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Studies on the Antitubercular Compounds. XX. Treatment of Experimental Tuberculosis of Mice with o-Aminophenol and its Derivatives

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An experiment was designed to examine the therapeutic *in vivo* effect of o-aminophenol and its derivatives that showed *in vitro* a marked antituberculous activity test, in comparison with 1314 Th.

The drug effectiveness was evaluated from the survival time against fatal tuberculous infection of mice, the drug administration was conducted *per os* either by the use of fine polyethylene tube, or by the mixture with feed.

In these experiments, the daily dose ef 1mg of PAP exserted no remarkable therapeutic effect compared with that of 1314 Th. However, 1mg of o-aminophenol exerted a slight favourable effect, though it was not reproducible.

These results suggest that a demonstration of significant effect of drug might depend upon many factors, especially, drug administration method and size of inoculum.

Studies on the Antitubercular Compounds. XXI. Synthesis and Antitubercular Activity of 2, 1, 3-Benzothiadiazole Derivatives

Isao SEKIKAWA

Thirty-seven kinds of 2, 1, 3-benzothiazole derivatives were prepared and thier antibacterial action against H37Rv strain of tubercle bacilli was tested in Kirchner medium containing 10 % bovine serum.

The most active compound was 2, 1, 3-benzothiadiazole-4-aldehyde isonicotinic acid hydrazone XXI and stronger than isonicotinic acid hydrazide.

Studies on Antibody Formation I. Antituberculoprotein Antibodies in Tissues of Tuberculous Animals as Detected by the Immunofluorescence Technique

Harue OKUYAMA, Eiji HAMADA and Kazuo MORIKAWA

Rabbits once subcutaneously inoculated with heat-killed tubercle bacilli received afterwards repeated intravenous injections of the same killed bacilli, and were sacrificed at given intervals. The visceral organs were removed and embedded in paraffin for section. The sections were stained by the sandwitch method for detection

of fluorescent antibodies, using as antigen a tuberculin-protein fraction prepared in our laboratory.

In the spleen, specific fluorescent cells were found to accumulate in the red pulp and in the surroundings of the white pulp, forming masses or scattering layers. Morphologically these cells were identified as plasma cells, which bore frequently Russel bodies. In the lymph nodes, as in the spleen, fluorescent cells were found scattered in the medullary cord. In the lungs, simillar cells containing fluorescent antibodies were observed in the surroundings of the tubercles. The same kind of cells were also detectable in the spleen and lymph nodes from tuberculous guinea-pigs.

In tuberculous rabbits the fluorescein-labelled tuberculoprotein injected into the skin disappeared more rapidly from the injection site than in normal rabbits, and 5 days later the fluorescent antibody against the tuberculoprotein became detectable arround the bundles of the subcutaneous collagen fibers.

However, some of the experimental data suggested that the fluorescent antibody thus detected in the tuberculous animals was not the antibody against the protein but against the minute polysaccharide contained in the tuberculoprotein samples.

Study on the Erythrocyte-Sensitizing Ability of the Phosphatide Fractions of the Tubercle Bacillus

I. Method for Preparing the Sensitizing Antigen and its Stability

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It has been shown that the phosphatide fractions of tubercle bacilli possess the erythrocyte-sensitizing ability and give passive hemagglutination reactions in the presence of tuberclous serum.

The present studies concerned with some agglutinating agents and methods for preparing the sensitizing antigens.

The following results were obtained.

1) The passive hemagglutination reaction was found to be the most suitable reaction system for the estimation of the activity of the phosphatide antigens, while Takahashi's kaolin agglutination reaction is advantageous for the estimation of the antibody titers.

2) As for the method for preparing the sensitizing antigens, the following method proved to be the most suitable: mixing the antigen previously swollen by water with equal volume of tetrahydrofuran, and diluting the resulting solution with phosphate buffered saline.

3) In case of one phosphatide fraction, using the antigens thus obtained, the minimum dose required to sensitize one ml of 2 % sheep erythrocytes suspension was found to be 2 μg. The serum antibody titers were constant over the range of various concentrations of the sensitizing antigen. The end point of the reaction was easy to be read.

4) Experimental date were obtained which support the hypothesis that the antigen in the phosphatide fraction is only one, and it is different from the Middlebrook-Dubos antigen.