

## A Fundamental study on Radioimmunoassay for Human Calcitonin

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Synthetic human calcitonin (Ciba) labelled with  $^{125}\text{I}$  was purified by gel filtration with Sephadex G-25 and G-100. Incubation mixtures containing  $200\mu\text{l}$  of antibody (Ciba) (1 : 10,000),  $100\mu\text{l}$  of aliquotes of standard or plasma samples and  $100\mu\text{l}$  of incubation buffer or pooled plasma from normal subjects were incubated for 3 days at  $4^\circ\text{C}$  followed by 24 hr incubation after adding  $100\mu\text{l}$  of tracer. The bound fraction was separated from free by charcoal-dextran adsorption and the radioactivity in both the precipitate and supernatant was counted in a gamma counter

This assay system was sensitive to about 10 pg added to the test tube with good results of recovery test and gave a parallel inhibition curve upon dilution of plasma from a patient with medullary thyroid Ca.

Basal plasma calcitonin concentration distributed less than 0.3 ng/ml in 21 normal subjects, from 98 ng/ml to 665 ng/ml in 6 cases with medullary thyroid cancer, normal to relatively high in 27 cases with chronic renal failure, 15 malignancy, 7 bone disease and 9 thyroid disease, and within normal range in 2 cases with parathyroid disease respectively. Plasma calcitonin in response to Ca infusion markedly increased in 2 cases with medullary thyroid cancer tested and one member of a family with medullary thyroid cancer, while no remarkable changes were seen in other cases tested including 2 cases with chronic renal failure and one with treated Graves' disease which showed increased basal calcitonin.

## Studies on the Immunoreactive Gastrin in Human Sera and in Gastrointestinal Mucosa

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In order to investigate the distribution and nature of gastrin (G) in the gastrointestinal mucosa, immunoreactive G (IRG) was determined by radio-immunoassay using double antibody technique in tissues obtained at autopsy or surgery, as well as in biopsy

specimen obtained under endoscopy. The largest amount of IRG was found in the antral mucosa among the tissues tested. A relatively large quantity of IRG was detected in the duodenal mucosa, but decreasing concentrations were found gradually towards the distal por-

tion. A little amount of IRG was detected in corpus of the stomach, ileum and pancreas. It was, however, interesting that the abundant IRG was detected in corpus of the stomach from a patient with pernicious anemia.

Distribution of big G (BG, Yalow & Berson) and little G (LG) in boiled extracts of gastrointestinal mucosa were studied by use of Sephadex G-50 gel filtration and starch gel electrophoresis. LG distributed mainly in the antral mucosa of the stomach (88.4%), and decreased towards distal portion of the duodenum. In contrast, BG increased gradually to the distal part; 53.4% of BG being found in the duodenal 3rd portion.

The similar studies on changes of IRG patterns of sera following intragastric acidifi-

cation and subsequent alkalization were carried out in a patient with pernicious anemia. Major component of serum IRG before stimulation was confirmed to be BG. After the acidification, BG decreased, but LG and IRG of smaller molecule than LG appeared. After the alkalization BG increased again and LG and smaller IRG disappeared.

These findings suggested that the molecular forms and distribution of IRG in sera and tissues may be closely related to release mechanism of gastrin.

In the present report, IRG patterns determined by CIS-gastrin kit and Dainabot gastrin kit were also compared with that obtained by double antibody method.

### **Simultaneous Determination for HB Antigen and Antibody by RIA Method**

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The RIA methods for detecting the HB antigen or antibody have been reported by several research groups. The solid-phase (SP) RIA method which has recently been developed by Abbott Laboratories is particularly excellent in sensitivity and reproducibility and has become widely utilized. Furthermore, the development of the antibody SP-RIA method is also required from the standpoint of epidemic researches, but it is not available yet due to the difficulty in purifying HB antibody and contamination of antigen in waste fluid.

In order to remedy all these difficulties, we attempted to work a process of placing antigen into a polyethylene tube of the kit

for detecting the HB antigen to form an antibody wall and an antigen wall and to develop the both reactions of antibody/unknown antigen/labelled antibody and unknown antibody/antigen/labelled antibody, which process permitted us to determine the antigen and antibody by the SP-RIA method and by taking advantage of those facts that, in the measurement of radioactivity, the cpm value of the antigen group rises and the cpm value of the antibody positive group falls, which cpm values of antigen antibody negative group and antigen antibody group are invariable.

Our present report describes the principle, the detection method and its results of the simultaneous determination for HB antigen