

iron and neutralization with sodium bicarbonate. Unbound iron ion in iron serum solution mixture was removed with a resin strip made of Amberlite IRA 400 rotating for 90 minutes, or with IRP-67 resin powder in 5 minutes. Binding reaction of iron free transferrin was instant and perfect.

TIBC values obtained by the present method was well correlated with those obtained by immunodiffusion method, and with those obtained by adding serum iron by colorimetry and unsaturated iron-binding capacity of the serum (UIBC) by radiometry. Although high reproducibility was observed in determining TIBC, it was not always good when UIBC was high as in iron deficiency anemia serum. Under-estimation of UIBC was observed when UIBC was high and iron content of iron solution was relatively low. The binding of iron to UIBC was accelerated by increasing iron content, or by adding sodium bicarbonate to iron solution, and the alkalinized iron solution was stable for 8 weeks of the test period. It seems that a hindrance substance exists in the sera of iron deficiency anemia. However it

might have been removed with serum iron by citric acid and CG-400 resin in the process of TIBC determination, since such hindrance of binding was not observed in determining TIBC. The tendency of under-estimation of UIBC using frozen serum was also disappeared by the addition of sodium bicarbonate to iron solution. On the other hand, over-estimation of UIBC was observed when test serum contained iron chelating agent, and when iron was adsorbed to the poorly coated glass vial. An accurate determination of TIBC disclosed the problems of UIBC determination and subsequently the method was improved.

High TIBC values were obtained in iron deficiency anemias and even in normal young female subjects. In 30 young normal female from 19 to 22 years old, TIBC was  $351 \pm 46$ , and UIBC was  $272 \pm 49$   $\mu\text{g}/100\text{ml}$  suggesting the trend of latent iron deficiency. Low TIBC was obtained in hemochromatosis in contrast.

TIBC and UIBC Kits were produced according to the above results.

### Radioassay of Vitamin B<sub>12</sub>

#### —Preparation of Solid Phase Intrinsic Factor and its Properties—

H. NATORI, M. FUKUDA, M. INABA and I. URUSHIZAKI

*Department of Medicine, Cancer Research Institute, Sapporo Medical College, Sapporo*

Determination of vitamin B<sub>12</sub> concentration in biological fluids have significant meaning in clinical diagnosis of hematological diseases, including pernicious anemia, blind loop syndrome, gastrectomized cases, and various myeloproliferative disorders. Optical method, microbiological assay, and radioassay were currently being used for determination of vitamin B<sub>12</sub>. Especially, the latter method was commonly used for microde-

termination of vitamin B<sub>12</sub> in biological fluids, because of its excellent sensitivity, accuracy, and specificity. The principle of radioassay of vitamin B<sub>12</sub> reported is competitive protein binding between radioactive Co labelled cyanocobalamin and sample B<sub>12</sub>, to intrinsic factor (IF) which is used as a specific binder for vitamin B<sub>12</sub>. Albumin coated charcoal method by Lau had been used as a standard procedure, however, inaccuracy was

caused often by inactivation or denaturation, and adhesion of IF to the glassware used during the assay procedure. First, we attempted to couple the IF to the insoluble particle by the covalent bond. IF monomer fraction obtained from Hog IF concentrate by gel filtration on Sephadex G-150, was coupled with Sepharose 6B or Sephadex G-25 by BrCN according to the method of Porath. This solid phase IF was stable and was found to have a good vitamin B<sub>12</sub> binding ability. Radioassay system was then tested. Steric hindrance and inhibition of conformational change of IF molecule on the binding between Sephadex G-25-IF and vitamin B<sub>12</sub> were suspected from the data of the time course studies of the system. On the other hand, Sepharose 6B-IF reacted more faster indicating lesser degree of these hindrances. Molecular sieve effect was not observed in both system tested. Separation between bound and

free vitamin B<sub>12</sub> by centrifugation was easier in the case of Sephadex G-25-IF. Both Sepharose 6B-IF and Sephadex G-25-IF were stable, judged from their vitamin B<sub>12</sub> binding capacity, at 4°C for more than one year. Procedures completed within 4 hours. Sensitivity was high, and vitamin B<sub>12</sub> concentration of 10 pg/ml order could be determined. Standard deviations of reproducibility experiments were less than 10% in the concentration range of 25–1600 pg/ml. Good correlation was obtained between the present method and charcoal method of Lau. Linear correlation could be obtained between vitamin B<sub>12</sub> concentration and radioactivity by coordination transformation according to hyperbolic equation. Therefore, vitamin B<sub>12</sub> concentration was calculated directly from the radioactivity data by the use of microcomputer of Programma 101 (Olivetti).

### Radioimmunoassay for $\alpha$ -fetoprotein in Human and Rat

M. ISHII and S. BABA

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

The development of radioimmunoassay for  $\alpha$ -fetoprotein (AFP) in human or rat by the method of two antibody or solid-phase was performed based on the fundamental study of specificity, sensitivity and reproducibility for the detection of AFP.

**Materials and Methods:** Preparation of Purified AFP: Highly purified human AFP was isolated from ascites of a patient with hepatoma by a technique of immune absorption against adulterant proteins using anti-human serum protein rabbit antiserum. Purified rat AFP was isolated from extract of rat fetal tissues by the same meth-

od as the isolation of human AFP.

**Preparation of AFP Antiserum:** Antisera to human or rat AFP were prepared by immunization of rabbits with their purified AFP in Freund's adjuvant at interval of 2 weeks for 2 months.

**Preparation of Radioiodinated Human or Rat Purified AFP:** Twenty  $\mu$ g of human or rat purified AFP was radioiodinated with 1 mCi of <sup>125</sup>I-Na by the method of Hunter and Greenwood, and <sup>125</sup>I-AFP was separated from residual unreacted <sup>125</sup>I-Na on Sephadex G-75 column chromatography.

**Radioimmunoassay:** Two antibody system was