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Cellular Fatty Acids during Fowlpox Virus Infection of Three Different Host Systems

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Fatty acid compositions of host cells during fowlpox virus infection have been studied by complementary thin-layer and gas–liquid chromatographic techniques. Three different host systems have been studied: the *in vivo* infection of the chick scalp, the *in ovo* infection of the chick embryo chorioallantoic membrane, and the *in vitro* infection of chick embryo fibroblast cell cultures. Extensive alterations in fatty acid composition of the host cell accompany the fowlpox infection of the chick scalp. The noticeable trends are toward greater unsaturation and a depletion of odd numbered fatty acids. In contrast, the fatty acid compositions of the chorioallantoic membrane and chick embryo cell cultures are not altered significantly following fowlpox virus infection. A slight increase in pentaenoic fatty acids was the only consistent effect of infection in these systems. To the extent that the fatty acid composition of a cell reflects its fatty acid metabolism, it can be concluded that the alterations in fatty acid metabolism observed in the chick scalp are not a constant feature of fowlpox virus infection, and therefore probably represent a host-determined response to infection.

Fowlpox virus contains an unusual lipid component for a virus, both in quantity and in quality. Lipids comprise 34% of the dry weight of the virion (*1, 2*), which is unusually high for a pox virus (*4*). In addition, the presence of squalene and cholesterol esters in the virus finds no counterpart in the lipid compositions of other viruses which have been studied (*2*). The unusual viral lipid composition reflects alterations in the lipid composition of the host cell upon infection with the virus (*2, 3*). An important tool in the study of viral effects on lipid metabolism has been to cultivate viruses in different cell types (*4*). The variety of situations under which fowlpox virus will replicate is somewhat limited, since it has only been cultivated in avian cells (*5*). However, even within the avian system a wide variety of responses to fowlpox virus is seen, depending on the conditions of cultivation. The three principal modes for cultivating fowlpox virus are direct inoculation of the chick scalp, inoculation of the chorioallantoic membrane of the chick embryo, or replication in cell culture using embryo fibroblasts. The alterations in cholesterol metabolism seen during fowlpox infection (*2, 3, 6*) appear to be characteristic of all three systems (*7*). This investigation was undertaken to determine whether the fowlpox-induced changes in host fatty acid composition observed in the chick scalp (*2*) are also a constant feature of fowlpox virus infection.

The methods of cultivation of fowlpox virus in the chick scalp, the chorioallantoic membrane, and chick embryo fibroblast cultures have been described (*3, 5*). Infected and mock-infected epithelial cells were isolated from chick scalp by treatment with trypsin (*8*). Epithelium was isolated from the chorioallantoic membrane by shaking with glass beads (*9*). Second-

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ary monolayer cultures of chick embryo fibroblasts were harvested and washed once with saline. The time of harvest was during the period of maximum virus production in each system, 5 days postinfection in the chick scalp (*10*), 4 days postinfection in the chorioallantoic membrane (*11*), and 2 days postinfection in the cell culture system (*5*). Cells were extracted with chloroform:methanol (2:1, v/v) according to the method of Folch *et al.* (*12*). Fatty acids in the total lipid were converted to the corresponding methyl esters by refluxing in 6% sulfuric acid in anhydrous methanol. The fatty acids were separated into unsaturation classes by argentation thin-layer chromatography (*13*) followed by repurification by thin-layer chromatography on Silica gel HR. Gas chromatographic analyses of the fatty acid methyl esters in each unsaturation class were done on a glass column (120 cm × 6 mm, o.d.) packed with 5% diethylene glycol succinate polymer coated on 80 to 100 mesh Diatoport S. Column temperature was programmed from 100–210° at 3° per minute. Helium flow rate was approximately 75 ml/min. An instrument equipped with a hydrogen flame ionization detector was used. In cases where the chain length of a particular unsaturated fatty acid was in doubt, an aliquot of the sample was hydrogenated (*14*) and the resulting saturated fatty acid mixture was analyzed by gas chromatography.

In each system studied, more than 30 different straight chain fatty acids were identified and quantitated. No branched chain members were detected. Chain lengths varied from 10 to 28 carbon atoms, and unsaturation ranged from 0 to 6 double bonds. To simplify the presentation of these results, the unsaturation class distribution of the fatty acid mixtures is presented in Table 1. Table 2 lists overall parameters characterizing the fatty acid

TABLE 1

UNSATURATION CLASS DISTRIBUTION OF FATTY ACIDS OF NORMAL AND FOWLPOX-VIURS-INFECTED CHICK CELLS

Fatty acid unsaturation class	Culture system					
	Chick embryo cell culture		Chorioallantoic membrane epithelium		Chick scalp epithelium	
	Control	Infected	Control	Infected	Control	Infected
	(Mole% of total fatty acids)					
Saturated	36.6	41.0	42.2	40.8	60.5	54.0
Monoenoic	43.2	38.6	27.3	26.0	24.7	31.1
Dienoic	2.6	2.0	12.4	13.8	13.2	12.4
Trienoic	4.5	3.8	1.4	1.2	1.02	0.88
Tetraenoic	9.0	10.1	13.1	12.0	0.56	1.67
Pentaenoic	1.8	2.4	1.1	1.9	–	–
Hexaenoic	2.0	2.2	2.2	2.9		

TABLE 2

OVERALL PARAMETERS OF THE FATTY ACID COMPOSITION OF NORMAL AND FOWLPOX-VIRUS-INFECTED CHICK CELLS

	Culture system					
	Chick embryo cell culture		Chorioallantoic membrane epithelium		Chick scalp epithelium	
	Control	Infected	Control	Infected	Control	Infected
Unsaturation index	119.0	118.9	127.8	132.0	56.5	65.2
Average chain length	18.3	17.5	18.0	18.0	17.4	17.4
Mole percentage odd-numbered fatty acids	0.93	0.98	1.05	1.16	7.28	4.36

mixtures, including unsaturation index (average number of double bonds per fatty acid molecule × 100) (*15*), average chain length, and percentage odd-numbered fatty acids.

The striking result of these data is that in only one system, namely the chick scalp epithelium, does fowlpox virus appear to alter significantly the fatty acid composition of the host cell. The changes brought about in this system, as noted previously (*2*), are an increase in overall unsaturation (in particular, an increase in the monoenoic fatty acid fraction) and a dramatic decline in odd-numbered fatty acids. Neither effect is seen upon infection of chorioallantoic epithelium or chick fibroblast cell cultures with fowlpox virus. The only differences noted in these two systems were slight elevations in the pentaenoic fatty acids in infected cells. The major pentaenoic fatty acid was 22:5ω6 and 22:5ω3 in the chorioallantoic membrane and chick embryo cell cultures, respectively.

To the extent that the fatty acid composition of whole cells reflects the fatty acid metabolism of these cells, it can be concluded that alterations in fatty acid metabolism are not a constant feature of fowlpox virus infection. It is possible that more detailed examination of subcellular distribution or lipid class distribution of fatty acids may reveal differences in infected chorioallantoic membrane and cell cultures. If such differences exist, they apparently are not dramatic enough to alter the overall fatty acid composition in these two systems. These observations suggest that changes in fatty acid composition observed in fowlpox-infected chick scalp epithelium may be a host response to infection rather than a virus-directed effect. Alternatively, the virus may be able to acquire the fatty acids it needs in chorioallantoic membrane and cell cultures from the fatty acids already existing in the cell. The fatty acid composition of the virus reflects the changes observed in the scalp epithelium, i.e., greater unsaturation and less odd-numbered fatty acids than normal scalp (*2*). The fact that the other two systems already have much more unsaturated fatty acids and fewer odd-numbered fatty acids may be significant in this regard.

The alterations in fatty acid composition observed in the chick scalp remain of interest, despite the fact that they find no counterpart in the other two systems studied. Investigation of this viral effect may reveal factors controlling fatty acid metabolism and how they may be altered in pathological states.

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