PLASMA PANCREATIC GLUCAGON IN PANCREATIC AND PRIMARY DIABETES, AND LIVER CIRRHOSIS: APPLICATION OF A CORRECTION TO THE RADIOIMMUNOASSAY FOR PANCREATIC GLUCAGON

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Summary
Evidence is present that plasma contains non-specific factors which interfere with the 30K glucagon assays. A correction can be made for these interference factors because the factors can be quantitated following absorption of glucagon with charcoal-dextran. Using a correction factor the range of fasting plasma immuno-reactive glucagon (IRG) in 12 totally pancreatectomized patients was below detectable limit.

Fasting levels of IRG were determined on the plasma from 25 liver cirrhotics complicated by abnormal GTT, 13 pancreatic diabetics with chronic calcified pancreatitis (CCP), 25 adult-onset primary diabetics and 25 healthy subjects. When all samples were measured using no correction factor, the mean levels of IRG were 358 ± 24 (mean ± SE), 170 ± 26, 178 ± 16 and 178 ± 7 pg/ml, respectively. Using a correction factor the mean levels of IRG were 177 ± 26, 16 ± 4, 39 ± 9 and 20 ± 4 pg/ml, respectively. The mean values of the interference factor were not significantly different among all five groups. During an arginine infusion the interference factor remained unchanged despite an increase in IRG.

It is available but not always necessary to apply a correction factor for 30K glucagon radioimmunoassay.

Key Words: 30K glucagon assay, interference factor, total pancreatectomy, chronic calcified pancreatitis, pancreatic diabetes.

Introduction
Highly specific antisera to pancreatic glucagon have no significant cross-reactivity with gut glucagon1-3). However, normal fasting levels of immunoreactive glucagon were reported in patients with total pancreatectomy despite the use of 30K which is one of the specific antisera4-6). An “interference factor” has been shown to the present in human plasma,
which can cause artifactual elevation of immunoreactive glucagon value conventionally determined by radioimmunoassay using antisemum 30K7). This factor does not seem to be pancreatic glucagon, because unlike pancreatic glucagon, it cannot be extracted from plasma by charcoal7). Accordingly, corrected pancreatic glucagon can be obtained by subtracting the "interference factor" value from the uncorrected glucagon value7).

In this study a glucagon radioimmunoassay using a correction factor has been compared with that using no correction factor in the fasting plasma in patients with pancreatic and primary diabetes, and liver cirrhosis complicated by glucose intolerance.

**Materials and Methods**

1. **Subjects:** 12 patients with total pancreatectomy, 13 with pancreatic diabetes caused by chronic calcified pancreatitis (CCP), 25 with liver cirrhosis complicated by abnormal oral GTT, 25 with adult-onset primary diabetes and 25 healthy subjects were studied. Means ± SE of fasting blood sugar were 241 ± 25, 181 ± 21, 109 ± 9, 205 ± 20 and 89 ± 2 mg/100 ml, respectively.

2. **Collection of samples:** After an overnight fast blood was drawn into a chilled glass tube containing 1000U of Trasylol® (Bayer, Japan) and 1.2 mg of EDTA per ml of blood. The tube was centrifuged at 4°C as soon as possible, and the plasma was separated and kept frozen until assay.

3. **Extraction of plasma with charcoal-dextran:** It has been demonstrated that charcoal can extract almost all of small amounts of either 125I-glucagon or unlabeled pork glucagon added to plasma7). We employed the Valverde's modified method8). A mixture of charcoal-dextran was added to each sample (final concentrations: 2.5% charcoal, 0.25% dextran). The combination was incubated at 4°C for 45 minutes (shaken every 15 minutes), centrifuged at 3000 rpm for 20 minutes and the supernatant was collected.

4. **Glucagon radioimmunoassay:** Procedure for plasma glucagon radioimmunoassay was followed by a modification in principle9). A standard curve was made using highly purified crystalline glucagon (Novo Research Institute, Denmark), 125I-glucagon (New England Nuclear, USA), antisemur 30K (Unger Foundation, USA), and Trasylol® in 0.2 M glycine buffer at pH 8.8. Unextracted plasma was assayed against this standard curve giving an uncorrected immunoreactive glucagon (uncorrected IRG) in pg/ml. Extracted plasma was measured against the same standard curve giving a quantification of "interference factor", also in pg/ml. The estimate for corrected immunoreactive glucagon (corrected IRG) was then obtained by subtracting the interference factor value from the uncorrected IRG.

Statistical analyses were made by the Students' t test. Probability less than 5% was taken as the level of significance. Results are presented as mean ± SE.

**Results**

1. **Fasting glucagon determinations**

Table 1 shows the values of uncorrected IRG, interference factor and corrected IRG in the fasting plasma in the 12 totally pancreatectomized patients. All the patients had measurable levels of uncorrected IRG (76–221 pg/ml), and appeared to have corrected IRG of near the zero (−13~10 pg/ml) which were below our detectable limit.

The mean values of uncorrected IRG in liver cirrhosis, pancreatic diabetes due to CCP, primary diabetes and healthy control were 358 ± 24, 170 ± 26, 178 ± 16 and 178 ± 7 pg/ml, respectively (Fig. 1). The mean values of corrected IRG were 177 ± 26, 16 ± 4, 39 ± 9 and 20 ± 4 pg/ml, respectively (Fig. 2). The uncor-