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Two-dimensional polyacrylamide gel electrophoretic pattern of duodenal tumour proteins

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ABSTRACT

We investigated the protein pattern of a surgically resected tumour in a case where it was difficult to distinguish between duodenal and pancreatic cancer The investigation was performed using two-dimensional polyacrylamide gel electrophoresis with silver staining Samples of the duodenal tumour, normal duodenal mucosa and normal pancreatic tissue from the same patient were compared. Each gel had *ca* 250–300 protein spots, and the tumour sample pattern more closely resembled that of normal duodenal mucosa than that of pancreatic tissue, suggesting that the tumour had arisen from duodenal mucosa There were three proteins identified only in the tumour sample gel, and these may have been tumour-specific proteins

INTRODUCTION

It is occasionally difficult to distinguish between advanced duodenal cancer and pancreatic cancer on clinical and histological grounds. For example, duodenal cancer can sometimes cause duodenal stenosis and obstructive jaundice, as can pancreatic cancer The most common presenting features of duodenal cancer are upper gastrointestinal bleeding and symptoms due to duodenal stenosis On histological examination of a biopsy specimen of the tumour it is very difficult to dinstinguish between duodenal cancer and pancreatic cancer, because most of these cancers are tubular adenocarcinomas.

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) of proteins was developed for research purposes in 1975, mainly by O'Farrell [1]. This technique has been used to compare normal and transformed cultured cells [2–5], but is less often used for solid tumours The present study was carried out to distinguish between advanced duodenal cancer and pancreatic cancer, using 2D-PAGE combined with silver staining, in a case where the diagnosis was uncertain clinically and histologically.

Case report

A 68-year-old female was admitted to hospital suffering from epigastric pain.

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She was found to have a duodenal tumour by fibergastroscopy and radiological examination of the upper gastrointestinal tract. Histological examination of a biopsy specimen of this duodenal tumour revealed moderately differentiated adenocarcinoma. Subsequently, the total bilirubin level gradually increased, and dilatation of the common bile duct was shown by ultrasonography. Abdominal computed tomography indicated that the intrahepatic and extrahepatic bile ducts were moderately dilated. Accordingly, percutaneous transhepatic biliary drainage was performed. She then underwent laparotomy, which revealed a Borrmann type 2 lesion (4 × 3 cm) situated 2.5 cm oral to the papilla of Vater. The tumour had invaded the pancreas and the main pancreatic duct was dilated moderately, but it appeared that it actually arose from the duodenum.

EXPERIMENTAL

Chemicals

Phenylmethylsulphonyl fluoride, pepstatin, benzamidine and Nonidet P-40 were purchased from Sigma (St. Louis, Mo, U.S.A.). Acrylamide, N,N′-methylenebisacrylamide, ammonium persulphate, N,N,N′,N′-tetramethylethylenediamine, trisaminomethane, glycine, urea, sodium dodecylsulphate (SDS) and silver reagent were obtained from Wako (Osaka, Japan). 2-Mercaptoethanol came from Nakarai Chemicals (Kyoto, Japan), and ampholine (pH 3.5–9.5) was purchased form LKB (Uppsala, Sweden)

Specimen acquisition

Specimens of the tissue resected at surgery were processed without delay Small pieces of tumour tissue (*ca.* 0.5 × 0 5 × 0.3 cm), normal duodenal mucosa and normal pancreatic tissue were cut by blunt and sharp dissection. Half of each specimen of the tumour, the normal duodenal mucosa and the normal pancreatic tissue was stored at −70°C The other half of each specimen was pinned to a cork plate, which was cut to approximately its size and then fixed in 10% buffered formalin Thin sections of the formalin-fixed specimens were cut and stained with hematoxylin and eosin for histological examination.

Sample preparation for electrophoresis

Samples were prepared for electrophoresis by a modification of the method of Tracy *et al.* [6] Frozen tissue was shaved with a cold scalpel until 100–200 mg had been collected. This was rapidly weighed and placed in a cold test-tube, and homogenization buffer (8 mol of urea and 50 ml of 2-mercaptoethanol per litre) was added at 600 μl per 150 mg of frozen tissue. To inhibit proteolysis, 10 μl of each of two solutions (25 mg of phenylmethylsulphonyl fluoride plus 1 mg of pepstatin per 1.4 ml of ethanol, and 16 mg of benzamidine per 1 ml of water) were added to 150 mg of frozen tissue The tissue was immediately homogenized (Econo-grind homogenizer; Radnoti Glass Technology, Monrovia, CA, U.S.A.). Af-

ter centrifugation in this homogenizer (1640 *g*, 10 min) 100 μl of the supernatant were removed for further processing, and the remainder was frozen. The sample was placed in a micro ground-glass homogenizer, and 33 μl of neutralization buffer [containing, per litre, 8 mol of urea, 80 ml of Nonidet P-40 surfactant and 50 g of ampholine (pH 3.5–9.5)] were added. The sample was again homogenized for 20 s, and then centrifuged at 15 860 *g* for 1 h (high-speed microcentrifuge MC-150; Tomy Seiko, Tokyo, Japan). A 100-μl syringe (Hamilton, Reno, NV, U.S.A.) was then used to aspirate *ca.* 50 μl from the middle of the tube, and 40 μl of this sample were immediately applied to the isoelectric focusing gel of electrophoresis.

Electrophoresis

The 2D-PAGE system of O'Farrell [1] was used, with some modifications. This system depends on isoelectric focusing under dissociating conditions (per litre, 8.5 mol of urea, 20 g of Nonidet P-40 sufactant and 20 g of pH 3.5–9.5 ampholine) in the first dimension and SDS gel electrophoresis in the second dimension. For isoelectric focusing, the gels were prerun at 200 V for 2 h, and then run at 300 V for 15 h and at 500 V for 1 h. For SDS gel electrophoresis, the stock gels were run at 24 mA and the analytical gels were run at 30 mA. The finished gels were stained with silver reagent, as previously described by Inoue *et al.* [7], and photographed. All of the gels were photographed with the alkaline side on the left.

RESULTS

Histological diagnosis

The tumour, normal duodenal mucosa and normal pancreatic tissue were examined histologically (Fig. 1). The tumour was a well differentiated adenocarcinoma.

Electrophoretic comparison of proteins

The results of electrophoresis of samples of the tumour, normal duodenal mucosa and normal pancreatic tissue are shown in Fig 2. The fixed amount of protein in the samples was *ca* 1 mg, as determined by the method of Lowry *et al.* [8]. About 250–300 spots appeared in the each gel, and the majority of spots on these gels were identical. A diagram of the gel from the normal duodenal mucosa is shown in Fig. 3. The black spots in the diagram are proteins common to the mucosa and tumour samples, and *ca.* 100 such spots were detected. A diagram of the gel from normal pancreatic tissue is shown in Fig 4. Again, the black spots in the diagram represent proteins that were also detected in the tumour sample gel, and *ca* 60 such spots existed. The gel of the tumour sample was thus more similar to that of the normal duodenal mucosa than to that of the normal pancreatic tissue. Fig. 5 shows a diagram of the gel from the tumour The three black spots

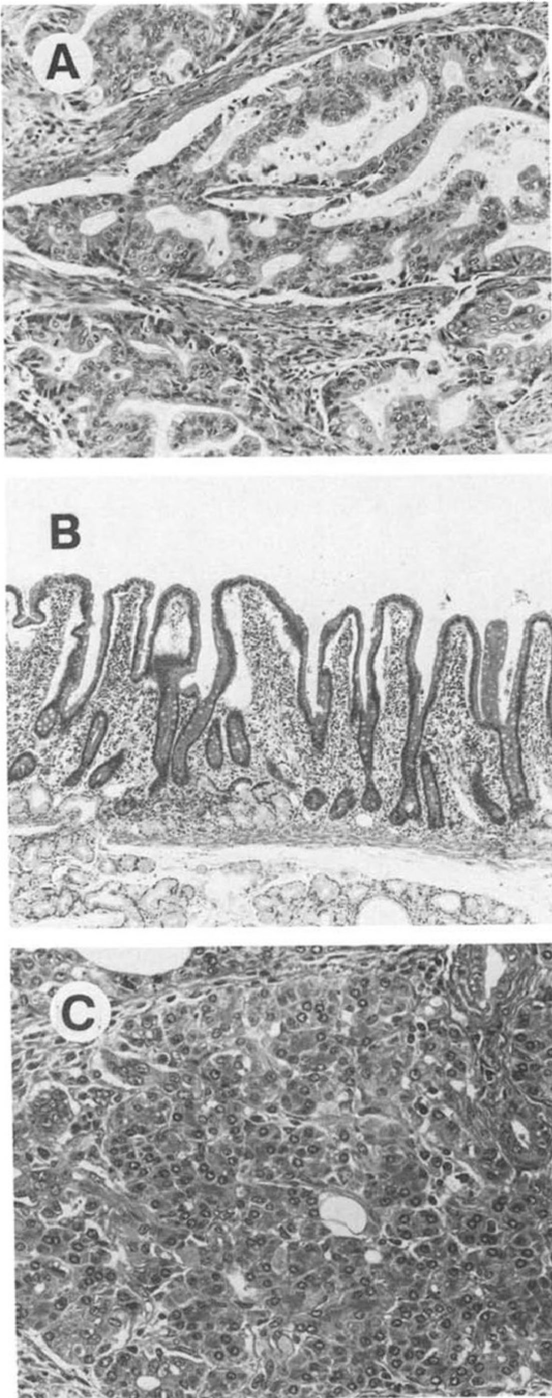

Fig 1 Histology of duodenal tumour, normal duodenal mucosa and normal pancreatic tissue Histological sections prepared and stained as described in the text (A) duodenal tumour, (B) normal duodenal mucosa; (C) normal pancreatic tissue.

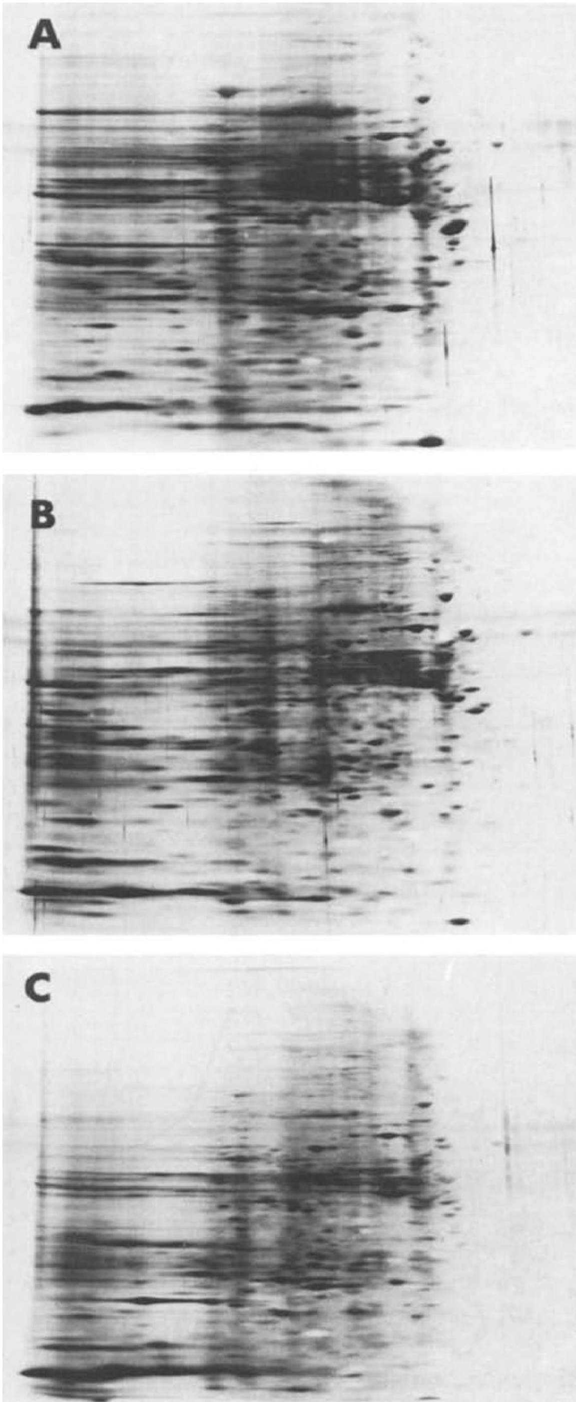

Fig. 2 Two-dimensional gels of (A) duodenal tumour, (B) normal duodenal mucosa and (C) normal pancreatic tissue

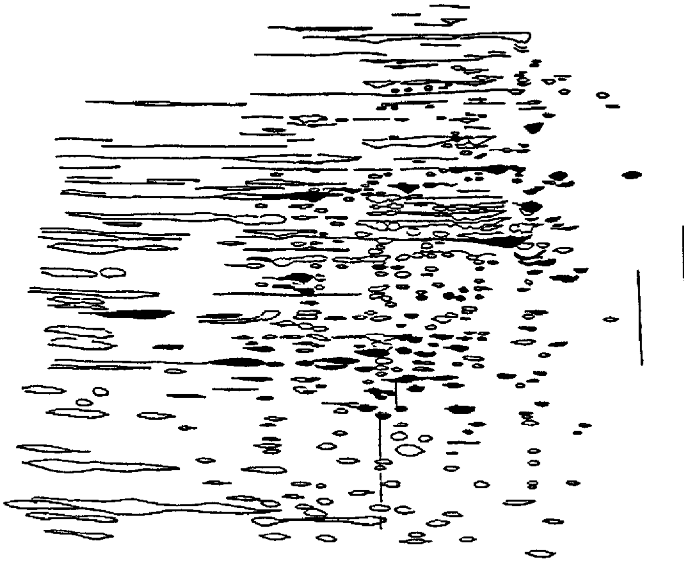

Fig 3 Diagram of the gel from the normal duodenal mucosa The black spots are common to protein spots in the gel from the tumour sample.

in the outlined area show proteins identified only in the gel from the tumour sample and not present in the gels from the other tissues (arrows, Fig. 5). Comparisons of the outlined area among the gels are illustrated at higher magnification in Fig. 6.

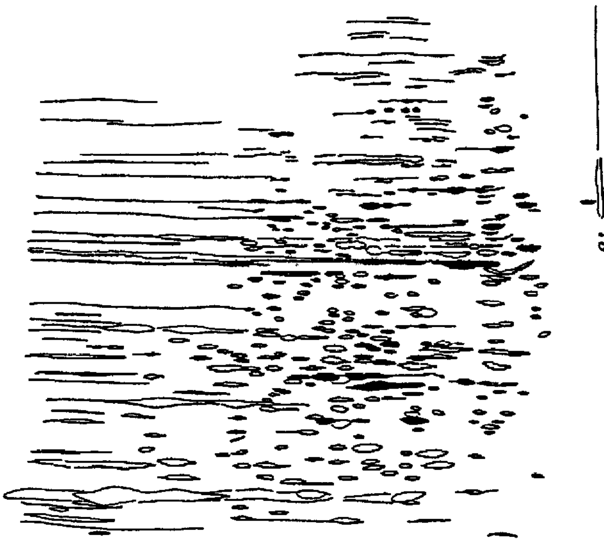

Fig 4. Diagram of the gel from the normal pancreatic tissue. The black spots are common to protein spots in the gel from the tumour sample

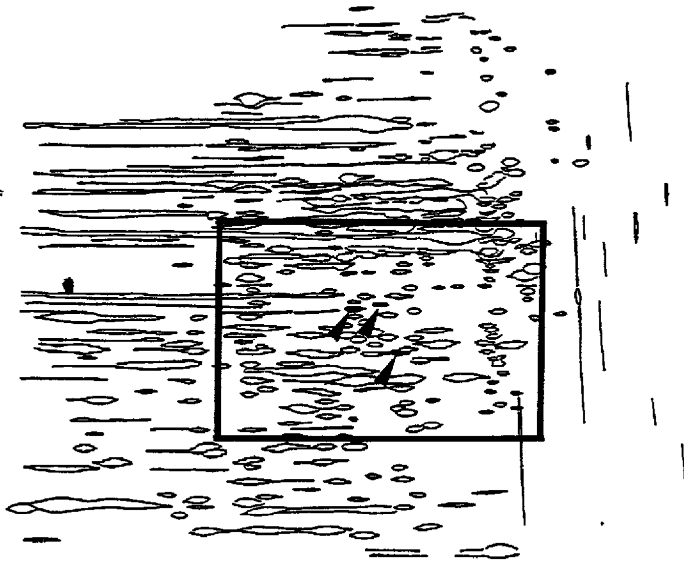

Fig 5 Diagram of the gel from the tumour sample Three black spots in the outlined area existed only in the gel from the tumour sample and not in the gels from the other samples

DISCUSSION

2D-PAGE is useful for the detection of cancer markers, because of its ability to separate and compare a large number of proteins [9,10]. Studies using 2D-PAGE have led to the identification of many transformation-specific peptides. Thorsrud *et al.* [11] mapped the pattern of proteins in the normal colonic mucosae, tubular adenomas and colonic carcinomas by 2D-PAGE. They reported that tubular adenoma and carcinoma had strinkingly similar protein patterns, which were different from that of the normal mucosa. We have previously reported on oesophageal carcinoma-associated proteins detected by 2D-PAGE. Four proteins were observed in all the oesophageal carcinomas studied that were not present in the normal oesophageal mucosae, and one protein was observed in all of the normal oesophageal mucosa samples that was not found in any of the oesophageal carcinomas [12].

The present study was carried out to distinguish between advanced duodenal cancer and pancreatic cancer, using 2D-PAGE. The protein pattern of the gel from the tumour sample was more similar to that of the normal duodenal mucosa than to that of pancreatic tissue, suggesting that the tumour had arisen from the duodenal mucosa. The three proteins detected that were only seen in the gel from the tumour sample may have been tumour-specific proteins Thus, 2D-PAGE was able to aid in the diagnosis of a tumour that was difficult to distinguish as either duodenal or pancreatic carcinoma.

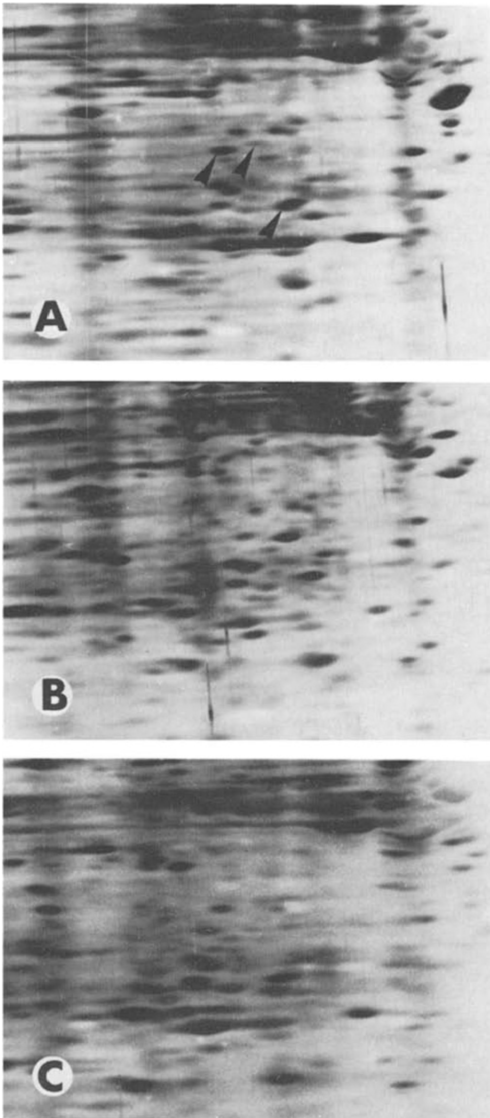

Fig 6 Electrophoretic patterns of duodenal tumour (A), normal duodenal mucosa (B) and normal pancreatic tissue (C): higher magnification of the outlined area in Fig. 5 The three spots show proteins that were present in the gel from the tumour sample but not in the gels from the other tissues.

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