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Argyrophilic and hormone immunoreactive cells in normal and hyperplastic pancreatic ducts and exocrine pancreatic carcinoma

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Summary. Scattered argyrophil cells were present in normal, large, medium-sized and small pancreatic ducts (ductules). There was marked increase in argyrophil cells in ducts with hyperplastic epithelium. Argyrophil cells were also found in 67.7% of all exocrine pancreatic carcinomas. In a well differentiated group including cystadenocarcinoma, mucinous carcinoma and well differentiated ductal adenocarcinoma argyrophil cells were found in all cases examined. Using four antisera (against insulin, glucagon, somatostatin and gastrin), insulin, glucagon and somatostatin cells were identified in 2.65%, 0.001% and 1.2% of normal ducts, and 7.5%, 2.4% and 4.6% of ducts with hyperplastic epithelium respectively and were also greatly increased in numbers in the latter group. Immunoreactive cells were present in 66.7% of exocrine carcinomas. Cells reactive for insulin were found in 7/15 cases; glucagon in 6/15 cases; somatostatin in 5/15 cases and gastrin in 2/15 cases. Eight cases contained two or more than two types of immunoreactive cells. The presence of argyrophil and hormone immunoreactive cells in pancreatic ducts and carcinomas is indicative of the close developmental relationship between endocrine and exocrine parts of the pancreas. The inter-relationship of response in the different cell types following stimulus suggests that injury to a common precursor may be involved.

Key words: Argyrophil cell – Hormone immunoreactive cell – Pancreatic carcinoma – Normal duct – Hyperplastic duct

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Introduction

The endocrine cells of the pancreas are not confined to the islet of Langerhans; they can be demonstrated in the exocrine pancreas (Larsson et al. 1974; Pellelier and Leclerc 1977).

The source of the neuroendocrine cells has been hotly disputed but an endodermal source has been gaining support recently at the expense of the idea of neural crest origin. The presence of these cells in tumours which are regarded as of endodermal origin lends support to the former view, but it could be argued that tumour cells have the ability to change their character as part of the neoplastic process. If endocrine cells have a common origin with ductal cells it might be expected that stimuli producing ductal hyperplasia would also produce an increase in endocrine cells. We have, therefore, determined the number and character of the endocrine cells in normal and hyperplastic pancreatic ducts and in exocrine pancreatic carcinoma.

Materials and methods

Thirty-one cases of pancreatic carcinomas were selected from the files of the Institute of Pathology, The London Hospital. Eight normal, well preserved pancreases from post-mortem cases were used as controls. All cases had been formalin fixed and routinely processed, and were embedded in paraffin. Four micron sections were stained with haematoxylin and eosin, Grimelius, modifed Masson-Fontana and diazo techniques. Small staining runs, sections of normal intestine were used as positive controls. The pancreatic carcinomas included 23 cases of ductal adenocarcinoma, 2 mucinous, 3 cystadenocarcinoma and 3 pleomorphic carcinomas. The tumours were classified on their morphological appearances, based on the classification by Chen and Baithun (1985), in which the term ductal carcinoma was applied to a tumour containing ducts. Ductal hyperplasia was divided into 3 categories, simple, papillary and atypical (Chen et al. 1985). The age of patients ranged from 19–26 years with a mean of 60.1 years. The male/female ratio was 1.5:1.

Table 1. Sources and dilutions of primary antisera

Antisera	Positive control	Dilution	Source
Rabbit anti-human glucagon	Normal human pancreatic tissue	1:300	DAKO
Guinea pig anti-human insulin	Normal human pancreatic tissue	1:400	DAKO
Rabbit anti-human somatostatin	Normal human pancreatic tissue	1:200	DAKO
Rabbit anti-human gastrin	Normal human duodenal mucosa	1:200	DAKO

Immunocytochemical methods. The twenty-one cases which contained argyrophil cells were further investigated by immunocytochemical staining for 4 antigens, listed in Table 1. Included within these cases were 16 cases of ductal adenocarcinoma (well differentiated 1, moderately differentiated 8, mucinous 2, and poorly differentiated 7), 3 cases of cystadenocarcinoma, and 8 normal pancreases.

Sections (4 um) were obtained from paraffin blocks and stained by the peroxidase anti-peroxidase technique. After completely dewaxing the sections in xylene and rehydrating them, endogenous peroxidase activity was blocked by incubation in methanol-hydrogen peroxide for 30 min. Sites of potential nonspecific binding of immunoglobulins were blocked by incubating the sections with a 1:5 dilution of normal swine serum for 10 min. The excess normal swine serum was drained off from the slides before incubation with specific primary antisera (Table 1). The slides were then sequentially incubated with 1:30 swine anti-rabbit IgG (DAKO) for 30 min and 1:20 rabbit peroxidase-anti-peroxidase (DAKO) for 30 min at room temperature with intervening rinses in Tris-buffered saline (TBS). Site of peroxidase activity were visualized by incubating the sections with 0.01% hydrogen peroxide and 0.05% diaminobenzidine tetrahydrochloride in 0.05 M Tris-buffer, pH 7.2 for 10 min. Sections were lightly counterstained with haematoxylin. The normal human pancreas (for insulin, glucagon, somatostatin) and normal human duodenum (for gastrin) were used as positive controls.

Negative controls were established by incubating the sections with the same dilutions of normal rabbit serum as those used for the corresponding specific antisera.

The number of argyrophil and hormone immunoreactive cells in the pancreatic carcinomas was counted in an arbitrary, but reproducible, manner (see Tables 3, 4):

+ only one or a few cells were present in 10 hp fields of the carcinoma

++ scattered positive cells were present but no more than 10 cells 10 hp fields

+++ positive cells are present in small groups, or were scattered, the number of the cells is more than 10/10 hp fields

The numbers of positive cells in each normal or hyperplastic duct were recorded (Tables 2, 3).

Results

The frequency of argyrophil cells in normal and hyperplastic ducts and exocrine pancreatic carcinomas are shown in Tables 2, 3. In normal ducts, argyrophil cells were scanty (Fig. 1a) but their

Table 2. The number of hormone IR cells and argyrophil cells in normal and hyperplastic pancreatic ducts

	Large				Medium				Small			
	Normal		Hyperplastic		Normal		Hyperplastic		Normal		Hyperplastic	
	Mean ± SD	n*	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n
Insulin IR cell	0.154 ± 0.533	13	0.889 ± 1.729	9	0.086 ± 0.251	222	0.237 ± 0.958	266	0.015 ± 0.124	858	0.219 ± 1.721	433
Glucagon IR cell	0.077 ± 0.266	13	0	9	0	222	0.293 ± 3.031	266	0	858	0.081 ± 0.732	433
Somatostatin IR cell	0.308 ± 1.066	13	0.111 ± 0.333	9	0.050 ± 0.288	222	0.128 ± 0.612	266	0.007 ± 0.1118	858	0.051 ± 0.292	433
Gastrin IR cell	0	13	0	9	0	222	0	266	0	858	0	433
Argyrophil cell (Grimelius)	1.60 ± 12.678	13	12.93 ± 18.16	9	0.191 ± 0.870	222	1.470 ± 492	266	0.032 ± 0.247	858	0.353 ± 1.797	433

*n = Number of ducts examined

Table 3. The frequency of argyrophil cells in exocrine pancreatic carcinomas

Grading positivity	Ductal adenocarcinoma (Differentiation)			Pleomorphic ca	Mucinous ca	Cystadeno. ca	Total
	Well	Moderately	Poorly				
+[a]	0	5[+]	5	0	2	1	13
++	1	3	2	0	0	0	6
+++	0	0	0	0	0	2	2
Total/Cases examined	1/1	8/10	7/12	0/3	2/2	3/3	21/31

[a] +, ++, +++ Grading of positivity refers to the relative number of positive cells
[+]; Number of positve cases

Table 4. The frequency of hormone-IR cells in exocrine pancreatic carcinoma

	Grading of positivity			Total positive cases/ Case examined
	+	++	+++	
Insulin-IR cell	4	1	2	7/15
Glucagon-IR cell	3	2	1	6/15
Somatostatin-IR cell	2	3	0	5/15
Gastrin-IR cell	1	0	1	2/15
Total positive cases/ cases examined	–	–	–	10/15

numbers increase in hyperplastic ducts (Fig. 1 b). (The mean increase in number of argyrophil cells over the normal is 8.1 times in large ducts, 7.7 times in medium-sized ducts and 11 times in ductules (Table 2). In 5 cases of dutular hyperplasia the argyrophil cells formed ductular structures (Fig. 1 c) with budding off from the ductules.

Argyrophil cells were also found in 67.7% of exocrine pancreatic carcinoma (Table 3). They were found in all cases of cystadenocarcinoma (3), mucinous carcinoma (2), and well differentiated ductal adenocarcinoma (1). In two of three cases of cystadenocarcinoma, some tumour cysts were partly formed by argyrophil cells (Fig. 2). However, in the poorly differentiated group, only a few argyrophil cells were seen and no positive cells were found in three cases of pleomorphic carcinoma.

The cells staining positively with the Grimelius technique were usually triangular or flask-shaped (Figs. 1 a–c) with a broad base on the basement membrane of the epithelium. The positively staining cytoplasmic granules were brown or black and uniformly small and round. They tended to be predominantly infranuclear but in a few cells the granules were dispersed throughout the entire cytoplasm.

Occasional argyrophilic staining in the margin of the ductal epithelium or in degenerating tissue was regarded as non-specific. Islets in the centre of carcinomas were carefully excluded.

Immunoperoxidase study

Using specific antisera against 4 antigens (insulin, glucagon, somatostatin and gastrin), hormone immunoreactive cells were identified in both normal and hyperplastic ducts. The details of the findings are listed in Table 3. In normal ducts, only a few insulin, glucagon and somatostatin immunoreactive cells were found in the ductal epithelium.

In total, insulin, glucagon and somatostatin immunoreactive cells were found in 29 (2.65%), 1 (0.001%) and 13 (1.2%) of 1093 normal ducts and 53 (7.5%), 17 (2.4%) and 32 (4.6%) of 708 hyperplastic ducts, respectively (Figs. 3–5). Three cases with normal ducts showed positively mainly for insulin and somatostatin, while 12 cases with hyperplastic ducts showed positivity for all hormones.

Hormone immunoreactive cells were found in 10 of 15 cases of exocrine pancreatic carcinoma which could be examined by the immunoperoxidase method (Table 4, Figs. 6–8). Eight cases contained two or more than two types of these cells. One of the two cases of cystadenocarcinoma contained numerous insulin-IR cells, and the other contained plentiful gastrin-IR cells. In some areas, the gastrin-IR cells formed tumour cysts mixed with mucin-producing cells (Fig. 7). Glucagon (Fig. 8) and somatostatin-IR cells were also found in this case.

Eight of 13 cases of ductal carcinoma contained hormone immunoreactive cells (Fig. 6). There were insulin positive cells in 5, glucagon positive cells in 5, somatostatin positive cells in 4 and gastrin cells in 1 case.

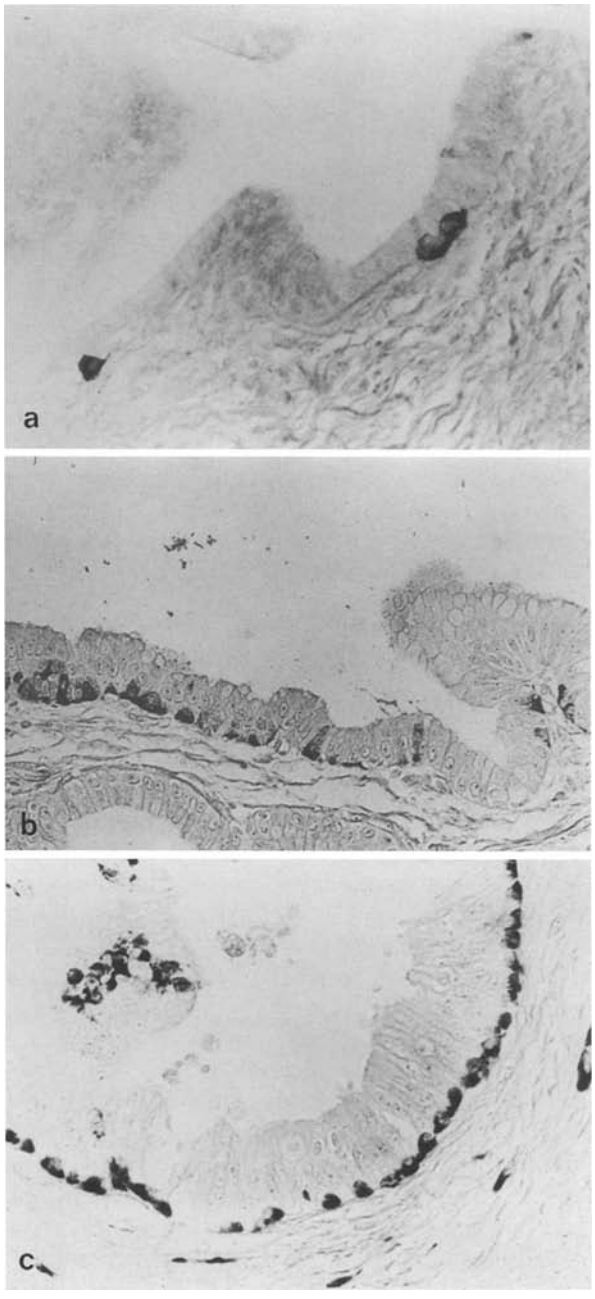


Fig. 1 a. Scanty argyrophil cells are present in normal medium sized pancreatic duct. The cells are triangular or flask-shaped and in basal location. Grimelius × 600. (**b**) Increased number of argyrophil cells in medium sized hyperplastic pancreatic duct. Grimelius × 600. (**c**) Hyperplastic small duct partly formed by argyrophil cells. Grimelius × 600

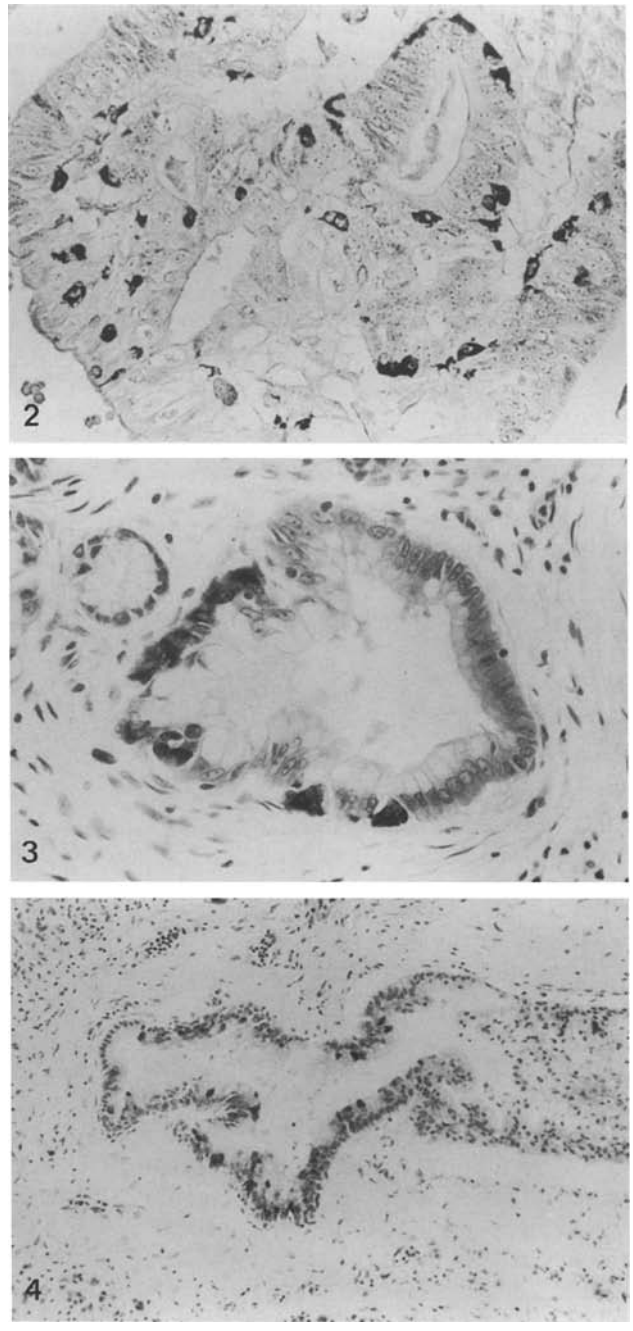


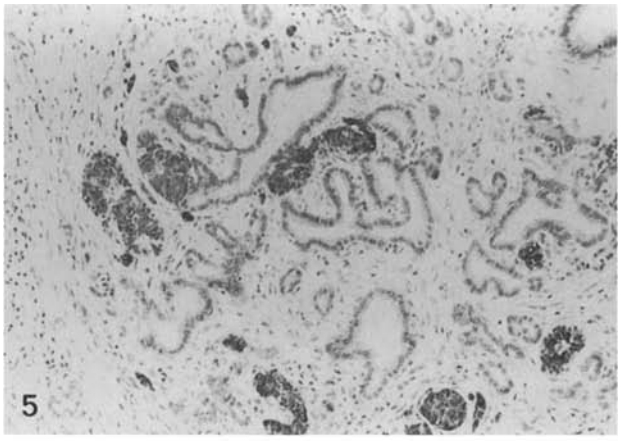
Fig. 2. Plentiful argyrophil cells in cystadenocarcinoma. The cells are mixed with mucin producing cells with a tendency of basal location. Grimelius × 600

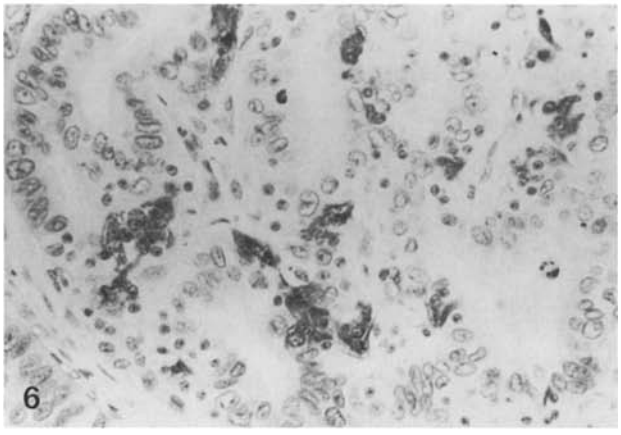
Fig. 3. A number of insulin immunoreactive cells in hyperplastic pancreatic duct. Immunoperoxidase × 600

Fig. 4. Glucagon immunoreactive cells in hyperplastic pancreatic duct. Immunoperoxidase × 200

Discussion

With the application of new techniques in immunocytochemistry it has been possible to identify endocrine cells at the cellular and ultrastructural levels and to identify hormones or peptides in tissue and plasma using specific antisera. Endocrine cells and tumours derived from them have been found in many tissues and organs, e.g. lung (Hamperl 1937; Yang et al. 1983), liver (Thomas et al. 1980),





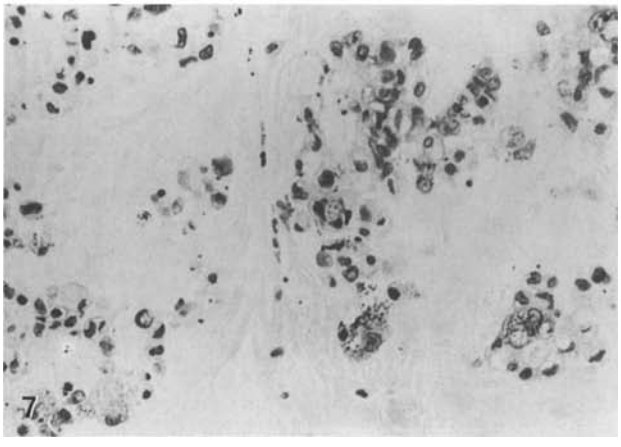


Fig. 5. Hyperplastic ductules with somatostatin immunoreactive cells. The islets are budding off from the ductules. Immunoperoxidase × 200

Fig. 6. Insulin immunoreactive cells in ductal adenocarcinoma. Immunoperoxidase × 600

Fig. 7. Plentiful gastrin IR cells in cystadenocarcinoma of the pancreas. Immunoperoxidase × 600

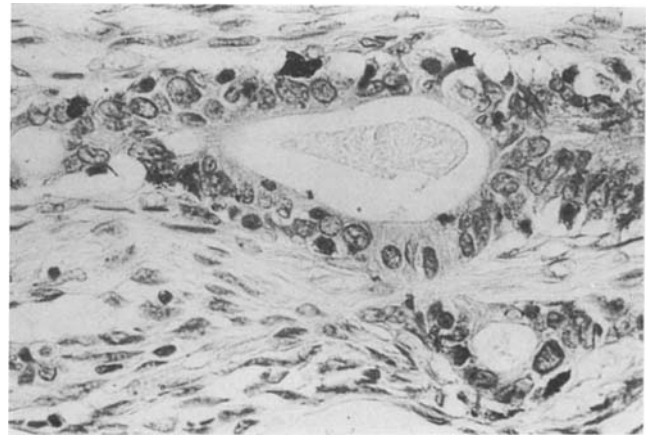


Fig. 8. Glucagon IR cells in cystadenocarcinoma of the pancreas. Immunoperoxidase × 800

breast (Cubilla and Woodruff 1977), kidney (Zak et al. 1983), bladder (Collino et al. 1985; Hamid et al. 1988), thymus (Rosai and Higa 1972), ovary (Robboy et al. 1975), endometrium (Sivridis et al. 1984; Aguirre et al. 1984; Delellis et al. 1984), uterine cervix (Habib et al. 1979), skin (Dijk and Seldam 1975) and a variety of other sites (Yalla et al. 1974; Kameya et al. 1980). The gastrointestinal tract has been recognised as a major endocrine organ in recent years (Dawson 1976; Pearse et al. 1977) and in the pancreas, islet cells and tumours derived from them have been studied extensively (Frieson 1979; Larsson 1978; Heitz et al. 1982; Mukai et al. 1982).

Up to 5 types of endocrine cells have been identified in the islets of Langerhans of the pancreas (Pearce 1977; Dawson 1984), but little attention has been paid to the existance and distribution of endocrine cells in the exocrine pancreas, although a few reports have been published on this subject (Feyrter 1953; Larsson 1977; Pelletier and Leclerc 1977; Larsson et al. 1974). In this study we have examined both normal and hyperplastic pancreatic ducts by using histochemical and immunohistochemical techniques, and have found a high frequency of argyrophil and hormone immunoreactive cells including insulin, glugagon, and somatostatin-IR cells in both normal and hyperplastic ducts (Table 3). We have shown that endocrine cells are increased up to 14 times normal in hyperplasia of ductal epithelium. Furthermore, budding of neuroendocrine cells from the ductules indicates local proliferation and supports the idea of a close embryonic relationship between the endocrine and exocrine parts of the pancreas.

Exocrine pancreatic carcinomas are commonly regarded as pure exocrine tumours. Recently a few cases of pancreatic exocrine carcinomas containing argyrophil (Compagno and Oertel 1978; Suda and Hashimoto 1979) and hormone immunoreactive cells (Mihas et al. 1978; Eusebi et al. 1981) have been reported. Compagno and Oertel (1978) reported four tumours with argyrophil cells from

41 cases of cystic neoplasms of the pancreas. Suda and Hashimoto (1979) drew further attention to this phenomenon describing argyrophil cells in a post-mortem series of 18 cases out of 41 unselected pancreatic adenocarcinomas. Eusebi et al. (1981) reported that 6 of 11 cases of primary pancreatic adenocarcinoma contained endocrine-paracrine cells. Included were 2 cases containing insulin-IR cells, one case containing 5-hydroxytryptamine and somatostatin-IR cells and one case having glucagon-IR cells. In our series, we found 21 of 31 cases of exocrine pancreatic carcinomas (67.7%) including ductal, mucinous and cystadenocarcinomas (67.7%) contained argyrophil cells and 10 out of 15 cases contained hormone immunoreactive cells. We were able to identify gastrin-IR cells in 2 cases, one a ductal adenocarcinoma, the other a cystadenocarcinoma in which many gastrin-IR cells were present throughout the tumour, intermixed with mucin-producing cells. Numerous glucagon and scattered somatostatin-IR cells were also found in the same case.

These findings, together with the widespread distribution of endocrine cells in the exocrine pancreas, support the idea of an endodermal origin of these cells.

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