Measles-virus-persistent infection in BGM cells

Modification of the incorporation of [^3H]arachidonic acid and [^{14}C]stearic acid into lipids

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In BGM cells chronically infected with measles virus, although the composition of the phospholipids is unaltered, the fatty acid composition is modified. Uninfected, lytic and persistently infected cells were labelled with [^3H]arachidonic acid and [^{14}C]stearic acid and their metabolic fate analysed. No difference in the total incorporation was observed in the different systems. However, the [^{14}C]stearic acid and [^3H]arachidonic acid were incorporated up to 2-fold and 13-fold respectively greater into the neutral lipid of persistently infected compared with that of uninfected cells. Both radioactive fatty acids were specifically accumulated in the triacylglycerol and non-esterified fatty acid fractions. Lytically infected cells were similar to uninfected cells. Although there was no significant difference in the incorporation of radioactivity into the total phospholipid in either system, there was a large decrease in [^3H]arachidonic acid incorporated into phosphatidylethanolamine and to a lesser extent phosphatidylcholine and phosphatidylinositol in persistently infected cells. [^{14}C]Stearic acid incorporation was also reduced in phosphatidylcholine and phosphatidylethanolamine fractions of persistently infected cells.

Several viruses are now implicated in persistent and latent infections in man and a number of animal models have been developed (for review, see Stroop & Baringer, 1982). These studies have been complemented by the establishment *in vitro* of cell lines persistently infected with virus (for review, see Ter Meulin & Carter, 1982).

In the co-habitation, both virus and cells appear to undergo modifications. The virus under the less selective conditions of a non-lytic system accumulates mutations, which lead to an attenuated or weakened population of viruses (Holland *et al.*, 1982). In turn, the cell, to compensate for energy diverted to virus replication, reduces some of its non-essential functions, e.g. hormone or neurotransmitter synthesis (Oldstone *et al.*, 1977). The mechanisms by which these ancillary functions are turned off is unknown. Recently, Munzel & Koschel (1982) showed that, in a measles-virus-persistent infection of C₆ glioma cells, although the number of β -adrenergic receptors was unaltered, there was a

total loss of catecholamine-stimulated (β -adrenergic-receptor-dependent) methylation of phosphatidylethanolamine. This in turn would lead to a loss of secretion of arachidonic acid, which is a precursor of the prostaglandins.

Our initial studies of measles-virus-persistent infections in BGM (African-green-monkey kidney) cells showed that, although the composition of the phospholipids remained unaltered compared with that in uninfected cells, the fatty acid composition was modified. There was an increase in the palmitic acid content of phosphatidylcholine and phosphatidylethanolamine and also an increase in the arachidonic acid content of the latter (Anderton *et al.*, 1981). In the present paper we have studied the incorporation and metabolism of stearic acid and arachidonic acid in lytically, persistently and uninfected BGM cells. We show that in persistently infected cells, arachidonic acid is not entirely incorporated into phospholipid, but most of it is channelled into triacylglycerols. This is the result of

a decreased acylation by arachidonic acid of phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol.

Methods

Cells

African-green-monkey kidney (BGM) cells or their measles-virus-(Hallé strain)-persistently-infected derivative (BGM-P) were grown as monolayers as previously described (Wild & Dugré, 1978). In lytic infections, cells were infected at a multiplicity of infection of 0.1 plaque-forming unit/cell. A cytopathic effect was first observed at 18–20 h after infection.

Radiolabelling

Confluent monolayers were incubated in Eagle's medium containing 2% foetal-calf serum, 0.5 μ Ci/ml of [$1\text{-}^{14}\text{C}$]stearic acid (sp. radioactivity 56 Ci/mol) and 5 μ Ci of [5,8,11,14- ^3H]arachidonic acid/ml (sp. radioactivity 120 Ci/mmol) as non-esterified fatty acid for periods up to 8 h. Lytically infected cells were labelled from 10 h after infection. All radiochemicals were purchased from Amersham International. To obtain a constant isotopic dilution of the radioactive fatty acids by the non-esterified fatty acids of the serum, the same batch of serum was used for experiments. All experiments were done in duplicate and repeated twice.

Lipid extraction and analysis

Cells were scraped into cold iso-osmotic saline (0.9% NaCl) containing 0.14 mg of delipidated bovine serum albumin/litre and recovered by low-speed centrifugation. The lipids were extracted in at least 20 vol. of chloroform/methanol (2:1, v/v) by a modification of the method of Folch, which included washing with 0.2% KCl (Folch *et al.*, 1957; Anderton *et al.*, 1981). After evaporation, the lipid species were separated by t.l.c. on pre-coated silica-gel G-50 plates (Merck). Neutral lipids were resolved as described previously (Anderton *et al.*, 1982) and phospholipids by the method of Portoukalian *et al.* (1978). Neutral lipids were detected under u.v. light with Primulin reagent or standards were chromatographed in parallel and stained with vanillin/ H_2SO_4 (Skipski, 1975). Phospholipids were detected with Dittmer's reagent (Dittmer & Lester, 1964). The individual lipids were scraped into scintillation vials and suspended in 4 ml of 45% ethanol and 10 ml of Instagel (Packard). Counting efficiency was determined by the external-standard channels-ratio method for double-labelling with ^3H and ^{14}C . Results are expressed in d.p.m.

To verify the position of the radioactivity incorporated into the diacylglycerol moiety of the

phospholipid, phosphatidylethanolamine and phosphatidylcholine, which had been labelled with [^3H]arachidonic acid and [^{14}C]stearic acid, were separated by t.l.c. as described previously (Zwingelstein *et al.*, 1978) and incubated with phospholipase A_2 (EC 3.11.4) from *Crotalus adamanteus* venom (Sigma) at 37°C for 2 h in 10 mM- Ca^{2+} , pH 7.4 (Haverkate & Van Deenen, 1965). The lysophospholipids, the residual phospholipids and fatty acids were separated by t.l.c. in diethyl ether and then, in the same direction, chloroform/methanol/acetic acid/water (25:15:4:2, by vol.). The radioactivity was determined in the corresponding spots.

Results

Incorporation of arachidonic acid and stearic acid

Previous studies have shown that BGM and BGM-P cells have the same phospholipid content and incorporate ^{32}P into phospholipids in a similar manner (Anderton *et al.*, 1982). The ^{32}P -labelled phospholipid composition of these cells contained only minor differences (Anderton *et al.*, 1981). However, [^3H]palmitic acid incorporation is increased up to 2-fold in persistently infected cells and the fatty acid composition was found to show a 30% increase in palmitic acid in the phospholipid with a corresponding decrease in the proportion of oleic acid present. Further analysis of the individual phospholipids revealed a 50% increase in the arachidonic acid content of phosphatidylethanolamine. No significant variation in the stearic acid content was found between infected and uninfected cells (Anderton *et al.*, 1981). This state may arise as a result of a block in the further metabolism of arachidonic acid once incorporated into the phospholipid, or may be due to an increased metabolism of the fatty acid.

In order to test these hypotheses, BGM, BGM lytically infected with measles virus (BGM-L) and BGM-P cells were doubly labelled with [^3H]arachidonic acid and [^{14}C]stearic acid for 2, 4 or 6 h. At each point, cells were harvested, the lipid extracted and the incorporation of radioactivity determined (Table 1). There was a difference in the uptake of the two isotopes into the different systems at the early times examined. Further, the ratio of $^3\text{H}/^{14}\text{C}$ radioactivity incorporated into the cells (16.3 ± 1.1 in BGM, 13.5 ± 1.8 in BGM-L and 16.2 ± 0.9 in BGM-P cells), which is a measure of their relative uptake and exchange within the cell, was not significantly different in the three systems. However, these values are different from the initial ratio in the incubation medium (9.8 ± 0.3).

The lipid extracts were resolved into their neutral and polar species by one-dimensional t.l.c. and the partition of the ^3H and ^{14}C isotopes determined (Fig. 1, Table 2). The incorporation of both [^3H]arachi-

Table 1. Incorporation of [^3H]arachidonic acid ($\text{C}_{20:4}$) and [^{14}C]stearic acid ($\text{C}_{18:0}$) into uninfected (BGM), lytically (BGM-L) and persistently (BGM-P) infected cells

The results are means $\pm$ s.d. Abbreviation used: ND, not determined.

Labelling period (h)	Incorporation (d.p.m./ μg of protein)					
	BGM		BGM-L		BGM-P	
	[^3H] $\text{C}_{20:4}$	[^{14}C] $\text{C}_{18:0}$	[^3H] $\text{C}_{20:4}$	[^{14}C] $\text{C}_{18:0}$	[^3H] $\text{C}_{20:4}$	[^{14}C] $\text{C}_{18:0}$
2	99 $\pm$ 12	4.9 $\pm$ 0.5	ND	ND	87 $\pm$ 9	4.8 $\pm$ 0.4
4	119 $\pm$ 12	7.6 $\pm$ 0.6	148 $\pm$ 12	10.7 $\pm$ 1.2	137 $\pm$ 8	9.1 $\pm$ 0.9
6	121 $\pm$ 13	8.8 $\pm$ 0.9	161 $\pm$ 18	12.3 $\pm$ 1.1	155 $\pm$ 16	10.9 $\pm$ 1.1
8	152 $\pm$ 14	10.7 $\pm$ 1.3	171 $\pm$ 8	13.4 $\pm$ 1.1	181 $\pm$ 12	11.3 $\pm$ 1.0

Fig. 1. Incorporation of [^3H]arachidonic acid (a and c) and [^{14}C]stearic acid (b and d) into the neutral (c and d) and polar (a and b) lipids of uninfected (BGM), lytically (BGM-L) and persistently (BGM-P) infected cells

Cells were radiolabelled for periods up to 8 h. At 2 h intervals duplicate samples were extracted and the lipids were separated into the neutral and polar components. The experiment was repeated twice.

donic acid and [^{14}C]stearic acid into the neutral lipids was greater in BGM-P than in either uninfected or lytically infected BGM cells. The difference in the incorporation was greater with [^3H]arachidonic acid (up to 10-fold increase) than with [^{14}C]stearic

acid (up to 2-fold). The $^3\text{H}/^{14}\text{C}$ ratio was 2.2–3.3 for uninfected and lytically infected cells and 13.8–15.9 for persistently-infected cells (Table 2). In contrast with the neutral lipids, the partition of ^3H and ^{14}C in the total phospholipids after the various labelling periods was similar [mean 16.4 (14.6–18.4) for uninfected and mean 14.6 (14.0–16.8) for persistently-infected cells].

Neutral lipids

In order to locate the increased incorporation of radioisotope in the different components of the neutral lipid of BGM-P cells, the various neutral lipids were separated and the distribution of radioactivity determined (Table 3). In BGM-P cells, there was a 63–67-fold increase in the incorporation of [^3H]arachidonic acid into triacylglycerols, a 37–61-fold increase into non-esterified fatty acids and a 4–6-fold increase into diacylglycerols. Similar changes were observed only into triacylglycerols when labelling with stearic acid; also the differences were not of the same order of magnitude. Comparison of the ratios of $^3\text{H}/^{14}\text{C}$ incorporated into the neutral lipid fractions (Table 3) shows that the triacylglycerols in persistently-infected cells had a ratio 20 times that of uninfected; the corresponding increase for non-esterified fatty acids was 10 times.

Phospholipid

Although there was no quantitative difference in the relative incorporation of [^3H]arachidonic acid and [^{14}C]stearic acid into the total phospholipid of uninfected and persistently-infected cells (see Table 2), we had previously observed differences in the fatty acid composition of the phosphatidylethanolamine in BGM-P cells (Anderton *et al.*, 1981). We therefore analysed the distribution of radioactivity between the individual phospholipids (Table 4). Compared with uninfected cells, there was a lower incorporation of [^3H]arachidonic acid into the phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine of BGM-P cells. Less [^{14}C]-

Table 2. *Distribution of [³H]arachidonic acid and [¹⁴C]stearic acid between neutral and polar lipids in BGM, BGM-L and BGM-P cells*

Results, the average values between the 2 h and 6 h labelling periods, are expressed as the percentages of radioactivity in neutral or polar lipids compared with that in total lipid ($\pm$ s.d.). *** indicates that there is a significant difference ($P < 0.01$) from the corresponding value for uninfected GBM cells.

	Neutral lipids			Polar lipids		
	[³ H]C _{20:4}	[¹⁴ C]C _{18:0}	³ H/ ¹⁴ C*	[³ H]C _{20:4}	[¹⁴ C]C _{18:0}	³ H/ ¹⁴ C*
BGM	3.3 $\pm$ 0.3	16.9 $\pm$ 1.4	3.4 $\pm$ 0.3	96.7 $\pm$ 0.3	79.2 $\pm$ 1.4	16.4 $\pm$ 0.6
BGM-L	3.1 $\pm$ 0.2	14.5 $\pm$ 0.3	2.9 $\pm$ 0.3	96.9 $\pm$ 0.2	85.5 $\pm$ 0.2	14.9 $\pm$ 0.8
BGM-P	34.7 $\pm$ 1.1***	41.6 $\pm$ 1.4***	16.3 $\pm$ 0.8***	65.3 $\pm$ 1.1***	58.4 $\pm$ 2.1	14.6 $\pm$ 0.8

* The ratio ³H/¹⁴C (means $\pm$ s.d.) represents the average values found between the 2 h and 6 h labelling periods.

Table 3. *Distribution of [³H]arachidonic acid and [¹⁴C]stearic acid in neutral lipids of BGM, BGM-L and BGM-P cells*

Results are expressed as the percentages of radioactivity in the neutral-lipid fraction compared with that in total lipid (means $\pm$ s.d.). The results are average values between the 2 h and 6 h labelling periods. Values are significantly different at: *, $P < 0.05$; ***, $P < 0.01$.

	³ H]Arachidonic acid (%)		
	BGM	BGM-L	BGM-P
Cholesteryl esters	0.38 $\pm$ 0.09	0.41 $\pm$ 0.10	3.05 $\pm$ 0.40***
Triacylglycerols	0.33 $\pm$ 0.08	0.28 $\pm$ 0.04	21.49 $\pm$ 1.56***
Non-esterified fatty acids	0.07 $\pm$ 0.01	0.15 $\pm$ 0.04	3.20 $\pm$ 0.49***
Diacylglycerols	0.73 $\pm$ 0.14	0.54 $\pm$ 0.08	3.81 $\pm$ 0.36***
Cholesterol	1.09 $\pm$ 0.24	1.34 $\pm$ 0.23	2.33 $\pm$ 0.35*
Monoacylglycerols	0.17 $\pm$ 0.02	0.19 $\pm$ 0.03	0.60 $\pm$ 0.36
	¹⁴ C]Stearic acid (%)		
	BGM	BGM-L	BGM-P
Cholesteryl esters	2.55 $\pm$ 0.82	1.87 $\pm$ 0.38	2.97 $\pm$ 0.84
Triacylglycerols	5.07 $\pm$ 1.05	5.75 $\pm$ 0.92	28.31 $\pm$ 4.19***
Non-esterified fatty acids	0.05 $\pm$ 0.02	0.06 $\pm$ 0.03	0.37 $\pm$ 0.15
Diacylglycerols	3.97 $\pm$ 0.43	3.65 $\pm$ 0.57	5.36 $\pm$ 0.98
Cholesterol	2.91 $\pm$ 0.47	3.04 $\pm$ 0.47	2.36 $\pm$ 0.51
Monoacylglycerols	0.50 $\pm$ 0.21	0.25 $\pm$ 0.08	1.42 $\pm$ 0.53
	Ratio of ³ H radioactivity/ ¹⁴ C radioactivity		
	BGM	BGM-L	BGM-P
Cholesteryl esters	1.9 $\pm$ 0.2	2.3 $\pm$ 0.3	18.7 $\pm$ 2.6***
Triacylglycerols	0.8 $\pm$ 0.1	0.5 $\pm$ 0.1	13.0 $\pm$ 0.6***
Non-esterified fatty acids	19.6 $\pm$ 2.0	31.7 $\pm$ 6.4	195.0 $\pm$ 19.6***
Diacylglycerols	2.1 $\pm$ 0.3	2.4 $\pm$ 0.8	13.8 $\pm$ 3.9*
Cholesterol	4.4 $\pm$ 0.8	4.7 $\pm$ 0.5	17.7 $\pm$ 2.4***
Monoacylglycerols	7.4 $\pm$ 1.7	8.3 $\pm$ 1.3	8.4 $\pm$ 2.7

stearic acid was incorporated into phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine. There was no difference in the incorporation into phosphatidylinositol.

The ratio of [³H]arachidonic acid [¹⁴C]stearic acid incorporated into phospholipids is shown in Table 4. For phosphatidylethanolamine, it was lower in BGM-P cells, but these cells showed a higher ratio for phosphatidylcholine than did other cells, although the latter results are at the limit of statistical significance. Lytically infected cells had slightly

lower ratios for phosphatidylcholine and phosphatidylethanolamine but were otherwise similar to uninfected cells.

To verify that the radioactive precursors were incorporated into the correct positions in the diacylglycerol molecule, i.e. arachidonic acid in *sn*-2 and stearic acid in *sn*-1 position, the radiolabelled phospholipids were hydrolysed with phospholipase A₂ and the released fatty acid was analysed. Of the ³H, 94% was found at the *sn*-2 position; 87% of the ¹⁴C was found at the *sn*-1 position. Despite the two

Table 4. *Distribution of radioactivity in phospholipid in BGM, BGM-L and BGM-P cells labelled with [³H]arachidonic acid and [¹⁴C]stearic acid*

Results are expressed as the percentage radioactivity in phospholipid compared with that in the total lipid (means $\pm$ S.D.). There is a significant difference from the corresponding value for uninfected BGM cells at: *, $P < 0.05$; **, $P < 0.02$; ***, $P < 0.001$. The results are average values between the 2 h and 6 h labelling periods.

	BGM	BGM-L	BGM-P
^{20:4} C			
Lysophosphatidylcholine	0.1 $\pm$ 0.1	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1
Sphingomyelin	0.1 $\pm$ 0.1	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1
Phosphatidylcholine	31.2 $\pm$ 0.9	27.9 $\pm$ 0.7	24.6 $\pm$ 0.6**
Phosphatidylinositol	33.4 $\pm$ 0.4	30.5 $\pm$ 0.7*	21.8 $\pm$ 0.1***
Phosphatidylserine	3.2 $\pm$ 0.2	4.8 $\pm$ 0.4	3.8 $\pm$ 0.1
Phosphatidylethanolamine	28.9 $\pm$ 0.8	33.2 $\pm$ 1.6	14.2 $\pm$ 1.0***
Bis(phosphatidyl)glycerol	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1	1.1 $\pm$ 0.2
^{18:0} C			
Lysophosphatidylcholine	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1
Sphingomyelin	1.4 $\pm$ 0.1	2.6 $\pm$ 0.4	2.6 $\pm$ 0.6
Phosphatidylcholine	45.2 $\pm$ 1.1	45.6 $\pm$ 1.4	19.7 $\pm$ 1.1***
Phosphatidylinositol	19.4 $\pm$ 0.7	19.5 $\pm$ 0.7	15.0 $\pm$ 1.2*
Phosphatidylserine	9.6 $\pm$ 0.6	10.0 $\pm$ 0.8	6.5 $\pm$ 0.8*
Phosphatidylethanolamine	18.3 $\pm$ 0.4	16.8 $\pm$ 0.8	14.6 $\pm$ 0.9**
Bis(phosphatidyl)glycerol	3.0 $\pm$ 1.5	2.1 $\pm$ 0.5	7.0 $\pm$ 2.1
³ H/ ¹⁴ C			
Lysophosphatidylcholine	7.0 $\pm$ 1.5	5.2 $\pm$ 1.1	4.1 $\pm$ 1.3
Sphingomyelin	2.2 $\pm$ 0.9	0.9 $\pm$ 0.2	0.4 $\pm$ 0.1
Phosphatidylcholine	12.9 $\pm$ 1.5	8.1 $\pm$ 0.3*	19.8 $\pm$ 1.3**
Phosphatidylinositol	29.3 $\pm$ 1.9	21.9 $\pm$ 1.3*	22.4 $\pm$ 1.4*
Phosphatidylserine	6.4 $\pm$ 0.6	6.6 $\pm$ 0.9	9.3 $\pm$ 0.9
Phosphatidylethanolamine	25.1 $\pm$ 0.6	26.7 $\pm$ 0.3	14.8 $\pm$ 0.6***
Bis(phosphatidyl)glycerol	2.8 $\pm$ 0.5	2.8 $\pm$ 0.9	2.8 $\pm$ 0.8

fatty acids being localized on different positions of the same diacylglycerol molecule, there is a difference in their relative turnover and this varies with the nature of the phosphate group.

Discussion

Recent studies on a number of cell types have shown that the physical properties of the membrane and the biological activity of the membrane enzymes are influenced by the composition and turnover of the acyl groups of the membrane phospholipids (Emmelot & Van Hoeven, 1975; Sandermann, 1978; Hirata & Axelrod, 1980). The results obtained in the present study on the phospholipids in measles-virus-persistently-infected BGM cells showed that the relative incorporation of [³H]-arachidonic acid and [¹⁴C]stearic acid into certain membrane phospholipids was greatly modified compared with uninfected cells. As the two fatty acids used are incorporated into two different positions in the diacylglycerol molecule, the difference in the relative incorporation of the two fatty acids in the same phospholipid would lead to an asymmetric metabolic state.

In order to measure these modifications in the relative incorporation of stearic acid (C_{18:0}) and arachidonic acid (C_{20:4}) in phosphatidylcholine and phosphatidylethanolamine, we have calculated the relative specific radioactivity of the fatty acids in the two phospholipids (See Anderton *et al.*, 1982). This ratio (specific radioactivity of the fatty acid in the phospholipid/specific radioactivity of the fatty acid in the total phospholipid) represents a measure of the relative turnover of the fatty acid in the phospholipid studied.

For phosphatidylcholine the above calculation shows that the relative turnover of the two fatty acids in uninfected and persistently-infected cells is similar (1.8 $\pm$ 0.4 in BGM and 1.9 $\pm$ 0.2 in BGM-P cells for C_{20:4} fatty acid and 1.4 $\pm$ 0.1 in BGM and 1.1 $\pm$ 0.1 in BGM-P cells for C_{18:0} fatty acid). In contrast, in lytically infected cells, there was a lower turnover of arachidonic acid (0.6 $\pm$ 0.1 for C_{20:4} and 1.0 $\pm$ 1 for C_{18:0} fatty acids).

The above calculation applied to phosphatidylethanolamine gives relative specific radioactivities of 0.40 $\pm$ 0.09 in BGM, 0.27 $\pm$ 0.06 in BGM-P and 0.70 $\pm$ 0.08 in BGM-L cells for C_{20:4} fatty acid and 0.47 $\pm$ 0.02 in BGM, 0.58 $\pm$ 0.06 in BGM-P and 0.51 $\pm$ 0.04 in BGM-L cells for C_{18:0} fatty acids.

The ratio of the relative specific activities of arachidonic and stearic acids in phosphatidylethanolamine is 0.84 ± 0.07 in BGM, 0.47 ± 0.02 in BGM-P and 1.37 ± 0.02 in BGM-L cells. This is the opposite to that found for phosphatidylcholine (1.29 ± 0.28 in BGM, 1.73 ± 0.04 in BGM-P and 0.55 ± 0.05 in BGM-L cells). There is a large reduction in the turnover of arachidonic acid compared with that of stearic acid in the diacylglycerol moiety of phosphatidylethanolamine in persistently-infected cells. In contrast, there is an increased turnover of arachidonic acid in lytically infected cells.

Thus in phosphatidylcholine, the parallel decrease of incorporation of both fatty acids (Table 4) in BGM-P cells probably results from a lower turnover of the entire diacylglycerol moiety as described by Vance & Burke (1974) in BHK cells infected with Semliki Forest virus. In contrast, there is an accentuated asymmetrical turnover in phosphatidylethanolamine of the arachidonic acid and stearic acid.

The decrease in the incorporation of arachidonic acid in lytic infections results from a selective decrease of its renewal in phosphatidylcholine, probably by inhibition of a specific deacylating-reacylating cycle, thus leading to an accumulation of arachidonic acid in the phosphatidylcholine fraction in the BGM-L cell phospholipid. Conversely, even though persistent infections slow down the renewal of arachidonic acid in phosphatidylethanolamine, the lytic infection activates it. This leads to an increased accumulation of arachidonic acid in phosphatidylethanolamine in persistent and to a decline in lytic infections.

The modifications observed in measles-virus-persistently-infected BGM cells could arise in several ways: they could be the result of (i) inhibition of incorporation of certain species of diacylglycerol into phosphatidylethanolamine after a modification of the specificity of ethanolamine phosphotransferase, (ii) a decline in the phospholipase A_2 arachidonic acid-liberating or -reacylating activity or (iii) a specific inhibition of the transmethylation of phosphatidylethanolamine to phosphatidylcholine, which occurs preferentially on the polyunsaturated species (Arvidson, 1968*a,b*). This pathway is sensitive to stimulation by certain hormones (Hirata *et al.*, 1979) and is rendered insensitive during measles-virus-persistent infections (Munzel & Koschel, 1982). The modification could also be the result of (iv) an activation of triacylglycerol syn-

thesis. The accumulation of radiotracer in the triacylglycerols was found with arachidonic and to a lesser extent stearic acids and also with palmitic acid and oleic acid (Anderton *et al.*, 1983).

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