A Case with Glucagonoma Syndrome
—Heterogeneity of Glucagon and Insulin—

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Abstract

The heterogeneity of glucagon and insulin in plasma and tissue extracts from a 57-year-old female with glucagonoma syndrome with surgically and autopsy verified islet-cell tumors was studied by Bio-Gel P-10 filtration. The preoperative plasma immunoreactive glucagon (IRG) level was 20.2 ng/ml, and plasma glucagon-like immunoreactivity (GLI) 25.8 ng/ml. The column chromatography of the preoperative plasma revealed three or four IRG components and four GLI components. Among these, peak II, the large glucagon immunoreactivity (LGI) peak, considered a candidate for proglucagon, was prominent, along with peak III.

The resected metastatic liver tumor contained an enormous amount of IRG and an appreciable amount of immunoreactive insulin (IRI), indicating that the elevated plasma IRG was mainly of tumor origin. The IRG pattern of the tumor tissue extract revealed a small quantity of IRG in peaks I and II, and a large amount in peak III; control pancreatic tissue extract manifested a similar elution pattern.

The IRI elution pattern of the tumor tissue extract revealed two major IRI peaks which migrated close to the elution volume of cytochrome C and insulin, respectively. This is a quite different pattern from the control pancreatic tissue extract in which the IRI peak was localized in the elution volume of the insulin.

We conclude that the present metastatic liver tumor produced not only enormous amounts of glucagon but heterogeneous peptides which contained immunological insulin determinants within their.

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mal glucose tolerance test, stomatitis, anemia and hypoproteinemia. Hypoaminoacidemia was present and fasting plasma IRG was always more than 20 times the normal level. A selective celiac angiogram revealed two round tumor stains (3.7 and 4.8 cm in diameter) in the liver but no lesion was detected in the pancreas.

Right hepatic lobectomy, partial resection of the pancreatic body and tail, and simultaneous splenectomy were performed on February 28, 1979. The resected portion of the liver contained two tumors but that of the pancreas contained no tumor. Hematoxylin-eosin, aldehyde-fuchsin and Grimelius silver staining of the tumor cells revealed them to be islet-cell carcinoma of the alpha-type. Electron-microscopy likewise showed alpha-like granules. The enzyme-labeled antibody method of Nakane and Kawai (1974) demonstrated glucagon but not insulin in the tumor cells. Aldehyde-fuchsin and anti-insulin immunohistochemistry of the resected pancreatic specimen showed the beta granules to be well preserved in the cells, but enzyme-labeled anti-glucagon staining failed to demonstrate alpha granules due to hydropic swelling.

On the 11th postoperative day, she died of cerebral hemorrhage. At autopsy, a tumor (1 x 1 x 1 cm) with hemorrhage and necrosis in the head of the pancreas was found. This was thought to be the primary lesion. Histologically, this tumor was very similar to the resected metastatic tumors of the liver. There was intracerebral hemorrhage due to rupture of the left middle cerebral artery.

**Plasma sample**

A blood sample was obtained from the antecubital vein early in the morning on February 27, 1979, the day previous to the laparotomy. It was placed in a chilled tube containing 1 mg of EDTA-2 Na and 500 KIU of aprotinin (Antagasan, Hoechst-Japan Co.) per ml of blood. The tube was rapidly centrifuged at 4°C. An aliquot of the plasma was separated for glucagon assay and the remaining sample was immediately frozen and stored at -20°C pending chromatography.

No postoperative blood sample was taken because of her serious general condition.

**Preparation of tissue extracts**

The surgically removed metastatic tumor of the liver and the resected pancreatic tail were immediately frozen and stored at -20°C until extraction. Both specimens, weighing approximately 0.5 g each, were extracted by the acid-alcohol technique of Kenny (1955). The dry extracts were homogenized in 2 ml of 0.1 M NH₄HCO₃ buffer (pH 8.8) and centrifuged. Aliquots of the soluble phase were separated for glucagon and insulin assays and the remaining samples were stored at -20°C pending chromatography.

The pancreatic tumor which was found at autopsy could not be made available for this study because of hemorrhage and necrosis.

The assays for vasoactive intestinal polypeptide (VIP) and substance P in both tissues were kindly carried out by Prof. N. Yanaihara (Yanaihara et al., 1977).

**Gel filtration procedures**

Properly diluted samples of the plasma and tissue extracts (each 2 ml) were chromatographed on 1 x 100 cm Bio-Gel P-10 (Bio-Rad Lab., Richmond) columns at flow rates of 10 ml/hour at 4°C. The elution buffer was 0.1 M NH₄HCO₃, pH 8.8, with 0.1% human serum albumin. Columns were calibrated with blue-dextran, cytochrome C, ¹²⁵I-insulin, ¹²⁵I-glucagon and ¹²⁵I-[Tyr¹]somatostatin. The column was calibrated with markers before and after chromatography of the plasma or the tissue extracts. Fractions (30 drops, 1.81 ml) were collected and frozen at -20°C waiting analysis.

**Radioimmunoassay of glucagon and insulin**

All samples were analyzed for immunoreactive glucagon by a dextran-coated charcoal separation method (Unger et al., 1959). In IRG assays we used porcine glucagon (Novo Co.) as the standard, ¹²⁵I-glucagon (Glucagon 125, Boehringer-Hoechst Co.) as the tracer, and antisera 30 K, furnished by Dr. Unger (Dallas), as the specific antisera for pancreatic glucagon. The assays measured 30 pg/ml of pancreatic glucagon with reliability.

On the fractions of plasma, we also determined immunoreactive glucagon with the Glucagon Kit (Daiichi Isotope Co.) with antisera OAL-123 and glucagon-like immunoreactivity by a dextran-coated charcoal separation method with antisera K-4023 (Yanaihara et al., 1979).

Using the Phadebas Insulin Test Kit (Shionogi Co.), insulin immunoreactivity was determined on the fractions of tissue extracts but not on plasma fractions because the plasma IRI level was too low to analyze.

**Radioreceptor assay of glucagon**

The radioreceptor assay for glucagon of extract from metastatic tumor tissue of the liver was kindly carried out by Dr. Y. Harano (Harano et al., 1981).
Results

Plasma glucagon fractions

Fig. 1 shows the IRG and GLI patterns on a Bio-Gel P-10 column utilizing 1ml of preoperative plasma when plasma IRG was 20.2 ng/ml and GLI 25.8 ng/ml. As shown in Fig. 1 (A), four peaks of 30K-IRG were seen, which is similar to those described by Valverde et al. (1976) and Recant et al. (1976). Peak I, the high molecular weight peak ("big plasma glucagon", BPG), present close to the void volume of the column; peak II corresponds to a molecular weight of 9000 (elution volume between cytochrome C and insulin), it is designated "large glucagon immunoreactivity" (LGI); peak III, corresponding to a molecular weight of 3500 (elution volume between insulin and somatostatin) compatible with pancreatic glucagon; peak IV has a molecular weight of less than 3500. As shown in Fig. 1 (B), however, peaks I-III were detected in IRG assay with OAL-123 but peak IV was not. In both assays, the percent IRG of peak II was calculated to be more than 25.

Four peaks of GLI, of which peak I and II were more prominent than those of 30K-IRG, were seen (Fig. 1 (C)). The percent GLI of peak II was calculated to be approximately 35.

Tissue concentrations of various peptides

The concentrations of various peptides in the tissues are listed in Table 1. The concentrations of IRG and IRI in the tumor tissue were higher than those in the con-

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<th>Table 1. Tissue concentration of peptides.*</th>
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<td>IRG</td>
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* (/g. wet tissue)
control pancreas tissue by 800 times and by one half, respectively, indicating that the content of IRG in the tumor was clearly greater than that of the control pancreas tissue.

The concentrations of both VIP and substance P were low in the tumor and control tissues.

**Glucagon fractions of tissue extracts**

In fractions from tumor and control tissue extracts, besides a large amount of peak III, two small IRG peaks were detected in the same elution zones as peak I (BPG) and peak II (LGI) in the plasma IRG pattern (Fig. 2). Peak IV, demonstrated in the plasma IRG pattern in the assay with 30K (Fig. 1 (A)) and in the plasma GLI pattern in the assay with K-4023 (Fig. 1 (C)), was not clearly discernible in either tissue extract.

**Insulin fractions of tissue extracts**

In fractions from pancreatic tissue extract, most of the insulin immunoreactivity was localized in the same zone as $^{125}$I-insulin. In the fractions from tumor tissue extract, besides the peak corresponding with

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**Fig. 2.** IRG elution patterns (IRG/g wet wt.) of acid-alcohol extracts of the metastatic liver tumor (A) and the pancreatic tail (B) on Bio-Gel P-10 column chromatography. Arrows indicate the elution volume of the markers (blue dextran, cytochrome C, $^{125}$I-insulin and $^{125}$I-glucagon).

**Fig. 3.** IRI elution patterns (IRI/g wet wt.) of acid-alcohol extracts of the metastatic liver tumor (A) and the pancreatic tail (B) on Bio-Gel P-10 column chromatography. Arrows indicate the elution volume of the markers (blue dextran, cytochrome C, $^{125}$I-insulin and $^{125}$I-glucagon).
$^{125}$I-insulin, another peak existed close to the elution volume of cytochrome C (Fig. 3). The percent IRI in the cytochrome C and $^{125}$I-insulin peaks of the tumor tissue extract were calculated to be 56.4 and 43.6, respectively.

**Radioreceptor assay of glucagon**

In Fig. 4, the displacement curve of $^{125}$I-glucagon produced by subjecting increasing amounts of tumor tissue extract in radioreceptor assay with isolated liver plasma membrane of rat, compared to the standard curve. The displacement and standard curves were parallel, indicating similar binding activity to isolated hepatocytic receptors for tumor tissue extract and human glucagon.

The values of glucagon measured by radioreceptor assay of peaks I-III of tumor tissue extract corresponded to 24.3, 8.2, and 51.3 percent of IRG value, respectively.

**Discussion**

The clinical features of this case were characteristic of a pancreatic alpha-cell glucagon-secreting tumor with metastases to the liver. All of the clinical manifestations of the glucagonoma syndrome which are characterized by dermatitis, weight loss, diabetes mellitus, anemia, hypoproteinemia and hypoaminoacidemia (Mallinson et al., 1974) were present. Histologically both primary and metastatic tumors were islet-cell carcinoma of the alpha-type. The resected metastatic tumor of the liver contained an enormous amount of IRG, and an appreciable amount of IRI. However, immunochemically, insulin was not, while glucagon was, demonstrated in the tumor cells. Of interest is the heterogeneity of glucagon and insulin in this case, for the heterogeneity of peptide hormones including glucagon (Danforth et al., 1976; Recant et al., 1976; Rigopoulou et al., 1976; Valverde et al., 1974 and 1976; Wier et al., 1975 and 1977), insulin (Beischer et al., 1975; Gorden et al., 1972; Roth et al., 1968; Steiner and Oyer, 1968; Steiner et al., 1968; Yalow and Berson, 1973a), calcitonin (Deftos et al., 1975; Sizemore and Heath, 1975), gastrin (Gregory and Tracy 1972), growth hormone (Gorden et al., 1973), prolactin (Rogol and Rosen, 1974; Suh and Frantz, 1974) and ACTH (Yalow and Berson, 1973b) has been demonstrated in plasma and in the tissue of origin and hormone-producing tumors.

On Bio-Gel P-10 column chromatography, we detected four components of plasma of this patient in IRG assay with antiserum 30K and in GLI assay, and three IRG components with antiserum OAL-123. Four different molecular weight components of 30K-IRG have been described in plasma of normal subjects and glucagonoma patients (Recant et al., 1976; Valverde et al., 1976).

Peak I, which is greater than 9000 daltons, may coincide with BPG. The presence of this molecule in plasma and in pancreatic tissue extracts has been reported by Valverde et al. (1974). These workers demonstrated that following incubation of plasma BPG with trypsin a glucagon immunoreactive peak appeared in a molecular weight
zone near the $^{125}$I-glucagon marker. Therefore BPG may represent a protein-bound glucagon, or a polypeptide containing immunological determinants within its structure.

Peak II, which has an approximate molecular weight of 9000 daltons, corresponds in size to LGI which is considered to be proglucagon in pancreatic tissue extracts, and the glucagon value of this peak of this patient by radioreceptor assay was low in comparison with peak I and peak III. In fact, Noe et al. (1975) demonstrated by gel filtration of tissue extracts, following islet incubation in the presence of $^3$H-tryptophan, that human LGI molecules contained covalently bound glucagon, suggesting that it may represent a precursor or an intermediate in human glucagon biosynthesis. Similar results were obtained when islets from guinea pigs (Hellerström et al., 1974), rats (O'connor et al., 1973), anglerfish (Noe and Bauer, 1971) and pigeons (O'connor and Lazarus, 1976) were used. Recant et al. (1976) reported that the proglucagon component represented approximately 17% of the total glucagon immunoreactivity in normal plasma. Elevation of this component has been reported in phloridzin hypoglycemia or poorly controlled alloxan-diabetes in dogs (Beischer et al., 1975; Gorden et al., 1972). The large immunoreactive moieties may represent precursors in patients with other functioning endocrine tumors (Deftos et al., 1975; Gorden et al., 1973; Gregory and Tracy 1972; Rogol and Rosen, 1974; Sizemore and Heath, 1975; Suh and Frantz, 1974).

In the present case, tumor and control tissue extracts manifested small IRG peaks in the same elution zones where BPG and LGI were detected on Bio-Gel P-10 filtration of preoperative plasma samples (Fig. 2). Valverde et al. (1974) reported that on Bio-Gel P-30 filtration, extracts from dog pancreas yielded IRG peaks corresponding to BPG and LGI, IRG of these peaks was 0.3% and 3% of the total immunoreactivity, respectively. However, Weir et al. (1977) did not note any clearly defined IRG peak corresponding to the specific plasma component in the tumor tissue extracts when they studied a patient with a glucagon-secreting tumor. The chromatographic patterns of tumor and control tissue extracts in our case were qualitatively, but not quantitatively, equal (Fig. 2).

In our case, more than 25% of the IRG and 35% of the GLI were associated with a component having a molecular weight of 9000 daltons, which was considered to be a different pattern from that found in normal plasma. The increase in LGI in our patient suggests that the secretory activity of the tumor was augmented due to either development or malignant transformation of the tumor. Inappropriately high levels of proinsulin are often found in patients with insulin-secreting islet-cell tumors of the pancreas (Beischer et al., 1975; Gorden et al., 1972). The large immunoreactive moieties may represent precursors in patients with other functioning endocrine tumors (Deftos et al., 1975; Gorden et al., 1973; Gregory and Tracy 1972; Rogol and Rosen, 1974; Sizemore and Heath, 1975; Suh and Frantz, 1974).

In the present case, tumor and control tissue extracts manifested small IRG peaks in the same elution zones where BPG and LGI were detected on Bio-Gel P-10 filtration of preoperative plasma samples (Fig. 2). Valverde et al. (1974) reported that on Bio-Gel P-30 filtration, extracts from dog pancreas yielded IRG peaks corresponding to BPG and LGI, IRG of these peaks was 0.3% and 3% of the total immunoreactivity, respectively. However, Weir et al. (1977) did not note any clearly defined IRG peak corresponding to the specific plasma component in the tumor tissue extracts when they studied a patient with a glucagon-secreting tumor. The chromatographic patterns of tumor and control tissue extracts in our case were qualitatively, but not quantitatively, equal (Fig. 2).

On the other hand, the IRI chromatographic patterns of tumor and control tissue extracts were quite different from each other (Fig. 3). The tumor tissue extract yielded two major IRI peaks near the elution volume of cytochrome C and insulin; the IRI peak in the control pancreas
tissue extract was localized in the insulin elution volume. We do not know whether these components corresponded to proinsulin and insulin, respectively, because we did not examine their natures. Gorden et al. (1972) have reported that the nature of the proinsulin-like component in at least some islet-cell tumor patients was different from that in non-tumor patients. The metastatic liver tumor cells of our patient did not demonstrate insulin immuno-histochemically, although the tissue extract from this tumor contained IRI recoverable by gel filtration.

Therefore, we conclude that this metastatic liver tumor produced not only an enormous amount of glucagon but heterogenous peptides which contained immunological insulin determinants within their structures. Furthermore, the possibility that this tumor also contained other peptides cannot be ruled out since islet-cell tumors containing several different peptides have been reported in the literature (Boden et al., 1977; Larsson et al., 1975; Tiengo et al., 1976).

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