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.

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

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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.

immune tissue, plasma from normal animals, plasma from immune animals, normal serum, and immune serum. The purpose of this paper is to describe briefly a method by means of which such studies may be conducted.

Inasmuch as the rabbit is susceptible to vaccine virus and also to *herpes febrilis* virus, and since the former agent produces cytoplasmic inclusions while the latter induces nuclear changes, rabbit tissue, vaccine virus, and herpetic virus were chosen for the work. Corneal epithelium was selected for the study, because in this tissue the intracellular changes caused by the viruses have been extensively investigated. Furthermore, the work of Aldershoff and Broers[2] indicated that the use of cornea *in vitro* would be attended by success.

The following is the technique employed. A rabbit is exsanguinated for plasma. Then the spleen is excised, from which a tissue extract is prepared. Finally, both eyes, intact, are enucleated and thoroughly washed in Ringer's solution to remove as many contaminating bacteria as is possible. With a sterile cataract knife the control eye is cross-hatched with closely spaced scarifications just deep enough to penetrate the epithelial layer. The test eye is treated in a similar manner with the exception that between each scarification the knife is dipped in a virus emulsion. The cornea is then removed from each eye and divided into 4 to 8 pieces. The pieces from the test eye are further inoculated by placing them for one hour in an emulsion of virus. In the bottom of a 50 cc. pyrex centrifuge tube a clot (3 cc.) of plasma and spleen extract is made. On top of this clot is placed one piece of normal or infected cornea which is then covered by another clot of plasma and tissue extract. The tubes, sealed with sterile corks, are placed in an incubator at 37° C. for 24 to 48 hours. Then the clots, containing their bits of cornea are carefully removed from the tubes and fixed in Zenker's fluid. Sections are stained with eosin and methylene blue or according to the method of Giemsa. In this work, a potent virus, freshly prepared and containing no preservative, must be used.

Many of the cultures made in the manner described above were free from bacteria, others contained a few colonies that did not interfere with the action of the viruses, while still others were sufficiently contaminated to render them valueless. In properly prepared cultures, abundant typical Guarnieri bodies appeared in the cytoplasm of corneal epithelial cells infected with vaccine virus, and numerous acidophilic inclusions occurred in the nuclei of cells injured by herpetic virus. Neither type of inclusion, however, was

[2] *Anns. Inst. Pasteur*, 1906, xx, 779.

found in control pieces of cornea. In infected and uninfected bits of cornea, definite evidence of growth of epithelial cells was frequently seen. The results of this work, including observations obtained through the use of tissues and plasma from immune animals, will be described in detail in a later communication.

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Respiratory Quotient of the Brain.

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We have studied the respiratory quotient of the brain of dogs by analysis of the blood entering and leaving that organ. Under amytal anesthesia, except for one animal, the superior longitudinal sinus of large female dogs was exposed and a sample of venous blood was drawn from it simultaneously with one of arterial blood from the femoral artery. The blood samples were analyzed for carbon diox-

TABLE I.
Respiratory Quotients of Intact Animal and of Brain.

Condition of Animal	Air R.Q. of Intact Animal	Blood R.Q. of Brain
Fasted 2 days	0.75	1.03
		1.01
Fasted 2 days. No anesthetic.	0.77	1.06
		0.97
Phlorizinized	0.69	0.97
		1.02
Depancreatized	0.67	0.94
		1.00
Depancreatized and Phlorizinized	0.69	0.96
		1.00
		1.00
		0.99

TABLE II.
Respiratory Quotients of Brain.

No. of Animals	Condition of Animal	Number of Observations	Average R.Q.
4	Normal	7	0.99
1	No anesthesia	4	1.04
4	Phlorizinized	12	1.00
4	Depancreatized	11	0.99
1	Depancreatized and Phlorizinized	4	0.99
14		38	1.00