the alpha-2 globulins whereas in the plasma of exposed workers and immunized guinea pigs, an additional peak between alpha-2 globulin and gamma globulin was evident. This additional peak was competitively inhibited by saturating amounts of unlabeled Alcalase. Rabbit antihuman gamma globulin serum or antiguinea pig gamma globulin serum precipitated significant amounts of radioactivity in those plasmas compared to control subjects confirming a gamma globulin is the binding protein to Alcalase. Thus, we have shown that exposed workers have a circulating gamma globulin capable of binding a component of labeled Alcalase.

Radioimmunoassay of Plasma Digoxin

H. KUBOTA, S. KAIHARA, M. IIO and H. MURAO

The Second Department of Internal Medicine, University of Tokyo, Tokyo

H. KUROSAKI

Daiichi Radioisotope Labs., Ltd., Tokyo

A rapid, sensitive method for measuring the plasma digoxin concentration has been developed by the radioimmunoassay technique.

Digoxin-BSA (bovine serum albumin) conjugate was prepared. Ten rabbits were immunized by the injection of the conjugate with six injections over 60 days period.

The digoxin specific antibodies were raised successfully.

The assay was performed by incubation in small test tubes, to which were added 0.2 ml of sample or standard serum, 0.1 ml of anti serum and 0.6 ml of buffer solution. Then tubes were shaken and 0.1 ml of ^{3}H-digoxin was added and incubated at room temperature for 15 min.

Separation of bound from free labeled digoxin was achieved by the dextran coated charcoal.

Supernatant phase was added to 15 ml of liquid scincillator, which consist of toluene and Triton X-100 and counted in a liquid scintillation counter.

Correction for quenching was made by automatic external standardization.

A standard curve was constructed for the solution of known concentration and unknowns were read.

The results of assays performed upon clinical cases who were administered various doses of digoxin showed digoxin levels in the range 0.0～4.5 mμg/ml.

There is a positive correlation between maintenance dosage and plasma digoxin concentration.

The digoxin specific antibodies show a high titer for digitoxin, and it permits their use in a radioimmunoassay system for digitoxin.

We has demonstrated their applicability in the assay of digitoxin.

A standard curve for digitoxin assay was prepared. A linear curve was obtained in the range 5-80 mμg/ml.