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pH Stability of Shope Papilloma Virus and of Purified Papilloma Virus Protein.

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We described[1] the isolation of a high molecular weight protein from virus-induced papillomas of western cotton-tail rabbits.[2] It was found that this protein could be extracted only from infectious suspensions of such papillomas and that virus activity was proportional to the amount of the protein present. This and other experiments, in which we have failed to dissociate virus activity from the protein, point to its being the purified agent responsible for the disease.

We have now completed a detailed comparison of the influence of pH on (1) the infectiousness of the virus and (2) the stability of the molecules of the protein. Stability of the virus was determined by dissolving the virus protein in buffer solutions of accurately determined pH and titrating the solutions in domestic rabbits[3] immediately, and after 6 hours, 24 hours, a week and a month. With the analytical air-driven ultracentrifuge,[4] the molecular constitution of these solutions was found from their sedimentation diagrams.

On the acid side of pH 7 the virus activity remains high until at a pH between 2.9 and 3.3 it suddenly is lost. The protein molecule splits at exactly this point. From pH 3.3 to pH 7, on both sides of its isoelectric point, the papilloma protein has a principal component with $S_{20^\circ} =$ ca 260×10^{-13} cm. sec.$^{-1}$ dynes^{-1}; there ordinarily appears also a faint secondary boundary corresponding to $S_{20^\circ} =$ ca 380×10^{-13}. At pH 1.85 the completely inactive protein sediments with the sharp boundary corresponding to a single molecular species with $S_{20^\circ} =$ ca 180×10^{-13}.

In contrast to conditions in acid solutions, full virus activity is not preserved for a long time at any pH greater than 7. Alkaline inactivation occurs in two ways. Above pH 10.1 virus solutions immediately become non-infectious. Below this point the titre of a virus solution gradually diminishes with time, at a rate that decreases

[1] Beard, J. W., and Wyckoff, R. W. G., *Science*, 1937, **85**, 201.

[2] Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607.

[3] Kidd, John G., Beard, J. W., and Rous, Peyton, *J. Exp. Med.*, 1936, **64**, 63.

[4] Biscoe, J., Pickels, E. G., and Wyckoff, R. W. G., *J. Exp. Med.*, 1936, **64**, 39; Wyckoff, R. W. G., and Lagsdin, J. B., *Rev. Sci. Instruments*, 1937, **7**, 246.

as neutrality is approached. Study of the stability of the protein molecule with the analytical ultracentrifuge demonstrates that it fragments at the same pH (10.1-10.2) at which immediate inactivation is observed. This molecular disintegration is more complete than the one seen in strongly acid solutions; the largest piece of the original protein that can be photographed in strong alkali has a sedimentation constant of only $S_{20}^{\circ} = \text{ca } 30 \times 10^{-13}$.

Below pH 10 the sedimentation constant of the papilloma protein molecule is not measurably changed nor is the molecular homogeneity visibly diminished even when solutions are kept until all virus activity has been lost. These solutions thus contain non-infectious protein material consisting of only slightly altered papilloma protein molecules. The biological properties of such non-infectious derivatives of the papilloma virus protein are being studied.

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Static and Kinetic Conditioned Reactions.*

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Former experiments of Spiegel[1, 2] and his coworkers (Aronson,[3] Price[4] and Spiegel) indicated that labyrinthine impulses may reach the cerebral cortex.

In order to investigate what part the connections of the labyrinth with higher centers play in the perception of position and motion, the study of conditioned reactions seemed promising. A special position table was built allowing dogs to be brought into any desired position. The dogs were slowly rotated from a sloping position through the horizontal plane into another oblique position. When the horizontal plane was passed, an electric shock (unconditioned stimulus) was applied to a leg, during the motion in one direction only; the defense reaction and the change in respiration were recorded. First, only conditioned reactions upon the horizontal position appeared. Later the animals learned also to differentiate be-

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1 Spiegel, E., *J. Nerv. and Ment. Dis.*, 1932, **75**, 504.

2 Spiegel, E., *Arch. Neur. and Psych.*, 1934, **31**, 469.

3 Aronson, L., *J. Nerv. and Ment. Dis.*, 1933, **78**, 250.

4 Price, J., and Spiegel, E. In press.