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Differentiation of Strains of Varicella-Zoster Virus by Changes in Neutral Lipid Metabolism in Infected Cells†

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Eleven isolates of varicella-zoster virus were tested for their effects on the incorporation of [^{14}C]acetate into lipids in infected human embryonic lung cells. By relative percent, all virus isolates demonstrated a shift from polar lipid synthesis to neutral lipid, especially triglyceride, synthesis. By data expressed as counts per minute per microgram of protein, the VZV strains could be separated into two groups: those strains which depressed lipid synthesis and those strains which did not depress, and may even have stimulated, lipid, especially triglyceride, synthesis. These results may be useful in understanding the development of lipid changes seen in children affected with Reye's syndrome following chickenpox.

The human herpesvirus varicella-zoster virus (VZV) is the etiologic agent for the childhood disease of chickenpox (varicella) and the recurrent disease shingles (zoster) (30). Although the disease is rarely life threatening in normal children, problems occur in children whose immune systems are compromised. Clinical trials of an attenuated virus vaccine in susceptible children with leukemia are in progress (15). A serious sequel of chickenpox in normal children is Reye's syndrome (23). Following apparent recovery from an initial viral illness (influenza B in epidemic cases, chickenpox in endemic cases [9]), the child develops the symptoms of Reye's syndrome: projectile vomiting, cerebral edema, and fatty infiltration of the viscera. At the present time, there is no accepted mechanism to explain this syndrome, although several hypotheses have been proposed. Since Reye's syndrome can follow chickenpox and involves changes in lipid metabolism in the affected child, we examined the effects of various isolates of VZV on the lipid metabolism of infected human cells.

MATERIALS AND METHODS

Cell cultures. The MRC-5 line of human embryonic lung cells, obtained from Whittaker M. A. Bioproducts, Walkersville, Md., was grown and maintained in Eagle minimal essential medium supplemented with 5% fetal bovine serum, 5% newborn calf serum, 0.2% $NaHCO_3$, 100 U of penicillin per ml, and 100 μg of streptomycin per ml (GIBCO Laboratories, Grand Island, N.Y.).

Viruses. Eleven strains of VZV were used in these studies. Four strains were obtained from P. Brunell, University of Texas Health Science Center at San Antonio: Ellen (EL), Lozano 26 (L26), Lozano 28 (L28), and Gretchen (GR). Two strains were obtained from S. Strauss and J. Ostrove at the National Institutes of Health: D. Luzak (DL) and Oka (OB). Five strains were isolated from clinical specimens in our laboratory: PVWT (PV), HSWT (HS), LCWT (LC), WT1 (WT), and JBWT (JB). Viruses were tested between passages 3 and 14. All virus strains were maintained as cell-associated virus in MRC-5 cells. Virus stocks were stored at −85°C. Initial studies determined that there was no significant difference in viral growth rates or titer of these 11 strains.

Lipid studies. For lipid analyses, MRC-5 cells were grown to monolayers in Costar (Cambridge, Mass.) 12-well cluster plates. An inoculum of VZV-infected MRC-5 cells (or control, uninfected cells) was added, and the plates were incubated at 37°C for 24 h to permit spread of virus; the cytopathic effect was approximately 2+ at that point. Then [1-^{14}C]acetate (New England Nuclear Corp., Boston, Mass.) was added at a concentration of 2 μCi/ml, and the plates were incubated for 24 h; at that point, the cytopathic effect was 4+. All wells were then harvested by trypsinizing the cells to form a suspension and then washing the suspended cells twice with phosphate-buffered saline. The drained cell

TABLE 1. Relative percentage of label incorporated into each lipid[a]

Virus[b]	% ^{14}C incorporated in:				
	CE	TG	FA	CH	DG
UC	14.3 ± 1.0	43.2 ± 4.5	15.9 ± 3.2	18.3 ± 0.7	8.5 ± 1.1
WT	7.1 ± 0.3	74.9 ± 0.2	9.2 ± 0.4	6.0 ± 0.4	2.5 ± 0.3
JB	6.8 ± 0.6	72.0 ± 2.1	11.6 ± 2.5	5.0 ± 0.5	3.4 ± 0.4
EL	6.1 ± 0.7	72.0 ± 0.5	9.5 ± 0.8	9.0 ± 0.8	3.2 ± 0.3
L28	5.4 ± 0.5	66.9 ± 1.3	9.4 ± 1.0	13.8 ± 0.6	4.9 ± 0.3
UC	13.3 ± 1.8	32.1 ± 4.0	20.3 ± 2.0	25.8 ± 2.3	8.5 ± 0.6
DL	5.2 ± 1.4	62.3 ± 6.0	11.6 ± 1.2	15.6 ± 4.2	5.3 ± 1.3
PV	5.0 ± 1.5	62.6 ± 2.4	11.0 ± 2.6	16.9 ± 1.6	4.5 ± 0.7
HS	4.5 ± 0.9	64.1 ± 4.3	14.4 ± 2.4	12.0 ± 1.3	5.0 ± 1.5
OB	3.9 ± 0.3	63.4 ± 1.1	13.7 ± 1.8	15.8 ± 1.7	3.2 ± 0.6
UC	9.7 ± 0.8	46.6 ± 5.6	28.2 ± 5.2	10.8 ± 0.4	4.8 ± 0.2
GR	7.1 ± 0.5	58.8 ± 2.4	21.0 ± 2.7	9.2 ± 0.6	4.0 ± 0.1
HS	6.3 ± 0.2	61.4 ± 3.8	20.1 ± 4.0	9.2 ± 0.8	3.2 ± 0.1
LC	6.5 ± 0.1	58.5 ± 4.2	22.1 ± 4.0	9.6 ± 0.4	3.4 ± 0.1
EL	5.8 ± 0.2	65.1 ± 4.6	18.5 ± 5.4	7.6 ± 0.7	2.9 ± 0.2
DL	6.6 ± 0.3	58.9 ± 5.6	21.2 ± 5.1	10.6 ± 0.6	3.7 ± 0.3
UC	8.5 ± 0.8	58.9 ± 2.3	18.8 ± 2.3	9.7 ± 0.4	3.4 ± 0.1
L26	6.1 ± 0.3	70.1 ± 3.7	11.9 ± 2.3	8.9 ± 0.3	3.0 ± 1.9
OB	5.8 ± 0.5	70.4 ± 1.1	12.9 ± 0.9	8.9 ± 0.5	2.2 ± 0.3
L28	5.4 ± 0.6	70.9 ± 0.7	12.5 ± 0.7	8.8 ± 0.3	2.4 ± 0.2

[a] CE, Cholesterol ester; TG, triglyceride; FA, free fatty acid; CH, cholesterol; DG, diglyceride.
[b] For full virus designations, see Materials and Methods; UC, uninfected control cultures.

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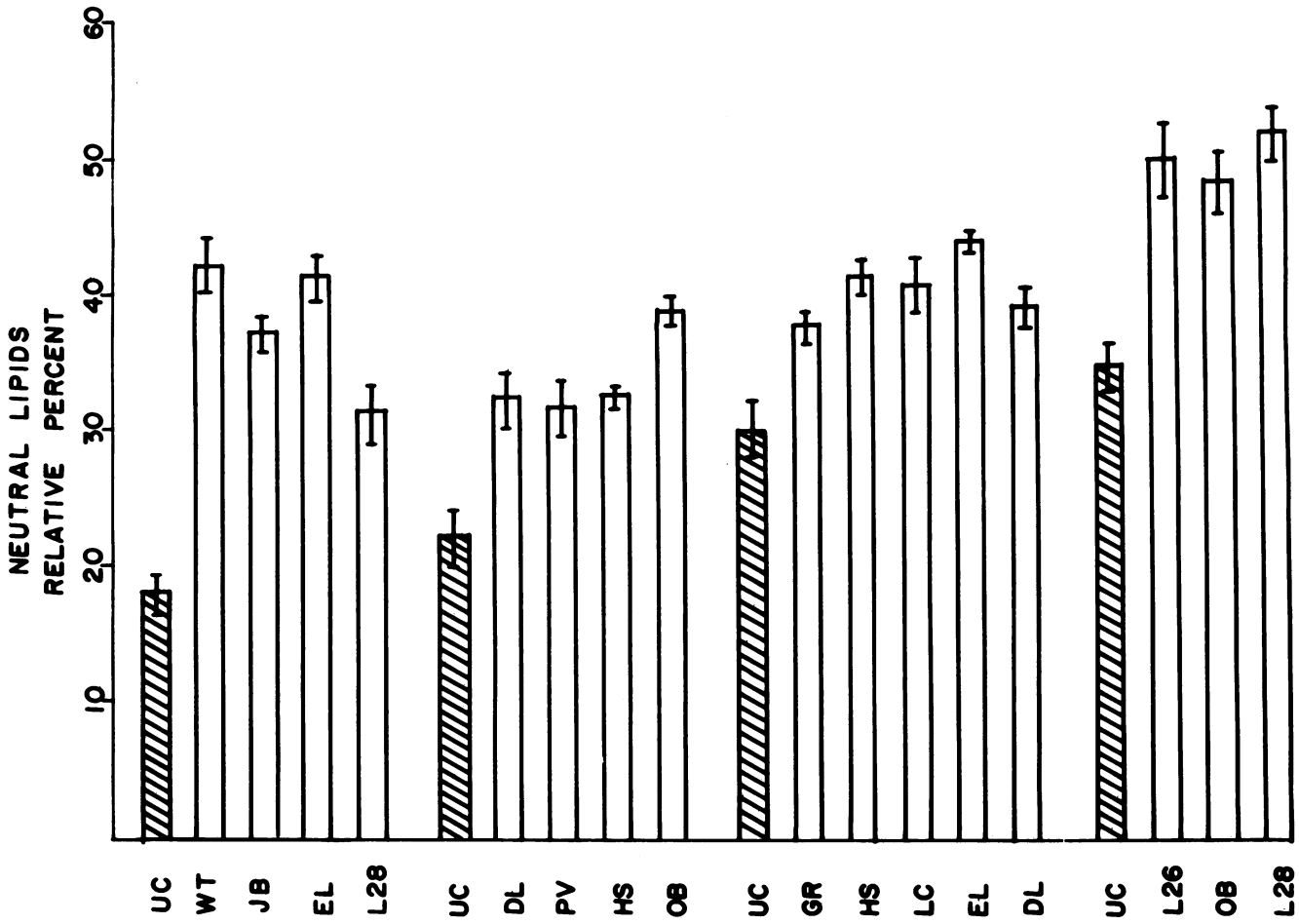

FIG. 1. Relative percent of ^{14}C-acetate incorporated into neutral lipids in MRC-5 cells infected with various strains of VZV (for full virus designations, see Materials and Methods; UC, uninfected control cultures).

pellet was frozen at −85°C until assay. The wells were assayed independently as follows: four wells for label incorporated into lipids, two wells for total protein content, and one well for virus infectivity.

Extraction of lipid. Frozen cell pellets were thawed and transferred quantitatively to 50-ml glass centrifuge tubes by suspension in several portions of phosphate-buffered saline. The final volume of suspended cells was 1 ml. Total lipid was extracted by the method of Bligh and Dyer (6) by adding 3.7 ml of chloroform-methanol (1:2), 1.3 ml of chloroform, and 1.3 ml of distilled water, vortexing between additions. At the beginning of the extraction procedure, 50 μl of chloroform containing approximately 1 mg of unlabeled total lipid from chicken liver was added to act as a carrier for the low levels of total lipid in the samples. The two layers were allowed to separate overnight at 4°C. The lower chloroform layer was removed with a Pasteur pipette, transferred to a glass tube with a Teflon-lined cap, and brought to dryness with a stream of nitrogen in a 45°C water bath. Lipid was dissolved in 100 μl of chloroform and stored in a freezer (−20°C) until chromatography.

Chromatography of neutral lipids. Neutral lipids were separated by one-dimensional chromatography on Whatman, Inc. (Clifton, N.J.), silica gel-loaded paper (SG81) by spotting 50 μl of the samples 2.5 cm from the bottom of each sheet (19 by 23 cm) and development in a heptane-diethyl ether-glacial acetic acid (80:18:1 [vol/vol]) solvent system at room temperature. Following development (approximately 2 h), the chromatograms were air dried, stained with 0.0012% aqueous rhodamine 6G, and viewed under UV light to identify lipids (21). For identification and comparison, known standards of cholesterol, cholesterol ester, triacylglycerol, diacylglycerol, monoacylglycerol, free fatty acid, and polar lipids were chromatographed in this system. Cholesterol ester ran at the solvent front, and polar lipids, which were predominantly phospholipid, remained at the origin. The spot for each lipid class was marked under UV light, and the chromatogram was dried. The spots were cut out and placed into scintillation vials. One milliliter of methanol was added first to each vial to extract lipid from the paper, and then 5 ml of scintillation fluid (Omnifluor; New England Nuclear) was added. Each sample was counted on a 7220 scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.).

Protein analysis. The amount of protein present was determined by the Lowry method (20) with bovine serum albumin as the standard. The amount of protein per well averaged between 97 and 159 μg in the four experiments reported. There were no significant differences between values within an experiment and between infected and control wells.

Infectious center assay. Levels of virus infectivity were determined by an infectious center assay described previ-

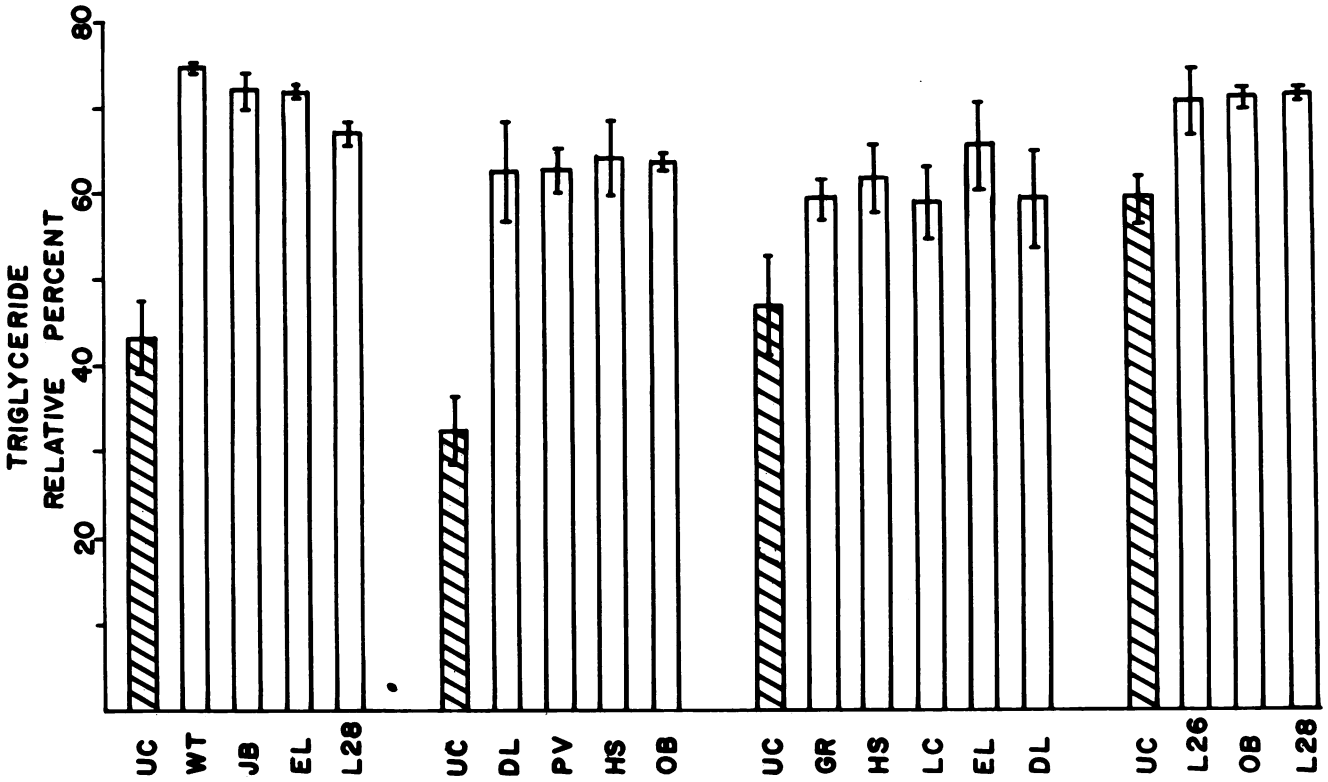

FIG. 2. Relative percent of ^{14}C-acetate incorporated into triglycerides in MRC-5 cells infected with various strains of VZV (for full virus designations, see Materials and Methods; UC, uninfected control cultures).

TABLE 2. Counts per minute per microgram of protein of neutral lipids[a]

Virus[b]	Cpm/μg of protein in:				
	CE	TG	FA	CH	DG
UC	17.0 ± 1.9	51.7 ± 5.8	18.5 ± 4.0	21.8 ± 1.9	10.1 ± 1.2
WT	1.6 ± 0.3	14.5 ± 1.2	4.6 ± 3.3	1.3 ± 0.2	0.5 ± 0.1
JB	1.8 ± 0.1	19.1 ± 1.3	3.5 ± 1.0	1.3 ± 0.1	0.9 ± 0.1
EL	1.6 ± 0.2	18.2 ± 2.4	4.0 ± 1.5	2.4 ± 0.5	0.9 ± 0.1
L28	7.5 ± 1.1	87.9 ± 11.4	17.5 ± 4.3	19.1 ± 1.9	6.8 ± 0.4
UC	7.8 ± 1.5	18.5 ± 1.5	11.9 ± 2.0	15.1 ± 2.7	5.0 ± 0.6
DL	7.2 ± 3.7	83.6 ± 28.1	15.0 ± 4.3	19.2 ± 2.8	6.5 ± 0.7
PV	3.6 ± 1.1	46.8 ± 8.5	8.4 ± 3.0	12.9 ± 3.4	3.5 ± 1.1
HS	4.0 ± 0.7	58.4 ± 9.8	13.0 ± 2.1	11.0 ± 2.1	4.4 ± 0.9
OB	2.5 ± 1.1	39.9 ± 16.1	9.1 ± 4.3	9.7 ± 3.8	2.0 ± 0.7
UC	7.0 ± 0.4	34.2 ± 7.9	20.0 ± 1.5	7.9 ± 1.1	3.5 ± 0.4
GR	10.6 ± 0.3	88.7 ± 9.7	31.5 ± 3.8	13.8 ± 1.8	5.9 ± 0.6
HS	11.4 ± 0.5	115.5 ± 11.1	37.5 ± 6.6	17.3 ± 1.9	5.9 ± 0.1
LC	11.1 ± 2.0	99.5 ± 18.3	37.5 ± 9.6	16.3 ± 3.0	5.7 ± 0.9
EL	2.4 ± 0.2	27.0 ± 2.3	7.7 ± 2.4	3.2 ± 0.4	1.2 ± 0.1
DL	8.9 ± 1.0	79.2 ± 14.3	28.5 ± 7.0	14.3 ± 1.9	5.0 ± 0.8
UC	8.4 ± 0.9	58.4 ± 3.5	19.5 ± 3.6	9.7 ± 1.2	3.4 ± 0.3
L26	9.4 ± 0.4	107.3 ± 4.7	18.4 ± 4.2	13.6 ± 1.0	4.6 ± 3.1
OB	9.3 ± 0.9	112.9 ± 9.3	20.6 ± 1.4	14.3 ± 1.7	3.4 ± 0.5
L28	6.4 ± 0.6	85.2 ± 10.7	15.0 ± 2.1	10.6 ± 1.1	2.9 ± 0.5

[a] CE, Cholesterol ester; TG, triglyceride; FA, free fatty acid; CH, cholesterol; DG, diglyceride.

[b] For full virus designations, see Materials and Methods; UC, uninfected control cultures.

ously (2). In each experiment, comparable levels of virus were obtained for the tested strains.

RESULTS

Isolation of virus strains. Since we were interested in a clinical disease (Reye's syndrome), we wished to obtain circulating virus strains. Local physicians were approached for clinical specimens; five isolates were obtained. However, we could not obtain enough specimens locally, and we also wanted virus strains circulating in other populations. Thus, four strains were obtained from P. Brunell, and two were from S. Strauss and J. Ostrove. Eleven strains were characterized in detail.

Lipid analysis. The MRC-5 cells grown to monolayers in 12-well cluster plates were infected with a suspension of VZV-infected cells and incubated for 24 h to permit the virus to spread; the viral cytopathic effect was 2+ at that point. ^{14}C-acetate was added, and the plates were incubated for 24 h, after which the viral cytopathic effect was 4+. Four wells were independently assayed for lipid by extracting the total lipid, separating the neutral lipid components by one-dimensional silicic acid paper chromatography, and determining the amount of label incorporated into each lipid group. Analysis of the resulting data was performed in two ways: by comparing relative percent and counts per minute per microgram of protein. Since factors such as cell passage number, degree of confluency, and stage of cell cycle may be responsible for variations in lipid composition observed in uninfected control cultures in replicate experiments, all comparisons of virally infected cells were made with the uninfected control of that experiment. Although trends could

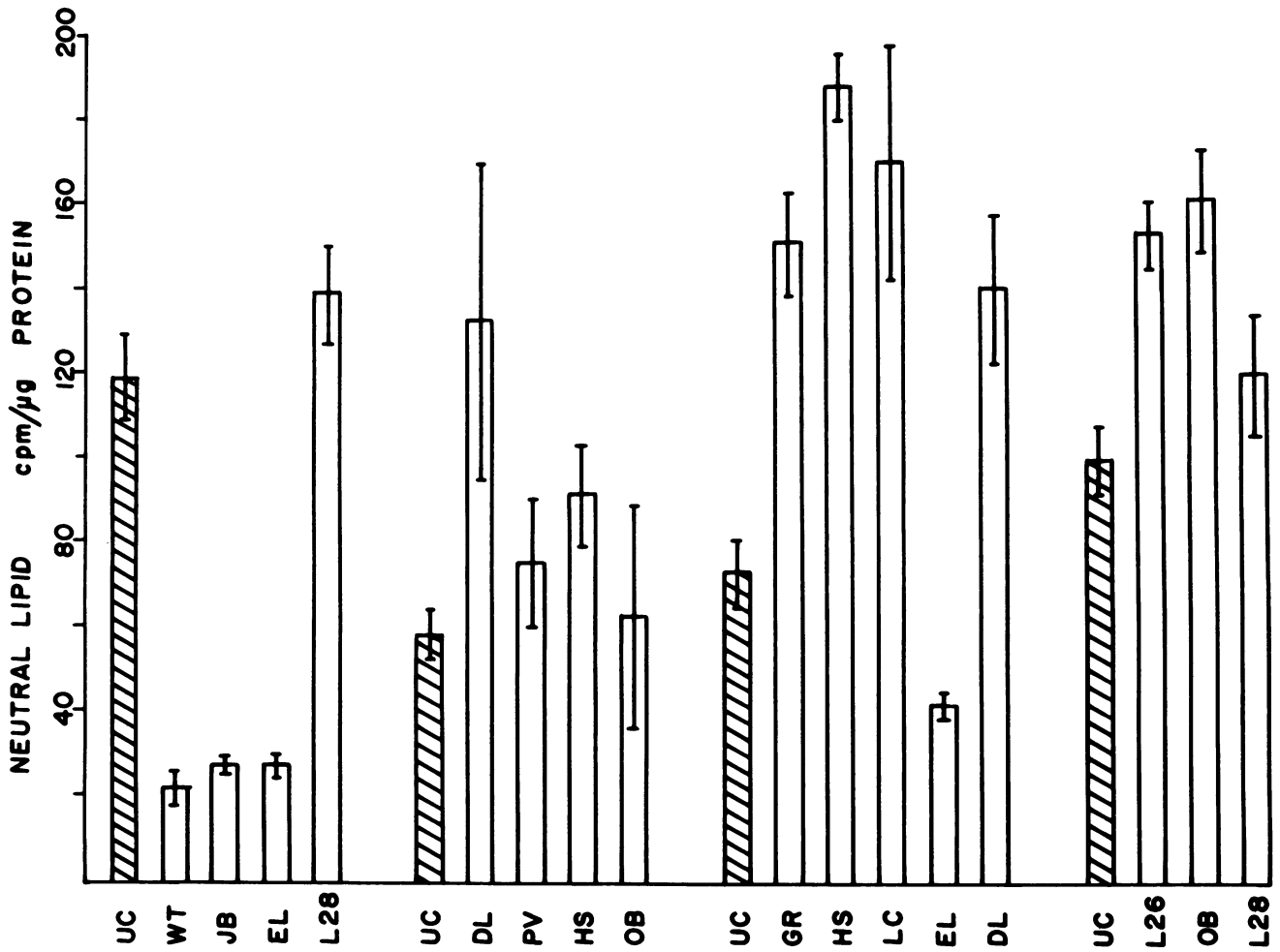

FIG. 3. Counts per minute of ^{14}C-acetate incorporated into neutral lipids per microgram of protein in MRC-5 cells infected with various strains of VZV (for full virus designations, see Materials and Methods; UC, uninfected control cultures).

be observed between experiments, actual values were compared only within an experiment.

Comparison of polar and total neutral lipids by relative percent. In this analysis, the total amount of label incorporated into lipids in each sample was calculated as 100%. The total amount of label incorporated into neutral lipids was determined by summation of the amount in each neutral lipid group; the total amount of label incorporated into polar lipids was determined from the amount of label present in the spot at the origin. The average and standard error were calculated for each lipid of each virus isolate and for uninfected control cells from four independently assayed samples. Each virus isolate was tested by this procedure at least twice. Representative experiments are included in this report.

With all virus isolates, there was an increase in the amount of label incorporated into neutral lipids compared with polar lipids (Fig. 1). The relative percent of neutral lipids in uninfected control cells ranged from 18 to 33%, whereas the amount in all infected cells ranged from 31 to 51%.

Comparison of individual neutral lipids by relative percent. Similar analyses by relative percent were done for each neutral lipid (Table 1). Although there were minor changes with various neutral lipids, the most consistent change was an increase in the relative amount of triglyceride in all infected cells (Fig. 2). Therefore, by relative percent, all 11 isolates of VZV caused a shift in lipid synthesis from polar lipids to neutral lipids, especially triglycerides.

Comparison of polar and total neutral lipids by counts per minute per microgram of protein. In addition to the analysis by relative percent, the average and standard error values obtained from the four independently assayed lipid samples were divided by the average of two independently assayed protein samples to provide a counts per minute per microgram of protein value for each isolate. The values obtained in this way for total neutral lipid synthesis are presented in Fig. 3. Similar trends were found when counts per minute alone were analyzed, indicating little variation in protein levels. In this analysis, the 11 isolates of VZV showed two different responses. One group of isolates (L28, DL, PV, HS, OB, GR, LC, and L26) had equal or greater incorporation of label into lipid per microgram of protein than did uninfected control cells. The other group of isolates (WT, JB, and EL) showed depressed levels of label incorporated into lipid compared with uninfected control cells.

Comparison of neutral lipid components by counts per minute per microgram of protein. When each neutral lipid was examined individually, a similar pattern was detected (Table 2). Synthesis of most individual lipids was similar to or greater than that in uninfected cells with eight isolates but much reduced in three isolates. Again, the largest change

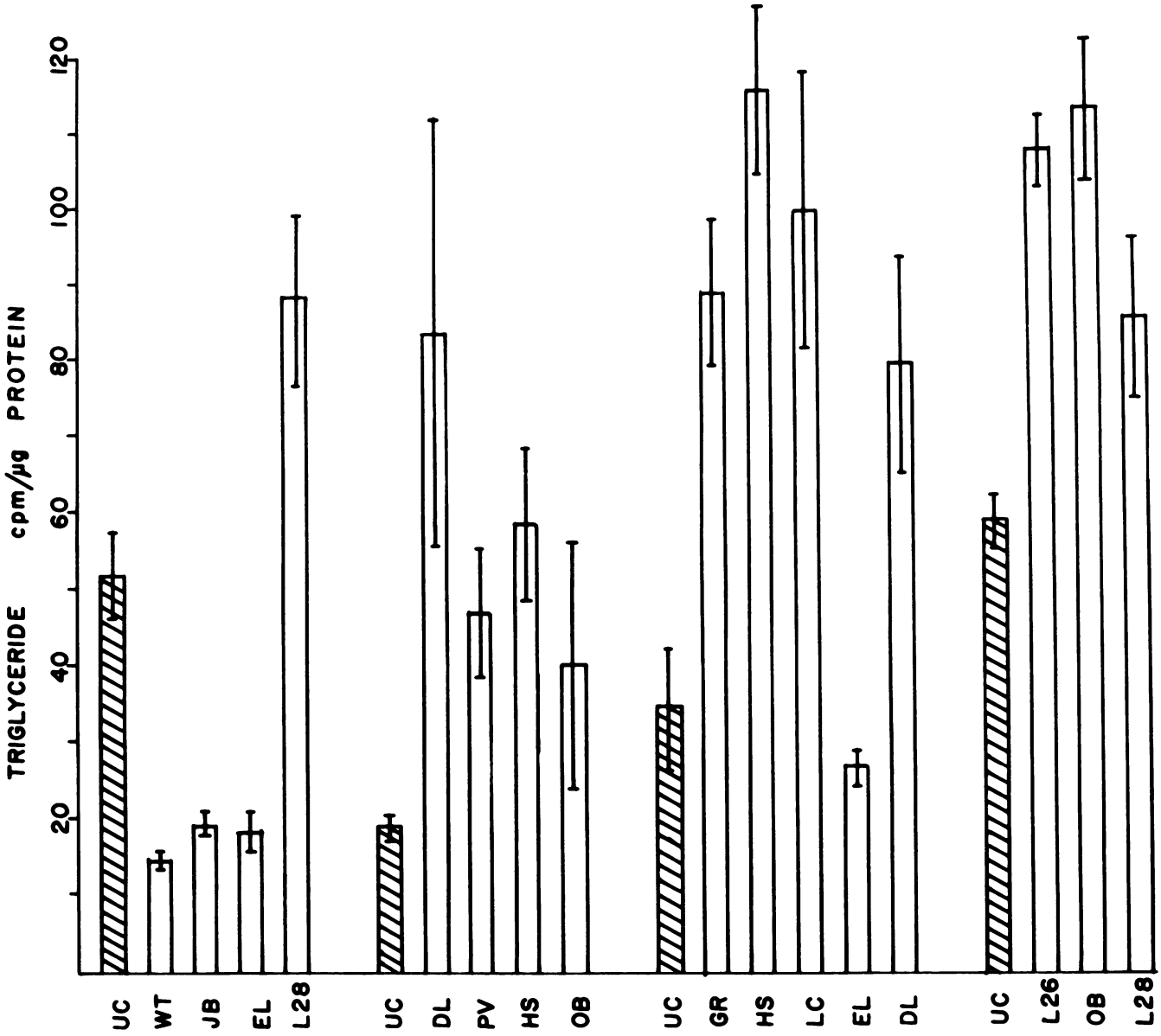

FIG. 4. Counts per minute of ^{14}C-acetate incorporated into neutral lipids per microgram of protein in MRC-5 cells infected with various strains of VZV (for full virus designations, see Materials and Methods; UC, uninfected control cultures).

was in triglyceride synthesis (Fig. 4); there was a dramatic increase with eight isolates, whereas the depression with the remaining three isolates was not as great as for other neutral lipids. Thus, counts per minute per microgram of protein analysis of neutral lipids separated the 11 VZV isolates into two groups: those strains that depressed lipid synthesis and those that did not do so and may even have stimulated the synthesis of some lipids, especially triglycerides.

DISCUSSION

An examination of the effect of VZV infection on lipid metabolism in MRC-5 cells indicated that VZV isolates alter the lipid metabolism of infected cells. There appeared to be a VZV-specific alteration in that, with all isolates, the relative amounts of incorporation of labeled precursors into neutral lipids, especially triglycerides, increased with respect to polar lipids. In addition, there was a strain-specific alteration in that some strains depressed lipid synthesis, whereas other strains did not depress, and may even have stimulated, lipid synthesis.

This is not the first time that herpesviruses have been shown to alter the lipid synthesis of infected cells. Early studies have shown the accumulation of cholesterol crystals in cells infected with a feline herpesvirus (14). Extensive studies with cell-associated viruses have been done by Fabricant et al. (13) with avian Marek's disease virus. They found that infection of chicken smooth muscle cells with Marek's disease virus caused accumulation of significant quantities of lipid compared with uninfected cells or cells infected with another related herpesvirus, herpesvirus of turkeys. These changes were demonstrated by oil red O staining and the appearance of cholesterol crystals only in Marek's disease virus-infected cells. Chemical analyses of lipid classes indicated increased amounts of cholesterol,

cholesterol esters, phospholipids, and squalene. Changes in the fatty acid composition of whole cells and in cholesterol esters were also found. This work indicated that two related cell-associated viruses can be significantly different in their effects on the lipid metabolism of infected cultured cells. In this report, two groups of cell-associated VZV strains were found to have markedly different effects on cellular lipid metabolism.

Non-cell-associated viruses have also been reported to alter cellular lipid components: glycolipid, phospholipid, and sphingolipid with herpes simplex virus infection (12, 25, 27, 28); phospholipid and phospholipid precursors with infections of Newcastle disease virus, fowl plaque virus, and Semliki Forest virus (8, 17); glycolipids with poxvirus infection (3); phospholipids with Sendai virus infection (5); phospholipid fatty acid composition with cells persistently infected with measles virus (4); and neutral lipid and lipid-bound sialic acid in monolayers infected with rabies virus (22).

This is not the first report of variation in VZV strains. We have previously shown that different strains of VZV react differently to the pesticide carbaryl; replication of some strains is enhanced, whereas that of others is not enhanced (18). We are presently comparing the groups separated by these two different approaches.

These studies may help to elucidate the mechanisms associated with Reye's syndrome, a disease associated with both VZV and changes in lipid metabolism. At the present time, there is no accepted mechanism for the development of Reye's syndrome following virus infection. An environmental stimulus has been suggested as a second trigger which permits the development of Reye's syndrome in certain exposed children. One model has suggested pesticide sprays as a possible second trigger (10, 11, 24). To further understand the mechanisms involved, we have developed an in vitro model system with the pesticide carbaryl and VZV (1, 2, 18). Other work has focused on aspirin as a second trigger (7, 16, 19, 26, 29). The work presented here suggests that, in addition to looking for these environmental stimuli, we may also have to investigate genetic differences in the virus itself which may cause various metabolic changes in host cells.

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LITERATURE CITED

1. **Abrahamsen, L. H., and M. Jerkofsky.** 1981. Enhancement of varicella-zoster virus replication in cultured human embryonic lung cells treated with the pesticide carbaryl. Appl. Environ. Microbiol. **41**:652–656.
2. **Abrahamsen, L. H., and M. Jerkofsky.** 1983. Characterization of varicella-zoster virus enhancement by the pesticide carbaryl. Appl. Environ. Microbiol. **45**:1560–1565.
3. **Anderson, R., and S. Dales.** 1978. Biogenesis of poxviruses: glycolipid metabolism in vaccinia-infected cells. Virology **84**:108–117.
4. **Anderton, P., T. F. Wild, and G. Zwingelstein.** 1981. Modification of the fatty acid composition of phospholipid in measles virus-persistently infected cells. Biochem. Biophys. Res. Commun. **103**:285–291.
5. **Blair, C. D., and P. J. Brennan.** 1972. Effect of Sendai virus infection on lipid metabolism in chick embryo fibroblasts. J. Virol. **9**:813–822.
6. **Bligh, E. G., and W. J. Dyer.** 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**:911–917.
7. **Bradford, W. D., and J. C. Parker, Jr.** 1971. Reye's syndrome. Possible causes and pathogenic pathways. Clin. Pediatr. **10**:148–153.
8. **Caric-Lazar, M., R. T. Schwartz, and C. Scholtissek.** 1978. Influence of the infection with lipid-containing viruses on the metabolism and pools of phospholipid precursors in animal cells. Eur. J. Biochem. **91**:351–361.
9. **Corey, L., R. J. Rubin, M. A. W. Hattwick, G. R. Noble, and E. Cassidy.** 1976. A nationwide outbreak of Reye's syndrome. Its epidemiologic relationship to influenza B. Am. J. Med. **61**:615–625.
10. **Crocker, J. F. S., R. L. Ozere, S. H. Safe, S. C. Digout, K. R. Rozee, and O. Hutzinger.** 1976. Lethal interaction of ubiquitous insecticide carriers with virus. Science **192**:1351–1353.
11. **Crocker, J. F. S., K. R. Rozee, R. L. Ozere, S. C. Digout, and O. Hutzinger.** 1974. Insecticide and viral interaction as a cause of fatty visceral changes and encephalopathy in the mouse. Lancet **ii**:22–24.
12. **Daniel, L. W., M. Waite, L. S. Kucera, L. King, and I. Edwards.** 1981. Phospholipid synthesis in human embryo fibroblasts infected with herpes simplex virus type 2. Lipids **16**:655–662.
13. **Fabricant, C. G., D. P. Hajjar, C. R. Minick, and J. Fabricant.** 1981. Herpesvirus infection enhances cholesterol and cholesterol ester accumulation in cultured arterial smooth muscle cells. Am. J. Pathol. **105**:176–184.
14. **Fabricant, C. G., L. Krook, and J. H. Gillespie.** 1973. Virus-induced cholesterol crystals. Science **181**:566–567.
15. **Gershon, A. A., S. P. Steinberg, L. Gelb, G. Glasso, W. Borkowsky, P. LaRussa, A. Ferrara, and the National Institute of Allergy and Infectious Diseases Varicella Vaccine Collaborative Study Group.** 1984. Live attenuated varicella vaccine. Efficacy for children with leukemia in remission. J. Am. Med. Assoc. **252**:355–362.
16. **Halpin, T. J., F. J. Holtzhauer, R. J. Campbell, L. J. Hall, A. Correa-Villasenor, R. Lanese, J. Rice, and E. S. Hurwitz.** 1982. Reye's syndrome and medication use. J. Am. Med. Assoc. **248**:687–691.
17. **Israel, A., F. Audubert, and M. Semmel.** 1975. Phospholipids in Newcastle disease virus infected cells. Biochim. Biophys. Acta **375**:224–235.
18. **Jerkofsky, M., and L. H. Abrahamsen.** 1983. Variation in human herpesvirus susceptibility to enhancement by the pesticide carbaryl. Appl. Environ. Microbiol. **45**:1555–1559.
19. **Linnemann, C. C., L. Shea, J. C. Partin, W. K. Schubert, and G. M. Schiff.** 1975. Reye's syndrome: epidemiologic and viral studies, 1963–1974. Am. J. Epidemiol. **101**:517–526.
20. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
21. **Marinetti, G. V.** 1964. Chromatographic analysis of polar lipids on silicic acid impregnated paper, p. 339–377. *In* A. T. James and L. J. Morris (ed.), New biochemical separations. D. Van Nostrand Co., New York.
22. **Portoukalian, J., M. Bugand, G. Zwingelstein, and P. Precausta.** 1977. Comparison of the lipid composition of rabies virus propagated in NIL 2 cells maintained in monolayer versus spinner culture. Biochim. Biophys. Acta **489**:106–118.
23. **Reye, R. D. K., G. Morgan, and J. Baral.** 1963. Encephalopathy and fatty degeneration of the viscera. A disease entity in childhood. Lancet **ii**:749–752.
24. **Rozee, K. R., S. H. S. Lee, J. F. S. Crocker, and S. H. Safe.** 1978. Enhanced virus replication in mammalian cells exposed to commercial emulsifiers. Appl. Environ. Microbiol. **35**:297–300.
25. **Schroeder, E. W., and J. M. Merrick.** 1979. Alterations in glycosphingolipid patterns in a line of African green monkey

kidney cells infected with herpesvirus. J. Virol. **32**:734–740.
26. **Starks, K. M., C. G. Ray, L. B. Dominquez, W. L. Stromberg, and D. F. Woodall.** 1980. Reye's syndrome and salicylate use. Pediatrics **66**:859–864.
27. **Steinhart, W. L., J. S. Busch, J. P. Oettgen, and J. L. Howland.** 1984. Sphingolipid metabolism during infection of human fibroblasts by herpes simplex virus type 1. Intervirology **21**:70–76.
28. **Steinhart, W. L., C. M. Nicolet, and J. L. Howland.** 1981. Incorporation of ^{32}P-phosphate into membrane phospholipids during infection of cultured human fibroblasts by herpes simplex virus type 1. Intervirology **16**:80–85.
29. **Waldman, R. J., W. N. Hall, H. McGee, and G. van Amburg.** 1982. Aspirin as a risk factor in Reye's syndrome. J. Am. Med. Assoc. **247**:3089–3094.
30. **Zaia, J. A.** 1981. Clinical spectrum of varicella-zoster virus infection, p. 10–19. *In* A. J. Nahmias, W. R. Dowdle, and R. F. Schinazi (ed.), The human herpesviruses. An interdisciplinary perspective. Elsevier, New York.