4360

The "Stained Slide" Microscopic Agglutination Test: Application to (1) Rapid Typing of Pneumococci; (2) Determination of Antibody.

ALBERT B. SABIN. (Introduced by William H. Park.)

From the Littauer Pneumonia Research Fund of New York University and the Department of Bacteriology of the New York University Medical School.

The importance of obtaining an early diagnosis of the type of infecting pneumococcus, especially in cases of lobar pneumonia to be treated with serum, has resulted in the development of many methods. The microscopic agglutination test just described, since it required very few organisms, was expected to reduce the time for a determination of type. Attempts to type the organisms directly from the sputum were unsuccessful. The fact that in the microscopic test the sputum was exposed to the immune serum for a very short time only, was at first thought to be responsible for the negative results. Small amounts of sputum were accordingly mixed with varying amounts of serum, and incubated in sealed capillary tubes at 37° C. for different periods of time; these mixtures were subsequently smeared and stained. The unsuccessful results are due possibly to the presence of some interfering factor or unfavorable physical condition in the substance in which the pneumococci are coughed up. Most of the sputa injected into the mouse's peritoneum are digested within 2 hours, and in 3-4 hours, sufficient organisms may be found to carry out a microscopic typing test. Broth may be used as a culture medium in the absence of mice, but the mouse is preferred because its peritoneum is somewhat selective for pneumococci, and because results can be obtained in a shorter time.

The procedure for microscopic typing is as follows: One cc. of a fresh sample of sputum is injected intraperitoneally into a mouse. 3-4 hours after injection some of the peritoneal fluid is obtained by capillary puncture. A glass slide is marked off into 4 parts, and a minute drop of the peritoneal fluid is expelled onto each one of the 4 partitions. The first is smeared with saline for control, and the others with a loopful of a 1-10 dilution of type I, of type II, and of type III, diagnostic serums respectively. This dilution of serum is chosen to largely eliminate group agglutinins. The smears are made thin, so that they dry rapidly; they are then stained for one-half minute with a fuchsin solution (10 cc. saturated alcoholic solution of basic fuchsin plus 90 cc. distilled water). The stain is washed off in running water, and the smears are examined with the

oil-immersion lens. If a specific agglutination reaction is observed in one of the smears with diagnostic serum, the organism is of the corresponding type. If no reaction occurs in any of the smears, and pneumococci are clearly seen, a diagnosis of group IV is made. When it is desired to know whether the organism is one of the fixed types of Group IV, a similar procedure is carried out with the corresponding diagnostic sera. Naturally occurring clumps of organisms differ in appearance from those produced by specific agglutination; they can be recognized by their occurrence in the saline control smear as well. Unless a fresh sample of sputum is used many of the organisms will have undergone autolysis and therefore more time must be allowed for growth. Since the mouse is not killed, another typing can be done if the first one should show insufficient organisms, and after death of the mouse the type may be confirmed. In the case of type III sufficient organisms for a microscopic typing are present as early as 2 hours after injection. The appearance of the specific reaction with type III, differs from that obtained with other types of pneumococci, primarily on account of the larger size of the capsule. More than 100 cases have already been typed by this method at the Harlem Hospital with perfect agreement as compared with the results obtained by the older methods.

Microscopic Antibody Test. It has been suggested[1] that the dosage of antipneumococcus sera be controlled by an antibody test on patient's blood. Taking advantage of the fact that the microscopic agglutination test requires only minute amounts of serum, it was performed on patients who had just recovered from lobar pneumonia or whose blood had been rendered anti-bacterial by therapeutic administration. Positive tests were obtained only against the homologous type of pneumococcus. The technique for carrying out the microscopic antibody test is as follows:

A drop or more of the patient's blood is taken with a capillary. This is either centrifuged or after coagulation and synaeresis, a minute amount of serum is smeared with a loopful of a heat-killed, saline suspension of the type of pneumococcus for which it is desired to determine agglutinins. The drops are thoroughly mixed and smeared very thin, allowed to dry in air, and stained for one-half minute with the fuchsin solution recommended for the microscopic typing test. The specific agglutination reaction is obtained here, but the clumps tend to be smaller. The sensitivity of this method was compared with the rapid macroscopic method of Arlyle

[1] Park and Cooper, *Trans. Sect. on Path. and Physiol., Am. Med. Assn.*, 1927.

Noble[2] and the regular macroscopic tube agglutination, and it was found to be 2½-3 times as sensitive as either of the other methods when a standard diagnostic serum was used for the comparison. Observations which aim to establish with the aid of the microscopic antibody test a more exact guide for the administration of anti-pneumococcus sera are being collected at Harlem Hospital.

The microscopic agglutination reaction has also been tried successfully with *B. typhosus* and *meningococcus*. Probably it might be applied as well wherever the older methods of demonstrating agglutination are used, and more advantageously in many instances where, without loss of time, the older methods are either unsatisfactory or impossible.

I wish to thank Dr. William H. Park and Dr. Jesse G. M. Bullowa for their kind cooperation and numerous valuable suggestions.

4361

A Method of Studying Virus Infection and Virus Immunity in Tissue Cultures.

T. M. RIVERS, E. HAAGEN AND R. S. MUCKENFUSS.

From the Hospital of The Rockefeller Institute for Medical Research, New York.

The fact that certain viruses, particularly vaccine virus, can be cultivated *in vitro* in the presence of susceptible living tissue is now well established. For the cultivation of viruses, small pieces of corneal or testicular tissue from the rabbit, or finely minced chick embryos have usually been employed. In only a few instances[1] has the claim been made that "inclusion bodies" were observed in tissues infected and cultivated *in vitro*. These claims, however, have not been sufficiently corroborated to be accepted generally.

The inclusions referred to above are characteristic of some diseases, *e. g.*, *vaccinia* and *herpes febrilis,* and, regardless of their nature, represent the visible intracellular changes incident to or resultant from infection or injury of cells by certain viruses. Therefore, it seemed likely that, if it were possible to develop a method by which susceptible tissues infected and cultivated *in vitro* regularly evidence these characteristic intracellular alterations, knowledge concerning infection and immunity might be obtained through the proper use of various combinations of viruses with normal tissue,

---

[2] Noble, A., *J. Bact.*, 1927, xiv, 287.

[1] Gins, H. A., *Zt. Hyg.*, 1916, lxxxii, 89.