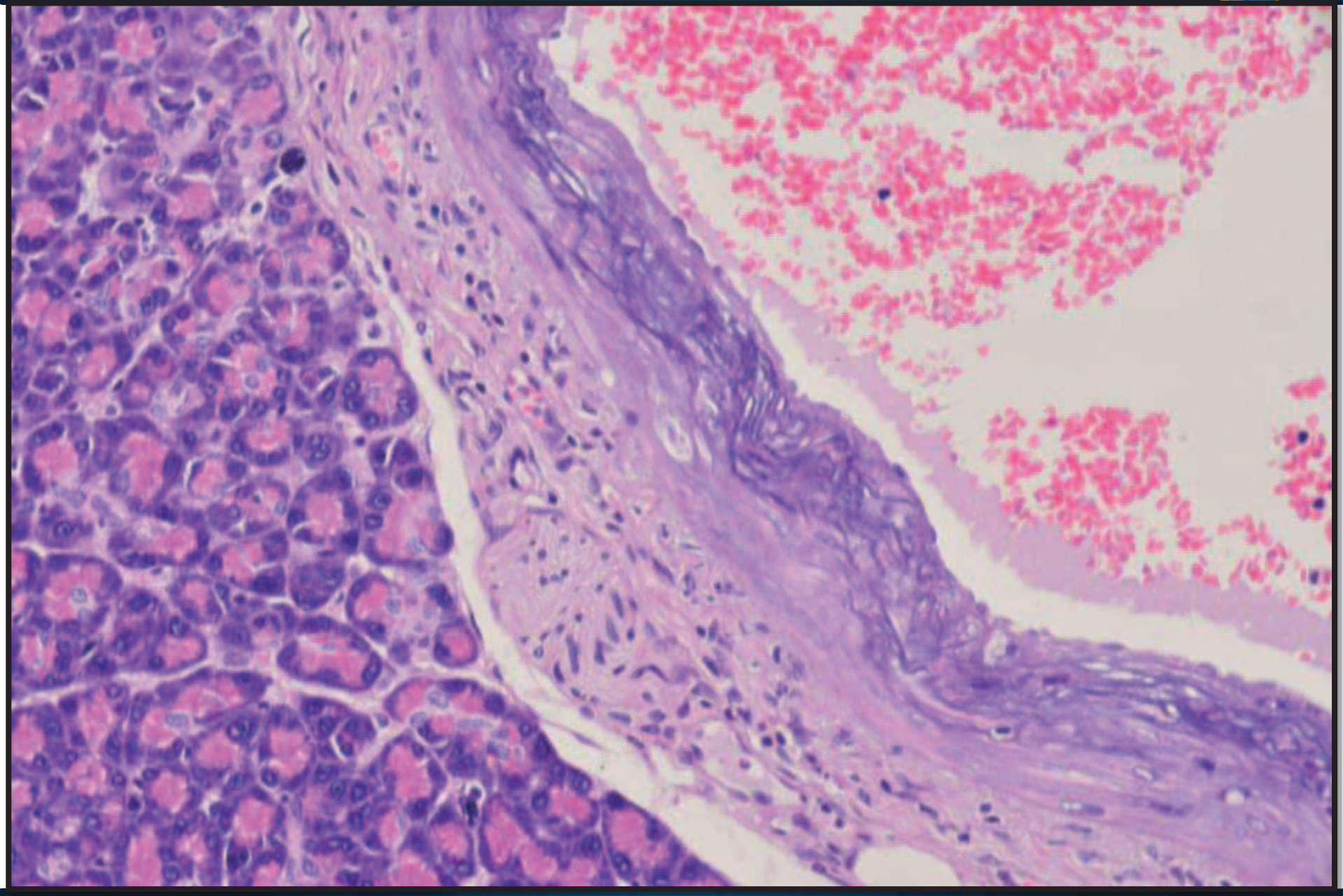
ATROPHY, ACINAR CELL



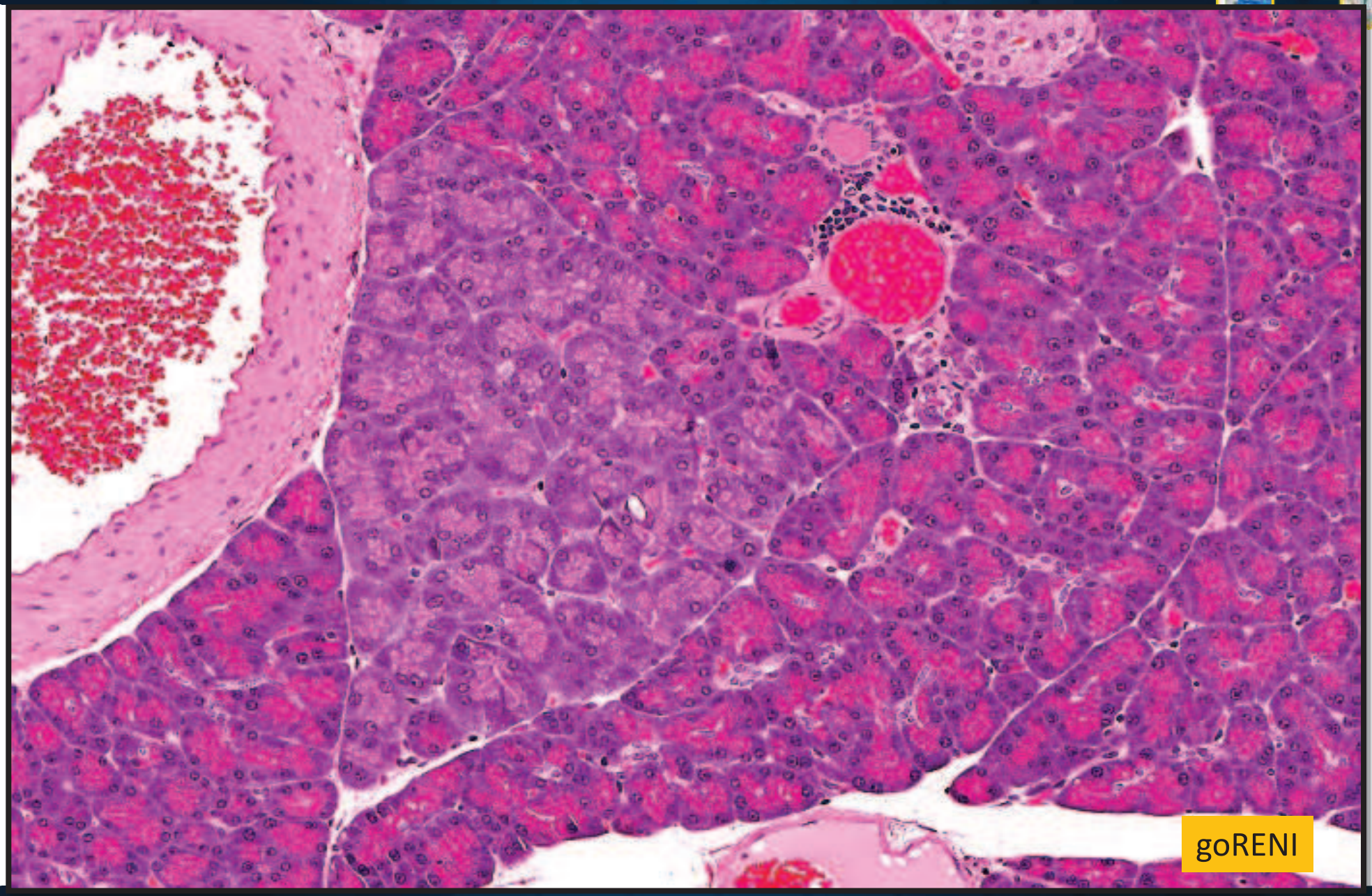
INFLAMMATION & HYPERTROPHY, VESSEL



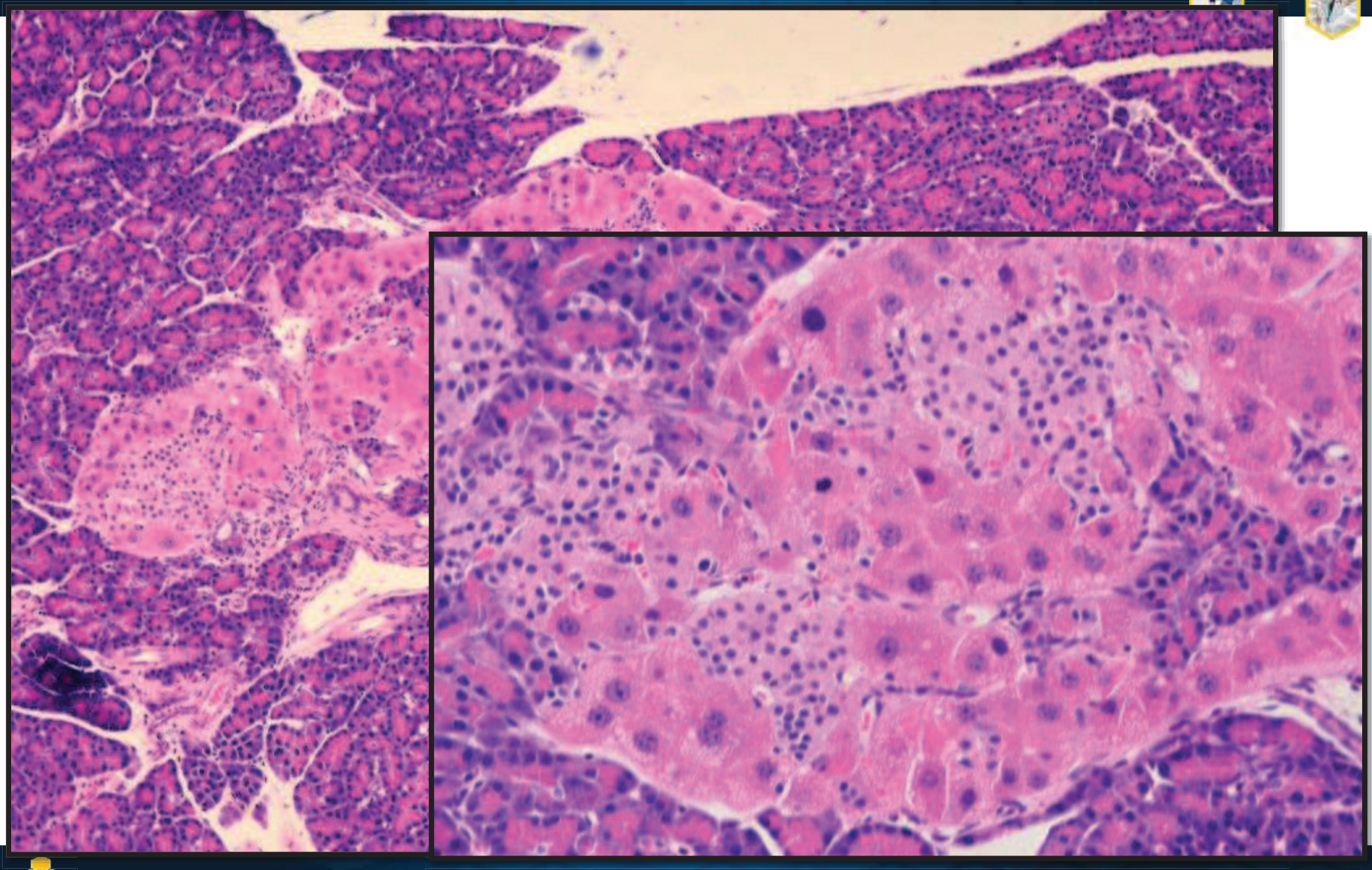
Mineralization- blood vessels



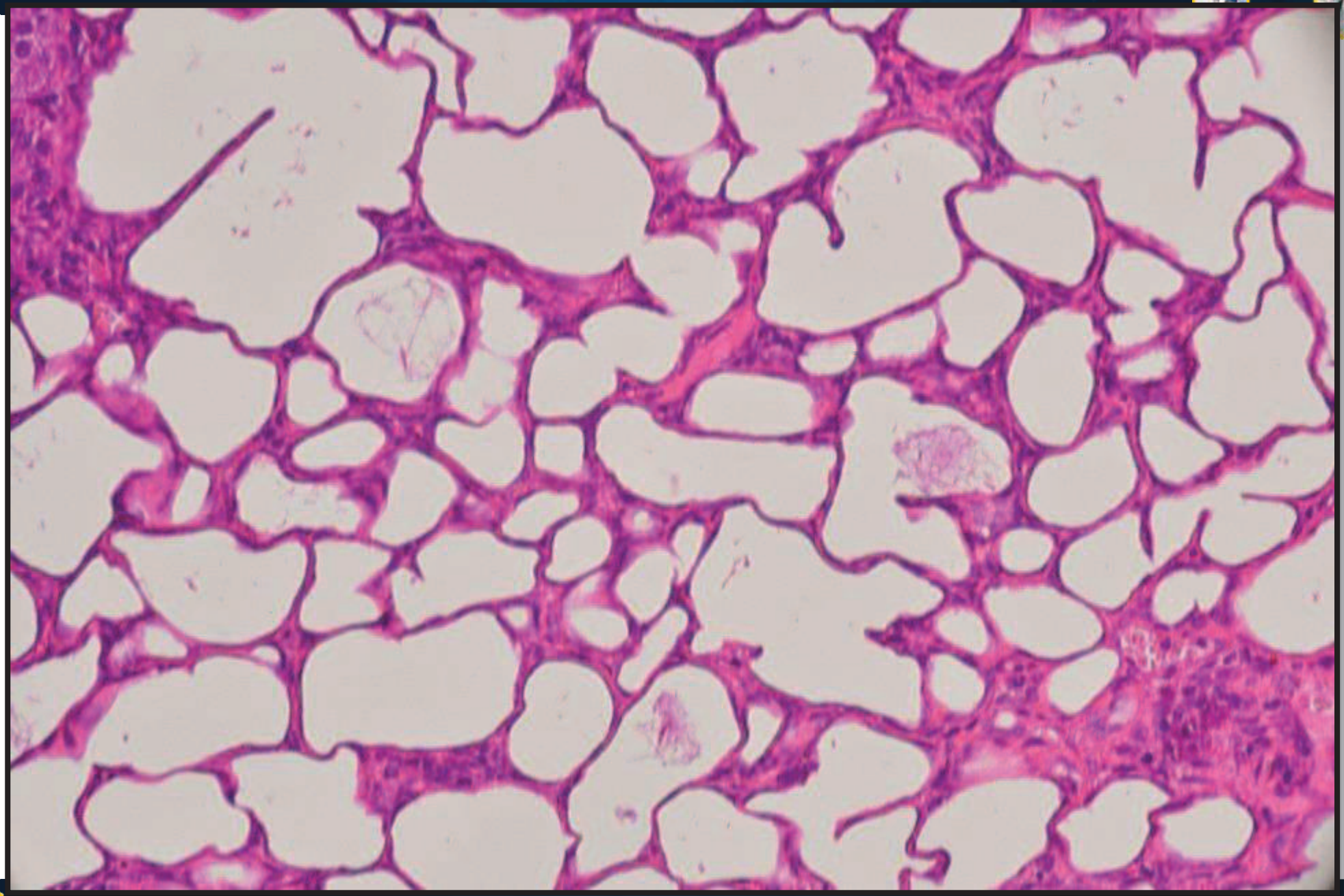
FOCUS, BASOPHILIC



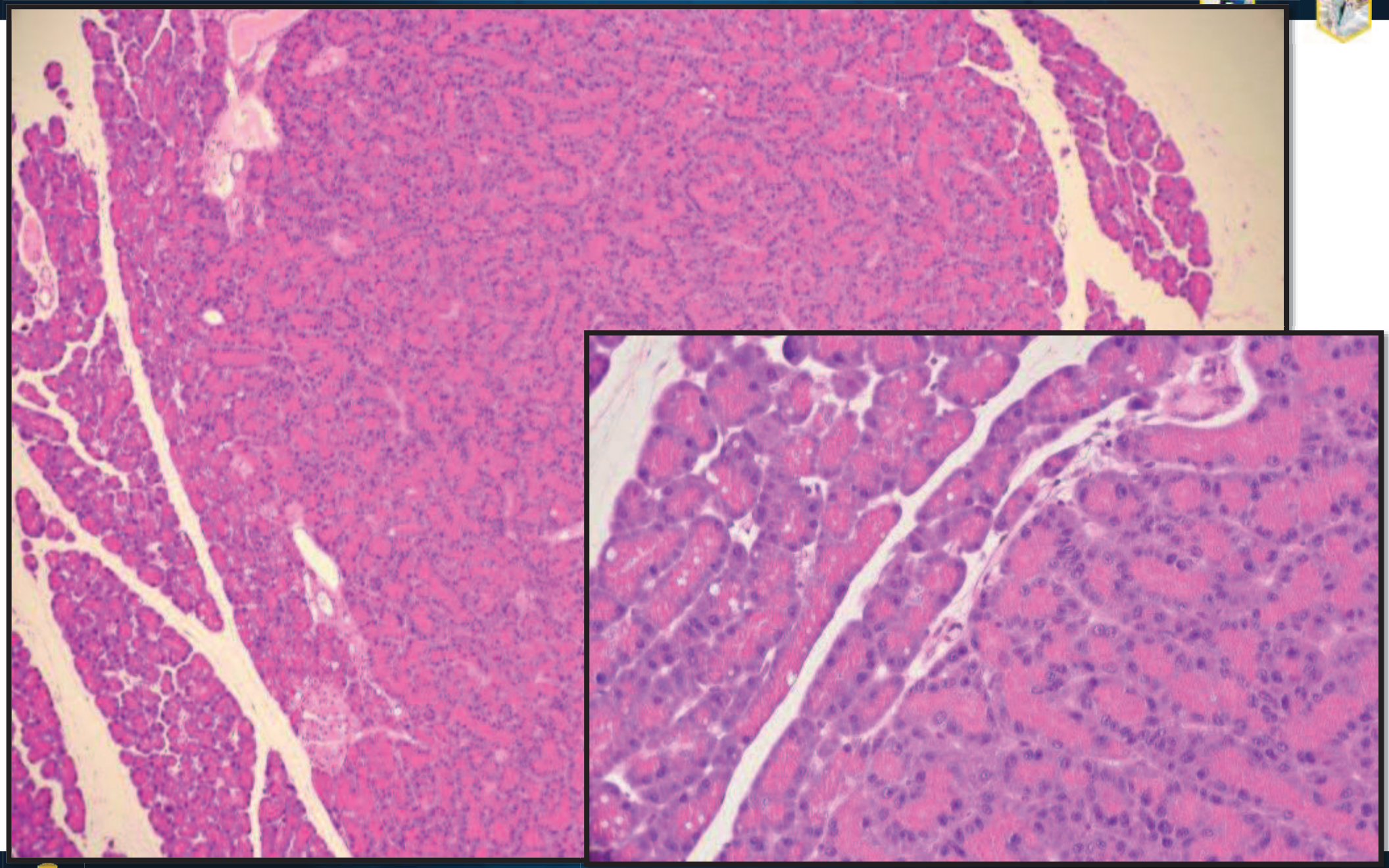
METAPLASIA, HEPATOCYTIC



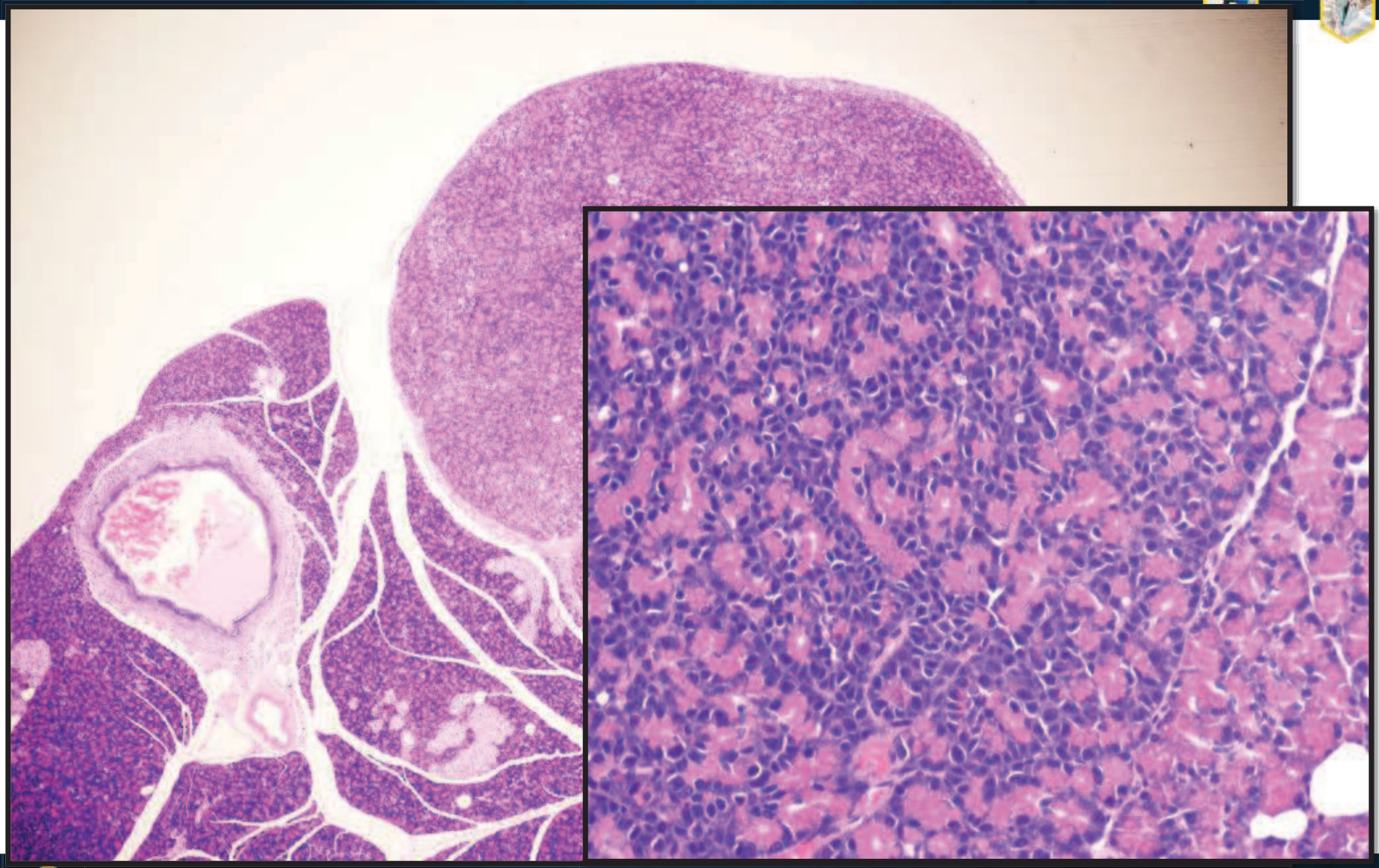
ECTASIA, DUCT



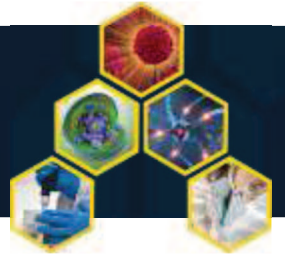
HYPERPLASIA, ACINAR CELL



ADENOMA, ACINAR CELL

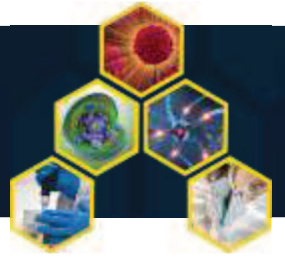


Endocrine Pancreas



- 1-2% of parenchyma receives 20% of blood supply
- Capillary network in islets is 10 time more fenestrated
- Several islet derived hormones and peptides influence directly exocrine function
- α cells – glucagon, β cells – insulin & amylin, δ cells - somatostatin, ε cells - ghrelin, PP or F cells- PP & adrenomedullin
  - Stimulatory – PACAP, NO, VIP, A II
  - Inhibitory – SST, PP. ghrelin, pancreastatin, adrenomedullin, galanin, CGRP, NPY and PYY

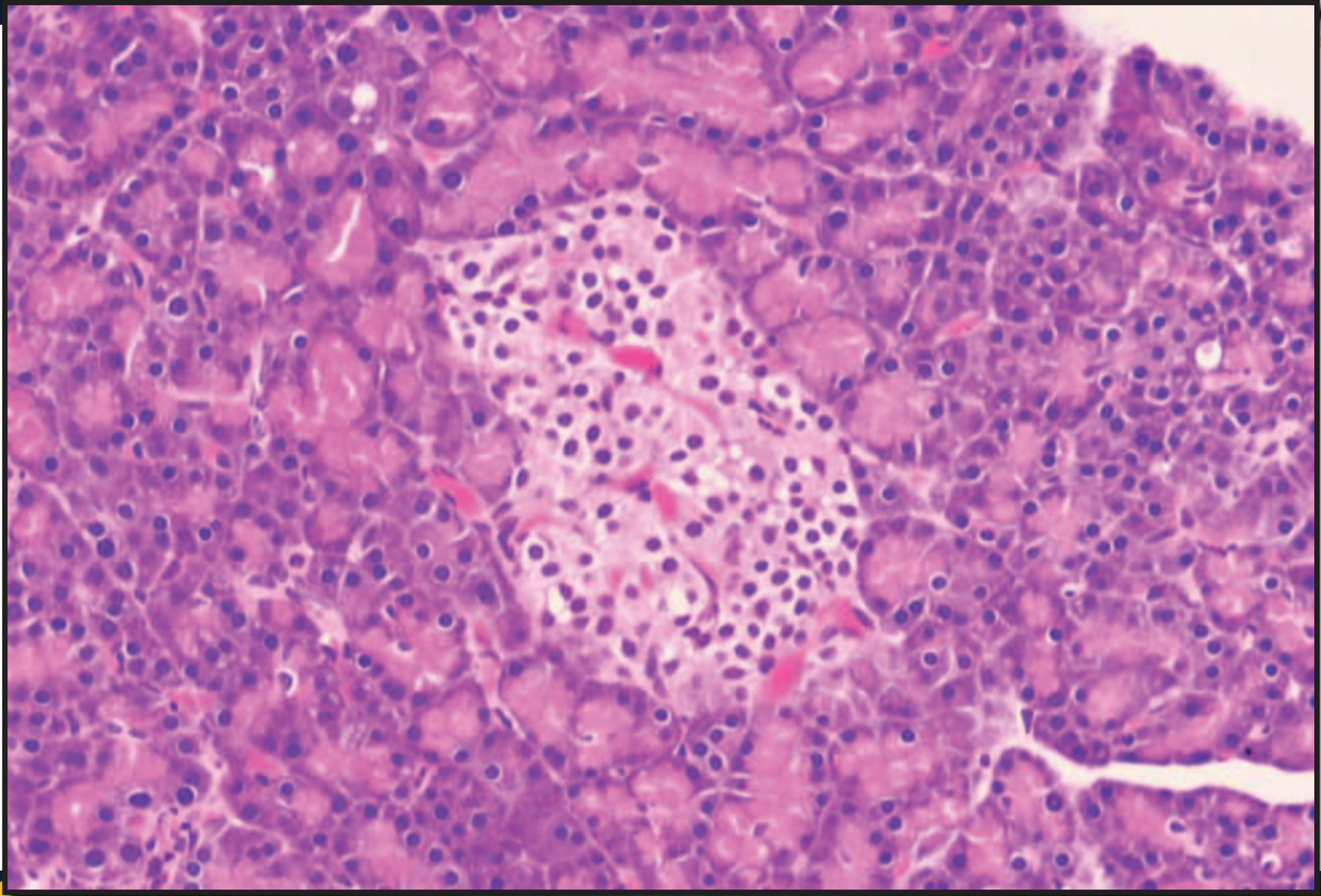
Endocrine Pancreas



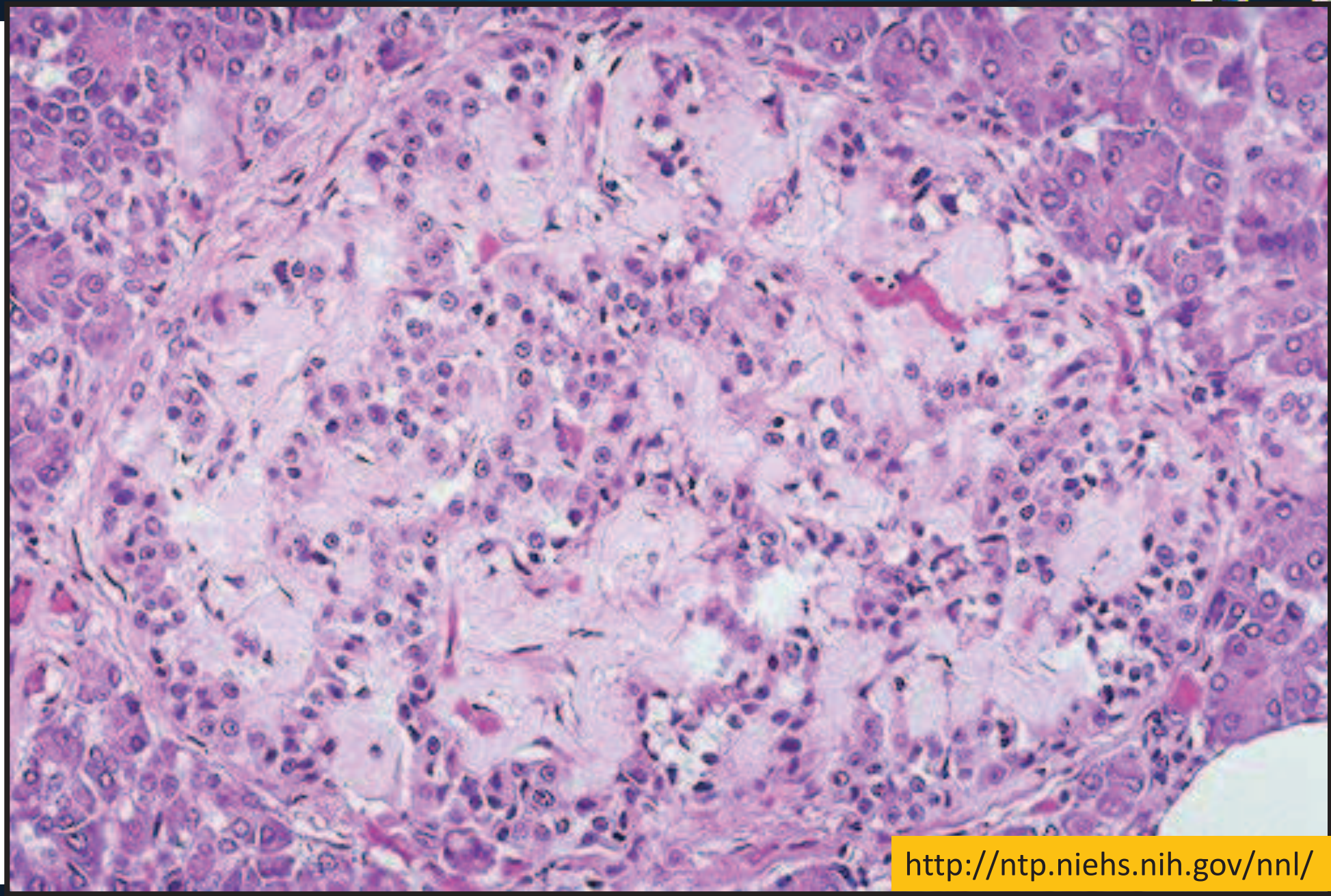
INHAND nomenclature and diagnostic critiera

- Non proliferative
  - Amyloidosis, islet
  - Angiectasis
  - Apoptosis, islet
  - Atrophy, islet
  - Degranulation, ß-cell
  - Fibrosis, islet
  - Infiltrate, inflammatory cell
  - Inflammation
  - Metaplasia, hepatocyte
  - Necrosis, single cell
  - Pigmentation, islet
  - Vacuolation, ß-cell
- Proliferative
  - Hyperplasia, islet cell
  - Adenoma, acinar-islet cell
  - Adenoma, islet cell
  - Carcinoma, islet cell

VACUOLATION, ß-CELL



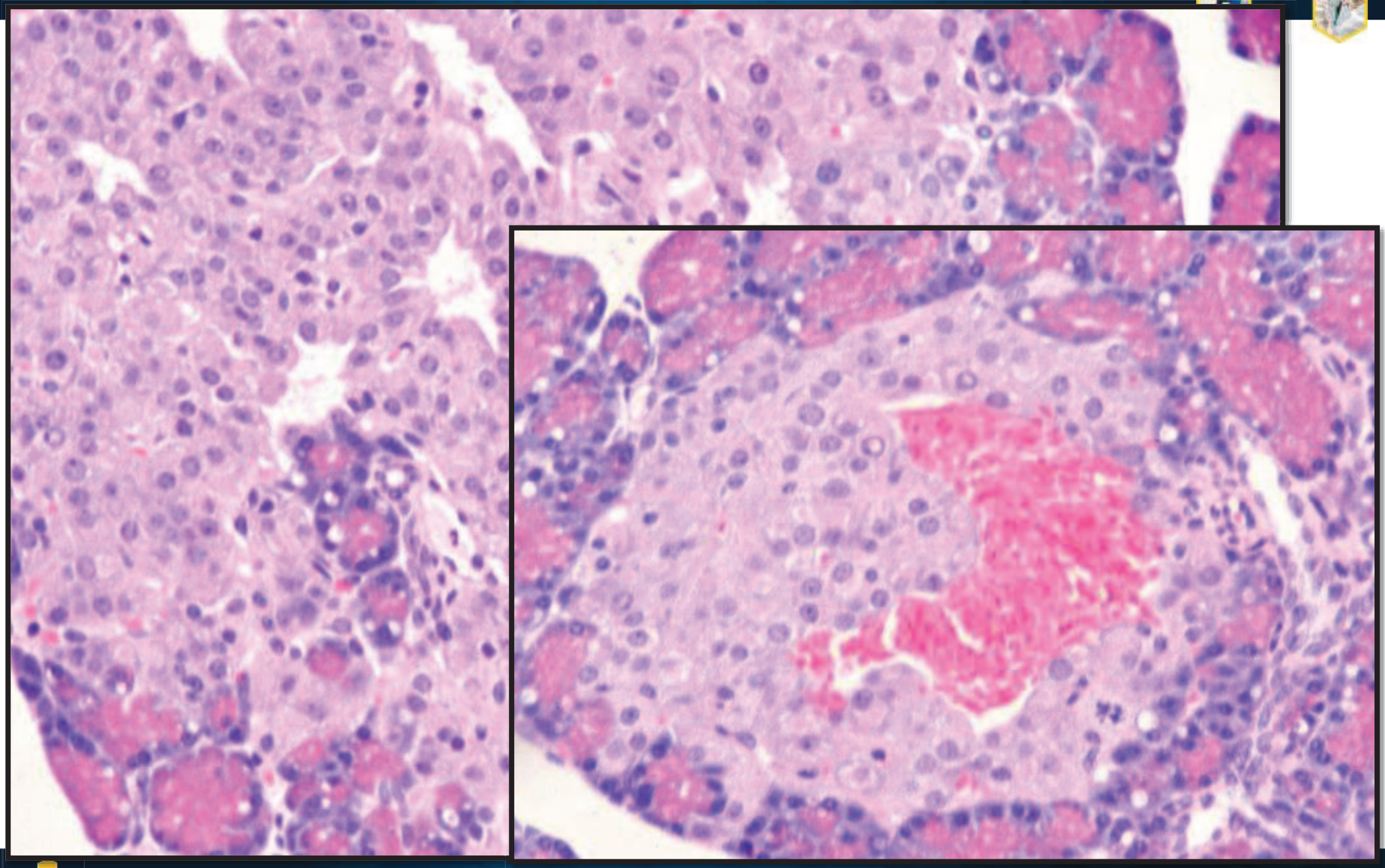
AMYLOIDOSIS, ISLET



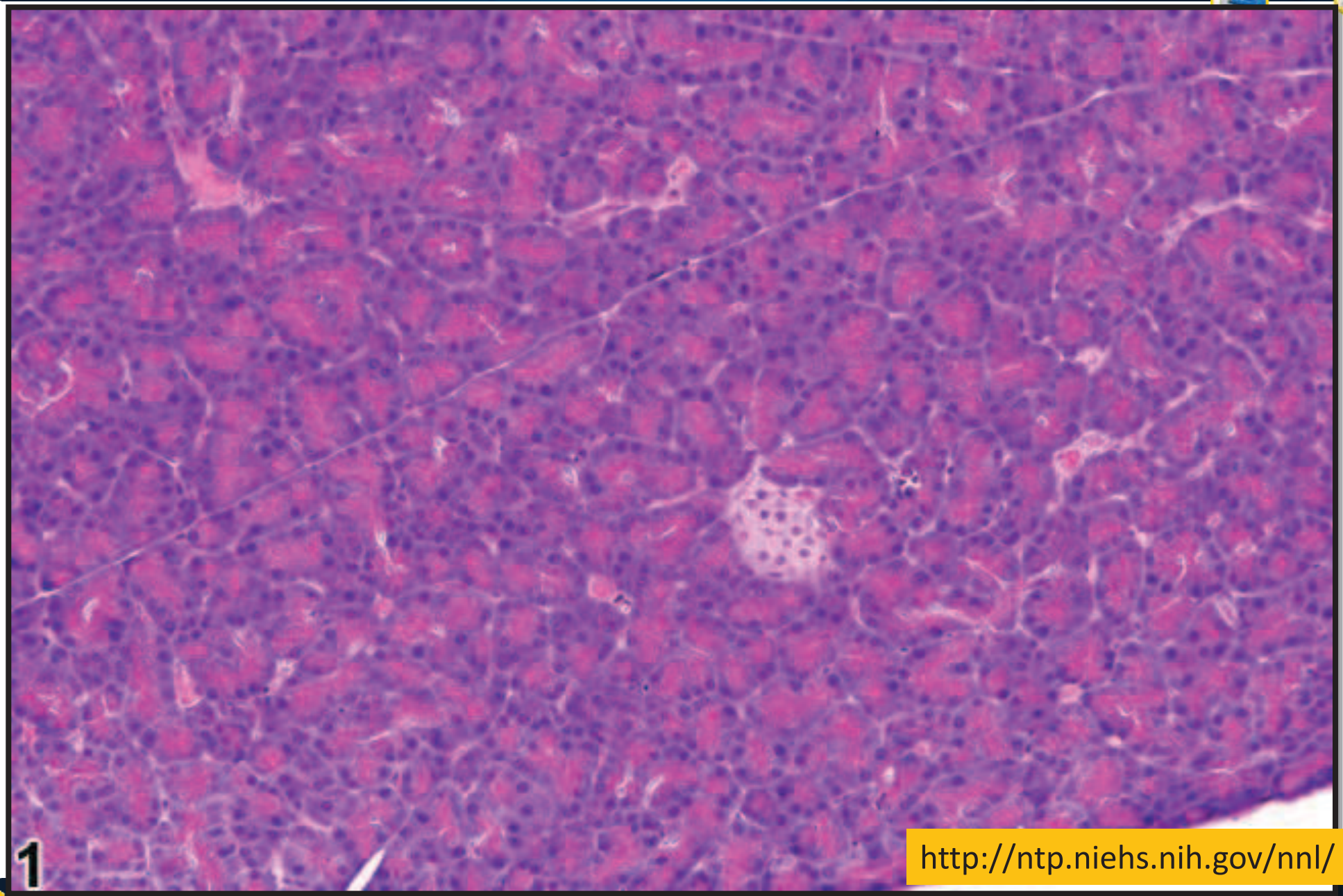
http://ntp.niehs.nih.gov/nnl/

Toxicol Pathol 1991 19: 123

ANGIECTASIS

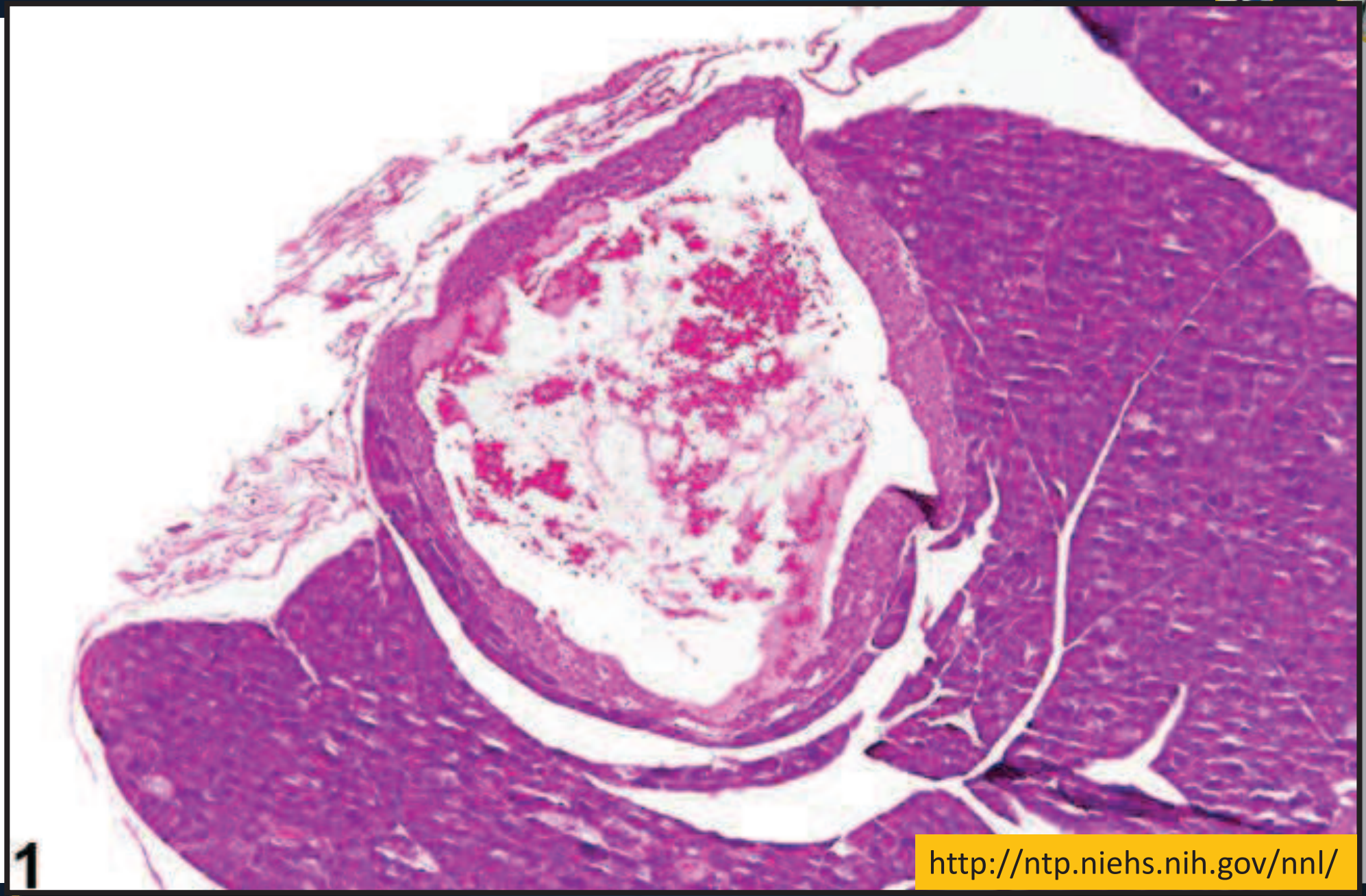


ATROPHY, ISLET



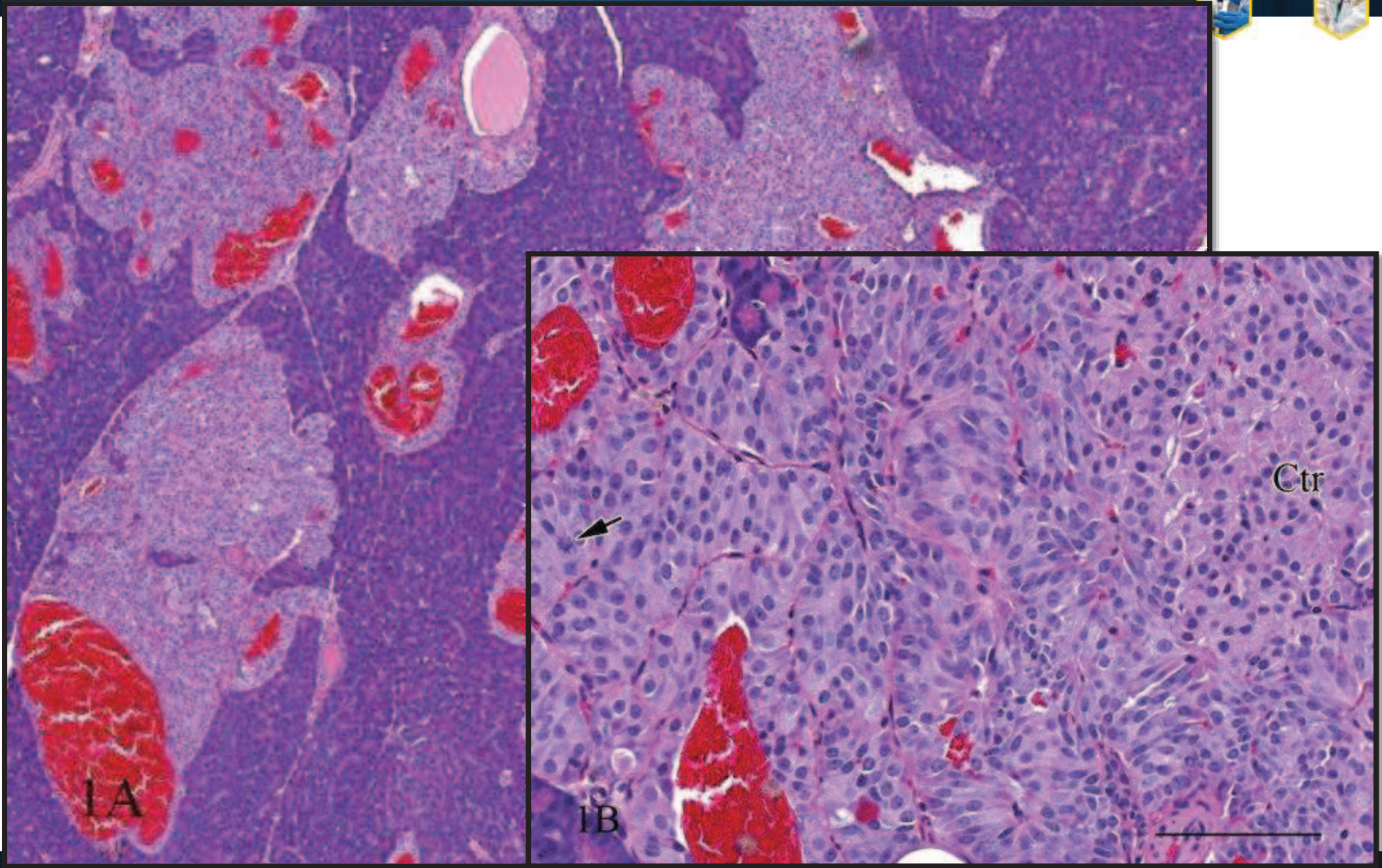
http://ntp.niehs.nih.gov/nnl/

Cyst



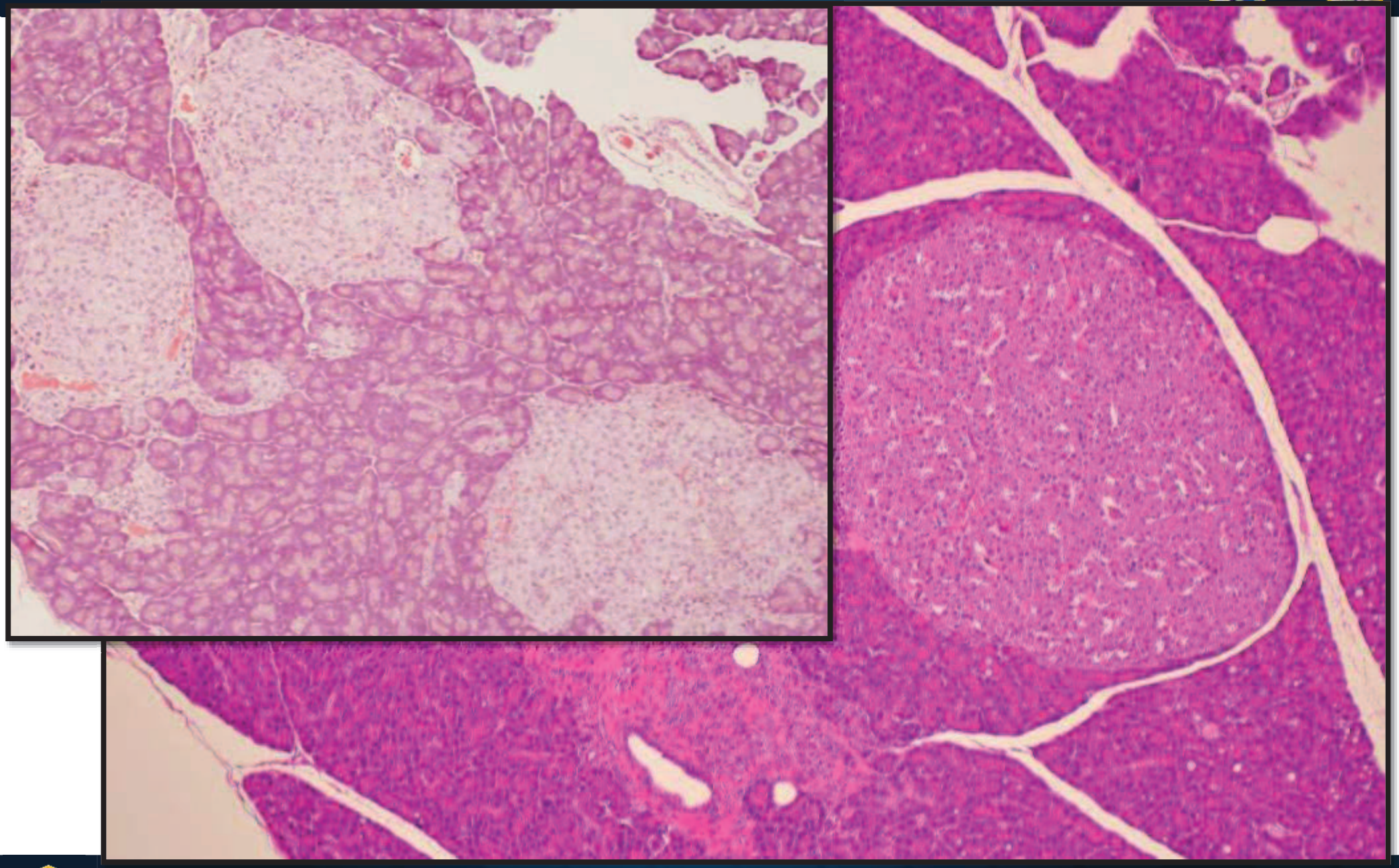
http://ntp.niehs.nih.gov/nnl/

Atypical Case of Islet Cell Hyperplasia

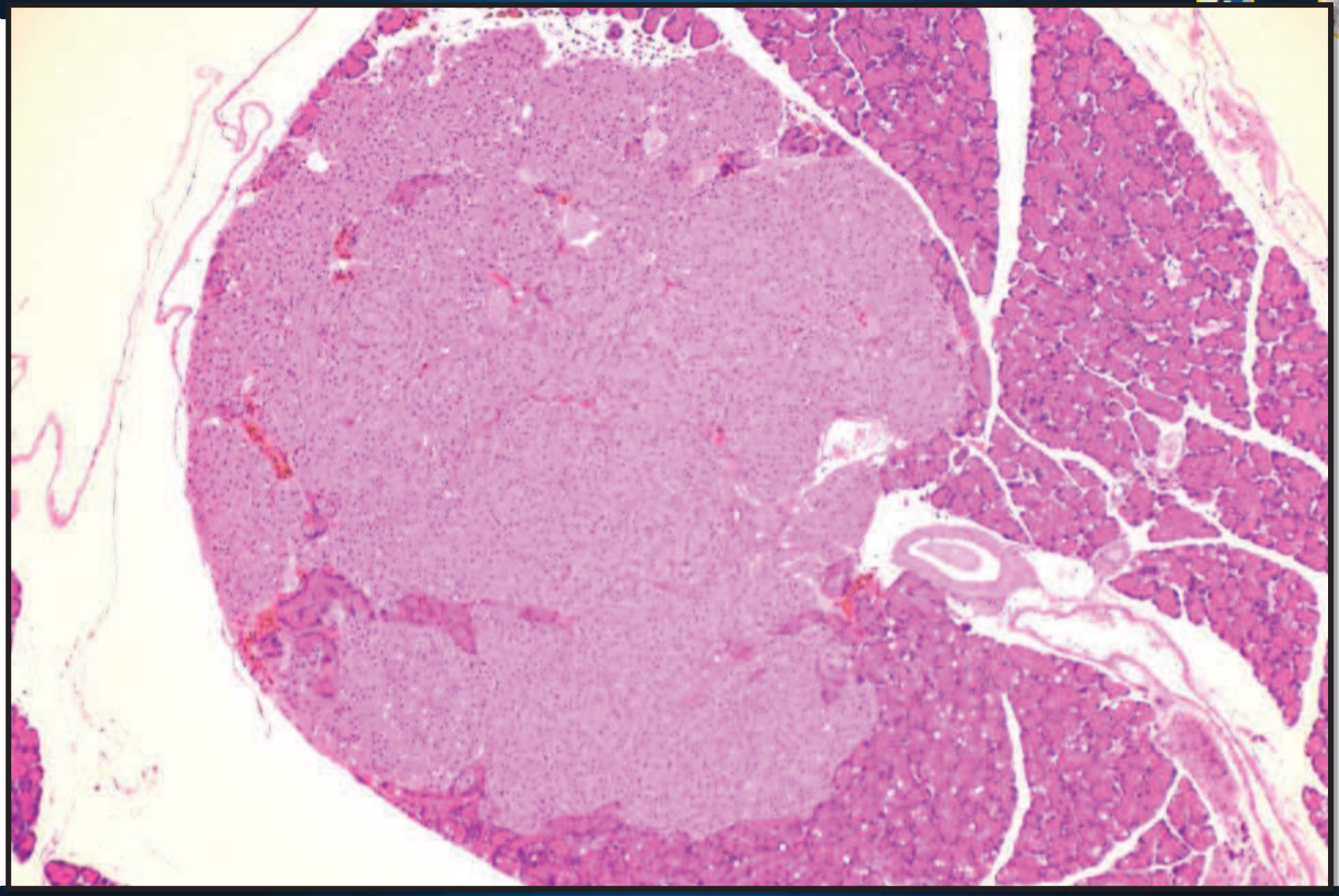


Shirai N et al. Toxicol Pathol 2012;40:819-822

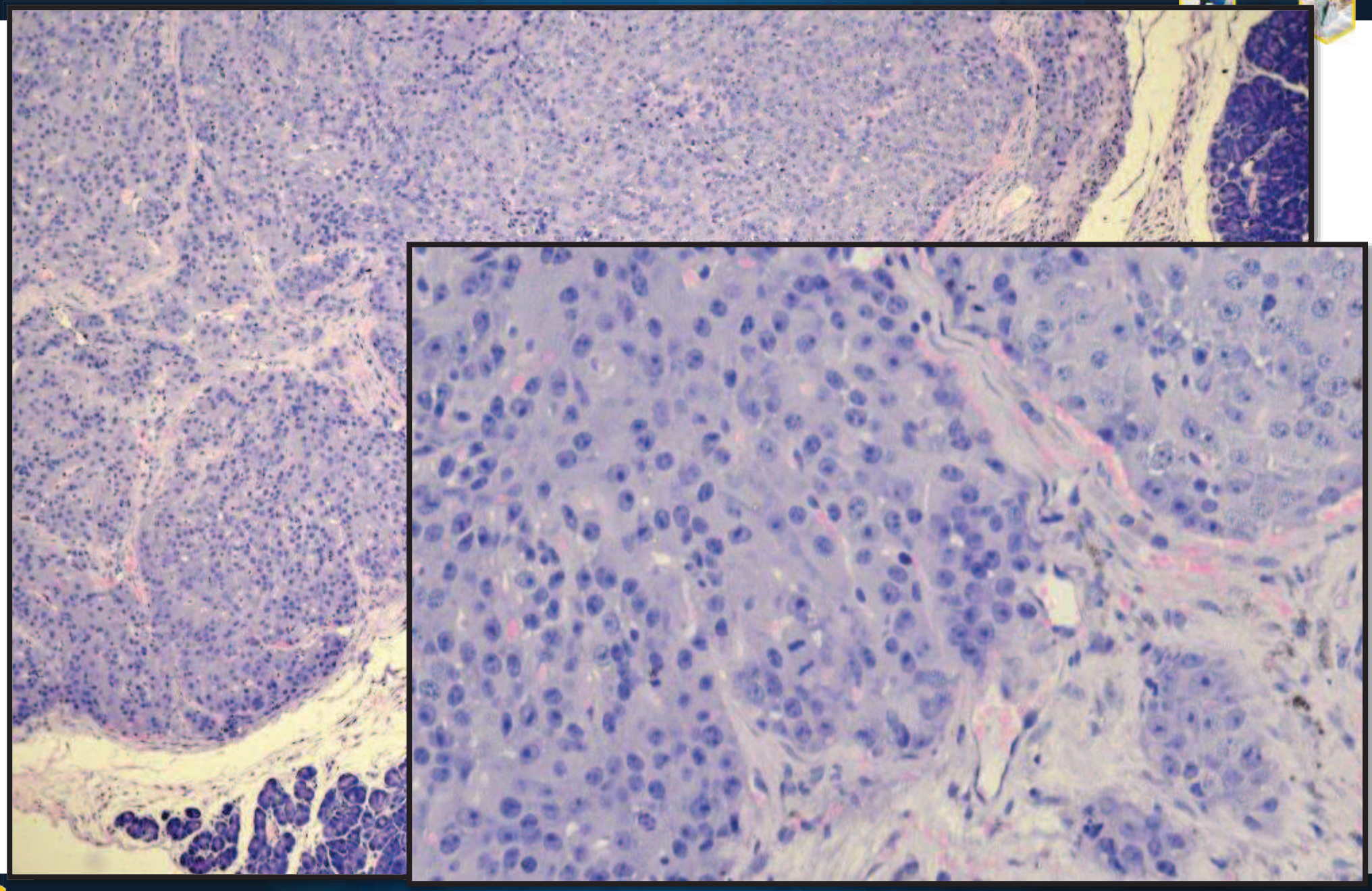
HYPERPLASIA, ISLET CELL



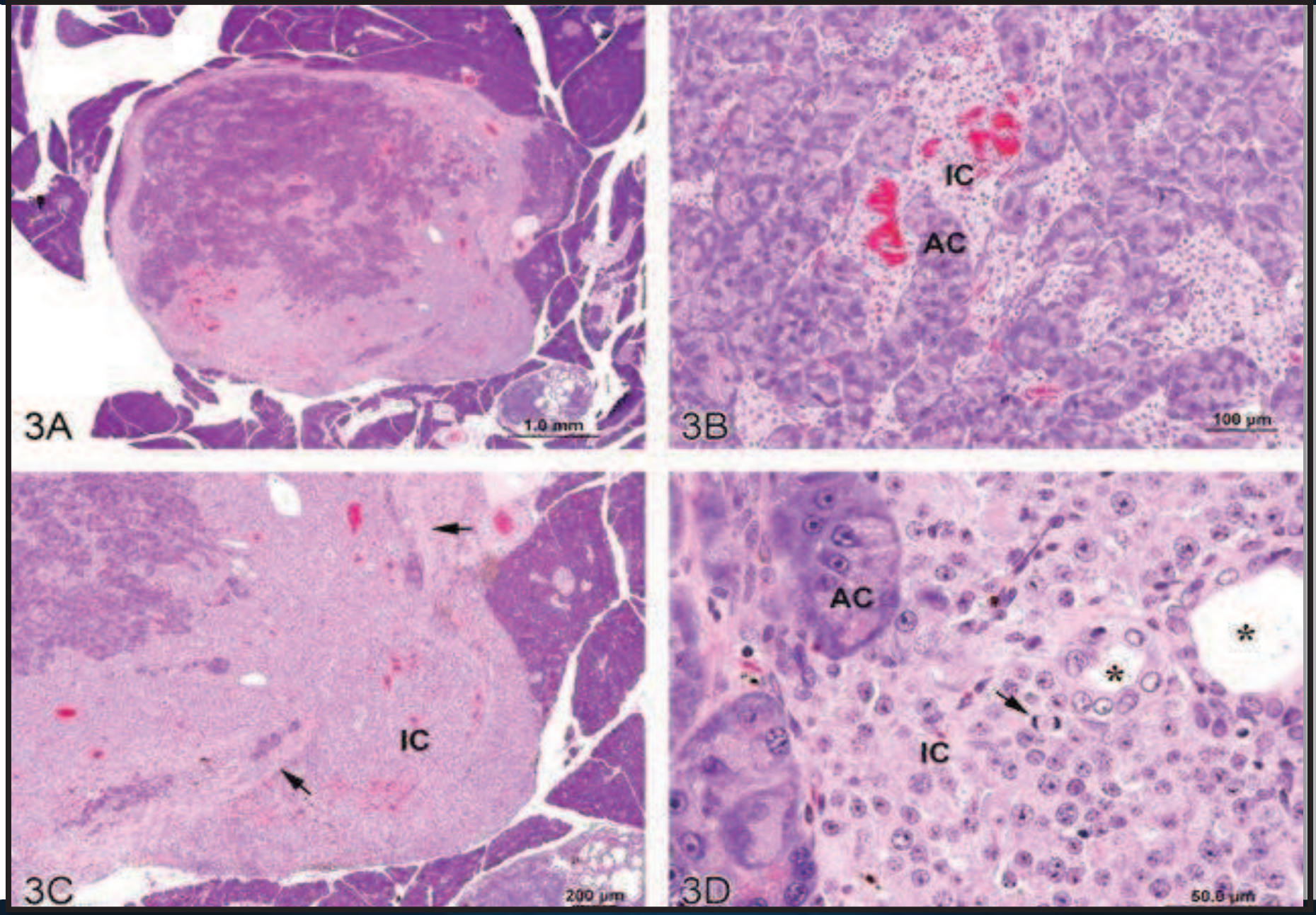
ADENOMA, ISLET CELL



CARCINOMA, ISLET CELL



ADENOMA, ACINAR-ISLET CELL



Adams E T et al. Toxicol Pathol 2010;39:240-266

ACKNOWLEDGEMENTS

AURIGENE

Accelerating Discovery

Thanks

Classification of pancreatic alterations with selected examples

Alteration	Xenobiotic
Exocrine	
• Vacuolation and autophagy (degeneration)	Crambene, ethanol, fumonisin B_1, organophosphates, puromycin, T-2 toxin
• Acinar cell apoptosis	Arginine, crambene, organophosphates, T-2 toxin
• Acinar cell necrosis followed by inflammation	Actinomycin D, arginine, azaserine, crambene, ethioinine, 4-hydroxyaminoquinoline-1-oxide (4-HAQO), lysine, organophosphates, puromycin, zinc
• Acute inflammation with variable acinar cell necrosis	alcohol, azathioprine, l-asparaginase, chlorthalidone, chlorothiazide, furosemide, 6-mercaptopurine, methyldopa, oral contraceptives, pentamidine, riftampicin, sulindac, tetracycline, thiazide diuretics, valproic acid
• Chronic interstitial inflammation	Alcohol
• Duct cell degeneration/necrosis	Dibutyl tin, Zinc
• Neoplasia (experimental animals) well-characterized	Azaserine, 7,12-dimethylbenz[α]anthracene, 4-hydroxyaminoquinoline-1-oxide (rat); *N*-nitroso (2-hydroxypropyl) (2-oxypropyl) amine, *N*-nitrosobis (2-hydroxypropyl) amine (rat and hamster); *N*-nitrosobis (2-oxypropyl) amine (hamster)
• Weak or sporadic association	Aflatoxin B1 (monkey); colfibrate, nafenopin, nitrofen, *N*-nitrosodimethylamine (rat); N_δ-(*N*-methyl-*N*-nitrosocarbamoyl)-L-ornithine (rat, hamster); *N*-nitroso-2,6-dimethylmorpholine, *N*-nitrosomethyl (2-oxopropyl) amine (hamster); *N*-methyl-*N*-nitrosourea (guinea pig, mouse), *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (dog)
Endocrine	
• Increased secretory granules	Benzothiodiazines (chlorothiazide, diazoxide, furosemide, trichlorothiazide, and hydrochlorothiazide)
• Degeneration, vacuolation and degranulation in β-cells	Cyclosporin, cyproheptadine
• Apoptosis	Streptozotocin
• Necrosis	Alloxan, α-amanitin, streptozotocin
• Insulitis (inflammation of islets)	Streptozotocin
• Neoplasia	Alloxan and streptozotocin (rat), azinophosmethyl, 6-diethylaminomethyl-4-hydroxyaminoquinoline-1-oxide, heliotrine