Recent Advance and Problem in Nuclear Medicine—In Vitro Test—

1-(1)

ANTIGEN, ANTISERA AND LABELLING. N.Yanaihara. Shizuoka College of Pharmacy. Shizuoka.

Processing of high molecular weight biosynthetic precursors is one of major explanations for the existence of molecular heterogeneity of regulatory peptides in blood and in tissues. Synthetic peptides of defined structures, which are related to the hormones and/or their precursors, serve not only as excellent immunogens for production of region-specific antisera but also as valuable substrates for characterization of the antisera. For example, synthetic glicentin (49-69) which corresponds to the carboxyl-terminal fragment of porcine glicentin was proved to be an valuable immunogen for the production of glicentin carboxyl-terminal specific antiserum, while the amino-terminal fragment of big gastrin (G34) was useful as an immunogen for eliciting G34 specific antiserum. Such region-specific antisera, as well as the synthetic peptides themselves, have effectively been used in immunohistochemical experiments and for development of highly sensitive and specific immunoassay of regulatory peptides. The structural features of labelled antigens also influence to the antigen-antibody interaction. Purposely-designed synthetic peptides were found to be useful as substrates for labelling. As an example, we have successfully utilized ^{125}I-hydroxyphenylpropionyl-[Gln^{1}]-serum thymic factor (STF) to develop the immunoassay system using the carboxyl-specific anti-SFT serum, especially, for measurement of blood level of the peptide.

1-(2)

RECENT ADVANCES IN IN-VITRO RI TEST IN ENDOCRINOLOGY. K. Miyai. Osaka University Medical School. Osaka.

Development of new assay methods of in vitro RI test results in progress in endocrinology as follows. For instance, a monoclonal antibody specific for hormone can be derived by the hybridoma technique and an antibody with low cross-reactivity can be prepared by D-GL (copolymer of D-glutamic acid and D-lysine) technique. Specificity of radioimmunoassay can be improved by utilizing such a specific antibody. Thorell developed a simple "homogeneous" radioimmunoassay using internal sample attenuator counting. Newly recognized hormones and hormone related substances such as neuropeptides,gut hormones and growth factors can be determined by radioimmunoassay. Development of several kits of radioimmunoassay for measuring free thyroxine and those of receptor assay for TSH binding inhibiting immunoglobulin promote laboratory diagnosis of thyroid abnormalities. The most important recent development in mass screening tests has been the introduction of radioimmunoassay for early diagnosis of congenital hypothyroidism and the adrenogenital syndrome. We developed TSH assay and free thyroxine assay in dried blood samples on filter paper. We screend 200,000 babies in the general population and found 30 patients with congenital hypothyroidism. These patients have grown normally with early treatment. Thus, in vitro RI tests are applied to preventive medicine.

2

STARATEGY OF FREE HORMONE ESTIMATES IN CLINICAL ENDOCRINOLOGY. Y.Shishiba. Division of Endocrinology, Toranomon Hospital, Tokyo.

It is well accepted that free hormone concentration is an important indicator of hormonal activity. To determine free T_4 and free T_3, various methods have been invented substituting the classical measurements of free hormone by equilibration dialysis. In the present study, a new free T_4 and free T_3 index based on the equilibration kinetics between T_4, T_3 and TBG were introduced and compared with those by direct measurements. Contribution of TBPA or albumin to the binding was assumed to be a constant. Both indices correlate with direct measurements by equilibration dialysis with the correlation coefficient more than 0.95. Both indices reflect clinical thyroid status without reflecting abnormalities in TBG. For the laboratory routine, we would like to recommend free T_4 and free T_3 calculation based on RIA of T_4, T_3 and TBG. When there is discrepancy between the calculated indices and clinical status, direct measurements by equilibration dialysis or by an appropriate method recently invented would be indicated. By the combination, abnormal binding of hormone by antibody or by abnormal protein (for example, binding of T_4 to abnormal albumin resulting in dysalbuminemic hyperthyroxinemia) would be correctly diagnosed with reasonable cost-performance efficiency.

3

RADIOIMMUNOASSAY OF TUMOR MARKERS. T.Imaeda. Gifu University School of Medicime. Gifu.

Several tumor markers were assayed in the same sera of 922 cancerous cases of lung, liver, biliary tract, pancreas and gastro-intestinal tract. The frequency of positive cases for tumor markers was compared according to the type of disease. When 138 cases of lung cancer were morphologically classified, a positive frequency of Calcitonin was high for small cell carcinoma and low for adenocarcinoma. The positive frequency of CEA was low for large cell carcinoma. As to ferritin, the positive frequency was high for large cell carcinoma and low for small cell carcinoma. All 10 cases, in which CEA was over 15 ng/ml and Calcitonin was over 200 pg/ml, belonged to stages III or IV. Among 561 cases of liver cirrhosis, in which AFP was assayed for 9 years since March, 1972, 16 cases indicated more than 400 ng/ml of AFP. 5 (31%) of these 16 cases developed hepatocellular carcinoma within 2 to 3 years. A tendency for an unusually high amount of Ferritin to be indicated in cases which AFP was low, was not observed. Among 44 cases of pancreatic carcinoma, the frequency of positive cases for CEA increased as the cancerous stage progressed. At stage IV, the frequency of positive cases for Elastase 1 dropped compared to stages I-III. All cases of pancreatic carcinoma, in which CEA was over 5.0 ng/ml belonged to stages III or IV. 81% of gastric cancer and 76% of colono-rectal cancer, in which CEA

exceeded 7.0 ng/ml were metastatic cases of liver or peritoneum.

Positive ratio of tumor markers in various cancerous diseases

(> normal values)

Cancerous diseases	No. of cases	Positive ratio of tumor markers				
		AFP	CEA	Ferritin	Calcitonin	Elastase 1
lung Ca.	138	– %	61 %	36 %	71 %	%
hepatocellular Ca.	263	79	42	54		
cholangio Ca.	7	0	29	43		
metastatic liver Ca.	135	19	84	49		
gall bladder Ca.	18	11	61	61		
extrahepatic bile duct Ca.	14	0	50	64		
pancreatic Ca.	44	0	80	61		70
esophageal Ca.	39	3	28	44		
gastric Ca.	156	12	47	23		
colonic Ca. & rectal Ca.	108	4	63	29		

4

DRUGS AND BODY COMPONENTS. Y.Sasaki. Toho University School of Medicine. Tokyo.

Drugs: The principle of the individualized therapeutic drug monitoring (TDM) was reviewed. The clinical role and importance of TDM were demonstrated illustrating representative cases treated with digoxin, deslanoside, tobramycin, teophyllin and phenobal. A new method of estimating digoxin blood levels at steady state was evaluated. Blood digoxin concentrations at transition state were used for the estimation of digoxin level at steady state based on the open linear one compartment model. The estimated plasma levels agreed well with measured blood concentrations at steady state. Deslanoside was measured with digoxin RIA kit utilizing the high cross reaction (76%) of antidigoxin antibody with deslanoside. Requirement for rapid assay of plasma drug concentrations were met by the successful stat assay of digoxin, which remarkably increased utilization of digoxin RIA. Assessment of assay methods for tobramycin comparing RIA, EIA, HPLC and bioassay revealed high sensitivity, good precision and reproducibility of RIA.

Body components: Clinical significance of measuring TBG, β_2m, βTG, PF4, tripsin, elastase 1 and bile acids were discussed demonstrating our clinical data. High insidence of elevated serum βTG was observed in patient with mitral stenosis and history of thrombosis. In advanced deabetic βTG and PF4 were elevated and tripsin was decreased.

5

MEASUREMENT OF HB VIRUS ASSOCIATED ANTIGEN AND ANTIBODY BY RIA. Kiyoshi Okada. Tokyo Metropolitan Okubo General Hospital. Tokyo.

The hemoagglutination method is widely used both in clinical practice and in research for the detection of hepatitis B virus associated antigen and antibody. However, this method has a low detection sensitivity for anti-HBs antibody, and it is necessary to use the RIA method for the detection of anti-HBs antibody after the administration of HB vaccine. To prevent babies from becoming HB virus carrier mothers due to maternal transmission of HB virus, the administration of HB immunoglobulin is effective, but by means of an assay of HBe antigen by the RIA method, it will be possible to decide more precisely than currently on the application of HB immunoglobulin administration.

6-(1)

APPLICATION OF RADIORECEPTOR ASSAY (RRA) IN ENDOCRINOLOGY. T.Tsushima. Tokyo Women's Medical College. Tokyo.

RRA has been proved to be very useful for detection, purification or measurement of various hormone-like substances (hormone agonists) and hormone-antagonists. Antibody to hormone receptors can be detected also by RRA-related technique. We have developed a new immunoprecipitation method for anti-insulin receptor antibody present in the serum of diabetic patient with type B syndrome. ^{125}I-insulin was chemically crosslinked to human placental membranes using disuccinimidyl suberate (DSS). ^{125}I-insulin crosslinked membranes were solubilized with Triton X-100 and incubated with anti-receptor serum and then with anti-human IgG rabbit serum. After centrifugation, radioactivity of the pellet was determined. All sera from 10 patients with type B syndrome were able to precipitate ^{125}I-insulin cross linked receptor dose-dependently. Immunoprecipitation was not affected by the presence of excessive amount of unlabelled insulin, suggesting the presence of antibodies that recognize the determinants outside the insulin binding sites on the receptor molecule. As compared with insulin-binding inhibition assay, the immunoprecipitation assay was much more sensitive. The effect of anti-insulin antibody could be removed simply by the addition of excess of unlabelled insulin. The immunoprecipitation will prove to be useful for detecting autoantibodies to hormone receptors.