INTRODUCTION

The clinical feature of bronchopulmonary aspergillosis varies depending upon the site and mode of the Aspergillus infection in the respiratory tracts. The most distinguishable type of bronchopulmonary aspergillosis is aspergilloma of fungus ball which shows the typical radiographic appearance of a round mass with air-containing halo. The definite diagnosis of aspergillosis is made by means of the mycological procedure. However, the detection of this fungus from the specimens was in some cases difficult. Therefore, it was necessary to establish the immunological diagnostic procedure by skin testing or serological technique.

Recently many investigators (10, 11, 12, 13, 17, 18, 21) have suggested that immunological techniques were available for the clinical diagnosis of aspergillosis. As an antigen of skin and precipitation tests, Longbottom et al. (17) reported the isolation of "C substance" from the mycelia of Aspergillus fumigatus. Biguét et al. (10) described that "C substance" was present in other Aspergillus species and also suggested the presence of another antigen which was designated "X substance" in Aspergillus species. However, the detail of chemical properties and immunological activities of these antigens were not investigated.

In previous papers (4—6) the authors have reported the isolation and purification of protein and polysaccharide fractions from Aspergillus fumigatus, and the chemical nature of these fractions.
was investigated in detail. It was shown that protein and polysaccharide, which were designated as AAP and APS-66, respectively, were useful for the antigens of skin and precipitation tests in rabbits or guinea pigs which were immunized with *A. fumigatus* and in patients with aspergillosis.

In the present experiments, the authors will describe the immunological properties of protein and polysaccharides obtained from *A. fumigatus* in immunized guinea pigs or rabbits and in patients with aspergillosis.

**Materials and Methods**

**Organisms** *Aspergillus fumigatus* (IFO No. 5840) was supplied by Research Institute for Microbial Diseases, Osaka University. *A. fumigatus* Itono strain was isolated from the patient with pulmonary aspergillosis. *Aspergillus nidulans* (IFO No. 5719), *Aspergillus niger* (IFO No. 4414), *Aspergillus terreus* (IFO No. 6123) and *Aspergillus flavus* (IFO No. 4053) were supplied by the Fermentation Institute of Takeda Pharmaceutical Co. Ltd., Osaka. The fungi were cultured in Czapek Dox synthetic medium at 30 °C for 71 hours and were lyophilized.

**Protein Fraction** Protein (AAP) fraction used for skin reaction antigen was extracted from the mycelia and spore of *A. fumigatus* (No. 5840), *A. niger*, *Penicillium* DC 11, *Streptomyces griseus*, *Cryptococcus neoformans*, acetone-dried *Mycobacterium tuberculosis* (Aoyama B strain), *Mycobacterium smegmatis*, *Mycobacterium fortuitum*, a typical mycobacteria P1 strain and *Nocardia asteroides*. The detail of the extraction and purification procedures was described in a previous paper (5).

**Polysaccharides** Two kinds of polysaccharides, glucan and galactomannan, were extracted and purified from the mycelia and spores of *A. fumigatus* (No. 5840) by the method described previously (5).

**Proteolytic Enzymes** All proteinase, trypsin (EC 3.4.4.4.), papain (EC 3.4.4.1.) and "pronase" were commercial products. "Pronase" is a peptidohydrolase obtained from *Streptomyces griseus* by Kaken Co. Ltd., Tokyo.

**Analytical Methods** The protein was determined by the method of Lowry (19). The determination of amino residue was carried out by ninhydrin method at 570 μ (15).

**Treatment of Protein (AAP) Fraction with Proteolytic Enzymes** Twelve mg of the AAP fractions were dissolved in 36 ml of 0.1 M phosphate buffer (pH 7.0), and 3 ml of pronase or trypsin solution (60 μg of enzyme/ml of buffer solution) were added to 27 ml of antigen solution. When papain was used as an enzyme, 3 ml of enzyme solution (30 μg of papain/ml of buffer solution) containing 0.01 M mercaptoethanol and 0.002 M ethylenediaminetetraacetic acid (EDTA) were added to the 27 ml of antigen