Quantitative Changes in Lipid Composition of Tissues Infected with Fowlpox Virus.*† (23942)

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Numerous investigations have been made of the effects of animal viruses on metabolism of their hosts. Bauer(1) and Putnam(2) have reviewed much of the previous work. Many of these studies have been concerned with respiration and with nucleic acid and phosphorus metabolism. No investigations have been concerned primarily with lipid metabolism. It has been demonstrated(3) that lipids in stainable quantities accumulate in the inclusion bodies found in chick skin or chorioallantoic membrane after infection with fowlpox virus. In order to understand better the pathogenesis of this infection a study was made of lipid distribution of normal and infected chick skin and chorioallantoic membrane. The results indicate that quantitative changes in lipid composition of these tissues accompany infection with fowlpox virus.

Materials and methods. Virus. A field strain of fowlpox virus obtained from Dr. E. R. Doll, University of Kentucky was employed. In this laboratory the agent had been passed 5 times in chick scalp. To procure a large amount of inoculum, the last passage was made in 4 dozen chicks. This stock material was stored in small amounts at –20°C until used. *Fertile eggs* were obtained from local hatchery. Eleven-day-old eggs were inoculated on the chorioallantoic membrane (CAM) by the classical window method and incubated separately from controls, both groups at 37°C. Control CAM were collected at 16 days. Whole membranes were collected from control eggs, whereas only lesions were collected from infected eggs. Analyses were made upon 5 groups each of normal and infected CAM. Each group consisted of from 75-85 membranes. *Day-old "Hyline" cockerels* obtained from local commercial hatchery were inoculated by scrubbing 1:5 or 1:10 saline dilution of the virus into the plucked scalp. Normal and infected tissues were collected from chicks at 7 days of age. Normal tissues consisted of scalps of 4 groups of birds (50-60/group), while infected scalps were obtained from 5 groups of infected chicks (50-60 group). Chicks were fed a balanced "starter" ration and water *ad libitum. Extraction.* Specimens of CAM and skin were frozen within 30-60 minutes after collection and maintained at –45°C. Subsequently they were dried to constant weight from frozen state by lyophilization. Extraction of lipid was performed by boiling the tissues 30 minutes in 95% ethyl alcohol followed by boiling for 30 minutes in Bloor's solvent and consequent continuous extraction for 48 hours by diethyl ether in a Soxhlet apparatus. Combined alcohol and ether extracts were evaporated to dryness under nitrogen, and lipids extracted exhaustively from the residue with petroleum ether (B.P. 30-60°C). After evaporation of petroleum ether under nitrogen, total lipids were dried to constant weight. The weighed lipids were redissolved in known volume of petroleum ether, usually 50 to 100 ml, and aliquots taken for various determinations. All *chemical determinations* were done in triplicate. *Cholesterol.* The Sperry-Webb(4) modification of Schoenheimer-Sperry method for cholesterol was used for determinations of free and total cholesterol. Esterified cholesterol was calculated as the difference between free and total cholesterol. The amount of fatty acid associated with cholesterol was estimated using the formula of Bloor(5). Color densities were read in Bausch and Lomb colorimeter at 625 mμ and compared with similarly treated samples of known cholesterol concentration. *Free fatty acids.* Suitable

* This investigation was supported in part by grant from Nat. Inst. of Allergy and Infect. Diseases, N.I.H., U.S.P.H.S.

† Presented in part at annual meeting of Soc. Am. Bact., Detroit 1957. Taken in part from Ph.D. research of W.M.T. at Vanderbilt Univ. School of Medicine.

TABLE I. Percentage Composition of Chick Skin Lipids.

Constituents	Normal skin	Infected skin	P*
Total lipid†	23.95 ±1.24‡	19.46 ± .68	.02
Total cholesterol	9.95 ±1.02	13.46 ± .75	.05
Free "	6.65 ± .72	7.82 ± .29	.10
Esterified "	3.28 ± .29	6.49 ± .40	.001
Phospholipid	18.48 ±2.21	23.18 ±1.54	.10
Free fatty acids	12.38 ± .40	15.78 ± .98	.025
Total " "	73.50 ±1.19	60.84 ±3.34	.02

* Student's t-test for significance. If P is less than .05, the change is considered significant.
† Calculated on basis of dry CAM wt.
‡ Mean ± stand. error.

aliquots of petroleum ether extracts were evaporated to dryness, dissolved in ethanol and titrated with 0.02 N NaOH. The factor used for converting titration results (milliequivalents) to mg was 0.282, the theoretical value for oleic acid. *Total fatty acids* were titrated as described above after saponification and extraction by modification of the method of Kelsey(6). *Concentration of phospholipid* was estimated by determining lipid phosphorus by the method of Fiske and Subbarow(7) and multiplying the value in mg by 25. The amount of fatty acids found in phospholipids was calculated from the phospholipid value using the formula suggested by Bloor(5). A check of the extraction procedure was performed by dissolving a weighed sample of tripalmitin in petroleum ether and subjecting it to extraction procedures previously described for the tissue samples. Titration of free fatty acids determined that there was no increase in fatty acids due to extraction procedure. To test for presence and activity of lipase in tissues, individual tissues from one group each of normal CAM and chick scalps, respectively, were frozen immediately after collection in containers immersed in dry ice and acetone. Two similar groups of control tissues were stood at room temperature for one hour after collection and then frozen. Both groups of tissues were carried through same procedure of lyophilization and extraction. Titration revealed that the content of free fatty acids was the same, within experimental error.

Results. Percentage composition of lipid extracted from normal and infected chick skin is given in Table I. Fractions undergoing significant changes after infection include total cholesterol, esterified cholesterol, and free fatty acids, all of which increased, and total fatty acids, which decreased. When results are calculated on a dry skin weight basis total lipid, esterified cholesterol and total fatty acid values show significant change. The range in concentration of other components reveals extent of physiologic variation.

Percentage composition of normal and infected CAM lipid is indicated in Table II. Concentrations of free cholesterol and phospholipid show significant decreases in infected membranes in contrast to increased values for esterified cholesterol. When results are calculated on basis of dry weight all components show significant change except phospholipid.

Discussion. Addition of individual lipid fractions reveals that 105% of the lipid in chick scalp, and 101% of that found in chorioallantoic membranes was recovered. These figures are within experimental error of the methods.

Statistical analysis of the data suggests that there is an alteration in lipid metabolism of tissues after infection with fowlpox virus, as indicated by quantitative changes in tissue lipids. That this effect may not be specific for fowlpox is suggested by the work of Cohn (8) who found decreased amounts of phospholipid phosphorus in CAM infected with influenza virus. Cornatzer(9) found a decrease in total lipid in papillomata produced in rabbit skin by the Shope virus, but phospholipid phosphorus increased in the lesion. Miroff, Cornatzer and Fischer(10) found while studying phosphorus metabolism of poliomyelitis-infected HeLa cells that although there was no net increase in phospholipid, there was an increased uptake of P^{32} into the phospholipid

TABLE II. Percentage Composition of Chorioallantoic Lipids.

Constituent	Normal CAM	Infected CAM	P*
Total lipid†	15.98 ± .74‡	23.72 ± .56	.001
Total cholesterol	17.60 ± .93	16.22 ± .50	.20
Free "	16.90 ± .86	4.30 ± .14	.001
Esterified "	.71 ± .09	11.85 ± .44	"
Phospholopid	53.36 ±1.38	32.04 ±1.50	"
Free fatty acids	5.38 ± .70	7.18 ± .29	.10
Total " "	47.66 ±4.00	54.14 ±3.72	.25

* Student's t-test for significance. If P is less than .05, the change is considered significant.
† Calculated on basis of dry CAM wt.
‡ Mean ± stand. error.

of cells. Other work of a similar nature has been summarized by Blank(11).

At present the site of metabolic alteration is not known. It is possible that infected cells are capable of metabolizing lipids in an abnormal fashion under the influence of the virus. An alternate possibility is that the liver of chick and embryo may have been metabolically damaged by the virus. Goodpasture(3) pointed out that there is a viremia in fowls infected with fowlpox, despite the fact that epithelium and mucous membranes are the only tissues which show lesions. Cohn (8) employed de-embryonated eggs in his study of phosphorus metabolism as affected by influenza virus infection and noted a decrease in CAM phospholipid phosphorus, the same effect noted in our study in which embryonated eggs were used. Gould and Taylor(12) have shown that the skin of certain species can synthesize cholesterol at a rate comparable to that in liver. Similar information concerning the CAM is not available.

In CAM, the virus seems to have produced a generalized increase in lipid with the exception of phospholipid and free cholesterol fractions. This increase may reflect an increased synthesis of lipid or a decreased ability of tissue to utilize this class of compounds. Changes in phospholipid and cholesterol may be explained by postulating that free cholesterol and the fatty acids formerly esterified to phospholipids have been used to form cholesterol esters. Apparently there has been an increased accumulation of fatty acids, some of which are present as free fatty acids.

Summary. 1) The normal lipid composition of chick skin and of chorioallantoic membrane is reported. Evidence is presented which indicates that there is an alteration in lipid metabolism of these tissues when infected with fowlpox virus. 2) This evidence consists of: (1) On dry weight basis, infection of the chick resulted in decreased amount of total skin lipids, the greater part referable to total fatty acid fraction. The level of esterified cholesterol became elevated. (2) Partition studies of chick skin lipid revealed that concentration of total cholesterol, esterified cholesterol and free fatty acids increased in contrast to lowered total fatty acid values. (3) Dry weight analyses of infected chorioallantoic membrane demonstrated a general increase in lipid components with the exception of free cholesterol and phospholipid, which decreased and were unchanged, respectively. (4) Partition of CAM lipid disclosed that free cholesterol and phospholipid decreased in percentage while esterified cholesterol exhibited a 15-fold increase during infection.

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Received March 7, 1958. P.S.E.B.M., 1958, v98.