

assays was $Y=1.06X+0.18$ and coefficient of correlation was 0.99. TSH crossreacted 15% with antiserum for LH, FSH also crossreacted less than 7% and prolactin 0.4%. GH and ACTH did not crossreact.

Correlationship between Kit A and Kit B: Regression equation was $Y(\text{ng/ml})=0.08X(\text{mIU/ml})+0.06$ ($X=0-300 \text{ mIU/ml}$) and Coefficient of correlation was 0.97. Plasma LH level in normal males calculated using this expression was $0.7 \pm$

0.17 ng/ml .

In these findings two problems were indicated. (1) Kit B was inadequate in observing the small LH changes near the resting level. (2) In both kits, especially Kit A, not only HCG but TSH and FSH remarkably crossreacted with antiserum for LH. When plasma samples containing high concentration of TSH or FSH are assayed, LH values may be modified by these hormones.

Basic Studies and Clinical Applications of Radioimmunoassay Kit for Human Prolactin

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Recently, a specific and sensitive radioimmunoassay (RIA) kit for human prolactin (PRL) has been developed by CEA-IRE-SORIN(CIS). By using this kit, the following basic and clinical experiments were performed.

The standard curve of this kit was almost completely paralleled with the standard curve by MRC 71/222 added in this kit. Moreover, the dilution curve of the plasma from a patient with hyperprolactinemia was also paralleled with this standard curve. The assay sensitivity of this kit was from 2.5 ng/ml to 200 ng/ml of serum or plasma. The cross-reactivity of with LH, FSH, TSH, GH, HCG, HPL, TRH, LH-RH and Somatostatin were not seen in this assay. Plasma samples with human PRL concentrations of 3 ng/

ml , 15 ng/ml , 120 ng/ml and 250 ng/ml had intra-assay coefficients of variation of 23.4%, 7.6%, 15.2% and 10.5% respectively. Inter-assay coefficient of variation of human PRL 20 ng/ml was 16.9%.

In clinical use, the fasting PRL level of normal male subjects ($n=20$) was $11.3 \pm 6.0 \text{ ng/ml}$. This value was almost coincided with the datum obtained from our NIH kit. The patients with hyperthyroidism, breast cancer and anorexia nervosa showed normal PRL values. However, patients with acromegaly and Cushing's syndrome showed a slightly high PRL values.

These data suggest that this kit is clinically a very usefull tool for measuring human PRL.

Studies of Gastrointestinal Hormones by Radioimmunoassay

Report II. Radioimmunoassay of Secretin

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In our previous papers we reported on the radioimmunoassay of gastrin and secretin. The present study was undertaken to investigate further fundamental aspects of secretin assay. The results obtained are reported in this paper.

For the study were employed an antiserum preparation with an antibody titer of 1: 6000 (supplied by Eisai K.K.), Schwarz-Mann 6-tyrosyl

secretin as labelled secretin and purified secretin from raw extracts as reference standard.

Under various conditions (involving dilution of antibody, one- or two-step method, concentration of protein added, addition or non-addition of trasylol, and pH value of buffer) calibration curves were prepared and compared.

For assay samples containing secretin at a con-

centration order of ng/ml, a valid calibration curve was obtained by using 1/15 M phosphate buffer of pH 6.4 containing 2.5% protein, 1:4000 antiserum added with trasylol and two-step method.

For those assay samples of a secretin concentration order of pg/ml, a nearly satisfactory calibration curve could be obtained by using two-step method with 1/15 M phosphate buffer of pH

6.4 containing 1.5% protein and 0.2 ml of 1:5000 antiserum added with trasylol. Since, however, this assay method still presents difficulties to be overcome especially regarding reproducibility and recovery test and is not sensitive enough to permit correct measurement of secretin in the blood, further studies along these lines are needed to improve the assay procedure.

Fundamental and Clinical Studies on an Anti DNA Radioassay Kit

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A sensitive and quantitative test for anti-double strand DNA antibody is quite desirable.

This paper reports fundamental and clinical studies of a new anti DNA radioassay Kit from Radiochemical center. This is an immuno-radio metric method, and ¹²⁵I labeled double strand DNA and heat treated serum were incubated, and formed immune complex was precipitated by 50% Ammonium sulfate. Antibody titer was expressed as unit by comparison to the provided standard serum. Heparinized or citrated plasma showed lower result than serum sample, and storage at 4°C was found good only for 1 week, but 50 day storage at -20°C resulted in 107±38% (inter assay variability) When pretreatment of serum at 56°C for 30 min was omitted or pretreated at 37°C, nonspecific binding of ¹²⁵-DNA was found. Intraassay variability at low and high anti DNA levels were 23% and 14%, respectively.

Dilution curve of potent serum paralleled to the standard curve. These results were considered to show some possible non specificity of the assay, however, under careful control of the assay, a reliable and quantitative assay could be achieved.

Clinically 109 samples were tested, and 25 normal subjects of the third and fourth decade ranged from -10 to 5 units/ml. Some patients with SLE showed extremely high values (max 860 u/ml, n=25, mean 107.3±188.4), but most patients with RA, other autoimmune disease and miscellaneous disease had values less than 25 u/ml. The results appeared to correlate with NBA tests, ESR, LE cell, LE test and hypergammaglobulinemia, but anti-DNA antibody by this method was found most specific to SLE.

Chronological observations of anti-DNA antibody titer also gave useful clinical information in individual cases.

Fundamental Studies and Establishment of the Double Antibody Radioimmunoassay Method for Human Ferritin

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The double antibody radioimmunoassay method for human liver ferritin was studied to establish a microassay. We purified serum protein-free ferritin from human livers, and made anti-

human liver ferritin rabbit serum by immunizing with the purified ferritin. For iodination of the ferritin, we used a modified method of Hunter and Greenwood, and ¹²⁵I-labeled ferritin was