D. Measurement III (Invitro Radioimmunoassay)

Studies on the Double-antibody Radioimmunoassay of Human β2 Microglobulin

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A double-antibody radioimmunoassay for β2 microglobulin in human body fluids was established and following results were obtained.

1. The standard curve showed linear over the range 1.95–62.8 μg/l and the detection limit was 0.49 μg/l. The coefficients of variation for intra-assay were 0.99–5.25% and for inter-assay were 1.42–8.6%, respectively. The cross-reactivities to Bence Jones protein (Kappa, Lambda) and human serum components except β2 microglobulin were not observed.

2. Correlation between the double-antibody method and the solid-phase method (Phadebas β2 Micro. Test.) in serum and urine showed good correlation, \( \gamma = 0.962 \) (n=38).

3. The mean concentration of β2 microglobulin in normal human serum, colostrum after normal delivery and cord blood serum at normal delivery were 1.71 ± 0.29 mg/l (m±SD, n=37), 37.3 ± 14.8 mg/l (m±SD, n=11) and 2.58 ± 0.28 mg/l (m±SD, n=11), respectively. The mean 24 hr urinary excretion of β2 microglobulin in normal human was 0.075 ± 0.036 mg/24 hr. (m±SD, n=10).

Radioimmunoassay of Cotinine


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To study the pharmacological effects of smoking in human we have developed a sensitive and specific radioimmunoassay of cotinine, a major metabolite of nicotine. Anti-cotinine antisera were made in rabbits by immunizing with N-aminoethylcotinine conjugated to BSA. 125I-labelled cotinine was prepared by radioiodination of N-aminoethylcotinine-CO-\( \bigcirc \)-O with the chloramine T method. After incubation at 4°C overnight, separation of antibody-bound from free tracer was done by the ammonium sulfate precipitation. Several nicotine and cotinine derivatives show slight cross-reactions with an anti-serum: d-nicotine 10.4%, l-nicotine 4.09%, nornicotine 0.52%, 6-(OH)-nicotine 0.16%, myosmine 0.10%, 6-(OH)-myosmine 0.10% and oxynicotine 0.02%. However, N-aminoethylcotinine and N-aminoethylcotinine-CO-\( \bigcirc \)-O cross-reacted with antisem more markedly than 1-cotinine (approximately 17 and 1460 times on molar basis, respectively), indicating that antibody is directed to not only the pyrrolidine ring but also the attached side chain of the pyridine ring. The lower limit of sensitivity of our assay is 1 ng per tube. Urinary cotinine was extracted with 10 times volume of chloroform since the direct addition of non-smoker's urine was found to interfere in subsequent radioimmunoassay. The mean recovery of 1-cotinine added in non-smoker's urine after extraction was 81 ± 20.3 (SD)%. The intra-assay coefficient of variation was 28%. The concentration of urinary cotinine of 11 smokers ranged from 0.06 to 18.5 μg/ml with

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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
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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 elsewhere (Folia endocrinol. jap. 53: 1106, 1977). The concentrations of immunoreactive (IR–) somatostatin detected by this assay were 0.1–5 ng/ml. 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 somatostatin. 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
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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 ml of Freund’s complete adjuvant, and injected subcutaneously into each rabbit at approximately 2-weeks intervals. About 100 days after the first immunization, serum from rabbit was collected.

Preparation of 125I-CCK-PZ: Using the chloro­min T method of Greenwood and Hunter, iodina­tion was performed with 3 mCi of Na125I and 2 μg of the synthetic CCK-PZ. Specific activity was 200–300 μCi/μg. 125I-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/ml-100 μg/ml.