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Regulation of Glycolipid Biosynthesis: Effects of Virus Infection and Drug-Induced Translational Inhibition on Glycolipid Metabolism†

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ABSTRACT: Suppression of HeLa cell protein synthesis by either viral infection or translation-inhibiting drugs induces alterations of cell glycolipid concentrations such that there is accumulation of the simple glycolipid species (mono- and diglycosylceramides) as well as a depletion of the more complex ones (triglycosylceramides and gangliosides). In addition, the cellular pool of free ceramides is increased two- to threefold over that found in control cells. The in vitro activities of UDP-glucose:ceramide glucosyltransferase and UDP-galactose:glucosylceramide galactosyltransferase in homogenates prepared from streptovitacin A treated HeLa cells were found to decline progressively with increasing times of drug treatment when exogenous ceramide or glucosylceramide was utilized as carbohydrate acceptor. However, if endogenous ceramide was used as glucose acceptor, the activity of UDP-glucose:ceramide glucosyltransferase in homogenates from cells pretreated for 6 h with translational inhibitor was approximately twofold higher than that found in control cell homogenates, presumably as a result of the increased ceramide concentration in drug-treated cells. The source of increased ceramide in such cells is uncertain but does not appear to be derived from an impairment of ceramide incorporation into sphingomyelin. The results suggest that one component of cellular control of glycolipid biosynthesis may be the regulation of free ceramide levels.

In a previous report from this laboratory (Anderson & Dales, 1978), it was shown that infection of HeLa or L cells with vaccinia virus produced quantitative changes in the cellular glycolipid compositions, in that those of simple, neutral glycolipids, particularly ceramide monohexoside (CMH[1]),

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[1] Abbreviations used: CMH, ceramide monohexoside; CDH, ceramide dihexoside; CTH, ceramide trihexoside; gangliosides G_{M2}, *N*-acetylgalactosaminyl(sialyl)galactosylglucosylceramide; G_{M3}, sialylgalactosylglucosylceramide; G_{D3}, sialylsialylgalactosylglucosylceramide; UDP-Gal, uridine diphosphogalactose; UDP-Glu, uridine diphosphoglucose; FCS, fetal calf serum; ME medium, minimal essential medium; PBS, phosphate-buffered saline; VSV, vesicular stomatitis virus.

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increased, while those of complex species such as ceramide trihexoside (CTH) and gangliosides declined. Furthermore, it was observed that similar, if not identical, alterations in glycolipid pattern could be induced in uninfected cells which had been treated with chemical inhibitors of protein or RNA synthesis. Since it is well documented that vaccinia infection is accompanied by a rapid decline in synthesis of host cell polypeptides (Becker & Joklik, 1964), it seemed likely that the observed changes in cellular glycolipid composition in vaccinia-infected cells were a consequence of virus-induced inhibition of cellular macromolecular synthesis rather than of a virally specified intervention into cellular glycolipid metabolism. Indeed, recent reports of increased CMH and/or decreased ganglioside contents associated with influenza (Huang, 1976) and herpes (Ruhlig & Person, 1977) viral infections suggest that such alterations in glycolipid composition may be common to cellular infections by a wide spectrum of virus species.

Since glycolipids, like glycoproteins, are cell surface molecules involved in various aspects of biological recognition (Fishman & Brady, 1976; Hakomori, 1975), it becomes of prime importance to probe the mechanisms whereby the concentrations of specific glycolipid species are altered in response to external and internal stimuli. Accordingly, the present report attempts to define, in part, the molecular mechanisms responsible for the elevation of CMH and for the depression of complex glycolipid levels which occur as a result of virus infection and/or translational inhibition.

Materials and Methods

Chemicals. Ceramide was obtained by phospholipase C (*Clostridium perfringens*, from Sigma) digestion (Kates, 1972) of beef brain sphingomyelin, isolated according to Dittmer & Wells (1969). Glucosylceramide was purchased from Miles Laboratories and ganglioside G_{M2} from Supelco, and hematoside (G_{M3}) was isolated from beef spleen according to Svennerholm (1972). Uridine diphosphoglucose (UDP-Glc) and uridine diphosphogalactose (UDP-Gal) were obtained from Sigma; streptovitacin A and pactamycin were purchased from Upjohn Co. Radioisotopes, [1-^{14}C]palmitic acid (55 mCi/mmol), [1-^{14}C]galactose (54 mCi/mmol), DL-[3-^{14}C]-serine (47.4 mCi/mmol), L-[^{35}S]methionine (523 Ci/mmol), uridine diphosphoglucose ([1-^{3}H]glucose; 4.84 Ci/mmol), and uridine diphosphogalactose ([U-^{14}C]galactose; 280 mCi/mmol), were purchased from New England Nuclear.

Cell Culture Conditions. Confluent 100-mm Petri dishes of HeLa or vero cells were incubated at 37 °C in 8 mL of minimal essential (ME) medium supplemented with 5% fetal calf serum (FCS) and containing either [1-^{14}C]galactose (0.1 μCi/mL), [1-^{14}C]palmitic acid (0.1 μCi/mL), or DL-[3-^{14}C]serine (0.1 μCi/mL). Incubation was carried out under conditions described in Results. For virus infections, 1 mL of virus inoculum was applied to each monolayer and allowed to adsorb for 1 h at 4 °C before the addition of medium. After the appropriate incubation periods, cells were harvested by scraping with a rubber policeman, spun into pellets for 10 min at 650*g*, and washed twice with cold phosphate-buffered saline (PBS).

For [^{35}S]methionine labeling of cell and virus polypeptides cultures were pulsed for 30 min with 5 mL of methionine-free ME medium containing [^{35}S]methionine (10 μCi/mL). Cells were harvested as above and cell pellets processed for polyacrylamide gel electrophoresis.

Polyacrylamide Gel Electrophoresis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Laemmli, 1970) of [^{35}S]methionine-labeled cell extracts was performed on linear gradients of 5–18% acrylamide, run for 4–5 h at 20 mA. After staining and destaining for visualization of standard molecular weight marker proteins, gels were dried and fluorographed (Laskey & Mills, 1975). Verification that cellular protein synthesis was inhibited in cells treated with either virus or drugs was obtained by excising cell polypeptide bands from fluorographed gels, solubilizing the gel bands in 0.4 mL of Protosol (New England Nuclear) and determining incorporated ^{35}S radioactivity by liquid scintillation spectrometry. For convenience, a major cell polypeptide of molecular weight 45 000, found to be present in both HeLa and vero cell extracts, and whose decreased isotopic labeling after translational inhibition was representative of cell polypeptides in general, was taken to reflect the relative degree of cell protein synthesis.

Glycosyltransferase Assays. HeLa cells grown to confluency in glass bottles (ca. 2×10^7 cells) for various time periods in the presence of streptovitacin A (10 μg/mL) were harvested as above and resuspended in 0.1 M sodium cacodylate buffer (pH 6.5) at a concentration of 10^8 cells/mL and disrupted by Dounce homogenization. Acceptor lipids, ceramide and glucosylceramide, were prepared as translucent sonicated suspensions consisting of ceramide or glucosylceramide (1 mg/mL), egg yolk lecithin (20 mg/mL), bovine serum albumin (1 mg/mL) in 0.1 M sodium cacodylate buffer, pH 6.5. Incubation mixtures contained in a final volume of 60 μL: 1 mg of cell homogenate protein, 13 μg of bovine serum albumin, 260 μg of egg yolk lecithin, 13 μg of ceramide or glucosylceramide, and 10.3 nmol of UDP-Glc ([1-^{3}H]glucose; 100 mCi/mmol) or UDP-Gal ([U-^{14}C]galactose; 12 mCi/mmol) in 0.1 M sodium cacodylate buffer, pH 6.5. After incubating for 20 min (for UDP-Glc:ceramide glucosyltransferase) or 60 min (for UDP-Gal:glucosylceramide galactosyltransferase), the assay mixtures were terminated by the addition of 80 μL of methanol and 160 μL of chloroform. Aliquots were removed from the resultant lower phases for thin-layer chromatography as described below for the preparative isolation of ceramide mono- and dihexosides.

Lipid Analyses. Cell pellets were extracted twice with chloroform–methanol (2:1, v/v) and once with chloroform–methanol (1:2, v/v). The combined extracts were treated with 0.2 N methanolic NaOH to destroy glycerolipids, neutralized with Rexyn H(101) cation exchanger (H^+ form) (Kates, 1972), filtered, evaporated under nitrogen, and partitioned according to Folch et al. (1957). Gangliosides, present in both upper and lower phases, were resolved by chromatography on silica gel H in chloroform–methanol–0.25% $CaCl_2$ (60:35:8, v/v; Van den Eijnden, 1971) with standard gangliosides G_{M3} and G_{M2}. The lower phase lipids were chromatographed in chloroform–methanol–water (65:25:4, v/v) to resolve mono-, di-, and triglycosylceramides and in chloroform–methanol–water (90:10:1, v/v) for the isolation of ceramide. After detection of labeled lipids by autoradiography or by iodine visualization of cochromatographed standard glycolipids, lipids were eluted with chloroform–methanol–water (95:95:10, v/v), dried in scintillation vials under nitrogen, and counted by liquid scintillation spectrometry.

Results

Effect of Virus Infection on the Incorporation of [^{14}C]-Galactose into Cellular Glycolipids. Since previous studies in this laboratory (Anderson & Dales, 1978) indicated that changes in cellular glycolipid levels could be induced by translational suppressants, the abilities of three viruses,

Table I: Incorporation of Label[a] from [^{14}C]Galactose into Glycolipids of Normal and Virus-Infected Cells[b]

glycolipid	uninfected HeLa cells	VSV-infected HeLa cells 4 h PI	VSV-infected HeLa cells 8 h PI	VSV-infected HeLa cells 12 h PI	polio-infected HeLa cells 4 h PI	polio-infected HeLa cells 8 h PI	polio-infected HeLa cells 12 h PI
CMH	480	760	1050	940	810	950	880
CDH	840	1150	1150	620	1020	820	560
CTH	1070	1040	750	560	1100	840	470
G_{M3}	340	310	310	220	290	300	210
G_{D3}	370	290	240	210	320	290	180

glycolipid	uninfected vero cells	VSV-infected vero cells 4 h PI	VSV-infected vero cells 8 h PI	VSV-infected vero cells 12 h PI	measles-infected vero cells 4 h PI	measles-infected vero cells 8 h PI	measles-infected vero cells 16 h PI	measles-infected vero cells 24 h PI
CMH	310	450	590	580	330	330	300	290
CDH	520	600	530	400	500	530	510	490
CTH	590	590	480	360	620	610	610	560
gangliosides	nd	nd	nd	nd	nd	nd	nd	nd

[a] In units of cpm of ^{14}C/mg of cell protein. [b] Virus infection was carried out at a multiplicity of infection of 5. Cultures in 100-mm dishes were maintained at 37 °C with 8 mL of ME medium supplemented with 5% FCS and were labeled with [^{14}C]galactose (0.1 μCi/mL) for 4 h prior to harvesting. Under the conditions used, cell protein synthesis after VSV or poliovirus infection was inhibited to 10% or less of control values within 4 h of inoculation. Measles-infected cells maintained 80% of normal translational levels even up to 24 h post inoculation (PI). Each result represents the average of determinations from three replicate cultures. Maximum deviation from average among replicates was 20%.

vesicular stomatitis virus (VSV), poliovirus, and measles virus, to shut off host cell polypeptide synthesis were checked. Pulse labeling of infected cells with [^{35}S]methionine revealed that cellular protein synthesis, as measured by isotopic incorporation into a major 45 000 molecular weight cellular protein, was decreased by more than 90% by 4 h post inoculation with either VSV or poliovirus. Measles-infected cells, on the other hand, continued to maintain approximately 80% of control polypeptide synthesis even after 24 h of infection. These results are consistent with those reported by other laboratories (Mudd & Summers, 1970; Franklin & Baltimore, 1962; Wechsler & Fields, 1978; Graves et al., 1978).

Monolayer cultures of HeLa or vero cells were incubated for 4-h periods with [1-^{14}C]galactose (0.1 μCi/mL) prior to and at various time points after high multiplicity infection (5 plaque forming units/cell) with either VSV, poliovirus, or measles virus. As shown in Table I, infection of HeLa cells with either VSV or poliovirus or of vero cells with VSV resulted in increased incorporation of labeled galactose into mono- and diglycosylceramides which were maximally labeled at approximately 8 and 4 h post infection, respectively. In contrast, the amounts of isotopic galactose incorporated into the more complex glycolipids, triglycosylceramide and gangliosides, declined progressively following virus infection. These results are very similar to those previously reported (Anderson & Dales, 1978) for vaccinia infection. Infection of vero cells with measles virus, on the other hand, produced very little change in galactose labeling of any glycolipid species even up to 24 h post inoculation. The results with measles virus, which is a relatively weak suppressant of host cell translation (see Results; Wechsler & Fields, 1978; Graves et al., 1978) are consistent with our previous contention (Anderson & Dales, 1978) that inhibition of host cell protein synthesis is necessary for the glycolipid alterations observed

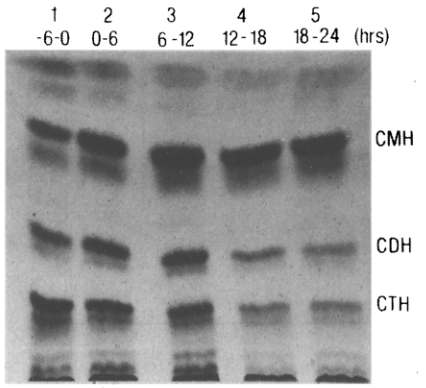

FIGURE 1: Radioautogram from a silica gel H plate of [^{14}C]-galactose-labeled glycolipids from HeLa cells grown in the presence of streptovitacin A (10 μg/mL) and labeled for the final 6 h before harvesting with [^{14}C]galactose (0.1 μCi/mL). Alkali-stable Folch lower phase extracts were chromatographed in chloroform–methanol–water (65:25:4, v/v). (Lane 1) Untreated cells; (lanes 2–5) cells treated for 6, 12, 18, or 24 h with streptovitacin A. This concentration of drug was sufficient to reduce cell protein synthesis by at least 95% of control levels within 6 h of exposure to cells.

Table II: Incorporation of Label from [^{14}C]Palmitic Acid into Sphingolipids of Normal and Translationally Inhibited HeLa Cells[a]

sphingolipid	cpm of ^{14}C/mg of protein: untreated HeLa cells	streptovitacin A treated HeLa cells	pactamycin treated HeLa cells
ceramide	2900	6100	5900
CMH	4300	7900	8100
CDH	5700	6600	6600
CTH	12100	8500	9100
gangliosides	7200	5100	5300
sphingomyelin	48500	40300	42000

[a] Confluent monolayer cultures of HeLa cells were maintained for 18 h in the presence of [^{14}C] palmitic acid (0.1 μCi/mL) with or without translational inhibitor, streptovitacin A (10 μg/mL), or pactamycin (1 μg/mL). At their respective concentrations, each drug inhibited cell protein synthesis to 5% or less that of control, untreated cells within 6 h after their addition.

in infections with such viruses as vaccinia and, as shown above, for VSV and poliovirus.

Effect of Inhibition of Cell Protein Synthesis on the Incorporation of [^{14}C]Galactose and [^{14}C]Palmitate into Cellular Glycolipids. Streptovitacin A and pactamycin, two translational suppressing antibiotics (Felicetti et al., 1966), were found, when used at concentrations of 10 and 1 μg/mL, respectively, to inhibit HeLa cell protein synthesis by at least 95% within 6 h of treatment. The pattern of incorporation of labeled galactose into HeLa cell glycolipids is noticeably affected within 6 h of the addition of streptovitacin A to monolayer cultures of HeLa cells. As shown in Figure 1, incorporation of galactose label into CMH and CDH is initially enhanced, while it is decreased into higher glycolipids, CTH and gangliosides. By 12 h after drug treatment, galactose labeling of CMH is maximal, whereafter the incorporation of isotope into all glycolipid species progressively decreases with time.

In order to determine the observed changes reflected true differences in the amounts of individual glycolipids and were not a result of altered turnover rates of glycolipid carbohydrates, cellular glycolipids were labeled in the ceramide moiety by culturing HeLa cells in medium containing [^{14}C]palmitic acid for 18 h in the presence or absence of protein synthesis inhibitor. As shown in Table II, suppression of translation with either streptovitacin A or pactamycin causes similar increases

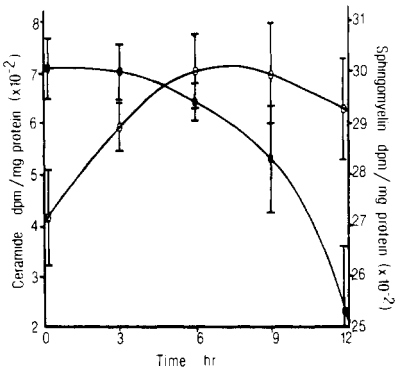

FIGURE 2: Incorporation of [^{14}C]palmitic acid into HeLa cell lipids in the presence of streptovitacin A. Cells were labeled for 15-min intervals with [^{14}C]palmitic acid (0.1 μCi/mL) at the times indicated after addition of streptovitacin A and then harvested for quantitation of labeled ceramide and sphingomyelin. Ceramide (O—O); sphingomyelin (●—●).

in the incorporation of [^{14}C]palmitate into simple glycolipids, CMH and CDH, while effectively reducing the labeling of CTH and gangliosides. Moreover, palmitate labeling of the glycolipid precursor, ceramide, is increased twofold in the drug-treated cells. Thus, it would appear that not carbohydrate turnover but rather ceramide biosynthesis is enhanced by translational suppression. This interpretation is consistent with our previous observation (Anderson & Dales, 1978) that vaccinia-infected cells display an elevated CMH content over control cells, when assayed for glycolipid-bound carbohydrate on a milligram cell protein basis.

Decreased Sphingomyelin Biosynthesis as a Possible Cause of Ceramide Accumulation. Since the observed increases in isotopic labeling of CMH and to a lesser extent, CDH, in translationally inhibited cells may be attributable to an increased ceramide pool, it is of interest to determine the source of the excess ceramide. In HeLa cells, ceramide is utilized both for the formation of glycolipids and for the biosynthesis of sphingomyelin. By labeling HeLa cells with [^{14}C]palmitic acid for 18 h, the ratio of isotopic activity in sphingomyelin to ceramide was found to be approximately 17:1, whereas this ratio is decreased to 7:1 in translationally suppressed cells (from data in Table II). In order to follow more closely the relationship between ceramide and sphingomyelin following inhibition of protein synthesis, monolayers of HeLa cells were pulsed for 15 min in the presence of [^{14}C]palmitic acid before and at various intervals after treatment with streptovitacin A. As shown in Figure 2, an increase in incorporation of label into ceramide is evident within 3 h after addition of drug and this increase is maximal within a further 3 h. Labeling of sphingomyelin, on the other hand, is altered only slightly during the first 3 h of drug treatment and thereafter progressively declines with time. Although not conclusive, this result suggests that the initial rise in ceramide labeling, following inhibition of cell protein synthesis, is not dependent on a reduction of ceramide incorporation into sphingomyelin.

By culturing HeLa cells in the presence of [^{14}C]serine for 6 h periods before and after addition of streptovitacin A, an enhancement in isotopic labeling not only of ceramide but also of sphingomyelin was observed within the first 6 h of drug treatment. A typical time course profile is shown in Figure 3. Although serine incorporation into the glycerophospholipids, phosphatidylserine, -choline, and -ethanolamine, is drastically suppressed after translational inhibition, labeling of the sphingolipids, ceramide and sphingomyelin, was initially stimulated from 0 to 6 h post drug treatment, before falling below control values.

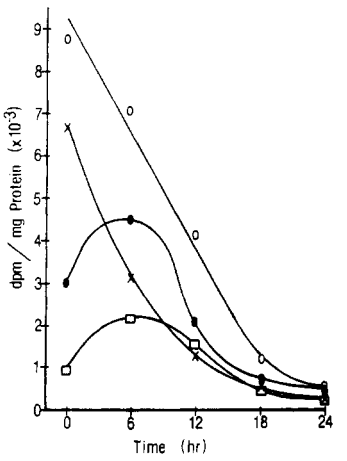

FIGURE 3: Incorporation of [^{14}C]serine into lipids of HeLa cells grown in the presence of streptovitacin A. Cells were labeled for 6-h periods with [^{14}C]serine (0.1 μCi/mL) until the times indicated after addition of streptovitacin A and then harvested for lipid analysis. Phosphatidylcholine + phosphatidylserine (O—O); phosphatidyl ethanolamine (X—X); sphingomyelin (●—●); ceramide (□—□).

Table III. Glycosyltransferase Activities in HeLa Cell Extracts[a]

time after addn of sa[d] (h)	exo cer added (μg)	exo CMH added (μg)	UDP-Glc:ceramide Glc-transferase[b]	UDP-Gal: Glc-ceramide Gal-transferase[b]
0			2.1 ± 0.7[c]	
6			3.5 ± 0.8[c]	
0	13		110	
3	13		85	
6	13		65	
0		13		315
3		13		270
6		13		185

[a] Ceramide and CMH were added as sonicated suspensions consisting of ceramide or CMH (1 mg/mL), egg yolk lecithin (20 mg/mL), and bovine serum albumin (1 mg/mL) in 0.1 M sodium cacodylate buffer, pH 6.5. Incubation mixtures contained, in volumes of 60 μL, 1 mg of cell homogenate protein, 13 μg of bovine serum albumin, 260 μg of egg yolk lecithin, 13 μg of ceramide or CMH, 10 nmol of UDP-Glc or UDP-Gal, and 6 μmol of sodium cacodylate buffer, pH 6.5. [b] In units of pmol (mg of protein)$^{-1}$ h^{-1}. [c] Results of seven determinations. [d] sa = streptovitacin; exo cer = exogenous ceramide.

Effect of Inhibition of Protein Synthesis on Glycolipid Glycosyl Transferase Activities. The increased incorporation of labeled palmitic acid and galactose into CMH and CDH observed in drug-treated cells (Figure 1; Table II) prompted an investigation of the activities of glycosyl transferases involved in the biosynthesis of these simple glycolipids. Cell homogenates prepared from HeLa cell monolayers after various times of treatment with streptovitacin A were incubated at 37 °C with or without appropriate acceptor lipids, ceramide or glucosylceramide, in the presence of isotopically labeled UDP-Glc or UDP-Gal. In the absence of exogenous ceramide, the activity of the UDP-Glc:ceramide glucosyltransferase was approximately twofold higher in cell homogenates derived from 6-h drug-treated cells than from control cultures (Table III). However, when ceramide was added to the incubation mixtures, UDP-Glc:ceramide glucosyltransferase activity decreased to 77% and 59% of control values in cell homogenates prepared after 3 and 6 h, respectively, of streptovitacin A treatment. In light of the observed stimulation of incorporation of labeled palmitic acid into free cellular ceramide in translationally inhibited cells (Table II), it seems likely that the apparent increase in endogenous-ceramide-

dependent UDP-Glc:ceramide glucosyltransferase activity is merely a reflection of the increased ceramide pool in these cells. By the addition of excess exogenous ceramide to cell homogenates, any inequities in the endogenous ceramide pools are eliminated, and it becomes evident (Table III) that UDP-Glc:ceramide glucosyltransferase activity progressively declines after inhibition of cellular protein synthesis. Similarly, it was found that the activity of UDP-Gal:glucosylceramide galactosyltransferase was substantially decreased within 6 h of translational inhibition (Table III). Such decreased activities of glycolipid glycosyltransferases are undoubtedly responsible for the observed decreases in isotopic labeling of the more complex glycolipid species, CTH and gangliosides, in protein-synthesis-inhibited cells.

It is, thus, apparent that the observed increased incorporation of labeled palmitic acid or galactose into simple glycolipids in translationally inhibited HeLa cells cannot be explained on the basis of altered activities of glycolipid glycosyltransferases. Rather it appears that the increased levels of CMH and CDH arise as a direct consequence of the enlarged intracellular pool of free ceramide.

Discussion

Previous investigations of glycolipid metabolism in cells infected with vaccinia virus showed an increased content of CMH and a decreased amount of gangliosides when compared with uninfected cells (Anderson & Dales, 1978). Moreover, analogous alterations in glycolipid composition could be induced in uninfected HeLa cells after inhibition of cell protein synthesis with a variety of drugs. Similar increases in CMH content have been observed in rat cerebellar cultures treated with cycloheximide (Benjamins et al., 1976), although, surprisingly, treatment with chloramphenicol, another suppressor of protein synthesis, did not alter the level of this glycolipid. The experiments reported here demonstrate that infection with either VSV or poliovirus, which, like vaccinia, efficiently inhibit host cell translation, also induces similar changes in glycolipid metabolism, whereas infection with measles virus, a poor suppressor of cell protein synthesis (Wechsler & Fields, 1978; Graves et al., 1978), results in very little change in the host glycolipid pattern. Quite possibly, the increased CMH contents associated with influenza (Huang, 1976) and herpes (Ruhlig & Person, 1977) virus infections are similarly consequences of virus-induced inhibition of cellular translation.

The present results provide evidence that the observed glycolipid changes are accompanied by an increase in the cellular ceramide pool as well as by a reduction in enzymatic activities of glycolipid glycosyltransferases. From two observations it appears that the increased level of free ceramide may be directly responsible for the enhanced synthesis of CMH in translationally inhibited cells: first, maximal ceramide accumulation temporally precedes peak CMH levels by roughly 6 h (cf. Figures 1–3); second, ceramide accumulation is responsible for the apparent increase in UDP-Glc:ceramide glucosyltransferase activity in homogenates from drug-treated vs. control cells, since the equalization of ceramide pools by the addition of excess, exogenous ceramide results in lower transferase activities in homogenates from drug-treated cells relative to those from controls (Table III).

Endogenous inhibitors of glycolipid glycosyltransferases have been recently reported in rat brain (Constantino-Ceccarini & Suzuki, 1978), suggesting a possible role for enzymatic suppressors in the regulation of glycolipid biosynthesis. On the basis of our determinations of glycosyltransferase activities in HeLa cells, however, it appears unlikely that suppression of cell protein synthesis leads to stimulation of glycolipid glycosylation by the depletion of some factor inhibitory for transferase activity.

At present, little is known about the cellular mechanisms involved in regulating glycolipid levels, although certain alterations in concentrations of individual glycolipids are known to take place as a result of a variety of situational changes such as: growth to confluency (Yogeeswaren & Hakomori, 1975), cell cycle variations (Chatterjee et al., 1973), morphological differentiation (Simmons et al., 1975) and transformation (Brady & Fishman, 1974; Hakomori, 1975). For the most part, these alterations appear to be caused by changes in enzymatic activities of specific glycosyltransferases or glycosidases rather than of enzymes involved in the biosynthesis of the core, ceramide moiety. In the translationally suppressed cell system described here, the enhanced synthesis of CMH appears to result directly from an increase in cellular ceramide, although it remains to be determined whether this arises from enhanced de novo ceramide synthesis or suppressed ceramidase activity. By using either [^{14}C]palmitic acid or [^{14}C]serine as sphingolipid precursors, little evidence was obtained that suppression of protein synthesis blocks ceramide incorporation into sphingomyelin. Indeed, within the first 6 h of streptovitacin A treatment, the incorporation of [^{14}C]serine into both ceramide and sphingomyelin increased substantially over that observed in untreated cells (Figure 3). Speculatively, one may suggest that sphingolipid biosynthesis, in general, is enhanced after translational inhibition as a result of increased availability of the amino acid, serine, which is no longer utilized for protein synthesis.

The phenomenon of ceramide-induced enhancement of glycolipid synthesis described here may be not unlike the condition seen in cases of Farber's disease (Moser et al., 1969) in which elevated levels of sphingomyelin and glycolipids are observed as a result of ceramide accumulation. Clearly, cellular levels of free ceramide are most determinatory for the total concentrations of both sphingomyelin and glycolipids, although the precise regulatory or compensatory interrelationships are poorly understood.

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Electrophoretic Behavior of Cytochrome *b* in a Partially Purified Preparation and Evidence for High Molecular Weight Associated Mitochondrial Translation Products†

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ABSTRACT: A partially purified preparation of cytochrome *b* lacking cytochromes *c*, c_1, and a–a_3 was isolated from yeast submitochondrial particles. The preparation contained 7 nmol of heme *b*/mg of protein and upon dodecyl sulfate gel electrophoresis separated into four major bands with apparent molecular weights of 31 000, 35 000, 48 000, and 50 000. The 31 000-dalton band, identified as cytochrome *b* by comparison with total mitochondrial translation products, showed normal migration behavior during dodecyl sulfate electrophoresis in different concentrations of acrylamide. Furthermore, this polypeptide migrated at a molecular weight of 31 000 when the preparation was heated in dissociation medium at 20, 37, 70, or 100 °C, when phenylmethanesulfonyl fluoride, the protease inhibitor, was present or absent, and in dodecyl sulfate–urea gels. By contrast, cytochrome *b* in the intact mitochondrial membrane displayed anomalous migration behavior in gels of different acrylamide concentrations. Two proteins of higher molecular weight are present in the immunoprecipitates of labeled mitochondria treated with the specific antiserum against cytochrome *b*. These polypeptides are products of mitochondrial protein synthesis as they are labeled in the presence of cycloheximide, not labeled in the presence of chloramphenicol, and absent in petite mutants. These mitochondrial translation products do not copurify with cytochrome *b* as they are not present in the partially purified cytochrome *b* preparation obtained from yeast cells labeled in the presence of cycloheximide. These proteins do not appear to be precursors of cytochrome *b* as the addition of a short or long chase of unlabeled amino acid did not alter the labeling of these high molecular weight proteins relative to cytochrome *b*. Furthermore, varying the time of pulse label in the presence of cycloheximide from 3 to 30 min also did not indicate any precursor–product relationship between these high molecular weight proteins and cytochrome *b*.

Our previous studies on the purification of cytochrome *b* from yeast mitochondria and its biogenesis on mitochondrial ribosomes raised two unanswered questions (Lin & Beattie, 1978; Lin et al., 1978). First, the actual molecular weight of cytochrome *b* was not conclusively demonstrated because proteolytic digestion to solubilize the proteins was a necessary step in the overall purification scheme. The purified cytochrome *b* polypeptide thus obtained had a molecular weight of 28 000 based on dodecyl sulfate gel electrophoresis and 28 800 based on sucrose gradient centrifugation (Lin & Beattie, 1978); however, when immunoprecipitates obtained from labeled yeast mitochondria treated with the specific antiserum against cytochrome *b* in the presence of phenylmethanesulfonyl fluoride ($PhCH_2SO_2F$[1]), the protease inhibitor, were analyzed by gel electrophoresis, the major labeled band in the immunoprecipitate migrated with a molecular weight of 31 000 (Lin et al., 1978). Alternately, the differences in molecular weight of the purified protein and the major band of the immunoprecipitate may have occurred because of the various conditions used for electrophoresis. Recently, several groups have reported that the migration of cytochrome *b* in b–c_1 complexes isolated from either beef heart (Bell & Capaldi, 1976; Marres & Slater, 1977) or yeast (Groot et al., 1978) mitochondria varies depending on the gel conditions used. Similarly, the molecular weight of cytochrome *b* varies when total yeast mitochondrial translation products were examined on polyacrylamide gels of different concentrations (Groot et al., 1978; Cabral et al., 1978).

In the present study, we have reevaluated the molecular weight of cytochrome *b* under various conditions of gel electrophoresis and solubilization using both intact mito-

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[1] Abbreviation used: $PhCH_2SO_2F$, phenylmethanesulfonyl fluoride.

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