## Quantitative determination of α-fetoprotein by radioimmunoassay in hepatic and other diseases

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We did radioimmunoassay of α-fetoprotein by salting-out method, in which sodium sulfate was used to precipitate the bound moiety. Following a method reported by Nishi et al., purification of α-fetoprotein was performed by DEAE and CM cellulose chromatography on serum of a patient with hepatoma.

In antigen solution obtained by this method, there was a trace amount of a normal serum constituent on immunoelectrophoresis analysis. Concentration of α-fetoprotein in the solution was measured immunologically utilizing single radial immunodiffusion method, and used it as antigen in radioimmunoassay.

Antiserum was prepared by immunizing rabbits with ascites from a patient with hepatoma, and after absorbing it with pooled normal sera, anti-α-fetoprotein antiserum was obtained which showed monospecificity for α-fetoprotein on immunoelectrophoresis.

Labelling of the antigen with $^{125}I$ was done by a method of Greenwood et al. Separation of antibody-bound from free labelled antigen was performed by salting-out method, as mentioned above.

Sensitivity of the measure was about 20 mμg/ml, and α-fetoprotein concentration of normal sera was below the sensitivity. Therefore, it was considered that the antiserum was specific for α-fetoprotein in a level of radioimmunoassay as well.

In hepatoma, there were many cases in which serum α-fetoprotein levels were so high that these could be detected even by Ouchterlong method. In some cases, α-fetoprotein was demonstrated only by the radioimmunoassay. In the remaining cases, its levels were below the sensitivity.

In each one case of cholangioma, metastatic liver carcinoma, stomach cancer and pancreas carcinoma, α-fetoprotein was not detected.

In liver cirrhosis, it was detected in 7 of 13 cases, showing 270 mμg/ml as a maximal level. In hepatitis, it was detected in 3 of 27 patients, and a maximal level was 175 mμg/ml. It was also detected in two pregnant women in the third trimester. In the healthy or non-hepatopathic patients, it was detected in one of 27 cases, whose disease was unknown.

## Measurement of serum digoxin concentration by radioimmunoassay

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The digoxin radioimmunoassay kit has been recently developed by Daiichi Radioisitope Laboratories. The assay procedure consisted of the following steps: (a) Incubation of the mixture of standard sample or serum, anti-digoxin serum and $^{3}H$-digoxin. (b) Separation of bound from free $^{3}H$-digoxin by dextran coated charcoal.

The equilibration between $^{3}H$-digoxin and an-

tibody was almost complete in 15 minutes.

A series of standards was run with each batch of unknowns. The standard curve was shown to vary vert little from batch to batch. It was possible to assay samples with one or two standards to check the standard curve and the method was applicable in situations where an early result was required.

The results from serial dilution of patient's serum with stock blood bank plasma were compared with the standard curve. The curves were almost parallel, suggesting that the substance in patient's serum being measured is immunologically indistinguishable from digoxin.

The standard deviation of replicate determinations within one assay was 1.75%. When repeated determinations were made on different days on aliquots of serum containing known amount of digoxin, the standard deviation was 6.28%.

There was a positive correlation between total daily dose and the serum digoxin concentration. Moreover, serum digoxin levels in patients judged clinically to be suffering from digoxin toxicity were higher than those in patients thought to be adequately digitalized. These differences were significant statistically.

Thus, the determination of serum digoxin may provide useful information to the clinician faced with the difficult problem of evaluating his patient's state of digitalization.

## The measurement of cyclic AMP by radioimmunoassay kit and competitive protein binding assay

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The measurement of cyclic AMP has been widely employed in many laboratories. We have compared two methods, radioimmuno-assay and competitive protein binding assay for measurement of cAMP in many tissues and solutions. Radioimmunoassay kit (Collaborative Research Inc.) contains anti-cAMP antiserum (rabbit), $^{125}$I-SCAMP-TME solution and anti-rabbit IgG antiserum (goat). cAMP radioimmunoassay, similarly with other peptide hormone radioimmunoassay, based upon competition of the cAMP with an isotopically labeled derivatives of the cAMP for binding sites on antibody for the cAMP. Free and antibody bound $^{125}$I-SCAMP-TME were separated by precipitation of antibody bound fraction with a second antibody. The displacement of $^{125}$I-SCAMP-TME by unlabeled cAMP when plotted as a semilogarithmic function was linear over a concentration range of 2–50 picomoles. The reproducibility of standard curves by several kits showed good results.

As to the competitive protein binding assay, cAMP binding protein was separated from rat liver (Kumon et al. 1970) and $^{3}$H-cAMP was purchased. This method, also, based upon the competition of the cAMP with an isotopically labeled cAMP for binding sites on binding protein specific for cAMP. Free and protein bound $^{3}$H-cAMP were separated by absorption with dextran coated charcoal. The displacement of $^{3}$H-cAMP by unlabeled cAMP when plotted as a semilogarithmic function was linear over a concentration range of 1–20 picomoles. The reproducibility of standard curves also showed good results. We compared values of same samples measured by radioimmunoassay and competitive protein binding assay, and those by competitive protein binding assay were slightly higher than phenomenon is under investigation. These methods were useful in measurement of cAMP in many tissues and solutions.