A Method for the Demonstration of Tissue Antibody.* (18522)

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A new technic is presented for the demonstration of tissue agglutinins before their appearance in circulating body fluids. Although the capacity of tissues to hold homologous antibody was shown by Kahn(1), no satisfactory method has heretofore been presented for the demonstration of tissue antibody. The present method was devised during a series of investigations concerning formation of antibodies *in situ*.

Preparation of tissues and organs. Small pieces of subcutaneous tissue from immunized mice were removed with forceps and ocular scissors, spread rapidly and thinly on clean glass slides and air-dried. The tissue was ringed with vaseline, homologous antigen added and the preparation sealed with a sterile coverslip. This direct spreading or smearing method for subcutaneous connective tissue was designed previously as a method for histological investigations. Organs to be examined were cut with a sharp scalpel or razor and the cut surface touched directly to a glass slide. The imprints thus formed were treated in the same manner as the subcutaneous spreads. All smears were made in duplicate in order to correlate immunological and histological studies. The slides for histological study were not sealed with vaseline but after air-drying were stained with May-Grünwald-Giemsa stains. Preparations from control groups of non-immune animals were run simultaneously with animals in various stages of immunization.

Preparation of antigens. Bacteria grown on a 0.5% dextrose nutrient agar base for 24 to 36 hours, were washed off with 0.5% HCHO in physiological saline and incubated overnight to insure sterility. When the bacterial suspensions were found to be sterile the organisms were washed in 0.025% HCHO dissolved in 0.85% NaCl and then centrifuged. This procedure was repeated 3 to 5 times. After washing, the organisms were diluted to a concentration of approximately 4 billion per milliliter. This was most conveniently obtained by allowing the original mixture to stand for 3 to 4 days at 4°C, after which time most clumps of organisms had settled out under gravitational influence and only the freely suspended and dispersed organisms remained. The suspension was examined microscopically to insure absence of bacterial aggregates.

Cellular antigens such as the sheep erythrocytes were washed and prepared in a 2% suspension in 0.85% NaCl and checked microscopically before use, for the presence of lysed stroma. The presence of lysed stroma in excessive numbers favors rouleaux formation and suspensions containing them are unreliable.

Agglutination technic. Two or 3 drops of antigen suspension plus equal volumes of physiological saline were added to the air-dried loose connective tissue and imprints. These preparations were incubated at 37°C for 1 to 2 hours and then examined microscopically. Final readings were made after remaining at 4°C overnight. When agglutinating antibody was present, well defined bacterial aggregates were seen adhering to the cells (Fig. 2, 4, 6). No such phenomenon was observed in similarly treated preparations taken from non-immunized control animals (Fig. 1, 3, 5). Positive results may be induced by microscopic fat globules. Such aggregates of bacteria differ from true agglutinations in that they are smaller, do not adhere tightly and uniformly and are found only in contact with the globular fat particles.

Discussion. The technic above can be used for the qualitative demonstration of agglutinating antibodies against *Salmonella ty-*

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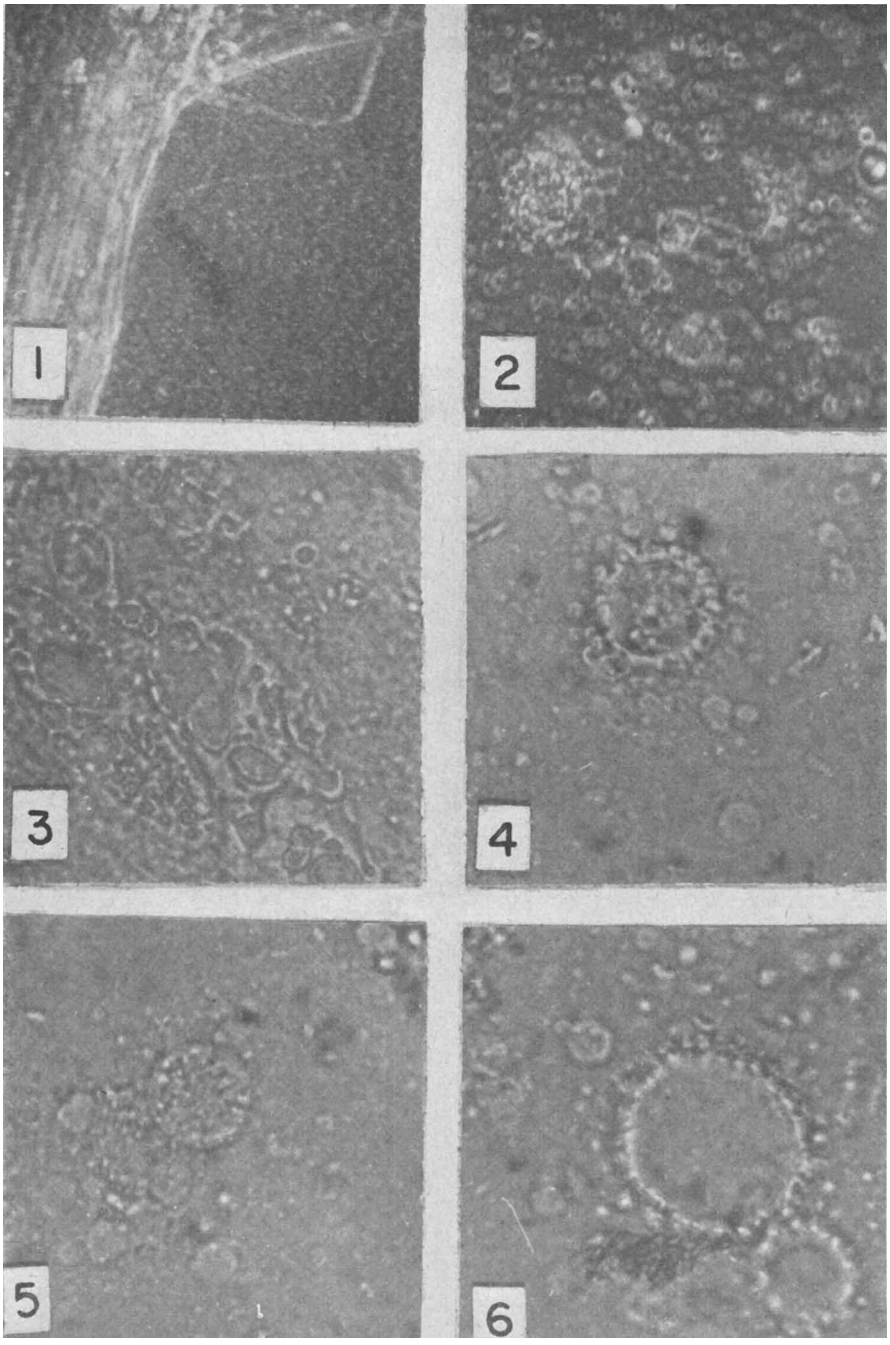

FIG. 1. Spread of loose subcutaneous tissue from non-immunized mouse. *Salmonella typhimurium* antigen added. Collagenous fibers and fibroblasts prominent with nuclei of latter visible among the fibers. Bacteria uniformly dispersed around tissue. No agglutination. Phase photomicrograph. Bright M contrast phase B, ×1000. Panatomic X film without filters.

FIG. 2. Spread of loose subcutaneous tissue from mouse 6 days after injection of approximately 9,000,000 *Sal. typhimurium organisms*, homologous antigen added. In the central part of the picture bacteria are agglutinated tightly to surface of lymphocytes while

intracellular areas show freely dispersed antigen particles. Phase photomicrograph conditions as given in Fig. 1.

FIG. 3. Spread of loose connective tissue from non-immunized mouse plus *Sal. typhimurium antigen*. Note uniform dispersion of antigen and prominent fibroblast cytoplasm and nuclei. No agglutination. Light microscope, ×2000.

FIG. 4. Spread loose subcutaneous tissue from mouse immunized 6 days previously with *Sal. typhimurium* antigen plus homologous antigen. Note "cartwheel" agglutinated bacteria around lymphocytes which is lacking in adjacent polymorphonuclear leucocytes. Light photomicrograph same as Fig. 3.

FIG. 5. Spread of loose subcutaneous tissue from non-immunized mouse plus *Sal. typhosa* antigen. Observe sharply defined cell outlines exhibiting none of the polar "cartwheel" attraction noted in Fig. 4. Light photomicrograph same as Fig. 3.

FIG. 6. Spread loose connective tissue of mouse immunized 6 days previously with *Salmonella typhosa* with homologous antigen added. Note strong polar attraction for bacteria by large lymphocyte in center of field. This effect is not shown by smaller more mature lymphocyte seen directly above. Light photomicrograph same as Fig. 3.

phosa, S. typhimurium, Pseudomonas aeruginosa and sheep erythrocytes. Agglutinating antibody to these antigens was detected in the loose connective tissue at the site of injection before it appeared in the blood. No antibody was found in the livers or spleens of immunized mice and rabbits before it began to circulate.

Although the technic described here is not adapted to quantitative measurement of antibody, it has value in detecting the presence of antibody in tissues and cells. It can, therefore, be used in investigations designed to secure information concerning various problems of immunophysiology.

The findings reported here support the previous observations that antibodies can be formed locally and also that the lymphocytes contain specific homologous antibodies(2,3). It is possible that this method for detecting cellular antibody could be adapted to practical problems concerned with the diagnosis of certain diseases, *e.g.* brucellosis in which circulating antibody may be extremely low.

Summary. A direct slide agglutination technic is described for determining the presence of agglutinating antibody in air-dried films of tissues and cells. This method has proved to be of value in correlating the appearance of antibody with cytological changes occurring at the site of antibody production.

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Maintenance of Denervated Taste Organs in Adult *Triturus v. viridescens.* (18523)

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Contrary to the widespread belief(1-6) that taste organs are dependent upon the trophic action of the gustatory nerve for development and maintenance, it has been demonstrated that amphibian taste organs can develop independently(7,8) and denervated organs can be maintained for at least 6 months (9). The present experiments not only

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