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# ACCUMULATION OF RADIOLABELLED FATTY ACIDS IN THE NEUTRAL LIPID FRACTION OF MEASLES VIRUS PERSISTENTLY INFECTED BGM CELLS

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The effect of measles virus infection (acute and persistent) on fatty acid metabolism has been studied in BGM (African green monkey kidney) cells. In persistently infected cells, there was an increase in the incorporation of radiolabelled fatty acids into the neutral lipid fraction. Compared to uninfected cells, the increase was up to 2-fold for palmitic, stearic and oleic acids and 8-14 fold for arachidonic acid. The lipid metabolism in measles virus acutely infected cells was unmodified. The radiolabelled fatty acids incorporated into the neutral lipids in persistently infected cells were principally associated with the triglyceride fraction. The implications of a virus- induced modification of lipid metabolism are discussed.

A number of factors are involved in the establishment and propagation of virus persistent infections in mammalian cells. To survive an otherwise lethal attack, it is necessary that the infection is attenuated. This can be achieved by the accompanying production of defective virus particles (1) or interferon (2). With the acquisition of the persistent state an equilibrium between the viral and host cell functions is established. In this state, the virus genome accumulates a number of mutations which lead to a biologically attenuated virus (3,4). However, despite known changes in the properties of the virus little is known of the modifications to the cell. In our previous studies of measles virus persistently infected cells, we found a

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modification of the fatty acid composition in the phospholipids. There was an increased incorporation of palmitic acid in certain phosphatides when compared to those synthesized from $^{32}$P-phosphate. The modifications of the phospholipid metabolism were accompanied by an increased incorporation of palmitic acid into the triglyceride of the persistently infected cells. These changes must arise from a modification of cellular metabolism.

In the present study we have examined the metabolism of radiolabelled fatty acids in uninfected, lytically and persistently infected cells to determine if all fatty acid metabolism is modified in measles virus persistently infected cells.

## MATERIALS AND METHODS

Cells : African green monkey kidney cells (BGM) or the measles virus (Hallé strain) persistently infected derivative (BGM-P) were grown as monolayer cultures as previously described (6). For lytic infections, cells were infected with the homologous virus at a m.o.i. of 0.1 p.f.u./cell.

Lipid extraction : Cell monolayers were washed twice in 0.14M NaCl, scraped down in the same solution, centrifuged and the lipid extracted as previously described (5).

Thin layer chromatography : Lipid extracts were resolved into their respective classes by one-dimensional thin layer chromatography on pre-coated silica gel plates G50 (Merck). The first 3 cm was chromatographed in diethyl ether followed by hexane, diethyl ether, acetic acid (90:10:1). The neutral lipid fractions were located by autoradiography. $^{3}$H was enhanced by spraying the chromatographic plate with 7 % (w/v) PPO/methanol prior to exposure. The position of the neutral lipids were verified by chromatography of standards.

Determination of radioactivity : The lipid fractions were scraped from the chromatography plates and suspended in 4 ml of ethanol-water (1:1) and 10 ml of Instagel (Packard). Counting efficiency was determined by the external standard channels ratio method.

Radiochemicals : All radioactive tracers were obtained from Amersham International.

Cell cultures were labelled with either 0.5 µc/ml (1-$^{14}$C) stearic acid (100 mc/mmol), 4 µc/ml $^{3}$H(9,10(n)) oleic acid (2 Ci/mmol) or 5 µC/ml $^{3}$H(5,6,8,9,11,12,14,15) arachidonic acid (120 Ci/mmol).

## RESULTS

Confluent monolayer cultures of BGM cells either uninfected (BGM), lytically (BGM-L) or persistently (BGM-P) infected were labelled

Table I

INCORPORATION OF FATTY ACIDS INTO NEUTRAL LIPIDS OF UNINFECTED (BGM), LYTICALLY (BGM-L) AND PERSISTENTLY (BGM-P) INFECTED CELLS

| Fatty acid | Period of radiolabelling (h) | % radioactivity neutral/total lipid | | |
|---|---|---|---|---|
| | | BGM | BGM-L | BGM-P |
| (1-$^{14}$C)stearic acid | 2 | 20.8±1.8 | | 44.3±1.7(*) |
| | 4 | 17.8±0.1 | | 43.9±3.0(*) |
| | 6 | 14.3±0.2 | 14.7±0.5 | 37.4±2.1(*) |
| $^{3}$H(9,10(n)) oleic acid | 2 | 19.8±0.2 | 15.0±0.1 | 27.5±0.2(*) |
| | 4 | 19.4±0.2 | 20.8±0.2 | 26.0±0.3(*) |
| | 6 | 13.6±0.3 | 15.3±0.5 | 27.6±0.3(*) |
| $^{3}$H(5,6,8,9,11,12,14,15) arachidonic acid | 2 | 4.3±0.1 | | 35.4±3.8(*) |
| | 4 | 3.2±0.1 | | 35.9±0.8(*) |
| | 6 | 2.3±0.1 | 3.4±0.4 | 32.8±0.9(*) |

BGM, BGM-L and BGM-P cells were incubated in medium containing one of the radioactive fatty acids which was added directly to the medium. At various time intervals, cells were harvested, lipids extracted and the neutral lipid fraction separated.
(*) Indicates that there was a significant difference in these values from that of uninfected BGM cells at $p < 0.01$ (student's t test). Three or four determinations of the fatty acid incorporation were used for each calculation.

with either $^{14}$C-stearic, $^{3}$H-oleic or $^{3}$H-arachidonic acids. At intervals, cells were scraped down and the total lipids extracted. The neutral lipids were subsequently separated by chromatography and the incorporation of radioactivity measured. In all cases a greater proportion of the radioactivity was incorporated into the neutral lipid fraction of persistently infected cells than uninfected controls (Table 1). This modification was not found in lytically infected cells.

The incorporation of the saturated fatty acid, stearic and the monosaturated oleic acid into the neutral lipids was up to two-fold greater in the persistently infected than non-infected cells. This difference was even more exaggerated with arachidonic acid where 8-14 fold differences were observed.

Table 1

DISTRIBUTION OF $^{14}$C-STEARIC ACID AND $^{3}$H-ARACHIDONIC ACID IN NEUTRAL LIPIDS AFTER INCORPORATION INTO BGM, BGM-L and BGM-P CELLS

(a) (1-$^{14}$C)-stearic acid

| | % of the radioactivity in neutral lipid | | | | | |
|---|---|---|---|---|---|---|
| Neutral lipid | BGM | | BGM-L | | BGM-P | |
| | 4 h | 6 h | 4 h | 6 h | 4 h | 6 h |
| Cholesterol esters | 19.9±6.8 | 12.4±4.8 | 12.2±4.4 | 13.3±3.8 | 6.4±1.4 | 8.0±4.3 |
| Triglyceride | 36.4±7.1 | 39.9±5.3 | 57.9±6.5 | 48.6±11.5 | 74.1±8.7(*) | 64.6±4.4(*) |
| Free fatty acid | 0.3±0.2 | 0.2±0.1 | 0.4±0.3 | 0.3±0.2 | 0.9±0.5 | 0.8±0.6 |
| Diglyceride | 25.1±1.5 | 28.5±3.5 | 23.8±7.4 | 26.2±4.1 | 10.2±3.2(*) | 16.4±1.6(*) |
| Cholesterol | 16.4±1.4 | 23.4±5.3 | 23.6±3.1 | 18.1±5.3 | 4.7±1.3(*) | 6.9±1.9(*) |
| Monoglyceride | 1.6±0.1 | 5.5±3.0 | 1.9±0.6 | 1.4±1.1 | 3.3±1.9 | 3.4±2.0 |

(b) ($^{3}$H)-Arachidonic acid

| | | | | | | |
|---|---|---|---|---|---|---|
| Cholesterol esters | 15.8±2.4 | 10.4±0.1 | 10.3±3.4 | 15.6±4.1 | 8.2±1.5 | 9.4±3.8 |
| Triglyceride | 11.0±0.9 | 12.2±7.1 | 7.2±0.5 | 11.0±1.0 | 63.5±5.6(*) | 61.1±3.2(*) |
| Free fatty acid | 2.8±0.3 | 2.0±0.4 | 3.9±1.5 | 5.3±2.1 | 9.2±1.2(*) | 9.1±5.9(*) |
| Diglyceride | 26.6±0.3 | 25.2±5.4 | 30.0±13.6 | 18.2±4.5 | 10.9±0.6(*) | 11.2±2.0 |
| Cholesterol | 36.7±1.0 | 42.5±7.1 | 41.5±8.3 | 43.0±3.7 | 5.5±0.7(*) | 8.1±1.2(*) |
| Monoglyceride | 5.4±0.3 | 6.3±1.5 | 6.1±0.3 | 5.5±0.7 | 2.6±1.9 | 1.3±0.3(*) |

*) Signifies that there is significant difference from the corresponding value for uninfected BGM cells.

To examine the nature of the accumulation of the fatty acids in the neutral lipids, the latter were subsequently separated by mono-dimensional thin layer chromatography. In the persistently infected cells, the radioactivity accumulates primarily in the triglycerides (Table 2). There is a significant reduction in the diglyceride and cholesterol fractions. In uninfected and lytically infected cells, 30-41 % of the $^{14}$C-stearic acid and 7-12 % of the $^{3}$H-arachidonic acid is found in the triglyceride fraction. In contrast, the persistently infected cells contained as much as 65-74 % $^{14}$C and 61-64 % $^{3}$H in the triglycerides. The same result was obtained with $^{3}$H-oleic acid.

The percentage of triglyceride in neutral lipid was greater in persistently infected than non infected cells. These results show that

in persistently, but not lytically infected cells, there is a virus-induced alteration of lipid synthesis resulting in the accumulation of fatty acids in the triglyceride fraction of the cell.

## DISCUSSION

During the establishment of virus persistent infections, cells undergo crises periods in which the equilibrium between lytic and persistent type infections is changed (7). Once a stable equilibrium is obtained and maintained between the virus and its host, the cells multiply at a similar rate to that of uninfected cells (4,8). The overall synthesis of DNA, RNA and protein is unchanged. However, in some host systems in which the cell has an ancillary product such as the synthesis of hormones, neurotransmitters and antibodies, their synthesis may be affected (8). However, the biochemical modifications in this case are unknown.

We have previously found (5) in measles infected cells a higher concentration of arachidonic acid, a precursor of prostaglandins, in PE than in PC. Thus the methylation of PE (9) which is very active in BGM cells would produce more unsaturated molecular species of PC than those synthesized by the Kennedy's pathway. However in measles virus persistently infected C6 glioma cells, a complete inhibition of the methylation pathway has been observed (10). We do not know at present whether the increased incorporation of arachidonic acid in infected cells (as reported in this paper), is linked to the fact that in BGM cells, measles virus does not affect the synthesis of PC by the Bremer's pathway or whether the infection changes the specificity of the acyl transferring enzymes involved in the synthesis of phosphatidyl-choline.

In the present study, we have shown that in measles virus persistently infected cells, the metabolism of fatty acids is greatly modified. There is an increased incorporation of both saturated and non-saturated fatty acids into the triglyceride fraction of neutral lipids.

In a further study, we (11) and also Lyons et al. (12) have observed an obesity phenomenon in mice persistently infected with canine distemper virus, which is closely related to measles virus. The accumulation of triglycerides which is the form in which animal fats are stored, may be relevent to the animal model. Studies on the lipid metabolism with adipocytes obtained from obese and lean persistently infected mice are now in progress to determine if a similar phenomenon to that observed in vitro occurs.

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