Quantitative Distribution of Phosphorus in Chorioallantoic Membrane as Affected by Infection with Influenza Virus.* (19448)

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In recent years interest has been focused on the chemical changes occurring in host tissues as a result of virus infection. Demonstration of chemical changes occurring in the host should make possible a more detailed study on the intermediate mechanisms of infection. This paper is a preliminary report dealing with the quantitative distribution of various phosphorus fractions in the chorioallantoic membrane as affected by infection with the PR-8 strain of influenza virus.

The multiplicity of organs and tissues present in the intact animal or embryonated egg make studies of this nature difficult and equivocal. The de-embryonated egg(1), with its isolated chorioallantoic membrane, was decided upon in an attempt to utilize a relatively more homogeneous cell population. Although this is essentially a tissue culture system, high titers of influenza virus can still be consistently produced in 24 hours, with little gross pathology evident in the membrane.

Material and methods. Eggs of 15 days incubation were de-embryonated and the adherent chorioallantoic membrane carefully washed with four 15 ml rinses of cold isotonic saline. The de-embryonated eggs were then partly filled with 10 ml of Hanks' solution(2), containing 10 units of penicillin and 40 μg of streptomycin per ml. In the virus experiments allantoic fluids from eggs infected with the PR8 strain of influenza A were diluted a 100-fold in the Hanks' solution which was added to each egg. These diluted fluids contained approximately 10 hemagglutinating units per ml. The eggs were capped and rolled (1 rpm) at 37°C. After varying intervals of incubation, the membranes of 2 eggs were removed from the shell, pooled, blotted in a constant fashion, and minced with fine scissors. The mince was then transferred to a tared 15 ml centrifuge tube and weighed on a chainomatic balance. This 2-membrane-pool then served as the material for subsequent analysis. Hemagglutination determinations were made on the suspending fluids at time of harvesting, according to the method of Salk(3). For the measurement of the phosphorus fractions the extraction procedure was the Schmidt and Thannhauser method(4), modified in that small amounts of tissue were used, and tissue residues were centrifuged. Phosphoprotein phosphorus was separated by the Delory(5) procedure. Aliquots of the various fractions were analyzed for total phosphorus by the method of Fiske and Subbarow(6).

Results. Table I includes the averages of the results of 5 experiments designed to show changes in the phosphorus content of the isolated chorioallantoic membrane after 12 and 24 hours incubation, both with and without virus. The slight difference in the nucleic acid fractions between the infected and normal membranes at 24 hours are not significant with the number of experiments completed. The striking decreases in the phosphorus content of the phospholipid fraction of the infected tissue seemed important enough so that further experiments were done at other intervals of infection.

Table II shows the values of phospholipid phosphorus for infected and noninfected membranes. In each case the value given is the average of 5 determinations. A rapid decrease in the phospholipid phosphorus occurred during the first 12 hours of infection. The great variations in the values for the 8-hour determination suggest a rapid flux at this period. Fig. 1 shows the relationship between the decrease in the phospholipid phosphorus and the rise in hemagglutinin titer.

Semi-quantitative determinations were performed on the fluid medium from the infected de-embryonated eggs in an effort to account for the phospholipid which disappeared from the membrane. The medium was dehydrated

* This work was supported in part by grants from the Rockefeller Foundation and the National Institutes of Health, U. S. Public Health Service.

TABLE I. Concentration of Phosphorus Fractions in Infected and Non-Infected Chorioallantoic Membranes at 12 and 24 Hr of Incubation.

Fraction	γ P/500 mg tissue 12 hr incubation		γ P/500 mg tissue 24 hr incubation	
	No virus	Virus	No virus	Virus
Total acid soluble	820	826	834	821
Lipid phosphorus	320	109	326	120
Total acid insoluble	376	384	380	410
Desoxyribonucleic acid	162	158	160	180
Ribonucleic acid	216	224	220	240
RNA/DNA ratio	1.33	1.41	1.38	1.33

TABLE II. Effect of Virus Multiplication on Phospholipid Phosphorus Content of Infected and Non-Infected Membranes.

Incubation time, hr	Phospholipid concentration, γ P/300 mg tissue	
	Non-infected	Infected
0	327 ± 16.3*	320 ± 19.2
8	324 ± 12.9	191 ± 28.7
12	334 ± 20	110 ± 5.5
16	326 ± 14.7	126 ± 8.82
20	320 ± 18.4	112 ± 6.7
24	325 ± 15.1	120 ± 6
36	309 ± 9.2	114 ± 5.1

* Stand. dev. $= \sqrt{\frac{\Sigma d^2}{N-1}}$.

in vacuo, and phospholipid was extracted according to the Schmidt-Thannhauser(4) method after the initial extraction with aqueous trichloracetic acid. The lipid extract was analyzed for phosphorus. The results indicate that at least 50-60% of the phosphorus liberated by action of the virus in the membrane remains lipid-bound in the medium.

Another group of experiments was performed, in which 5 μc of P^{32} was added per egg. The membranes were minced and extracted in the standard manner, and both P^{31} analysis and P^{32} counts were done on each fraction. There was a marked increase in the specific activity of the phospholipid fraction, as shown in Table III. The RNA values showed increases in activity in the infected group, but this cannot be considered too significant, since small and variable amounts of contamination by phosphoprotein phosphorus would considerably alter the values.

Discussion. With the procedures utilized, the nucleic acid phosphorus concentrations following infection were not significantly altered during the time periods studied. The rapid decreases in phospholipid phosphorus, and the presence of considerable quantities of lipid-bound phosphorus in the culture medium, suggest that phospholipid molecules as such or in combination with other cellular constituents are liberated from a cell infected with the influenza virus. Since not all of the liberated phosphorus could be accounted for in the culture medium as lipid-bound phosphorus, it may be that some is liberated in the form of inorganic phosphorus.

The increased uptake of P^{32} into the phospholipid fraction following infection may be explained either by increased phospholipid synthesis, or possibly as a result of a phosphoroclastic type of reaction.

It would be of utmost importance if one

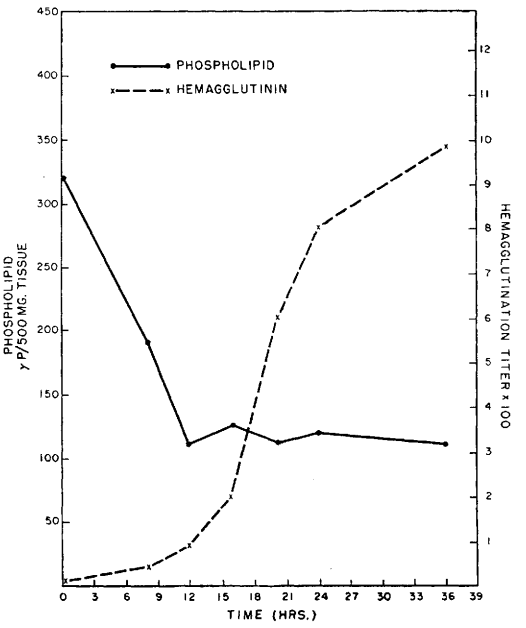

FIG. 1. Relation between decrease in phospholipid content and rise in hemagglutination titer.

TABLE III. Effects of Virus Multiplication on Relative Specific Activity of Phosphorus Fractions at 24 Hr Incubation.

Fraction	Relative specific activity*	
	Non-infected	Infected
Phospholipid	3.15	5.86
Desoxyribonucleic acid	.65	.67
Ribonucleic acid	3.60	4.20

* Rel. spec. activity—Specific activity (counts/minute/μg of P^{31}) of each fraction as % of specific activity of inorganic P fraction.

could ascertain the site of the reacting phospholipid in the cell. It has been shown by Friedkin(7) that mitochondria, which contain large amounts of phospholipids, take up P^{32} and incorporate it into their phospholipid fraction. In view of Friedkin's work, it is an interesting consideration that the observed changes in P^{32} uptake of infected tissue may occur in the mitochondria.

Since it is also known that considerable phospholipid is found in cell membranes, there may be a relation between phospholipid liberation and the penetration of the virus particle into the cell. The work of Howe(8) who found large amounts of the red cell receptor in a stroma complex rich in phospholipid, seems pertinent.

Because it has been shown(9) that large differences in the proportion between the hemagglutinating and infectious properties of influenza virus may occur in de-embryonated eggs, nothing can as yet be postulated concerning these findings and the infectious form of the virus.

Summary. It has been shown that infection of the chorioallantoic membrane by the PR-8 strain of influenza virus results in: 1. The decrease in the phospholipid phosphorus concentration of the membrane; 2. The presence of large amounts of lipid-bound phosphorus in the fluid medium; and 3. A more rapid uptake of P^{32} into the membrane.

The author wishes to express his sincere gratitude to Dr. Boris Magasanik for his assistance in the course of this work, and to Dr. A. K. Solomon for his help in the procurement and analysis of the radioactive materials.

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Received February 14, 1952. P.S.E.B.M., 1952, v79.

Lack of Interference of Chloramphenicol with Penicillin in a Hemolytic Streptococcal Infection in Mice. (19449)

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Considerable interest has been shown in the recent demonstration by Jawetz and coworkers that aureomycin, terramycin, and chloramphenicol interfere with the early bactericidal action of penicillin(1-6). When any of the 3 antibiotics was combined with penicillin *in vitro,* susceptible organisms were destroyed more slowly than when exposed to penicillin alone. Interference also occurred in mice infected with group A streptococci; animals that received both chloramphenicol and penicillin had a higher mortality rate than those treated with penicillin alone. Effective blood levels were present for less than 3 hours, however, so that death or survival of the animals depended upon brief antibiotic