Depressants of the barbiturate series are known to suppress respiration and transform mammals into poikilothermous animals.[11, 12] In contrast to chloretone it would seem that nembutal, a barbiturate, may bring about narcosis by shifting some enzymatic reaction whose presence can be detected by the measurement of the oxygen consumption as a function of temperature.

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Study of the Papilloma Virus Protein with the Electron Microscope.*

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Information relative to the physical character of the papilloma virus protein[1] has been obtained by means of ultracentrifugation,[2] electrophoresis,[3, 4] diffusion and viscosity.[2] Recently, more direct studies of this animal virus have been undertaken with the electron microscope. In the present paper are described the preliminary observations dealing chiefly with data gathered with this instrument relative to the appearance and size of the virus.

Papilloma virus protein isolated in 0.05 M phosphate buffer pH 6.5 by previously described ultracentrifugal procedures[1] was diluted with water to concentrations of the order of 0.05 to 0.5 mg per cc. The salt concentration in the final solutions was approximately 0.005 M. Preparations for study were made by pipetting the solutions onto collodion membranes supported on 200-mesh wire gauze. Free fluid was removed with the pipette, and the resulting

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thin film allowed to dry in the air. For comparison, examinations have been made also of mixtures of papilloma and tobacco mosaic viruses,† the latter in concentration of about 0.4 mg per cc of the mixture.

The essential findings are illustrated in Figs. 1 and 2. In Fig. 1 are shown the results obtained with papilloma virus alone. Repeated micrographs of 3 different batches of the purified protein have resulted consistently in observation of the circular images shown in Fig. 1. When the protein concentration was kept in the region of approximately 0.1 mg per cc, the images were for the most part single, though grouping was frequent as seen in Fig. 1. In preparations of higher concentration, large groups of particles occurred and fewer single images were seen. A regular arrangement of images in the groups was not observed. Evidence of material in the preparations other than that revealed in the circular images was remarkably slight. Since the material examined behaved initially as the papilloma virus, it appeared likely that the images seen were micrographs of the virus particles. In Fig. 2 is shown a mixture of

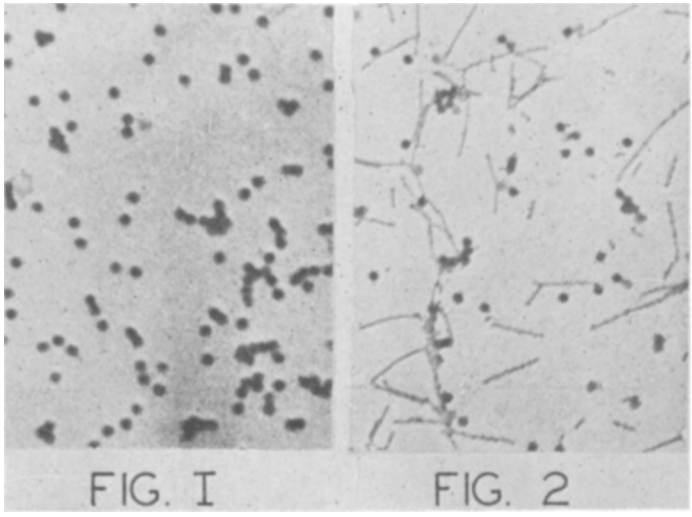

FIG. 1. Rabbit papilloma virus protein. (29,000 ×.)

FIG. 2. Mixture of rabbit papilloma virus and tobacco mosaic virus. (27,000 ×.)

These electron micrographs were made at 55 kilovolts, and the magnification in the final pictures was calculated on the basis of measurements on the tobacco mosaic virus rods which have been reported to be 15 mμ in diameter.

† The tobacco mosaic virus was available through the generosity of Dr. W. M. Stanley, Rockefeller Institute for Medical Research, Princeton, N.J. The virus had been purified at least 2 months prior to examination.

the papilloma virus protein with tobacco mosaic virus. The appearance of the latter was similar to that described by Stanley and Anderson.[5] Besides the rod-shaped images, there were others which had the appearance of fragments[5] of tobacco mosaic virus rods. These were probably not associated with the papilloma virus as they were not seen in preparations of this material in the absence of tobacco mosaic virus.

Measurements have been made of the diameter of 21 papilloma virus images in the presence of tobacco mosaic rods as in Fig. 2. Similar measurements were made also on the width of 21 tobacco mosaic rods. The values obtained for both varied rather widely. In the instance of the papilloma virus images, the standard deviation was 10.8% of the mean as compared with 12.2% for the diameter of the rods. The ratio of the mean diameter of the circular images to mean rod width was 2.93. The width of the rods of tobacco mosaic virus has been reported to be 15mμ as determined both by X-ray and electron microscope.[5] On this basis the mean diameter of the papilloma virus images was 44.0 mμ.

From the studies thus far made the preparations of the papilloma virus protein appear to be of considerable homogeneity with respect to particles comparable in size and shape as revealed by the electron microscope. Data previously obtained[2] by diffusion, viscosity and sedimentation studies indicate that the papilloma virus particle behaves as if it were an oblate ellipsoid with an axial ratio of 11 if hydration is neglected. In the present work, images of the papilloma virus particle have been consistently round, a finding compatible with the results of the earlier studies. On the other hand, if the particles were actually disc-shaped, then no particles were encountered that were not lying flat in the focal plane. Only the round form has been seen in aggregates where "rouleau" formation might well have been expected though the specimens were dried. The electron microscope data are not sufficient to permit a definite estimate of the shape of the papilloma virus particle. It is of interest, however, to consider the values obtained in the light of previous findings by indirect methods. The molecular weight of the papilloma protein,[2] calculated from sedimentation and diffusion data is 47,100,000, and the density by pycnometer measurement is 1.32. A spherical particle of these characteristics would have a diameter of 48.2 mμ, a value in good agreement with that of 44.0 mμ found in the present work. A flat ellipsoid of the same weight and density would have a major axis considerably greater than 44.0.

[5] Stanley, W. M., and Anderson, T. F., *J. Biol. Chem.*, 1941, **139**, 325.