marked than in the case of β-carotene. The decreased effect of β-carotene, when injected into the blood stream, cannot be explained on the basis of passive filtration in the lungs since injections, when made directly into the portal system, were no more effective than when they were made into the heart or into the jugular vein.

Summary. No chemical evidence was obtained that taurocholic acid, glycocholic acid, and decholin form compounds with β-carotene. When β-carotene, together with taurocholic acid, glycocholic acid, or decholin, was fed to bile fistula vitamin A deficient rats, the β-carotene was not utilized. When β-carotene, in the form of a suspension, was injected intravenously, it was less effective than when administered orally. A less marked difference was noted in the case of vitamin A.

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Ultrafiltration of Psittacosis Virus.

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Levinthal[1] using the "gradocol" membranes of Elford, estimated the particle size of the virus of psittacosis to be 220 to 330mμ. Sir Henry Dale[2] has referred to unpublished experiments of Elford, in which the virus was found to be 275 mμ in diameter. No data are available on the details of filtration or the type of material used in these experiments. Microscopic measurements show the elementary body to range in size from 200 to 300 mμ,[3] while microphotographic studies give the smallest elementary bodies a diameter of 240 to 300 mμ.[4] In connection with psittacosis studies being conducted in this laboratory, it was considered of interest to determine the size of the infective particle under controlled conditions.

The virus strain used was isolated from infected shell parakeets in 1934 and has had no known connection with a human case. The virus was carried according to routine in white mice until December, 1935, when the strain was established on the chorio-allantoic mem-

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1 Levinthal, W., *Lancet*, 1935, **1**, 1207.

2 Dale, H. H., *Huxley Memorial Lecture*, 1935.

3 Lillie, R. D., *U. S. Pub. Health Rep.*, 1930, **45**, 773.

4 Coles, A. C., *Lancet*, 1930, **1**, 1011.

brane of the developing chick. Over 190 consecutive bacteria-free passages have been made to date, with the virus maintaining an unaltered infectiousness for susceptible white mice. All virus used for ultrafiltration studies was obtained from membranes inoculated subsequent to the 43rd passage.

The virus suspensions were prepared from 72-hour membranes. After aseptic removal from the egg, the infected tissue was placed in 50 cc. Erlenmeyer flasks containing 2 cc. of the indicated diluent per membrane. After agitating for 20 minutes in a mechanical shaker, the mixture was centrifuged for 20 minutes at 3000 RPM and the clear supernatant fluid removed for ultrafiltration. Control mice receiving 1 cc. of a 10^{-6} dilution of this material intraperitoneally succumbed in 9-12 days with typical findings for psittacosis. Routine staining of infected membranes and mouse organs was done by the original method of Castaneda,[5] and the presence of L.C.L. bodies was demonstrated in all cases.

Ultrafiltration was conducted at room temperature under a positive pressure of nitrogen; the method of Elford,[6] as modified by Bauer and Hughes[7] was used. Pressures varied from 18 to 30 cm. Hg, and the volume of filtrate ranged from 5 to 12 cc. All filtrations were complete in 4 minutes or less. Membrane thicknesses ranged from .136 to .152 mm. Use of a 1-100 dilution of the original supernatant fluid obviated the necessity of a preliminary filtration to remove gross tissue particles. *Chromobacterium prodigiosum* was added before filtration and was absent from filtrates in all cases. All membranes were satisfied by the preliminary passage of 5 cc. of the diluting fluid.

The infective agent passed readily through membranes with average pore diameters of 645, 454, 436 and 410 mμ; hormone broth pH 7.3 and buffered water† pH 7.3 were used as the suspending and diluting media. Filtrates showed practically no decrease in titer when inoculated mice were compared with those receiving unfiltered material. With one inconclusive exception, the virus was completely retained by membranes with an average pore diameter of 383 mμ or less.

On the basis of these results, the filtration end-point is approximately 400 mμ. By the application of Elford's correction factor for this range, the diameter of the infective agent of psittacosis is found to be 200 to 300 mμ, a figure in close agreement with microscopic and microphotographic measurements of the elementary body.

[5] Castaneda, M. R., *J. Infect. Dis.*, 1930, **47**, 416.

[6] Elford, W. J., *J. Path. and Bact.*, 1931, **34**, 505.

[7] Bauer, J. H., and Hughes, T. P., *J. Gen. Physiol.*, 1934, **18**, 143.

† McIlvaine's standard, diluted 1-50.