conjugates of the incitant.[5] There are, however, statements in the literature to the effect that sensitive human beings have been partially desensitized by ingestion of the specific allergen.[6,7]

Summary. Through the feeding of certain allergenic compounds to the non-sensitive subject, a state of resistance may be established against subsequent experimental sensitization of the skin by the same substance.

[5] Landsteiner, K., and Chase, M. W., *J. Exp. Med.*, 1937, **66**, 337.

[6] Park, R. G., *Brit. Med. J.*, 1944, **2**, 816.

[7] Stevens, F. A., *J. Am. Med. Assn.*, 1945, **127**, 912.

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Rabbit Papilloma and Vaccinia Viruses and T_2 Bacteriophage of *E. coli* in "Shadow" Electron Micrographs.*

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Although electron micrography has added greatly to knowledge of the shape, size and other characters of viruses, the findings leave much to be desired. The capacity of viruses to absorb and scatter fast electrons is relatively small, and, consequently, images of the particles are likely to be of low contrast and indistinct, especially at the periphery. Contrast in influenza virus images may be enhanced slightly by treatment of the virus with osmic acid;[1] use of calcium chloride, though obscuring internal structure, aids greatly in micrography of equine encephalomyelitis[2] and influenza viruses[3] but not in the instances of the rabbit papilloma and tobacco mosaic viruses. In addition, the images are flat, and judgment of particle shape must be based solely on contour portrayed in 2 dimensions. A considerable advance in the electron micrography of viruses is the application of the shadow-casting technic recently reported by Williams and Wyckoff.[4,5,6] In this process, the molecules of metals evaporated *in vacuo* fall on the virus particles at a grazing angle and cast "shadows" which give the appearance of 3-dimensional contour to images in electron micrographs. The shape and length of the shadows cast on the background and the contour of the images afford a much better basis for judgment of the shape of the particle than the unshadowed images. Studies with this technic have been made of the vaccinia and rabbit papilloma viruses and the T_2 bacteriophage of *E. coli*. The results‡ with these 3 viruses are reported in the present paper.

Materials and Methods. The viruses were

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[1] Sharp, D. G., Taylor, A. R., McLean, I. W., Jr., Beard, D., Beard, J. W., Feller, A. E., and Dingle, J. H., *J. Immunol.*, 1944, **48**, 129.

[2] Sharp, D. G., Taylor, A. R., Beard, D., and Beard, J. W., *Arch. Path.*, 1943, **36**, 167.

[3] Taylor, A. R., Sharp, D. G., Beard, D., Beard, J. W., Dingle, J. H., and Feller, A. E., *J. Immunol.*, 1943, **47**, 261.

[4] Williams, R. C., and Wyckoff, R. W. G., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 265.

[5] Williams, R. C., and Wyckoff, R. W. G., *Nature*, 1945, **156**, 68.

[6] Williams, R. C., and Wyckoff, R. W. G.,

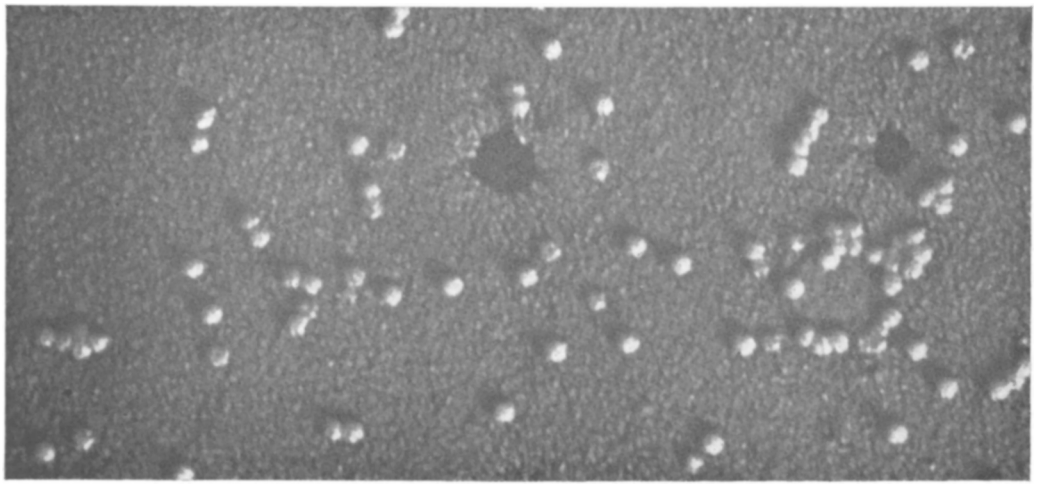

Fig. 1.
Rabbit papilloma virus shadowed with 10 mg of gold at the angle tangent 2/7. Magnification 42,700 x.

employed only in purified preparations. Vaccinia[7,8] and rabbit papilloma viruses[9,10] were obtained as previously described. The bacteriophage,§ the T_2 strain[11] parasitizing *E. coli*, was cultivated on the host grown in broth and purified by ultracentrifugal procedures which will be described elsewhere.[12] Purified virus suspensions containing about 10^{12} particles per ml were placed on the collodion film support, and, after about half a minute, the excess fluid was removed with a fine pipette. The films after drying were viewed immediately in the electron microscope or, more often, lightly washed by flooding once or twice with or dipping in distilled water. When the films were again dry, they were ready for observation and electron micrography in the ordinary way, or for application of evaporated metal. In some instances the films were observed and photographed, then shadowed and examined again.

Gold was used for the papilloma and vaccinia viruses and chromium for the bacteriophage. The weighed metals were evaporated in a 10-turn conical helix of 5 mil tungsten wire, through which alternating current was passed from a 500-volt-ampere transformer at 20 volts. Enough heat was generated to complete the evaporation in 1 to 3 minutes. The vacuum chamber was a glass tube 1.5 inches in diameter and 12 inches long. It was continuously evacuated with a Cenco Hivac pump and a 2-stage mercury diffusion pump, giving an ultimate vacuum of the order of 10^{-5} mm of Hg. The metal was deposited on the papilloma and vaccinia viruses at the angle whose tangent was 2/7 and on the bacteriophage at the angle of tangent 1/5. The distance from the evaporator coil to the virus was 7 cm. Assuming evaporation equally in all directions, the metal thickness on the side of the virus particle normal to the beam would be given by the equation

$$\text{Thickness} = \frac{W}{4\pi r^2 \rho}$$

Science, 1945, **101**, 596.

‡ This work was described in part at the meeting of the Electron Microscope Society of America, December 1, 1945, at Princeton, N. J.

7 Craigie, J., and Wishart, F. O., *Brit. J. Exp. Path.*, 1934, **15**, 390.

8 Beard, J. W., Finkelstein, H., and Wyckoff, R. W. G., *J. Immunol.*, 1938, **35**, 415.

9 Beard, J. W., Bryan, W. R., and Wyckoff, R. W. G., *J. Infect. Dis.*, 1939, **65**, 43.

10 Taylor, A. R., *J. Biol. Chem.*, 1946, **163**, 283.

§ We are greatly indebted to Dr. Max Delbrück, Vanderbilt University, for the strain of bacteriophage and its host and for advice in the problems of cultivation.

11 Anderson, T. F., *J. Cell. and Comp. Physiol.*, 1945, **25**, 17.

12 Hook, A. E., Taylor, A. R., Sharp, D. G., and Beard, J. W., to be published.

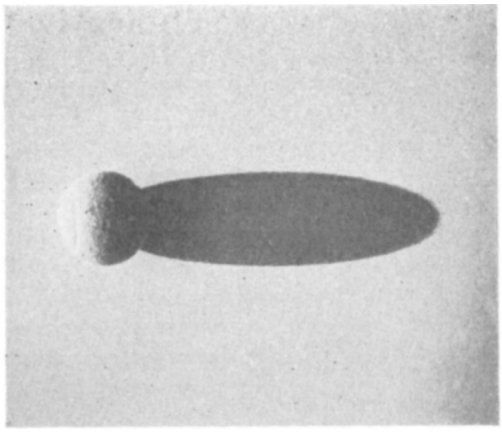

Fig. 2.
Photograph of sphere (tennis ball) and the shadow cast by light striking at the angle tangent 2/7.

in which W = weight of metal evaporated in grams; r = distance (7 cm) from the metal to the virus preparation and ρ = the density of the metal (19 for gold, 7 for chromium). The thickness of the deposit on the film lying at an angle φ to the beam was less by the factor tan φ, which in this case was 2/7 or 1/5. Under the conditions of the experiments the thickness of the layer of gold on the normal surface was about 8.5 Å per mg of metal evaporated; the values for chromium were greater by the quotient of the densities, or 2.7 times as much.

For purposes of comparison, a photograph was made of a spherical object, a tennis ball, and the shadow produced by light striking it at the angle of tan 2/7.

Results. The rabbit papilloma virus in conventional electron micrographs[13] is seen as circular or rounded images of low contrast which merge indistinctly into the background at the periphery. On the basis of apparent uniformity in the size and shape of the images, the virus was judged to be essentially spherical in shape. In Fig. 1 there is shown the appearance of the virus shadowed with 10 mg of gold. Most of the images are highly uniform in shape and size; others, which are irregular in size and shape, have the appearance of representing fragments of virus particles.

The characters of the shadow cast by a sphere, a tennis ball, lighted at an angle of tangent 2/7, are shown in Fig. 2. On comparison of Fig. 1 with Fig. 2, it is seen that the shadows of the virus particles differ greatly from the shadow cast by the ball. The shadows of the virus are nearly triangular in shape, relatively very broad at the base, and the length is, on the average, about ⅔ of what it would be if the height of the particles were equal to the diameter of the images. Judging from these characters, it is clear that the particles, at the moment of shadowing, were not spherical, but were flattened on the under and possibly on the upper sides as well.

Vaccinia virus deposited on the collodion membrane from 0.005 M phosphate buffer and washed by dipping in water is pictured in Fig. 3. As described by Green, Anderson and Smadel,[14] the images are approximately rectangular in shape and give evidence of differentiation in the internal structure of the particle as shown by the presence of rounded regions of density greater than that of the remainder of the particle. The particles lightly washed seem to be coated with a sticky material which causes coherence of some of the particles and in which bubble-like structures appear.

Vaccinial elementary bodies shadowed with 8 mg of gold are seen in Fig. 4. In contour in the horizontal plane the images of Fig. 4 are similar to those of Fig. 3 except for roughness and unevenness about the edges, which may be related either to uneven deposit of gold or possibly to coating of the bubble-like structures seen in Fig. 3. The bodies are clearly flattened in varying degree, as shown both by contour and length of the shadows. The shadows are not sharp in the region of the apex where the metal shades far into the shadow. One of the images seen in Fig. 4 is almost circular and of very high contrast. The shadow associated with it is much longer than the shadows of the other particles. This appearance suggests, as discussed below, that the image represents

[13] Sharp, D. G., Taylor, A. R., Beard, D., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 205.

[14] Green, R. H., Anderson, T. F., and Smadel, J. E., *J. Exp. Med.*, 1942, **75**, 651.

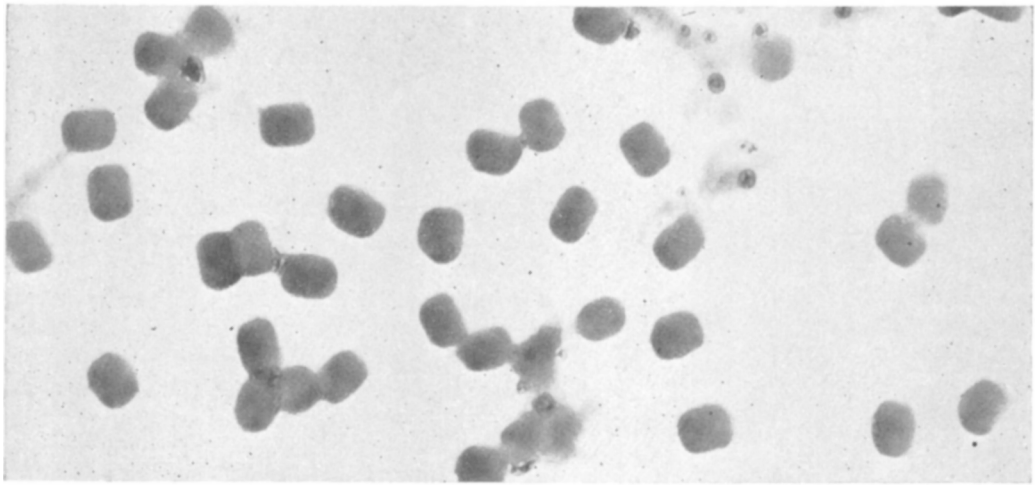

Fig. 3.
Conventional electron micrograph of vaccinial elementary bodies. Magnification 25,000 x.

one of the particles standing on end. In many of the images there are seen relatively broad and low mound-like protrusions that may represent the effects of internal structure, possibly the material of the regions of high density of Fig. 3, extending up beneath the surface.

The T_2 bacteriophage of *E. coli* is shown in the electron micrograph of Fig. 5. The purified bacteriophage was suspended in physiological saline solution, and the concentrate was diluted ⅕ with 0.023 M $CaCl_2$ for preparation of the film. Similar to the findings of Luria and Anderson,[15] the images of the bacteriophage are tadpole-shaped, showing the presence of a large head and a stubby tail tipped with a ball- or disc-shaped knob. The heads, which are of high capacity to absorb electrons, are uniform in size and hexagonal in shape. The tails, which show low electron-stopping power, are uniform in length but appear to vary in width. No internal structure was visible in the presence of the calcium salt.

In Fig. 6 are shown bacteriophage particles shadowed by the evaporation of 3.7 mg of chromium. The heads do not give the shadows either of ideal spheres, or even of regular polyhedrons, but rather those of short, somewhat flattened rods with conical caps. The stubby tail, of uneven thickness, terminates in a ball- or disc-shaped structure. The tail shadows are likewise short, indicating a flattened condition consistent with the observed low electron-stopping power. The degree of flattening is not the same in all preparations nor has it been the same for the individuals of a given picture, as may be seen in Fig. 6.

Discussion. The application of the shadow technic adds greatly to the results of direct observation of viruses in the electron microscope. Coating of the virus preparation is effected *in vacuo* under conditions not greatly different from those to which the preparation is subjected in the course of conventional electron micrography. Thus the characters portrayed with 3-dimensional effect in the shadow pictures are those of the dried and possibly shrunken and distorted virus particles and are probably not quantitatively representative of the particles in their native state in aqueous suspension. Measurements of shadows[6] of tobacco mosaic virus rods yield values of rod height similar to results obtained for rod width by means of X-ray examination[16] and measurements in electron micro-

[15] Luria, S. E., and Anderson, T. F., *Proc. Nat. Acad. Sci.*, 1942, **28**, 127.

[16] Bernal, J. D., and Fankuchen, I., *J. Gen. Physiol.*, 1941, **25**, 111.

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

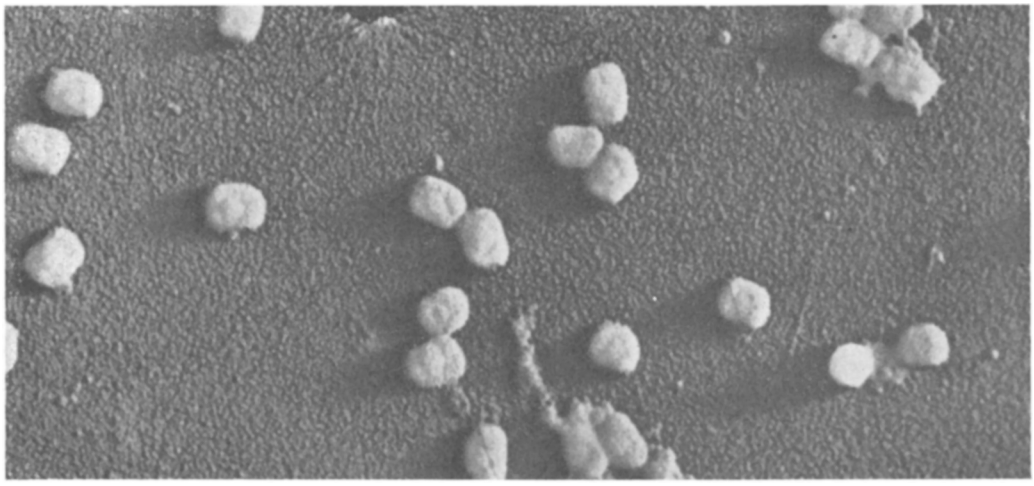

Fig. 4.
Vaccinia virus shadowed with 8 mg of gold at the angle tangent 2/7. Magnification 25,000 x.

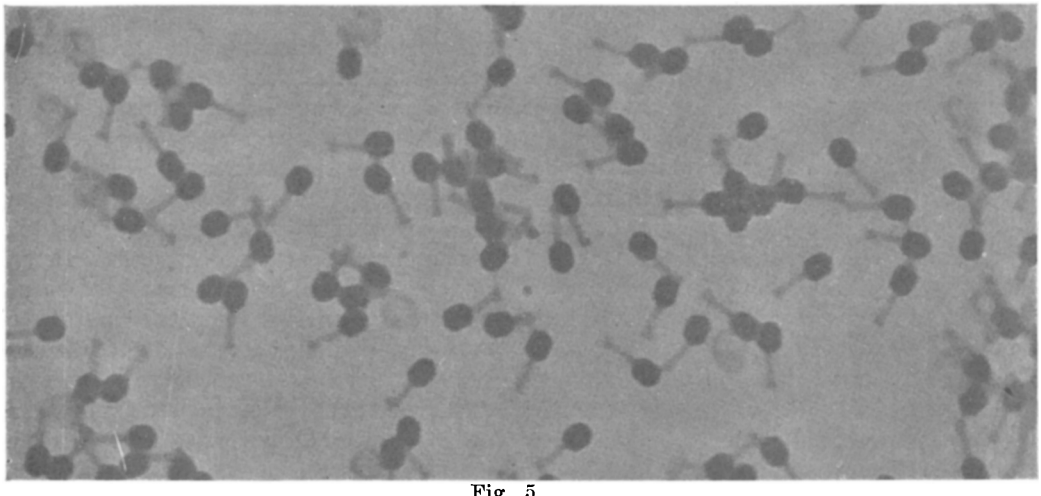

Fig. 5.
Conventional electron micrograph of the T_2 bacteriophage of *E. coli*. Magnification 42,700 x.

graphs.[17] That shrinkage of other viruses occurs under these conditions, however, is indicated by the observation of particle diameter in electron micrographs smaller than values obtained in studies of the virus in aqueous suspension.[18]

Shadow electron micrographs of the papilloma virus corroborate evidence of uniformity of particle size and shape obtained in sedimentation studies[19] and from conventional electron micrographs.[13] In the shadow micrography, the apparent height of the particles is about ⅔ that expected of spheres, a value much greater than that for oblate spheroids of axial ratio 11-1 previously predicted[19] from sedimentation, diffusion and viscosity data on the assumption that the particle is unhydrated. Recent studies[20] of

[18] Sharp, D. G., Taylor, A. R., McLean, I. W., Jr., Beard, D., and Beard, J. W., *J. Biol. Chem.*, 1945, **159**, 29.

[19] Neurath, H., Cooper, G. R., Sharp, D. G., Taylor, A. R., Beard, D., and Beard, J. W., *J. Biol. Chem.*, 1941, **140**, 293.

[20] Sharp, D. G., Taylor, A. R., and Beard, J. W., *J. Biol. Chem.*, 1946, **163**, 289.

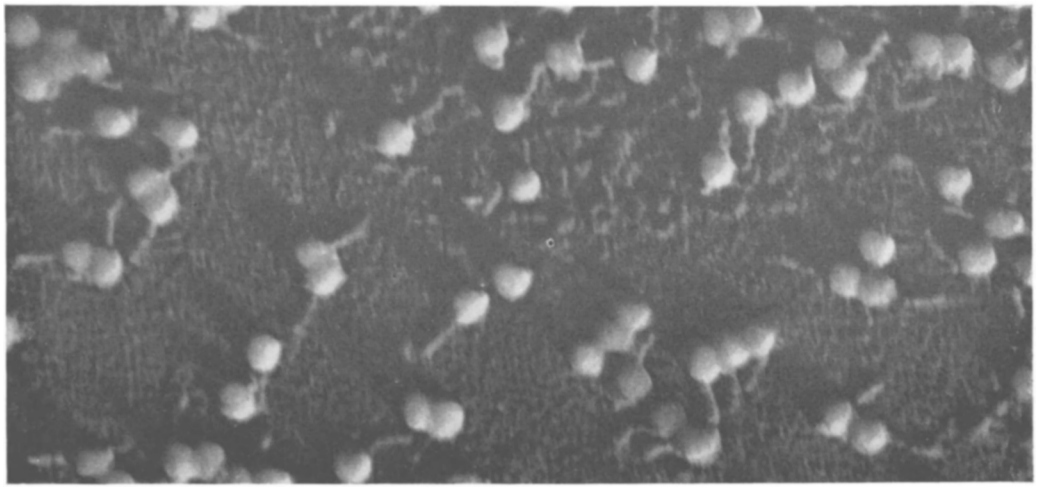

Fig. 6.
Bacteriophage shadowed with 3.7 mg of chromium at the angle tangent 1/5. Magnification 42,700 x.

the density of the papilloma virus in aqueous suspension indicate that the particles are 58% water (by volume) and, if spherical in shape, about 66 mμ in diameter. The difference between this value and 44 mμ observed in electron micrographs[13] is probably, in part, a measure of the shrinkage occurring in the plane of the picture. Greater shortening of the vertical diameter than of that in the horizontal plane is not improbable, since settling and spreading of the wet particle on the collodion membrane might be expected. This is consistent with the obvious flattening of the particles on the under side indicated by the shadows broad at the base. Quantitatively, the shadow pictures show that the dry papilloma virus particles are slightly flattened spheroids; the finding is not incompatible with the view that the virus is essentially spherical in the wet state and flattens on drying in contact with the collodion membrane.

The elementary bodies of vaccinia have been described as "brick-shaped"[14] which implies a difference between the height and the width of the particles lying on the collodion membrane. Judging from the contour in the shadow pictures, the dry bodies seem greatly flattened, but this appearance is not wholly consistent with the length of the shadows. This discrepancy and the lack of sharpness of the shadows may be explained, at least in part, on the assumption that shrinkage of the particle continues during the process of metal-coating. The particles, even after drying, are clearly well-rounded about all diameters. There is some evidence that the particles are not brick-shaped but are essentially short cylinders or rods. In a large number of micrographs, the width or short dimension of particles has been relatively uniform, and no evidence has been seen of bodies lying on edge. On the contrary, circular images of high contrast and of diameters similar to the short dimension of the rectangular images occur with a frequency of about 1 or 2 per hundred. A shadow image of this sort is shown in Fig. 4. The circular shape, and the relatively high contrast of the image, together with the greater length of the shadow, may be interpreted as evidence that this particle is a short rod or cylinder standing on end. The consistency of the findings suggests that the body casting this image differs from the others only in orientation, and that the vaccinia virus may be essentially rod-shaped.

The mound-shaped protrusions seen in the shadow images are interpreted as representing the intraparticular material of high contrast in conventional electron micrographs. The structure appears relatively large and of greater density and resistance to shrinkage than the remainder of the particle.

The highly uniform width, the well-rounded contours and the length of the shadows are characters suggesting that the headpieces of the bacteriophage are likewise probably short cylinders. The hexagonal shape in ordinary electron micrographs indicates a conical shape of the ends of the rods. In the absence of information of the density, size and water content of the bacteriophage in aqueous suspension, no estimate can be made of the possible degree of shrinkage on drying.

Summary. Electron micrographs of the rabbit papilloma and vaccinia viruses and the T_2 bacteriophage of *E. coli* were obtained with the metal-shadowing technic. The papilloma virus dried *in vacuo* for application of the metal appeared spheroidal in shape, flattened especially at the region of contact with the film. The vaccinia virus was greatly flattened and showed the presence of a denser internal material bulging beneath the surface. The bacteriophage was a tadpole-shaped structure with a headpiece of well-rounded contours and a stubby tailpiece. The findings were discussed with respect to their bearing on the shape of the viruses in the wet state.

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Attempts to Propagate Murine Poliomyelitis Virus on Various Intestinal Bacteria and Protozoa.*

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Studies of microbial metabolism have amply demonstrated that many bacteria and protozoa are endowed with a complex equipment of enzymes that are utilized for cellular propagation. It is therefore not surprising that numerous attempts have been made to grow certain pathogenic viruses in symbiosis with bacteria or other free-living cells.[1] Without passing on the merits of these observations, the relationship between the virus of poliomyelitis and the intestinal flora seemed to us of particular interest in this connection. As is well known, human virus—with sufficient neurotropic virulence to permit intracerebral transfer to monkeys—can often be isolated from the faeces of cases or healthy contacts. More important yet, normal albino mice harbor regularly in the intestinal tract neurotropic murine virus (Theiler virus) which apparently persists for the lifetime of the animal. Additional evidence of the remarkable affinity of poliomyelitis virus for the alimentary canal is furnished by experience with the mouse-adapted strain of human SK poliomyelitis virus which could be recovered from the faeces of 3 different infected hosts, natural or experimental, *i.e.*, man,[2] monkey[3] and guinea pig.[4] How a supposedly strictly neurotropic virus can maintain itself for any protracted period in the absence of living nervous material is dif-

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1 a. Nicolau, S., and Lwoff, A., *Bull. Soc. Path. Ex.*, 1932, **25**, 721; b. Silber, L. A., and Wostruchowa, E. I., *Centralbl. Bakt. Orig.*, 1933, **129**, 389; 1933, **129**, 396; 1934, **132**, 314; c. Silber, L. A., and Dosser, E. M., *Centralbl. Bakt. Orig.*, 1933, **131**, 222; d. Silber, L. A., and Timakow, W. D., *Centralbl. Bakt. Orig.*, 1935, **133**, 242; e. Poppe, K., and Busche, G., *Centralbl. Bakt. Orig.*, 1936, **136**, 385.

2 Trask, J. D., Vignec, A. J., and Paul, J. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **88**, 147; *J. Am. Med. Assn.*, 1938, **111**, 6.

3 Trask, J. D., and Paul, J. R., *Ann. Int. Med.*, 1942, **17**, 975.

4 Ibanez, J. S., Trabajos del Instituto Cajal de Investigaciones Biologicas, 1944, **36**, 137.