

LATS-IgG, LATS serum, anti-thyroglobulin antibody (Anti-TG) IgG from a patient with Hashimoto's thyroiditis resulted in a dose-response inhibition of the binding of the label. This binding system is proved highly specific or anti-microsomal antibody (Anti-M), and permits a quantitative estimation of this antibody with a sensitivity of about 100 times that of immunofluorescent technique, and is able to detect the potency of antibody in only 0.05  $\mu\text{g}$  standard LATS IgG.

Substituting purified thyroglobulin for microsomes and Anti-TG IgG for LATS-IgG, a quantitative binding assay for Anti-TG with comparable sensitivity was also devised. By using these two methods, serum antibody levels were studied in patients with various diseased conditions.

Of 55 untreated Graves' patients, all but one were found to have elevated serum Anti-M, and 89% had abnormal Anti-TG values.

Eighteen of 19 chronic thyroiditis were positive in Anti-M, and 86% were also abnormal

in Anti-TG. All the untreated Graves' patients and patients with chronic thyroiditis were proved to have at least one of the two antibodies in their sera.

Primary hypothyroidism was also associated with high incidences of serum antibodies, however, all but one of simple or adenomatous goiter were negative in both antibodies. Anti-M were also measured in 330 hospitalized controls and their results were analysed respect to their clinical diagnoses. Thirty-one of them had moderately high serum Anti-M and 29 of them were found to be associated with either thyroid disorders, collagen or autoimmune diseases, widespread neoplasms, renal diseases, and endocrine-metabolic disorders. Elevated serum values were observed in 79 cases and 71% of them had above listed conditions, and were significantly different from the disease distribution in negative Anti-M group. Only 31% of negative group were associated with these diseases.

## Demonstration of Antibody to "Alcalase" in the Laundry Detergents by Radioimmunoassay Technic

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The laundry detergents containing bacterial enzymes (Alcalase) derived from cultures of *Bacillus subtilis* have been marketed since several years ago. Respiratory symptoms such as asthmatic attacks, cough and chest pain were observed among workers adding powdery enzyme into the detergent. This study was undertaken to determine whether antibodies to enzymatic material could be detected in the plasma of exposed workers. Radioimmunoassay technic has proved successful in detecting low concentrations of antibody because of its higher specificity and sensitivity than any other immune systems. Alcalase was dissolved and used for label-

ing and immunization of guinea pigs. Labeled Alcalase was purified on sephadex G 75 column and three peaks were obtained. These three components were incubated with 4 groups of plasma samples (exposed workers, unexposed control subjects, immunized guinea pigs and control guinea pigs) for adequate period. Incubation mixtures were applied on paper for electrophoresis, afterwards papers were scanned and contacted with film for radioautograph and finally stained with Amido Schwarz. Among three components only void volume component showed the difference as follows. In controls radioactivity migrated as a single peak in the region of

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 antiginea 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

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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  $^3\text{H}$ -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 scintillator, 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  $\text{m}\mu\text{g}/\text{ml}$ .

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  $\text{m}\mu\text{g}/\text{ml}$ .