

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 <sup>125</sup>I 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 <sup>125</sup>-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 <sup>125</sup>I-labeled ferritin was

separated through a sephadex-G75 column and repurified through G-200 column before assay. Specific radioactivity of the labeled ferritin ranged from 0.3 to 0.4 mCi/mg. We adopted the double antibody system as the assay procedure. All dilutions were made with 1/15M phosphate buffered saline, pH 7.5, containing 1% bovine serum albumin (1% BSA-PBS). One hundred  $\mu$ l of serum sample or standard ferritin was added to 100  $\mu$ l of anti-human liver ferritin (1:8000) and 500  $\mu$ l of buffer. Then,  $^{125}$ I-labeled ferritin (10,000 cpm = 20 ng) was added. The solutions were mixed and incubated at 4°C for 48 hours. One hundred  $\mu$ l of the normal rabbit serum (1:50) and 100  $\mu$ l of the second antibody (1:10) were added to the solutions, followed by incubation at 4°C for 24 hours.

The above assay conditions appeared the best. Then, the total and precipitated counts were measured by a Packard auto- $\gamma$ -counter. The standard ferritin was diluted in both the 1% BSA-PBS and ferritin-free human serum. The ferritin-free serum was produced by sepharose 4B affinity chromatography, using the anti-ferritin rabbit serum. The standard curves in these diluents were almost the same. The ferritin-free human serum protein did not interfere with the assay system. Using this, we measured the ferritin concentration in normal human serum.

The minimal ferritin amount detectable by this assay method was 31 ng/ml. The mean concentration of ferritin in normal human serum was 135 ng/ml for male (36 samples), and 80 ng/ml for female (30 samples).

### **$\alpha$ -Fetoprotein Measurements in Brain Tumors**

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The result of the measurement of  $\alpha$ -fetoprotein (AFP) by radioimmunoassay (RIA) and hemagglutination on 34 brain tumors was reported. Preliminary experiments with the  $\alpha$ -feto-RIA kit testing dilution curve, recovery test and reproducibility were satisfactory. The data obtained by the hemagglutination method on 15 cases of brain tumor were all negative (below 200 ng/ml).

Brain tumors were divided into two groups, a glioma group (16 cases), and a non-glioma group (18). The former contained glioblastoma multiforme (5 cases), astrocytoma (5), oligodendroglioma (1), medulloblastoma (1) and pinealoma (4); the latter, meningioma (9), pituitary adenoma (4), acoustic neurinoma (1), craniopharyngioma (1), hemangioblastoma (1) and metastatic tumor (2). Although the AFP values above 20 ng/ml were considered as positive according to the reports made by others, the AFP values did not exceed 20 ng/ml in our cases.

In glioma group, 2 out of 5 glioblastomas and one out of 5 astrocytomas showed the AFP values above 9 ng/ml, while those of 19 normal adults

represented the mean plus 2 sigma measured.

In non-glioma group, one out of 9 meningiomas and 3 out of 4 pituitary adenomas showed the AFP values above 9 ng/ml.

#### **(Results)**

The mean AFP values were  $4.6 \pm 4.82$  ng/ml in glioma group,  $5.1 \pm 4.37$  ng/ml in non-glioma group. Neither of these values was significantly different from the mean AFP value in normal adults  $3.3 \pm 2.68$  ng/ml.

#### **(Conclusion)**

Recently there is a paper, suggesting a possibility of glioma and embryonal carcinoma producing AFP, because of high AFP values were frequently measured not only on glioma or embryonal carcinoma but also on the tissue or content of the cyst of such glioblastomas. Our data, however, showed no positive AFP values even with a much higher sensitive RIA method. Our study indicates that the clinical usefulness and significance in measuring of serum AFP in brain tumor are disappointing.