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 α-fetoprotein in Human and Rat

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The development of radioimmunoassay for α-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 method 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 µg 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 unreached <sup>125</sup>I Na on Sephadex G-75 column chromatography.

Radioimmunoassay: Two antibody system was
based on a modification of the coprecipitation-inhibition technique described by Morgan et al. Otherwise, solid-phase system was based on the ability of antibody-coated polymers to bind tracer antigen.

Results: Radioactivity of $^{125}$I-AFP; $^{125}$I-AFP of specific activity of 10 to 20 mCi was obtained, and radioimmunoelectrophoretic pattern of human or rat $^{125}$I-AFP against their AFP-antiserum showed a single radioprecipitin line.

Radioimmunoassay: Two Antibody System; From the standpoints of sensitivity and reproducibility for the detection of AFP, the most reasonable conditions for assay were investigated and determined as follows. The reaction mixture of 0.1 ml of sample, 0.1 ml of a 1:4,000 dilution of AFP-antiserum and 0.5 ml of diluent (phosphate buffered saline pH 8.6 containing 1% BSA) was incubated in each tube at 4°C for 12 hr, after which 0.1 ml of $^{125}$I-AFP (10,000 cpm, 1–2 ng of AFP) was added and then incubated for another 36 hr. At the end of this period, 0.1 ml of a 1:100 dilution of normal rabbit serum and 0.1 ml of a 1:10 dilution of anti-rabbit $\gamma$-globulin goat serum were added and further incubated for 24 hr. After this incubation, each tube was centrifuged at 3,000 rpm for 30 min and the $^{125}$I-AFP content of the precipitate was counted in a scintillation counter. The sensitivity for the detection of human or rat AFP allowed 8 ng of AFP/ml of serum and a standard curve at the range of 8 to 256 ng of AFP/ml was useful for the quantity of AFP. The maximum deviation of determined AFP value in test sera was approximately 10%.

Solid-Phase System: From the same points as two antibody system, the fair satisfactory conditions for assay were selected as follow. Polystyrene tube was coated with 1 ml of 1:4,000 (human) or 1:1,000 (rat) dilution of AFP-antiserum in 0.1 M bicarbonate buffer, pH 9.6 at 4°C for 2 hr. To each antibody-coated tube 1 ml of a 1:10 dilution of sample was added and incubated at room temp. for 12 hr, and then 0.2 ml of $^{125}$I-AFP (50,000 cpm, 5–10 ng of AFP) was added at 12 hr after an initial period of incubation. Furthermore, the incubation was followed for 36 hr. After aspiration of the solution, each tube was washed 5 times with 2 ml of 0.15 M NaCl solution and radioactivity of $^{125}$I-AFP was counted. A standard curve at range of 1 to 16 ng of AFP/ml was obtained. The maximum deviation of determined AFP was 30%. A dose response curve obtained by addition of the AFP-free serum prepared by affinity chromatography using AFP-antiserum to each AFP standards was closely coincident in two antibody and solid-phase system a standard curve given with only the AFP standards. A dose response curve obtained by serial dilution of AFP positive serum was parallel to a standard curve in both system. No cross reactions were demonstrated between human and rat AFP by the radioimmunoassay for AFP.