

system or sexual apparatus, although occasionally, there was slight interstitial deposit in the testis. The intestinal crura were essentially negative except for fairly frequent scant punctate granular deposits in the outer rim of cytoplasm of the lining cells.

Male and female worms showed a similar distribution of glycogen. However, the amount was distinctly less in the female than in the male and sometimes apparently out of proportion to difference in size. This is explained by the less well-developed musculature and parenchyma of the female worm and also by the relatively more abundant sexual apparatus which is glycogen negative.

The histological studies permitted only a rough quantitative estimate of the amount of glycogen present. In spite of this, the quantity of glycogen in male worms from the longer periods of infestation usually exceeded that present in the early stages. In the

former, the glycogen was often found in coarser, more compact granular form within musculature and body parenchyma. However, the location of the glycogen remained the same.

*Summary.* From 13.6 to 29.0% of the dry matter of the males of *S. mansoni* consisted of glycogen. In females the amount varied between 2.7 and 5.0%.

The glycogen in both male and female worms was deposited principally in the musculature and parenchyma. The relatively small amount in the female is explained by the less well-developed musculature and parenchyma and the more abundant sexual apparatus which is glycogen negative.

Histological studies were in general accord with the quantitative observation that the amount of glycogen in the male tends to increase with age.

Received March 10, 1950. P.S.E.B.M., 1950, v73.

### Studies of Influenza A (PR8) Infected Tissue Cultures by Electron Microscopy.\* (17756)

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The growth and development of a virus may be studied in the cells upon which it is parasitic. In the course of such studies, by means of a tissue culture technic(1,2), we have found that there is a very close association between the formation of the typical spheres of influenza virus and the elongated rod-like filaments previously shown in centrifuged influenza infected allantoic fluid(3-6).

\* Supported in part by a grant in aid from the National Institutes of Health, U.S.P.H.S.

<sup>†</sup> National Institutes of Health Fellow.

1. Porter, K. R., Claude, A., and Fullam, E. F., *J. Exp. Med.*, 1945, v81, 161.

2. Bang, F. B., and Gey, G. O., *Proc. Soc. Exp. Biol. and Med.*, 1949, v71, 78.

3. Taylor, A. R., Sharp, D. G., Beard, D., Beard, J. W., Dingle, J. H., and Feller, A. E., *J. Immunol.*, 1943, v47, 261.

4. Moseley, V. M., and Wyckoff, R. W. G., *Nature*, 1946, v157, 263.

Previous observers have noted that rod-like forms occur in ultracentrifuged allantoic fluids infected with several strains of influenza virus including newly isolated strains (4,7,8) and that they adsorb and elute from red cells along with the spherical virus particles(7). Moseley and Wyckoff(4) have noted the occurrence of segmentation on the rods and consider this as evidence for a conversion between rod-like and spherical forms.

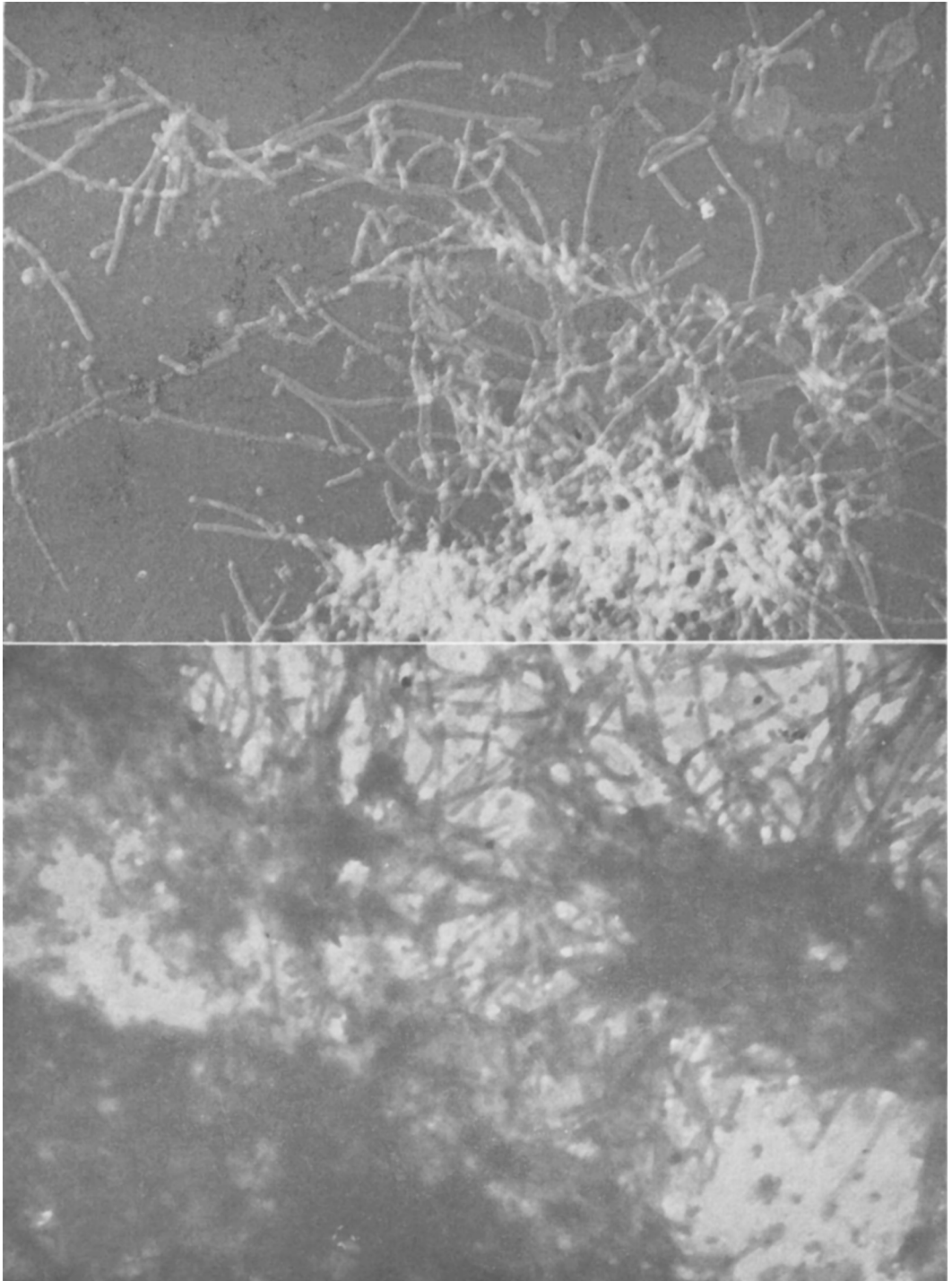
We have confirmed these observations and have noted rod forms in all three major types (Influenza A PR8, Weiss,<sup>‡</sup> W. S.,<sup>‡</sup> and FM

5. Heinmets, F., *J. Bact.*, 1948, v55, 823.

6. Dawson, I. M., and Elford, W. J., *Nature*, 1949a, v163, 63.

7. Chu, C. M., Dawson, I. M., and Elford, W. J., *Lancet*, 1949, vi, 602.

8. Isaacs, A., Edney, M., Donnelley, M., and Ingram, M. W., *Lancet*, 1950, vi, 66.



**FIGS. 1 and 2.** Electron micrographs from areas of Influenza A (PR8) infected tissue cultures where cell destruction was observed. Spherical and filamentous forms are seen in close association. *Mag.*  $\times 12,900$ . (Fig. 2 is unshadowed).

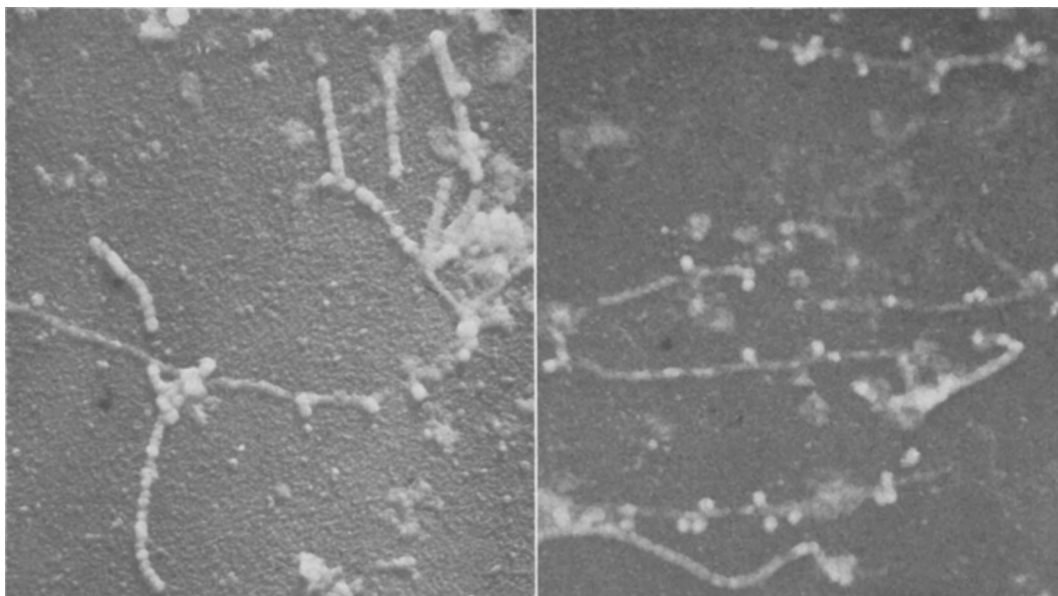


FIG. 3 (left). Electron micrograph of an area from an infected tissue culture with segmentation of filaments showing varying periodicities. ( $\times 23,000$ ).

FIG. 4 (right). Another area showing suggestive evidence of a breakdown of the filaments and a release of spherical particles. ( $\times 23,000$ ).

No. 1,<sup>†</sup> Influenza B Lee,<sup>‡</sup> and Swine Influenza V15) but not in allantoic fluid from normal eggs or those infected with Mumps, Newcastle disease virus, or Eastern Equine Encephalitis virus. We have extended this study to observations made directly on cells infected with influenza A (PR8) virus.

*Method.* Maximow flying cover slip cultures were used exclusively in this study following the technic of Porter, Claude and Fullam(1). Originally, plasma clot cultures were used, introducing the virus at the time the cultures were started, but after 10 experiments, averaging 4 infected and 4 control cultures each, we had seen only very occasional evidence of virus. At this point, in order to obtain a very high cell infection rate and to reduce the time in tissue culture to a minimum, the following method was adopted.

Seven or 8 day old chick embryos were inoculated onto the chorio-allantoic membrane with 0.2 cc of a suspension of influenza A (PR8) virus containing  $10^8$  infective units per cc (10% allantoic fluid). This strain had

been originally obtained from Dr. R. E. Shope in 1946 and maintained by about 15 embryo passages since then. The eggs were incubated at 35°C for 24 or 48 hours and the chorio-allantoic membrane was removed, cut into small pieces, and set up in Maximow cultures using Simms' ultra-filtrate medium(9). The cultures were incubated at 35°C for 24 hours and fixed with 2% osmium tetroxide vapor for from 2 to 7 hours, washed, dried and shadowed with chromium. Five experiments have been done totaling 9 cultures from influenza infected eggs and controlled with 6 active and 6 inactive preparations of a pox virus, probably herpes simplex, and 6 active and 4 inactive preparations of Newcastle Disease (CG) virus. Inactivation was performed by heating to 65°C for 30 minutes.

*Results.* Fig. 1 and 2 show masses of the elongated forms mentioned above and seen in most of our tissue cultures in areas in which the cells were destroyed. The co-existence of typical spherical influenza particles and filaments is seen. Isolated instances of clumps

<sup>†</sup> Courtesy of Dr. T. G. Ward, Johns Hopkins University School of Hygiene.

9. Simms, H. S., and Sanders, M., *Arch. Path.*, 1942, v33, 619.

of almost pure rods have been seen and one wandering cell, presumably a macrophage, showed a large preponderance of spherical forms, but the vast majority of infected cells show the co-existence of the two shapes.

In some favorable areas it was possible to see gradations between filaments and spheres. Fig. 3 shows rods with segmentation varying in period, the largest being approximately 100 m $\mu$  or the same as the spherical particles. Some seemingly "degenerate" filaments are shown in Fig. 4 with spheres spaced along them which suggest that segmented filaments may break down releasing spherical forms.

It is important to note that the number of segmented or transitional forms seen is much higher in these tissue cultures than in influenza virus preparations from allantoic fluid. Neither spherical nor rod-like forms have been seen in the controls.

**Discussion.** There is good evidence that the elongated filaments seen in influenza virus infections of the chick embryo are intimately connected with the development of the virus for 4 reasons. First, the rods have been seen in 6 old strains of influenza virus and in several newly isolated ones(7,8), all strains examined having shown them. Normal allantoic fluid from embryos infected with other viruses have not shown them; second, other

investigators(7) have shown that the filaments adsorb and elute from red cells in the same way that the spherical virus particles do, an observation which we have confirmed; third, we have shown in this article that on direct examination of tissue culture epithelial cells bearing the infection, filaments and spheres appear in close association with each other; fourth, segmented or beaded forms in various stages suggesting a transition from filaments to spheres have been demonstrated.

**Summary.** 1. Epithelium from influenza A infected chick chorio-allantoic membranes were grown in tissue culture and examined with the electron microscope.

2. The infected cells showed spherical virus particles in close association with elongated rod-like structures like those seen in ultracentrifuged allantoic fluid from influenza infected eggs.

3. Various gradations between unsegmented rods and beaded chains were seen in certain areas strongly suggesting a link between the two forms.

The authors express their thanks for the aid given them in the use of this tissue culture technique by Drs. K. R. Porter and P. Vanamee of the Rockefeller Institute in New York.

Received March 10, 1950. P.S.E.B.M., 1950, v73.

## Thyroid Hormone and Tissue Cholesterol Distribution.\* (17757)

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A number of investigators have demonstrated, both in humans and in experimental animals, that there is an inverse relationship between the activity of the thyroid gland and

the plasma cholesterol level(1). The influence of the thyroid hormone on the cholesterol concentration of other tissues, however, has been studied only to a very limited extent(2). In view of the importance of this problem for

\* This study was supported by grants from the Life Insurance Medical Research Fund and the National Heart Institute, National Institutes of Health, U. S. Public Health Service. The authors wish to express their appreciation for the use of the facilities of the Hancock Foundation. Contribution No. 248, from the Department of Biochemistry and Nutrition, University of Southern California.

1. Reviewed in: Peters, J. P., and Van Slyke, D. D., *Quant. Clin. Chemistry, Interpretations*, I, Baltimore, 1946, 497.

2. (a) Forbes, J. C., *Endocrinol.*, 1944, v35, 126; (b) May, L. G., Moseley, R. W., and Forbes, J. C., *Endocrinol.*, 1946, v38, 147; (c) Fleischmann, W., and Shumacker, H. B., *Bull. Johns Hopkins Hosp.*, 1942, v71, 175.