D. Measurement III (Invitro Radioimmunoassay)

Studies on the Double-antibody Radioimmunoassay of Human $\beta_2$ Microglobulin

Nobuhiko Mizuno, Kiwamu Okada, Shigeki Takase and Shigeaki Baba

The Second Department of Internal Medicine, Kobe University School of Medicine, Kobe

A double-antibody radioimmunoassay for $\beta_2$ 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 $\mu$g/l and the detection limit was 0.49 $\mu$g/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 $\beta_2$ microglobulin were not observed.

2. Correlation between the double-antibody method and the solid-phase method (Phadebas $\beta_2$ Micro. Test.) in serum and urine showed good correlation, $\gamma=0.962$ (n=38).

3. The mean concentration of $\beta_2$ 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 $\beta_2$ microglobulin in normal human was 0.075±0.036 mg/24 hr. (m±SD, n=10).

Radioimmunoassay of Cotinine

S. Matsukura*, N. Sakamoto*, Y. Seino*, K. Takahashi*,
H. Matsuyama**, H. Muranaka** and H. Imura***

*Third Division, Department of Medicine, Kobe University School of Medicine, Ikuta-ku, Kobe
**Kyoto Hospital of the Japan Tobacco and Salt Public Corporation,
Higashiyama-ku, Kyoto
***Second Division, Department of Medicine, Kyoto University School of Medicine, Sakyoku-ku, Kyoto

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. $^{125}$I-labelled cotinine was prepared by radioiodination of N-aminoethylcotinine-CO–$\rightarrow$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%, I-nicotine 4.09%, nor nicotine 0.52%, 6-(OH)-nicotine 0.16%, myosmine 0.10%, 6-(OH)-myosmine 0.10% and oxynicotine 0.02%. However, N-aminoethylothnicotine and N-aminoethylcotinine-CO–$\rightarrow$O cross-reacted with antiserum more markedly than 1-cotinine (approximately 17 and 1460 times on molar basis, respectively), indicating that antibody is directed 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 $\mu$g/ml with