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THE LIPID CONSTITUENTS OF INFLUENZA VIRUS,
CHICK ALLANTOIC MEMBRANE,
AND SEDIMENTABLE ALLANTOIC PROTEIN

L. H. Frommhagen, N. K. Freeman, C. A. Knight

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Virus Laboratory and Donner Laboratory of Medical Physics
University of California, Berkeley, California

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PR8, DSP (an A strain kindly provided by Dr. L. Hoyle), and Lee strains of influenza virus, grown in chick embryos, were purified by a combination of differential centrifugation and adsorption on and elution from a column of aluminum phosphate-silica gel. Our modification of the column procedure (1) also yielded as a by-product, relatively large amounts of purified sedimentable material which appears to be identical to the "sedimentable protein" from normal allantoic fluid (2), and hence we have termed it sedimentable protein from infectious allantoic fluid. These and related materials have now been compared with respect to character and content of lipid.

Lyophilized and dried preparations of PR8, DSP, and Lee virus, sedimentable proteins from normal and infectious allantoic fluid, and intact chick allantoic membrane were extracted with chloroform:methanol (2:1) for 8-30 hours with constant stirring. The residue was centrifuged off and washed with diethyl ether several times. The total extract was taken to dryness and re-extracted with petroleum ether.

The petroleum ether extract was chromatographed on a silicic acid micro column and the fractions analyzed by infra red spectroscopy for esterified and unesterified sterols, triglycerides ("neutral fat"), and phospholipid by a modification of the method of Freeman et al (3) for determination of serum lipids.

The sum of the extraction and fractionation data, infra red determinations, and phosphorus analyses has led to the following conclusions:

(1) The phospholipid content of all three strains of influenza virus (PR8, DSP, and Lee) is $11.5 \pm 1.0\%$. Previous estimations of phospholipid (2, 4, 5), based upon the phosphorus content of the lipid extracts, are thus slightly lower than the value now obtained by a direct infra red method.

(2) All three strains of influenza virus contain at least three phospholipids in approximately the same ratio: cephalin (40%), sphingomyelin (30%), and lecithin (15%). In addition, another compound lipid having the characteristics of a glycolipid, was detected in an amount of 5% of the total phospholipid.

(3) All three strains of influenza virus contain $6.5 \pm 0.6\%$ unesterified cholesterol.

(4) The "neutral fat" (triglyceride) of all three strains of influenza virus is negligible (less than 0.3%).

(5) The petroleum ether insoluble fraction of the $28 \pm 2\%$ extracted by the chloroform:methanol has been shown to consist of a portion of the total sphingomyelin, and proteinaceous material.

(6) The sedimentable protein of normal allantoic fluid and the sedimentable protein of infectious allantoic fluid resemble each other, but are contrasted from the virus, by a low content of phospholipid (about 7%) and cholesterol (about 4%). They are analogous to the virus, however, in possessing, in the same proportions, the same phospholipids and a negligible quantity of triglyceride.

(7) The phospholipids of the allantoic membrane match those of the virus and sedimentable proteins in type. In addition, the ratios of the individual phospholipids and the proportions of phospholipid:cholesterol:triglyceride are of the same order as those of the influenza virus preparations. The latter findings support Hoyle's postulate (6) that the lipid of influenza virus is derived from the cells in the allantoic membrane.

Earlier investigations (2, 5) reported the presence in influenza virus of 0.85-0.97% phosphorus, but 12% phospholipid and 1% nucleic acid (4, 7) account for only 0.6% phosphorus. This discrepancy remains to be explained, although some of our data suggest that the higher phosphorus values of the early investigations may have resulted from the variable absorption of inorganic P from the media. For example, influenza virus purified by the aluminum phosphate column method has a phosphorus content of 1.4%, whereas influenza virus purified by conventional methods in the absence of phosphate possesses only 0.74% phosphorus. However, even the latter figure possibly includes some inorganic P derived from allantoic fluid which contains about 160 μg m of inorganic P per ml.

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