distribution of $B_{12}$ values in same serum between the three method.

The best result was obtained from the method C.

There were a significant corelation between the vitamin $B_{12}$ values evalueted from microbiological assay using L. leichmannii and this phadebas radioassay.

From these results, it is concluded that this assay method of serum vitamin $B_{12}$ is very useful and simple method, and the evaluated $B_{12}$ values are quite accurate and can be used clinically as the same meaning of the value obtained from microbiological assay.

## Competitive Radioassay of Serum Vitamin $B_{12}$ Significance of Serum Vitamin $B_{12}$ Estimation in Liver Disease

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Serum vitamin $B_{12}$ levels by radioassay were demonstrated to increase, compared with those in normal subjects and patients with gallstone or chronic pancreatitis, in patients with liver disorders, especially acute hepatitis and liver cancer. In patients with acute hepatitis, a highly significant correlation between serum vitamin $B_{12}$ and transaminase was observed, but no significant relationship was found between serum vitamin $B_{12}$ and either serum bilirubin or iron concentration etc. In hepatoma as well as metastatic liver tumor originated from pancreas, serum vitamin $B_{12}$ was shown to marked increase. From these results, clinical usefulness of serum vitamin $B_{12}$ determination by radioassay kit was confirmed in the diagnosis of primary and metastatic liver tumor from pancreas as well as in diagnosis of course of acute hepatitis.

## Fundamental Studies on Radioimmunoassay for Digitoxin and Digoxin using $^{125}I$ Labeled Antigen

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We have already presented our study on digitoxin and digoxin radioimmunoassay using $^{3}H$ labeled antigen and are selling digoxin radioimmunoassay kit.

Now we performed fundamental studies on digitoxin and digoxin radioimmunoassay using $^{125}I$ labeled antigen.

**Materials and Methods**

$^{125}$I-labeled antigens were prepared by the modified method of Greenwood et al using chloramin T. Digoxin-BSA conjugate in complete Freund's adjuvant were used to induce antiserum in rabbits, which is also used in $^{3}$H-digoxin RIA kit.

Our assay procedure was as follows: Add 0.1ml of standard or sample, 0.1 ml each of diluted antiserum and carrier serum and 0.5 ml of phosphate buffer into test tube, and mix well.

Then, add 0.5ml of dextran coated charcoal and mix well. After centrifugation of the mixture, remove the supernatant and count the radioactivity of precipitate in test tube by well type scintillation counter.

The present study is for evaluating mainly immunoreaction rate, amount of dextran coated charcoal and recovery rate of digitoxin and digoxin.

**Results**-digitoxin-

$^{125}$I labeled digitoxin and digoxin antibody were equilibrated by 10 minutes of immunoreaction. The dextran coated charcoal having the charcoal concentration of 0.25—0.5%, and incubation time for 30 minutes after addition of it were adequate.

By adding labeled antigen at the final stage of procedure the best dose-response curve could be obtained. Moreover, the recovery rate was fairly good in that case. Five ng/ml of digitoxin could be detected.

-digoxin-

$^{125}$I labeled digoxin and digoxin antibody were equilibrated by 5 minutes of immunoreaction.

The other results except for the sensitivity observed almost similar to those of digitoxin radioimmunoassay.

**Discussion**

Sensitivity and precision in these assay systems were good.

Those methods using $^{125}$I labeled antigens have an advantage to be able to omit the troublesome procedure of counting the sample required in the case of radioimmunoassay using $^{3}$H labeled antigens.

We consider that these methods could be adapted for digitoxin and digoxin radioimmunoassay in plasma.

Compounds for labeled antigens synthesized by Dr. S. Miyano and Dr. N. Abe (Faculty of Pharmaceutical Sciences, Fukuoka University) were utilized in our experiments.

## Radioimmunoassay for B-Methyldigoxin ——Use of Digoxin RIA Kit——

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Recently, attention has been called for the clinical use of β-methyldigoxin because of its better absorbability from the intestines as compared with digoxine. With the purpose of evaluating the clinical use of β-methyldigoxine, Validation was mede for the use of commercially available digoxin RIA kit to measure the serum concentration of β-methyldigoxine.

Digoxine RIA kit which was developed by