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## Studies on the Complement-Fixing Activity of Rabbit $\gamma$ M Antibody against Human Serum Albumin (HSA)

Tohru OHARA, Takuro KIMURA & Masahide SHIMIZU.

- 1) Rabbit antibody against HSA did not show any complement-fixing activities in the presence of specific antigen. The situation was the same regardless of the modification of experimental conditions such as concentration of  $\gamma$ M antibody, conversion of the antigenic configuration into aggregated state or of the change of reaction temperature to 37°C instead of conventional 4°C.
- 2) In the case observed above, it does not necessarily mean that the  $\gamma$ M antibody can not combine with HSA, but the former might form invisible complexes with the latter. This was proved by the hemagglutination inhibition test, because the original hemagglutinating titers of the  $\gamma$ M antibodies were obviously reduced by the addition of soluble HSA.
- 3) When the reaction mixtures of the  $\gamma$ M antibody and its corresponding antigen, HSA labelled with I<sup>131</sup> were subjected to gel filtration by Sephadex G-200 column, considerable amounts of radioactivities were proved to exist at the  $\gamma$ M eluting position. This fact is also considered to show the formation of soluble complexes between  $\gamma$ M antibodies and I<sup>131</sup>-labelled antigens.
- 4) The mechanism of the lack in the reaction mixtures of  $\gamma$ M antibody and HSA of the formation of visible Ag-Ab complexes is not clear as yet, but it is considered to be possible that this fact has something to do with the non-complement-fixing activity of the  $\gamma$ M antibody against HSA.

## Serum Factors Influencing on MI Phenomenon

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The migration of alveolar macrophages of tuberculous rabbits from capillary tubes was found to be inhibited by tuberculoprotein and more intensively inhibited by adding rabbit anti-tuberculosis serum in the migration medium. The more antisera of high precipitin titer was added, the more intensively the migration inhibition occurred even without tuberculoprotein. The migration of normal alveolar macrophages was not inhibited with antiserum, however, was clearly inhibited in the presence of tuberculoprotein. The same phenomenon was also observed under the condition of the BGG-anti-BGG system. The inhibitory effect on migration by tuberculopolysaccharide was about the same as by tuberculoprotein.

Anti-tuberculosis sera were fractionated with ammonium sulfate. The addition of the tuberculoprotein and the fraction which precipitated by 60~80% ammonium sulfate indicated moderate migration inhibition. This fraction further fractionated by DEAE-Sephadex A-50 column chromatography and Sephadex G-75 gel filtration.

The fractions which demonstrated immunoelectrophoretal mobilities in albumin and  $\alpha$ -globulin showed moderate migration inhibition in the presence of specific antigen. On the contrary, when control normal sera were fractionated by the same procedure of antisera, the fractions which demonstrated albumin and  $\alpha$ -globulin on immunoelectrophoresis showed no migration inhibition in the presence of antigen.

## Simplification of the Arylsulfatase Test for the Differential Typing of Mycobacteria

with special reference to inoculum size and incubation period

Ken-ichi YAMAMOTO and Yoshio TAKAHASHI

In order to simplify the arylsulfatase test, arylsulfatase activity has been studied of 62 different strains of acid-fast bacilli. It has been shown that this test may be simplified as follows.

- 1) For inoculum, cultures of bacilli from Ogawa's egg media can be used instead of those from Dubos Tween-albumin broth.
- 2) Several milligram of bacilli can be directly inoculated with a platinum loop into broth tubes with substrate.
- 3) In this method the incubation period can be shortened. The period of 5 to 7 days may be suitable.

## Studies on the Influences of Cyclophosphamide (CPA) on Experimental Tuberculosis

Setsuro NAKAMOTO

In rabbits sensitized with killed tubercle bacilli (10 $\mu$ g of the strain H37Rv in Arlacel-Drackeol adjuvant intramuscularly), the development of the tuberculin skin reaction was almost completely or completely suppressed by daily subcutaneous injections of CPA at the doses of 10 and 15 $\mu$ g/kg, while the formation of the circulating antibodies (antipolysaccharide, antiprotein and antiphosphatide) was not deeply influenced. The decrease in body weight of the animals was proportional to the injected amounts of CPA.

In rabbits experimentally infected with live bovine-type tubercle bacilli (0.8 and 1.0 $\mu$ g of the strain Miwa intravenously), combined treatment with CPA and streptomycin was found most effective as compared to single treatment with streptomycin alone, the formation of tubercles in visceral organs having been remarkably suppressed. Even though the injected amount of streptomycin was smaller than its minimum effective dose, the combination of CPA notably increased its curative effect. On the contrary, single treatment with CPA alone considerably enhanced the process of the disease. In case of the combination of CPA, the development of the tuberculin skin reactions was delayed from 2 to 3 weeks, but not suppressed.