

# Early Morphological Changes After Total Gastrectomy in the Rat Pancreas

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**Abstract:** Total gastrectomy is widely used as a major surgical treatment for gastric cancer. The aims of this study were to investigate the possible effects of total gastrectomy on pancreas morphology, and to determine the immunolocalisation of chromogranin A (Cg A) and synaptophysin (SYP) antigens, described as specific tumour markers for neuroendocrine tumours.

Ten rats underwent total gastrectomy via oesophago-duodenostomy. After a month the rats were killed, and tissue samples were taken from the pancreas, processed for light microscopy and stained with haematoxylin-eosin. In addition, 5-7 µm thick paraffin embedded tissue sections were immunohistochemically stained with Cg A and SYP.

The acinar cells showed abundant cytoplasm which contained numerous zymogen granules in the experimental group animals in comparison with the control group. The islets of Langerhans showed significant hyperplasia postoperatively when compared to the control group. Strong immunoreactivity for Cg A and SYP was seen in the islet cells.

It may be suggested that total gastrectomy causes adverse morphological changes in the pancreas. Strong Cg A and SYP immunoreactivities may be a relevant marker for pancreatic cancers in the early postoperative period after total gastrectomy.

**Key Words:** Pancreas, total gastrectomy, chromogranin A (Cg A), synaptophysin (SYP).

## Total Gastrektomi Sonrası Erken Dönemde Sıçan Pankreasındaki Morfolojik Değişiklikler

**Özet:** Total gastrektomi mide kanserlerinin tedavisinde çok yaygın bir şekilde kullanılmaktadır. Bu çalışmada, total gastrektominin pankreas morfolojisi üzerine etkisinin ve nöroendokrin tümör markırı olarak tanımlanan kromogranin A (Cg A) ve synaptophysin (SYP) antijenlerinin lokalizasyonlarının belirlenmesi amaçlanmıştır.

Çalışmada 10 adet rata, özofago-duodenostomi yolu ile total gastrektomi uygulanmış, 30 gün sonra alınan pankreas doku örnekleri rutin protokolü takiben hematoksilen-eosin ve immünohistokimyasal olarak da Cg A ve SYP ile boyanmıştır.

Deney grubu kontrol grubu ile karşılaştırıldığında, deney grubu asinar hücrelerinin çok sayıda zimojen granülleri ile bol sitoplazma ve Langerhans adacıklarının da önemli derecede hiperplazi gösterdiği belirlenmiştir. Adacıklarda kuvvetli Cg A ve SYP immünreaktivitesi gözlenmiştir.

Sonuç olarak total gastrektominin, pankreasta morfolojik değişikliklere neden olduğu ve total gastrektomiyi takip eden erken postoperatif dönemde kuvvetli Cg A ve SYP immünreaktivitesinin pankreas kanseri için bir işaret olabileceği öne sürülebilir.

**Anahtar Sözcükler:** Pankreas, total gastrektomi, kromogranin A (Cg A), synaptophysin (SYP)

## Introduction

Since carcinoma of the pancreas is so difficult to cure and its aetiology remains obscure, it is important to investigate potential risk factors such as total gastrectomy. Several reports have indicated an increased

incidence of pancreatic cancer in patients undergoing gastric resection (1,2).

In gastric carcinoma total gastrectomy is frequently performed with curative intent. This is the most common and usually the first treatment for patients with gastric

cancer. The removal of part or all of the stomach is a major change to the digestive tract. Numerous nutritional, metabolic and hormonal disturbances and glucose intolerance develop after total gastrectomy. Weight loss, diarrhoea and other gastrointestinal symptoms are common after radical gastric surgery, despite dietary support and symptomatic treatment (3,4). Numbers of clinical studies have been reported on glucose intolerance and pancreatic endocrine function after gastrectomy (1,2,5,6). Watanapa and Williamson (7) have reported that partial gastrectomy stimulates pancreatic growth and enhances carcinogenesis, probably by means of increased cholecystokinin (CCK) release.

Total gastrectomy in rats has been used as a model to study postgastrectomy syndromes. Total gastrectomy causes adverse morphological changes throughout the oesophago-duodenal anastomatic region (8-10). Studies in experimental animals have shown that gastric surgery (total gastrectomy) can induce adenocarcinoma of the oesophagus without the use of carcinogens (9). In the alimentary canal, carcinogenesis can be enhanced by luminal factors acting directly on the mucosa to produce hyperplasia (11,12) but in the pancreas any such influence seems to be exerted indirectly through humoral and/or neural mechanisms.

Surprisingly, few histopathological studies have been performed on the pancreas in patients after gastrectomy. The premise of this study was that post gastrectomy pancreatic symptoms should be evidenced by morphological and histopathological changes. The aims of the present study were to investigate the possible effects of total gastrectomy on pancreas morphology, and to detect chromogranin A (Cg A) and synaptophysin (SYP) antigens immunohistochemically, described as specific tumour markers for neuroendocrine tumours in the pancreas.

## Materials and Methods

### Experimental Animals

Fifteen adult male Wistar albino rats each weighing 350 to 400 g were kept in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals and the experiments were approved by the Cumhuriyet University Medical Faculty Animal Care Committee. They were given a standard pellet diet and tap water. The animals were starved for 24 h prior to

surgery. After the operations, the rats were deprived of food and water for 24 h, and then were fed cow's milk for 2-3 days.

### Surgical Procedures

Before surgery, all rats were anaesthetised by intraperitoneal injection of xylazine (3-10 mg/kg body weight) and ketamine chloride (90 mg/kg body weight). Gastrectomy was performed by resecting the stomach after ligation of the blood vessels and by anastomosing the oesophagus and the duodenum end-to-end. The oesophagus was anastomosed to the duodenum using 6-0 polysorb synthetic absorbable lactomer sutures. The abdominal cavity was closed with 3-0 silk sutures. Five rats were subjected to sham operations in which the abdominal cavity was opened for approximately 45 min, the same length of time as that required for the gastrectomy procedure.

### Histological analysis

On the 30<sup>th</sup> day the rats were killed, and tissue samples were taken from the pancreas and fixed in 10% buffered formaline, embedded in paraffin, cut into 5 µm sections, and stained with haematoxylin-eosin (13). Immunohistochemical staining was simultaneously performed on serial sections from the paraffin-embedded tissue. Briefly, 5 µm sections were deparaffinised in xylene and then rehydrated. Endogenous peroxidase activity was blocked with 1% H<sub>2</sub>O<sub>2</sub> in methanol. Subsequently, sections were washed in phosphate-buffered saline. Primary antibodies chromogranin A (Cg A) (Novocastra Laboratories Ltd, United Kingdom) and synaptophysin (SYP) (Quartett, Berlin, Germany) were applied for 2 h. Subsequently, sections were incubated with a secondary antibody (Biotinylated goat anti-polyvalent). The reaction products were visualised with amino ethylcarbazole (AEC) (Lab Vision, Fremont, CA, USA) as the chromogen, and sections were counterstained with haematoxylin-eosin.

## Results

### Sham-operated group

There were no alterations in the histology of the exocrine and endocrine pancreas in the sham-operated animals in contrast with those seen in the experimental group acinar and endocrine cells (Figures 1-5). The acini had a rounded structure consisting of mainly pyramidal

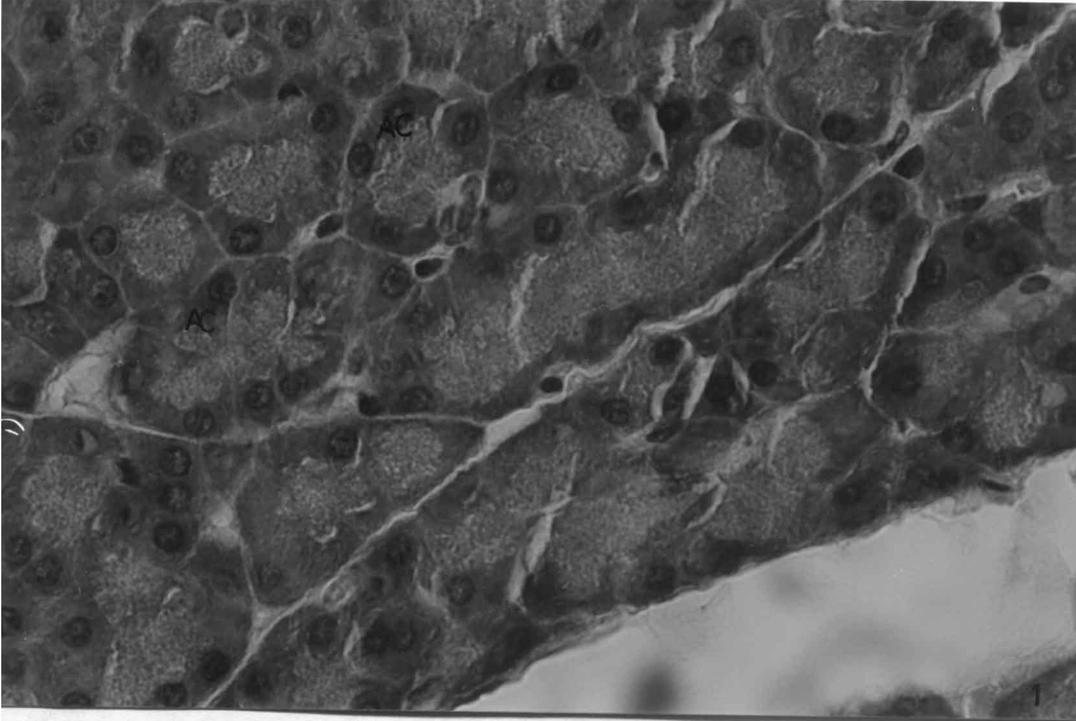


Figure 1. There is no alteration in the histology of the exocrine pancreas in the sham-operated group. The exocrine acinar cells (AC) show normal structure (H-E; X100).

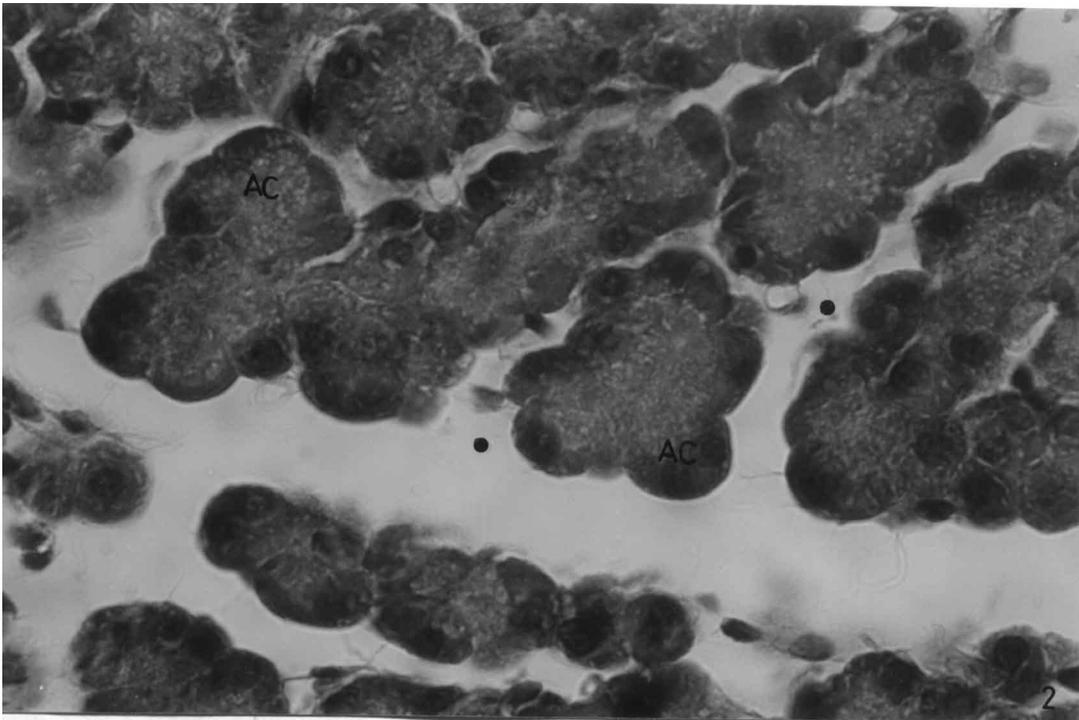


Figure 2. The acinar cells (AC) demonstrate abundant cytoplasm which contains numerous zymogen granules in the experimental group animals. There are inter acinar spaces (●) between irregular glands (H-E; X100).

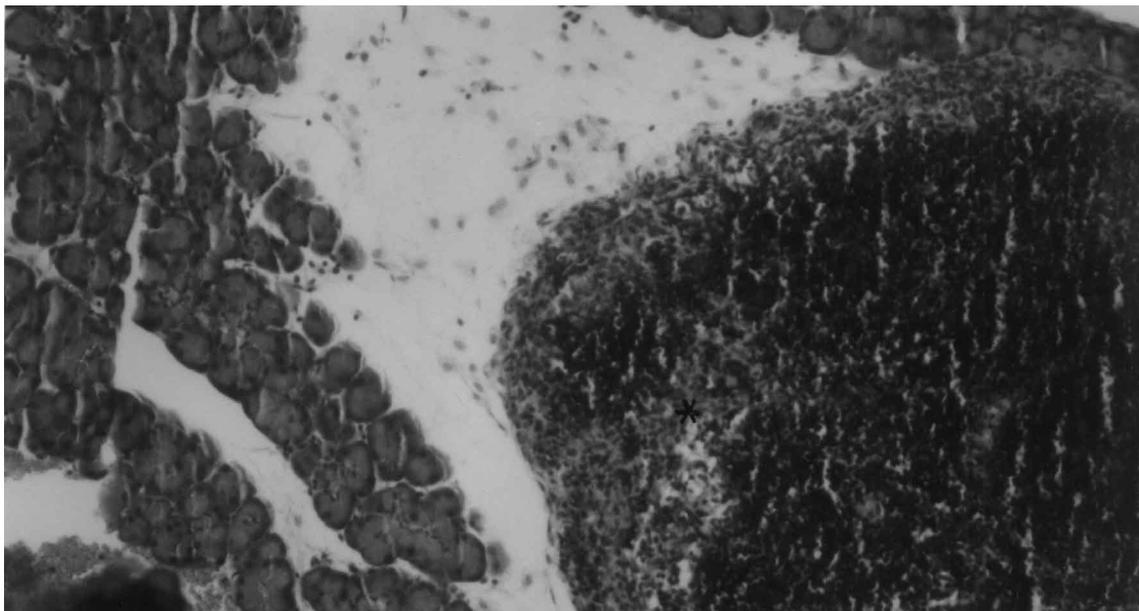


Figure 3. Intense infiltration of lymphocytes, neutrophil and eosinophil leukocytes (\*) are seen in the pancreatic tissue 30 days after total gastrectomy (H-E; X10).

epithelial cells. The nuclei of the acinar cells were situated at the basal region of the cells. The apical portion of the cells contained numerous zymogen granules (Figure 1). Weak immunoreactivity for Cg A was seen in only a few of the islet cells while the acinar cells were unstained (Figures 4a,b). Weak immunoreactivity for SYP was seen in the islets of Langerhans. The exocrine acinar cells were unstained (Figures 5a,b).

#### Experimental group

The acinar cells showed abundant cytoplasm which contained numerous zymogen granules in the experimental group animals in comparison with the control group. There were inter acinar spaces between irregular glands in the experimental group animals (Figure 2). Histological examination showed intense lymphocyte, neutrophil and eosinophil leucocyte infiltration in the pancreatic tissue (Figure 3). The islets of Langerhans showed significant hyperplasia postoperatively in comparison with the control group (Figure 4c). Strong immunoreactivity for Cg A was seen in the islet cells. The exocrine acinar cells were unstained (Figures 4c,d). The strongest immunoreactivity for SYP was seen in the islets of Langerhans in this group while the exocrine acinar cells were also stained (Figures 5c,d).

#### Discussion

Several reports have indicated an increased incidence of pancreatic cancer in patients undergoing gastric resection (1,2). Total gastrectomy is commonly performed for gastric malignancies in humans. However, numerous nutritional, metabolic and hormonal disturbances may develop after total gastrectomy. Therefore, clinical and experimental studies have frequently been carried out for the evaluation of those disturbances (14,15). Several experimental studies in rats have shown that a close functional interaction exists between the stomach and the pancreas. Significant exocrine pancreatic trophism has been reported after partial or total gastrectomy in rats (16,17). Buchler et al. (18) suggested that vagotomy in rats induced pancreatic hyperplasia, enzyme dissociation, and decreased basal amylase discharge in vitro. In the present study, the acinar cells showed abundant cytoplasmic zymogen granules in the experimental group when compared to the control group. During total gastrectomy, most gastropancreatic nerves are destroyed. Together with truncal vagotomy, this operative procedure results in a nearly complete extrinsic denervation of the pancreas. Several studies in patients with truncal vagotomy have

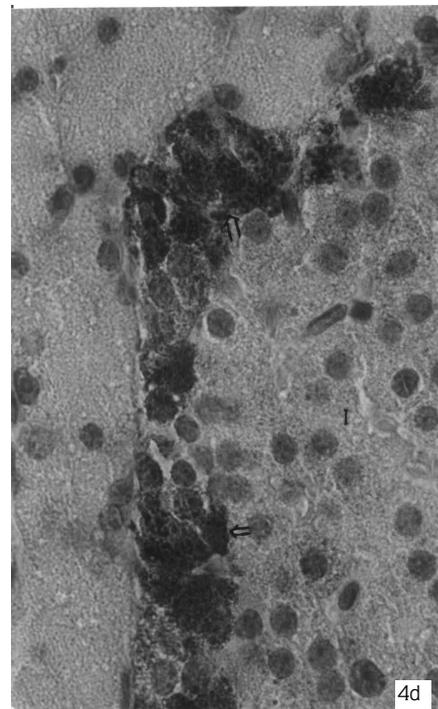
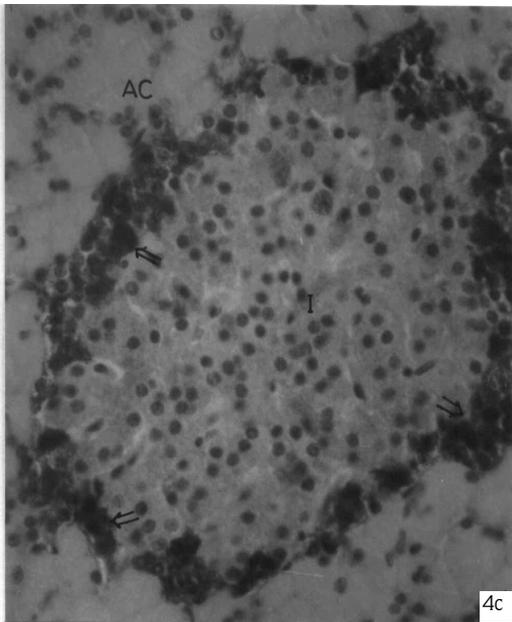
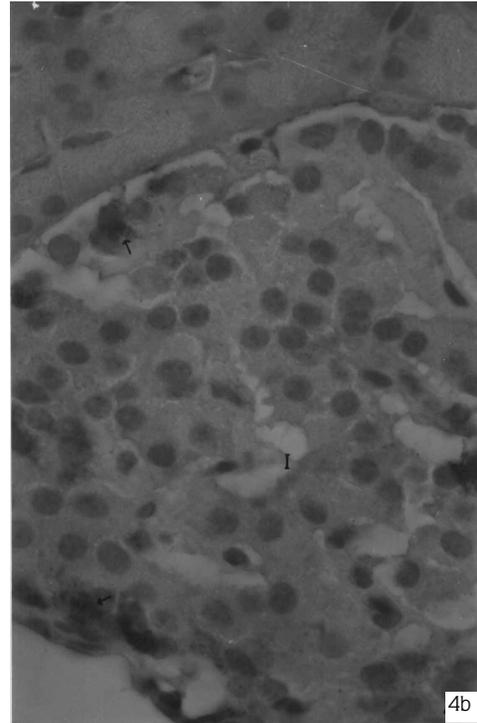
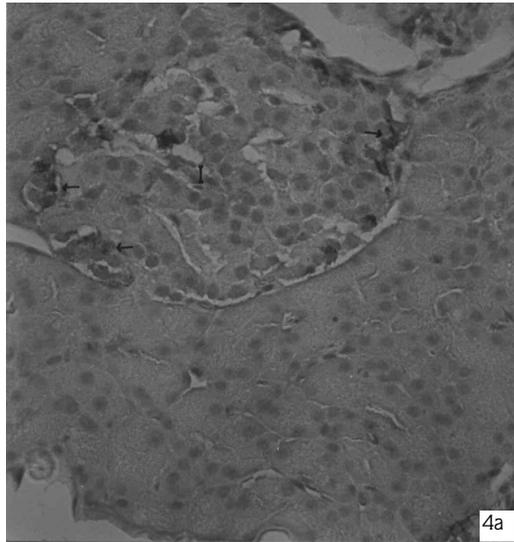


Figure 4. Immunohistochemical staining for Cg A.  
 a, b) Weak reaction (→) in the islets of Langerhans (I) in the sham-operated group. The exocrine acinar cells (AC) are unstained X40, X100. c,d) Hyperplasia of endocrine cells in islets of Langerhans (I) in the experimental group compared with the sham-operated group. There is strong immunoreactivity (⇒) to Cg A in the islets of Langerhans (I) but negative immunoreactivity in acinar cells (AC) 30 days after total gastrectomy X40, X100.

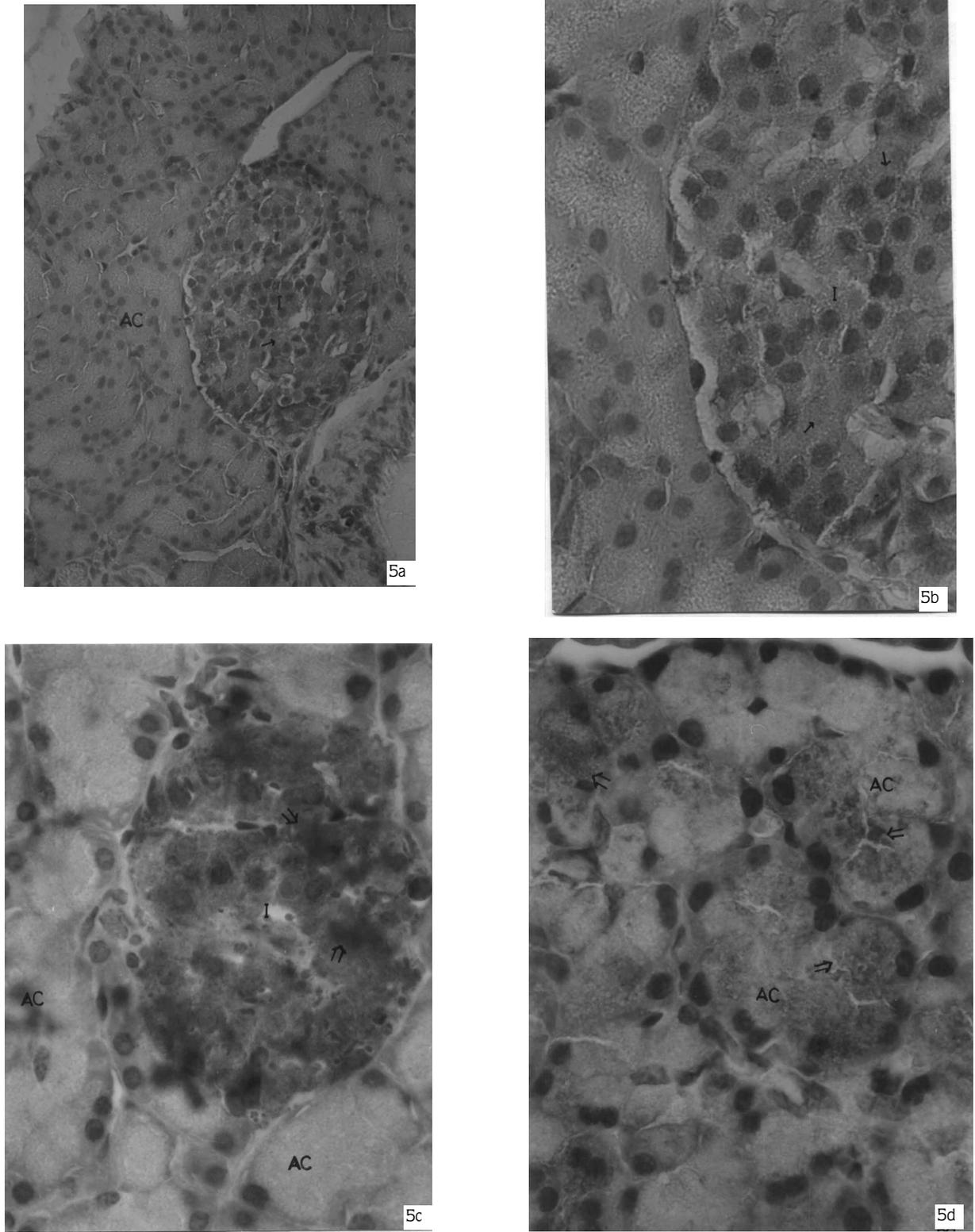


Figure 5. Immunohistochemical staining for SYP.  
a, b) Weak reaction (→) in islets of Langerhans (I) in the sham-operated group. The exocrine acinar cells (AC) are unstained X40, X100.  
c, d) Strong immunoreactivity (⇒) for SYP in islets of Langerhans (I) and exocrine acinar cells (AC) in the experimental group X100.

shown that truncal vagotomy alone leads to exocrine pancreatic insufficiency, with significant reductions in secretin-stimulated trypsin output (61%) and lipase secretion (53%) (19). SYP, described as a specific tumour marker for pancreatic neuroendocrine tumours, showed strong immunoreactivity in the exocrine acinar cells of the experimental group. Gastrectomy stimulates pancreatic growth and enhances carcinogenesis by increasing cholecystokinin (CCK) release (7). CCK is considered a major regulatory hormone for the exocrine pancreas (20). The mechanism by which gastrectomy may contribute to the development of pancreatic cancer is unclear, but increased concentrations of CCK after gastrectomy might mediate pancreatic carcinogenesis.

The islets of Langerhans showed significant hyperplasia postoperatively in comparison with the control group. In animals, the vagus nerve is involved in insulin secretion (21); because vagotomy is performed in most gastric operations, this may contribute to postprandial hyperglycaemia. Vagotomy may alter the release of gastrointestinal peptides from the mucosa of the small intestine, which, in turn, affects absorption and glucose tolerance (22). After total or partial gastrectomy, a dumping syndrome followed by secondary hypoglycaemia may occur. The early dumping syndrome is due to the rapid passage of ingested food. The rapid absorption of carbohydrate produces marked

hyperglycaemia, an excessive stimulus (23) that causes the islets to become hyperactive, and this, finally, results in their hyperplasia.

SYP and Cg A are markers for neuroendocrine tumours (24-26). Cg A is a glycoprotein found in neuroendocrine cells and which could be used as a tumour marker for neuroendocrine tumours (26). SYP is a glycoprotein isolated from the presynaptic vesicles of bovine neurones. Initial studies have demonstrated its presence in neurones in the brain, spinal cord and retina, and in adrenal medullary cells. A subsequent study demonstrated its presence in pancreatic islet cells and certain neuroendocrine neoplasms, including several pancreatic islet cell tumours (24). In the present study, the strongest immunoreactivity for synaptophysin was seen in the islets of Langerhans and in the exocrine acinar cells.

In conclusion, strong Cg A and SYP immunoreactivities may be relevant markers for pancreatic cancers in the early postoperative period following total gastrectomy.

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