9073 P

# A Complement-Fixation Reaction Involving the Rabbit Papilloma Virus (Shope).

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The rabbit papilloma virus[1] is a notably stable and specific pathogenic agent which lends itself readily to quantitative immunological work.[2] The present paper reports a complement-fixation reaction with extracts of the papillomas caused by this virus and sera effective against it.

The technic used was essentially that described for viruses by Bedson and Bland[3]; it is based largely on the principles established by Bordet,[4] Dean,[5] and Merrill.[6] To 0.2 cc. of the appropriate dilution of inactivated serum was added 0.2 cc. of saline containing 2 units of guinea pig complement (titrated immediately beforehand), and then 0.2 cc. of the proper dilution of antigen. The antigen consisted of a centrifugalized, or filtered, saline suspension of glycerolated, or frozen and dried, papilloma tissue which had been ground with sand in a mortar and extracted overnight (or longer) in the refrigerator. The antigen was heated at 56°C. for 30 minutes immediately before use. Occasionally antigen was put into the tubes first and serum third, but complement was always added second. These reagents were then gently shaken and allowed to stand 2 hours at room temperature. 0.4 cc. of a suspension consisting of 0.2 cc. of 5% sheep cells mixed 10 minutes beforehand with 2 units of amboceptor in 0.2 cc. saline was then added to the tubes, which were again shaken and incubated at 37°C. for 30 minutes. Readings in terms of fixation were made immediately after the incubation and again after the tubes had been in the refrigerator overnight.

*Serum Tests.* The sera of 15 domestic and 5 wild cottontail rabbits bearing papillomas caused by the Shope virus were found to bind complement in the presence of antigen, whereas the sera of 6 normal domestic and 3 normal cottontail rabbits gave no fixation when tested concurrently. In further experiments the sera of 6

1 Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607.

2 Kidd, John G., Beard, J. W., and Rous, Peyton, *J. Exp. Med.*, 1936, **64**, 63, 79.

3 Bedson, S. P., and Bland, J. O. W., *Brit. J. Exp. Path.*, 1929, **10**, 393.

4 Bordet, J., and Gengou, O., *Ann. Inst. Pasteur*, 1901, **15**, 289.

5 Dean, H. R., *Z. Immunitätsforsch.*, 1912, **13**, 84.

6 Merrill, M. H., *J. Immunol.*, 1936, **30**, 169.

domestic rabbits bearing papillomas induced by tar, as well as specimens from 10 domestic rabbits resistant to the fibroma or myxoma virus,* have failed to fix complement on test with known complement-binding antigens.

Comparative tests were made of the virus-neutralizing and complement-fixing properties of the sera of 6 domestic rabbits bearing Shope papillomas and these properties were found to vary together. Two of the sera showed marked capacity to bind complement, and these neutralized the virus most effectively; 2 had little power to bind complement or to neutralize the virus, and the remaining 2 proved intermediate as regards both titers.

*Tests with Extracts of Various Papillomas.* Complement-binding antigens have been procured from the glycerolated Shope papillomas of wild cottontail rabbits, jack rabbits, snowshoe hares, and domestic rabbits. These differed notably in effectiveness, however, the complement-binding power of an extract being roughly proportional to its infective titer. Non-infective extracts from the papillomas of a number of domestic rabbits have failed to bind complement when tested in various dilutions with strong antisera. Zone phenomena have sometimes been observed with inhibition of fixation when the proportion of antigen to antibody has been too great.†

Heating 2 antigenic filtrates for 30 minutes at 60°C. did not notably affect their complement-binding power, but this was greatly diminished or destroyed by heating at 63°, 66°, or 69°C. Comparative tests indicate that the infective Shope virus contained in these filtrates was likewise unaffected at 60°C. but was largely or completely destroyed by heating for 30 minutes at 63°, 66°, or 69°C.

The following experiment provided further indications of the existence of a relation between the complement-binding and infective capacities of papilloma extracts. Five per cent extracts of glycerolated papillomas from 2 cottontail rabbits were kept 7 and 21 days respectively in the refrigerator, after which 5 cc. samples of each were put through filters of widely different pore size. The filtrates (1.5-2.0 cc. in total amount) were compared for complement-binding power and for ability to produce papillomas on the skin of susceptible rabbits. The Berkefeld V filtrates fixed complement completely, while the Berkefeld W filtrates bound it only slightly if at all when tested with the same antiserum, and the extracts filtered through single Seitz E K discs were without perceptible

* These fibroma-myxoma antisera were generously given by Dr. R. E. Shope, and the amboceptor by Dr. Kenneth Goodner.

† In a collateral test done recently with a potent complement-binding antigen and undiluted antiserum, visible precipitation occurred.

power to fix complement. Inoculations showed that the infective virus had passed through the Berkefeld V candles in large amount, whereas little had come through the Berkefeld W and Seitz filters.

*Summary.* A complement-fixation reaction is described, with extracts or filtrates of papillomas containing infective Shope virus and antisera effective against the latter. The implications of the work are being studied.

9074 P

## Intradermal Venom Reaction. A New Method of Determining Capillary Fragility.

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The intradermal injection of a potent moccasin snake venom in sufficient concentration into animals or human beings is followed by a local ecchymosis. However, dilute solutions, such as 0.1 cc. or 0.2 cc. of 1 :3000 will not normally be followed by ecchymosis.[1]

The dry scales of moccasin venom are dissolved in physiologic saline with merthiolate added as a preservative. The titration of the venom is carried out by the use of the chicken embryo.[2] The lowest dilution of venom in 1 cc. amounts that produces hemorrhage of the vascular tree in a four-day-old chicken embryo in a 3-hour period is considered to contain 10 hemorrhagin units. This is usually a 1 :3000 solution. Thus 0.1 cc. of standardized venom contains one hemorrhagin unit. Such standardized venom solution will not show deterioration in 4 months at icebox temperature.

The intradermal venom test consists of injecting 0.1 cc.-0.2 cc. (1 or 2 hemorrhagin units) of standardized moccasin venom intradermally and reading the reaction within 30 minutes to one hour, 0.1 cc.-0.2 cc. of physiologic saline injected at the same time serving as a control. A positive test is one in which there is a definite capillary rupture or ecchymosis at the injection site within an hour. The ecchymosis may be one centimeter or more in diameter. A delayed positive reaction is one that shows a diffuse ecchymosis in 12

1 Peck, Samuel M., *Arch. Derm. and Syph.*, 1933, **27**, 312.

2 Witebsky, C., Peck, S. M., and Neter, E., PROC. SOC. EXP. BIOL. AND MED. 1935, **32**, 722.