

An enteroglucagon tumour

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SUMMARY A hormone-secreting renal tumour that affected small bowel structure, motility, and function has been described by Gleeson *et al* (1971). The present studies show that the large amount of glucagon-like immunoreactivity which could be extracted from this tumour had the molecular weight, action on plasma insulin and glucose *in vivo*, and immunological reactions of the gut hormone enteroglucagon. This is the first enteroglucagonoma to be reported.

The development of a radioimmunoassay for glucagon (Unger, Eisentraut, McCall, and Madison, 1961a) led to the unexpected discovery of two sources of glucagon immunoreactivity, one from the pancreas and the other from the gastrointestinal tract (Unger, Eisentraut, Sims, McCall, and Madison, 1961b). These are released by different physiological stimuli. The peripheral blood level of glucagon-like immunoreactivity was found to rise after oral glucose (Samols, Tyler, Marri, and Marks, 1965). Using triple-catheter dogs this was shown to be due to material released by the gastrointestinal tract (Unger, Ohneda, Valverde, Eisentraut, and Exton, 1969). Oral glucose causes a suppression of pancreatic glucagon release (Unger *et al*, 1969). Hypoglycaemia, on the other hand, stimulates the release of pancreatic glucagon and causes a fall in the level of gastrointestinal glucagon-like immunoreactivity (Persson, Gyntelberg, Heding, and Boss-Nielsen, 1971). A few of the antisera raised to pancreatic glucagon do not crossreact with the gastrointestinal material (Eisentraut, Ohneda, Parada, and Unger, 1968), which must differ in part of its amino acids sequence. The evidence therefore suggests that there are two functionally distinct types of glucagon-like immunoreactivity. The glucagon-like immunoreactivity of gastrointestinal origin is called 'enteroglucagon' for convenience. Extraction studies have shown that enteroglucagon exists in two forms, the larger of molecular weight 7000 and a smaller amount of molecular weight 3500 (Valverde, Rigopoulou, Exton, Ohneda, Eisentraut, and Unger, 1968). These two forms of enteroglucagon occur throughout the gastrointestinal tract and both are released into the blood stream by oral glucose (Valverde, Rigopoulou, Marco, Faloona, and Unger, 1970). The mucosal

endocrine cell producing enteroglucagon has recently been localized by immunofluorescence and shown to have only minor histological differences from the alpha cell of the pancreas (Polak, Bloom, Coulling, and Pearse, 1971).

In 1971 Gleeson, Bloom, Polak, Henry, and Dowling described a patient with marked gastrointestinal abnormalities and a renal tumour, which was found to contain cells histologically similar to the pancreatic alpha cells. This tumour was rich in a glucagon-like material and studies will now be described which suggest that the tumour produced enteroglucagon.

Methods

A calibrated 1 metre G 50 Sephadex gel column was used to purify a crude extract of the tumour (supernatant of 20% w/v saline homogenate) and also to give an approximate molecular weight of the immunoreactivity. Human surgical specimens of ileum and pancreas were extracted by the method of Kenny (1955) for use as standard solutions. A proportion of the ileal extract was purified on the gel column into the larger high molecular weight enteroglucagon and smaller low molecular weight enteroglucagon fractions (Valverde *et al*, 1968).

The action *in vivo* of the tumour material on insulin and glucose release was tested by the intravenous injection into the jugular vein of 200 g Porton rats under nembutal anaesthesia. Crystalline porcine pancreatic glucagon (Novo) was used as a standard.

Antisera were raised in rabbits to pancreatic glucagon polymer (Heding, 1969) and one, R15, was relatively specific for pancreatic glucagon, cross-reacting little with enteroglucagon. Antisera to Sephadex-purified tumour enteroglucagon were raised in two guinea pigs.

Pancreatic glucagon and purified tumour extracts were iodinated by the method of Greenwood, Hunter, and Glover (1963). Immunoassay techniques were based on those described by Albano and Ekins (1970) and employed a charcoal separation.

Results

The main peak of immunoreactivity eluted from the gel column (Fig. 1) coincides with the 7000 molecular weight position. It is identical with the elution volume of the high molecular weight fraction of enteroglucagon from human ileal extracts. A faster moving peak was also present suggesting the presence of some material of slightly higher molecular weight.

Intravenous injection in the rat of crude tumour extract (the pancreatic glucagon immunoreactive equivalent of 3000 ng per rat) caused a rise of both plasma glucose and insulin. Injection of gel-purified material in more physiological doses (immunoreactive equivalent of 200 ng per rat) produced neither a rise in insulin nor in glucose. The control injection of pancreatic glucagon (80 ng per rat) produced a more rapid and larger rise of both insulin and glucose than the crude tumour injection. It also achieved blood levels of immunoreactive glucagon which were lower than that following injection of gel-purified material (Fig. 2).

Using an antiserum to pancreatic glucagon, R59,

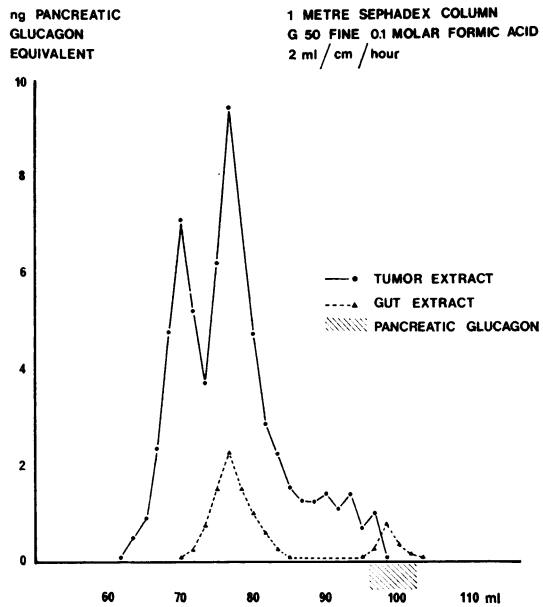


Fig. 1 Gel filtration of tumour extract.

Immunoreactivity of eluate is measured in pancreatic glucagon equivalents using antiserum R59 which reacts with pancreatic and enteroglucagon. The eluate volumes of the two peaks found in gut extracts and that of pancreatic glucagon are indicated.

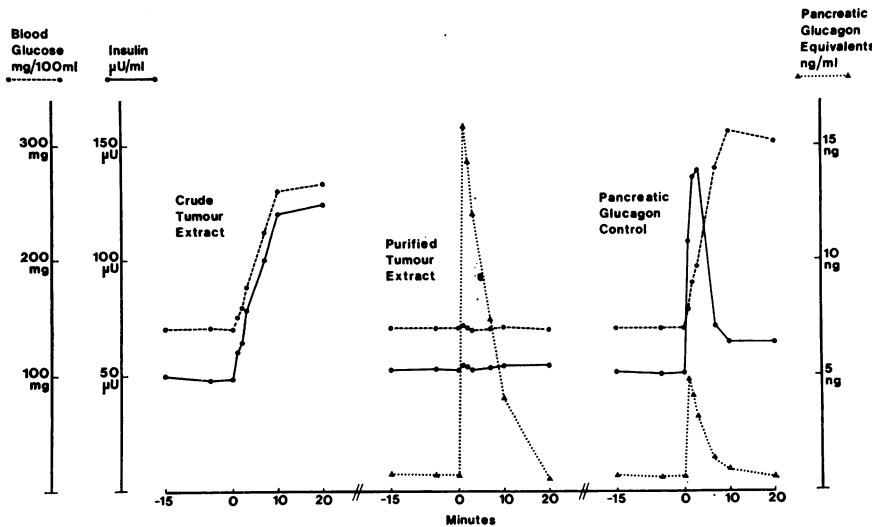


Fig. 2 Response of rat plasma glucose and insulin to injected tumour glucagon-like immunoreactive material and pure pancreatic glucagon.

The crude tumour extract graph is the mean of two animals and the purified tumour and pancreatic glucagon graphs means of three animals. Glucagon-like immunoreactivity is measured with antiserum R59.

which crossreacts strongly with enteroglucagon, the immunoreactive pancreatic glucagon equivalent of 150 μg per gram tumour wet weight was found (Gleeson *et al.*, 1971). The patient's preoperative fasting plasma contained 6000 pg/ml (Gleeson *et al.*, 1971) and postoperatively it contained 400 pg/ml. Immunoassay of the tumour extract, using a relatively specific antipancreatic glucagon antiserum, R15 (Fig. 3), shows little displacement of the pan-

creatic glucagon label and this suggests that the material is similar to enteroglucagon.

An antiserum, Gp. R from a guinea pig immunized with tumour, bound iodinated highly purified tumour immunoreactivity (Byfield, Bloom, Dowling, and MacIntyre, 1971). This labelled tumour was easily displaced by high molecular weight human ileal enteroglucagon, though much less well by the low molecular weight fraction, and very little by human pancreatic extract. It was also greatly displaced by human plasma samples taken following oral glucose, presumed to have elevated enteroglucagon (Unger *et al.*, 1969), but not at all by plasma taken during arginine infusion with elevated pancreatic glucagon levels.

Discussion

In this extrapancreatic alpha-cell-like tumour the glucagon immunoreactivity could either have been similar to pancreatic glucagon or to enteroglucagon. Its molecular weight and lack of action on plasma glucose and insulin levels in the rat are both more compatible with it being enteroglucagon. More definite evidence that it is like enteroglucagon is provided by its immunological reactions. It fails to combine with an antiserum which reacts only with pancreatic glucagon. Also, human plasma when it contains enteroglucagon, but not when it contains pancreatic glucagon, is able to prevent the binding of radioactive tumour glucagon to an antiserum to tumour glucagon.

Enteroglucagon is of considerable interest in that it is the only small intestinal peptide hormone whose level can be confidently measured in peripheral blood. It has been found to be maximally released by actively transported sugars (Unger *et al.*, 1969). Its actions, however, are not yet established. It has proved so far too labile to purify enough for meaningful administration experiments. Initial suggestions that it caused insulin release have not been substantiated (Marco, Faloon, and Unger, 1971). The action of low molecular weight enteroglucagon extracts in causing glycogenolysis does not appear to play an important physiological role (Marco *et al.*, 1971). It has recently been suggested, on circumstantial evidence, that it may inhibit pancreatic bicarbonate output (Dyck, 1971).

The patient whose tumour is discussed had several notable intestinal abnormalities, including severe colonic and jejunal stasis, malabsorption, and small intestinal villous hypertrophy (Gleeson *et al.*, 1971). These abnormalities all disappeared following removal of the tumour, concomitant with the return to normal of the previously elevated plasma enteroglucagon levels. This is the first enteroglucagon-

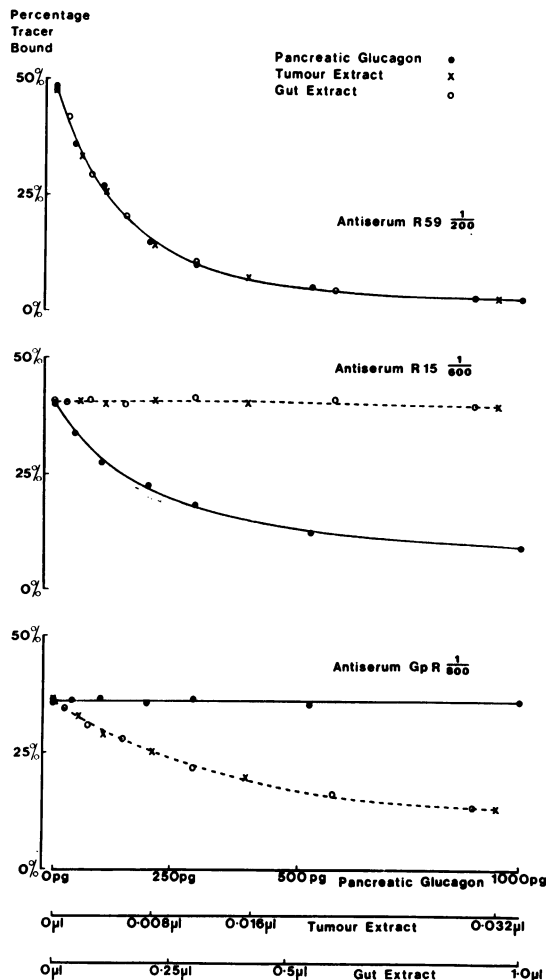


Fig. 3 Radioimmunoassay curves of pure pancreatic glucagon, tumour extract and human gut extract using three different antisera.

Antiserum R59, which crossreacts strongly with enteroglucagon, and antiserum R15, which crossreacts very little with enteroglucagon, were raised to pancreatic glucagon and are used with iodinated pancreatic glucagon tracer. Antiserum Gp R was raised to tumour extracts and is used with iodinated tumour glucagon tracer.

producing tumour to be described and the findings in the patient are probably the best indication so far of the actions of this enigmatic hormone.

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