the mean of 4.8±1.7 (SE). The urinary excretion of cotinine in smokers increased after smoking 3 cigarettes for 30 min. Daily urinary excretions of cotinine reflected roughly the amounts of cigarettes smoked per day. These results indicate that the measurement of daily cotinine excretion by radioimmunoassay may be a good indicator of cigarettes consumption and is considered to be a useful tool for investigating the smoking effects in human.

Radioimmunoassay of Somatostatin and Its Clinical Application

Mitsuyasu ITOH, Terunori MITSUMA and Akio TOMITA
First Department of Internal Medicine, Nagoya University School of Medicine

The establishment of radioimmunoassay for somatostatin is important for the assessment of its role in terms of humoral regulation. There are, however, several problems about the assay and extraction method. We developed a new assay system, and the assay procedure was already described elswhere (Folia endocrinol. jap. 53: 1106, 1977). The concentrations of immunoreactive (IR-) somatostatin detected by this assay were 0.1–5 ng/m*l*. In this assay method, we examined the extraction method, and further investigated the clinical application.

IR-somatostatin in the tissue was extracted by aceton, methanol, ethanol, or a mixture of 2N acetic acid and methanol. The average recovery rate was approximately 65, 68, 75, more than 90% respectively. The dilution curve of IR-somatostatin extracted from each sections of the rat brain was paralleled to the standard curve gained by synthetic somatostain. The extracts from the rat brain were identified by synthetic somatostatin on Sephadex G-25 column chromatography. We detected 31±2 ng (M±SE) IR-somatostatin in rat hypothalamus and also other regions in a rat. Then we apply this method to the clinical use. The extracts from a human pancreatic tissue of insuloma were identified by it through the same chromatography. IR-somatostatin in C.S.F. was also detected by this assay. So this assay and extraction method would be able to use for the experimental and clinical application.

A Radioimmunoassay for Cholecystokinin-pancreozymin

Eiichi TSUKIE, Hiroshi NISHIKAWA, Fumitake ISHIHARA, Koji SHIBATA and Haruo KAMEDA
The First Department of Internal Medicine Tokyo Jikeikai University School of Medicine, Tokyo

A sensitive and specific radioimmunoassay for cholecystokininpancreozymin (CCK-PZ) has been developed using rabbit antiserum to synthetic porcine (27-Tyr) CCK-PZ.

Preparation of antibody: The immunogen was conjugated with BSA. 0.7 mg of CCK-PZ was emulsified in 1 m*l* of Freund's complete adjuvant, and injected subcutaneouly into each rabbit at approximately 2-weeks intervals. About 100 days after the first immunization, serum from rabbit was collected.

Preparation of ^{125}I-CCK-PZ: Using the chloramin T method of Greenwood and Hunter, iodination was performed with 3 mCi of Na^{125}I and 2 μg of the synthetic CCK-PZ. Specific activity was 200–300 μCi/μg. ^{125}I-CCK-PZ was separated from free iodine by gel filtration using Sephadex G-50 superfine.

The double antibody technic was used to examine the dilution-effect and specificity of the rabbit antiserum. Bo% showed 58%, so this result indicated that the rabbit antiserum had a high titre enough to be used at the dilution of antiserum of 1:10000.

In this studies Gastrin, Secretin, Glucagon and Enteroglucagon showed no significant cross reaction at each concentration of 10 pg/m*l*-100 μg/m*l*.

In the inhibition curve of CCK-PZ, minimum detectable concentration was about 200pg/m*l*, and measurable range was 200–3000pg/m*l* in this assay system.

We are now studing the measurement of CCK-PZ level in human serum.

Studies of Gastrointestinal Hormones by Radioimmunoassay
Report III Radioimmunoassay of Secretin

Akiko MORIYAMA, Hiroyasu MURAYAMA, Jujiro OKAMOTO and Yoji HARADA*
The Department of Nuclear Medicine, Cancer Center and the 4th Department of Internal Medicine, Tokyo Medical College*

We carried out and reported previously a series of fundamental studies on secretin RIA. Supplied with Secretin RIA Kit through the courtesy of Daiichi Radioisotope Laboratories, we conducted both fundamental and clinical investigations with this device. The results are presented in this paper.

In order to determine the optimal incubation time and temperature specimens were incubated at 4°C or room temperature for 1 to 5 days. A nearly identical calibration curve was obtained both from specimens incubated at 4°C for 3 to 5 days and from those incubated at room temperature for 2 to 4 days. Values for CV at different points on calibration curve ranged from 4.2 to 13%, 8% on the average, indicating an excellent reproducibility of calibration curve, with a significant difference in CV observed between levels of 0 and 50 pg/m*l*. The recovery test also yielded a satisfactory result with an average recovery rate of 101%. Mean CV for intra-assay of 11 serum specimens was 2.9% while a corresponding value for interassay was 8.1%. At low concentration levels of below 100 pg/m*l* CV was found at an average of 7.3%. From combined consideration of these figures and the reproducibility of calibration curve it seems possible to determine a secretin concentration level of down to 50 pg/m*l* with reasonable accuracy by this particular technique.

Average secretin levels in the fasting blood as measured by the radioimmunoassay method in a total of 40 subjects of 4 distinct categories, i.e. healthy state, gastric ulcer, doudenal ulcer and duodenal ulcer scar, were 113 pg/m*l*, 102 pg/m*l*, 117 pg/m*l* and 102 pg/m*l*, respectively, hence with no significant differences observed between these disease states. In a further study the blood level of this hormone was investigated for its eventual correlationship with gastrin levels in circulating blood. At the present stage, however, no definite correlation has been shown to exist.

Clinical Evaluation for Secretin Radioimmunoassay kit

Shigeki NAKATA*, Kunisaburo MORIDERA*, Keishiro INOUE*, Yutaka SEINO**, Kozaburo MORI**, Tomohiko TAMINATO**, Yuzuru KATO**, Shigeru MATSUKURA** and Hiroo IMURA***
**Hyogo Prefectural Tsukaguchi Hopsital*
***Third Division, Internal Medicine Kobe University School of Medicine*
****Second Division, Internal Medicine Kyoto University School of Medicine*

Sensitive and specific radioimmunoassay kit for secretin (Daiichi radioisotope laboratory Japan) was developed using antiserum raised in rabbit against synthetic secretin (synthesized by Yanaihara, Shizuoka pharmaceutical college Japan). Human insulin, pork monocompornent glucagon, C-peptide, human gastrin I, GIP, VIP, motilin and substance P did not cross-react with this antiserum. Labelled hormone was obtained by conventional chloramine-T method using [Tyr^1] secretin. Separation of bound label from free label was made by double antibody techni-