B. In Vitro Assay

1401

EVALUATION ON SERUM IRI DETERMINATION BY SOLID PHASE RADIOIMMUNOASSAY. Y. Takahara A. Ishibashi, J. Satoh, Y. Sasaki, S. Yamashita, M. Kondoh and Y. Yanahara. The 2nd Tokyo National Hospital. Tokyo

We have measured serum IRI concentration in diabetes mellitus, diseases of liver and pancreas, during 50g OGTT using a newly established Insulin RIA KIT II (Dinabot) with the antibody-coated plastic beads.

Under consideration of the effect of incubation time on the reaction rate, the preferred incubation time was studied at 25°C. There was not so difference at 24 hours and 48 hours. At 4, 25 and 37°C degradation of B/T % continued along with elevation of temperature. The influence to the measuring value using automated pitetting equipment was notable in the test tube followed by the sample-tube. The intrassay--and interassay-reproducibility, recovery test and dilution test proved to be satisfactory. The effect to the measuring value of EDTA-2Na and heparin was a little, however, sodium fluoride (NaF) was very notable. In diabetic group, pancreatitis and pancreas cancer, there was low responses to glucose administration and △IRI/△BS (30 min.).

1402

DETECTION OF INSULIN ANTIBODY LIKE SUBSTANCE BY PRECIPITATION METHOD WITH PEG AND CELLULOSE ACETATE MEMBRANE ELECTROPHORESIS. S. Nogami, K. Suzuki, K. Nakashikiryoh, N. Ishizuka, H. Kayanuma, M. Ogasawara, T. Kondoh, M. Fukushi and T. Uchikawa Department of Radiology and Internal Medicine, Tokyo Metropolitan Komagome Hospital, Tokyo.

In routine test of blood IRI, we found some cases whose IRI values were under zero by PEG method but not extremely high by double antibody method. This cause was investigated by measurement of nonspecific binding ratio(NSB%) and cellulose acetate membrane electrophoresis. NSB% of sera were measured according to the assay protocol of blank described in the publication of the Insulin immunoassay kit(Kaken Chemical Co.). Besides sera which were reacted with 125-I insulin overnight were applied in a cellulose acetate membrane and electrophoresed in barbital buffer(pH 8.6,μ 0.06) at 1 mA/cm for about one hour. After stain of serum protein, the membrane was cut into small pieces and the radioactivity of them were measured. In all cases whose IRI values were under zero by PEG method, NSB% were definitely high of 35-79% (about 7% in normal sera). The radioactivity peak was observed in γ-globulin fraction and most of the radioactivity moved to the same fraction as the reference serum by addition of nonradioactive insulin. It was indicated by these results that those sera have endogenous specific binder to insulin corresponding to γ-globulin, probably insulin antibody. Accordingly it was suggested that PEG method is superior to double antibody method in detecting the patient sera containg insulin antibody like substance and enable us to obtain an accurate interpretation to date.

1403

RADIORECEPTOR ASSAY OF INSULIN. M. Hamazu, E. Yabumoto, T. Yamazaki, M. Kobayashi, Y. Shigeta. Department of Radiology and Medicine 3, Shiga University of Medical Science , Ohtsu

Since Berson and Yalow invented radioimmunoassay(RIA), insulin has been measured by detecting immunologically active site of insulin. However, RIA cannot differentiate biologically inactive one from active . In order to detect biologically active insulin , we performed radioreceptor assay(RRA). Placenta membranes were prepared according to Posner's method and mono-iodo insulin was prepared by chloramine T method of Freychet. After membranes were incubated with labeled and unlabeled insulin for 60 min at 15 C, a portion of incubation mixture was transfered to microfuge tubes containning an oil mixture with a specific gravity between those of the buffer and membranes. The pellet of membranes was separated by cutting through the oil phase and was counted for radioactivity. With this method, proinsulin has only 4% activity of insulin, whereas RIA showed 13% of normal. Abnormal insulin, [Leu^{B-25}]-insulin,has normal activity in RIA, whereas RRA showed only 10% of mormal. Whereas [Leu^{B-24}]-insulin has only 20% activity of normal in RIA, [LeuB-24] has 50% of normal in RRA. Thus, with this simple method, we can estimate the levels of abnormal insulin or proinsulin and can measure only biologically active hormones, which conventional RIA cannot do.

1404

FUNDAMENTAL STUDY OF C-PEPTIDE KIT "DAIICHI" II - COMPARISON WITH CURRENT KIT AND SHIONOGI'S KIT -. **M. Usami, Nuclear Medicine Clinical Laboratory, Faculty of Radiology, Okayama Saiseikai General Hospital.**

We obtained a good result from a study of the kit - reproducibility of standard curve; C.V.=0.8~5.1 % (8 replicate), Bo/T %; 34.1~39.2 %, intra-assay precision; C.V.=2.08~4.19 %, inter-assay precision; C.V.=3.89~6.98 %.
The difference between expected value and measured value of diluted sera (1/2~1/16) with distilled water or saline was less than ±1.01 % and recovery rate was 103.65±2.55 % (M±1SD).
Concerning incubation temperature and time, the best result was obtained when the assay was performed according to the "directions for use".
Correlation with C-peptide Kit "Daiichi" was r=0.988, y=0.015+1.075x, and that with Shionogi's Kit was r=0.973, y=-0.046+1.111x, both showed high correlation.
Normal fasting value was 0.86~2.82 ng/mℓ (1.52±0.54 ng/mℓ, N=30) and no difference was found between male and female.
These results of fundamental study indicate C-peptide Kit "Daiichi" II using synthesized human C-peptide has been greatly improved from the current kit in every aspect and is the easiest to handle of the three kinds of kits used for comparison.
From the preceding, it can be concluded the kit is more desirably improved one for ordinary clinical examination.