Glycosphingolipids are membrane-bound components containing biologically active oligosaccharide moieties which confer blood group specificity on erythrocytes (1), and probably function as tissue antigens (2). The sialoglycosphingolipids contain biologically active oligosaccharide moieties which confer blood group specificity on erythrocytes (1), and probably function as tissue antigens (2). The sialoglycosphingolipids have also been postulated to have a role in neuronal function (3) and sulfatide GL-1bS; a role as carrier or receptor in hormone-dependent sodium ion transport (4). Although much is known of glycosphingolipid biosynthesis (5, 6) and catabolism (7–9) in vitro, little is known of their in vivo metabolism. Some insights into their biosynthesis and catabolism have been gained from the study of changes resulting from viral transformation of mammalian cell lines in tissue culture (10, 11), and the pattern of glycosphingolipid accumulation in genetic diseases characterized by mutant lysosomal hydrolases (7, 9). It is difficult to carry out in vivo studies on humans because of restrictions on the amount of radioactive isotope that may be used and the complexity of studies with stable isotopes (12). However, glycosphingolipid metabolism can be studied in human skin fibroblasts by a procedure first established for studying the turnover of glycosphingolipids in porcine blood (13). This paper reports the glycosphingolipid composition of normal human skin fibroblasts and the use of D-[U-14C]glucose to study their metabolism.

**EXPERIMENTAL PROCEDURES**

**Fibroblast Culture Conditions—**Skin fibroblasts, which had undergone three to six transfers, were cultured as previously described (14) in modified Eagle’s medium (Gibco) supplemented with ascorbic acid (100 mg per liter), streptomycin (75 mg per liter), and penicillin (100 units per ml). The medium was further supplemented with fetal calf serum (10%) and calf serum (10%) unless otherwise stated. When grown under these conditions in Falcon 100 mm diameter petri dishes at 37°C under a CO2 tension of 10% (pH fluctuation of 7.2 to 7.4) a confluent monolayer of cells (2.5 x 10⁶ per plate) was obtained in approximately 4 weeks. Mycoplasma infection was monitored by routinely culturing cells and media in mycoplasma agar medium (Gibco). All studies described in this paper were carried out on cells judged by this criterion to be free from mycoplasma. Prior to harvesting, cells were washed with 0.15 M NaCl to remove the culture medium and then scraped from the plate with a rubber policeman, washed with 2.0 ml 0.05 M acetate buffer, centrifuged at 600 x g for 10 min, and the pellet lyophilized.

**Isolation of Glycosphingolipids—**Total cellular lipids were extracted from the lyophilized powder (10³ cells; approximately 100 mg, dry weight) by stirring first in methanol (100 ml) and acetate buffer, centrifuged at 600 x g for 10 min, and the pellet lyophilized.
then adding chloroform (2 x 100 ml) over a period of 2 hours. Following the Folch (15) procedure, the resulting lower phase was fractionated into neutral, polar, and individual glycosphingolipids on a silicic acid column by the method of Vance and Sweeley (16). The crude glycosphingolipid fraction (acetone-methanol, 9:1) was treated with 0.6 N NaOH in methanol (1.0 ml) in the presence of 1.0 ml of chloroform for 1 hour. After neutralization with concentrated HCl (0.05 ml) the mixture plus washings (chloroform-methanol, 2:1) was diazylated against several changes of distilled water for 24 hours. Detailed studies (17) have shown that there is no loss of glycosphingolipids during dialysis; the dialysis tubing was washed with 2 x 10 ml of chloroform-methanol (2:1) as a precaution against such loss. Individual glycosphingolipids were resolved by thin layer chromatography in chloroform-methanol-water (11:4:0.6). The upper aqueous phase (from the Folch procedure) was evaporated to dryness, subjected to alkaline methanolysis as described above, and dialyzed exhaustively. The crude gangloside fraction was separated by thin layer chromatography using chloroform-methanol-2.5 N NH₄OH (60:40:9) (two sequential developments). Individual glycosphingolipids were visualized by iodine and a permanent record of the thin layer chromatography plate was made on diazo projection paper (B. K. Elliott Co., Pittsburgh, Pa.). The diazo paper is placed over the thin layer chromatography plate and illuminated from below on an x-ray viewer for approximately 5 min. Exposure to ammonia vapor gives the blueprint, examples of which are reproduced in Figs. 1 and 2. The glycosphingolipid bands were scraped, transferred to a sealing tube with reduced ends and a Kimflow fritted disc of coarse porosity (Kimble Products, Owens, Ill.) and eluted with 20 ml of chloroform-methanol-water (10:4:0.5).

Analysis and Identification of Glycosphingolipids—After addition of mannitol (0.04 to 0.2 μmoles), glycosphingolipids were converted to methyl glycosides by methanolysis in 1.0 N HCl (16, 18) neutralized with silver carbonate and re-N-acetylated if necessary (18). Quantitative estimation and identification was carried out in a single procedure by gas-liquid chromatography of the trimethylsilyl derivatives (16, 17) using a Hewlett-Packard F and M model 402 equipped with a flame ionization detector and a 6 foot x 1/4 inch 3% OV-1 on Supelcoport (100 to 120 mesh) column. Glycosphingolipids were identified by their Rₑ value and the ratio of the constituent sugars obtained from the gas-liquid chromatography trace. The fatty acid composition was also estimated by gas chromatography of the fatty acid methyl esters resulting from methanolation, using a 15% EGA (6 foot x 1/4 inch external diameter) U-shaped glass column. Since di- and tri-unsaturated fatty acids were minor constituents of fibroblast glycosphingolipids, a 3% OV-1 column was used for routine fatty acid analyses. The sphingosine base composition was determined by the method of Polito et al. (19). The structures of the major glycosphingolipids were confirmed by direct mass spectrometry using an LKB 9000 combined Gas-Liquid Chromatograph-Mass-Spectrometer (20).

Incorporation of d-[U-14C]Glucose into Fibroblast Glycosphingolipids—d-[U-14C]Glucose in 10 ml of modified Eagle's medium (14) was added to 35 100 mm petri dishes (10 μCi per plate, specific activity 270 μCi per μmole); each of which contained approximately 2.0 x 10⁶ cells (three to seven passages) in a confluent monolayer culture. Studies with skin fibroblasts at a density of 1.25 x 10⁵ cells/100 mm plate showed that the initial level of glucose in the medium (400 mg per liter) is reduced to 300 after 24 hours, 200 after 48 hours, and 100 after 120 hours; medium is replenished every 72 hours for all studies unless stated otherwise. After 6 hours the medium from five of the plates was discarded, the cells were washed with saline, harvested, and lipid fractions isolated as described above. The hexose, sphingosine, and fatty acid moieties of the glycosphingolipids were counted separately as previously described (13). Since preliminary experiments had shown that maximum incorporation occurred between 24 and 48 hours, cells were harvested at 6, 24, 36, and 48 hours. After 48 hours, fresh media was added to chase the d-[U-14C]glucose from the remaining plates of cells and further cells were harvested at 72, 102, and 168 hours.

RESULTS

Glycosphingolipid Composition—Fibroblasts cultured from human skin were found to contain GL-1α, GL-2α, GL-3, GL-4 (Fig. 1), G₂₃ and G₂₅ (Fig. 2); GL-1β was a minor component (Fig. 3). These glycosphingolipids were identified by their cochromatography with authentic standards (isolated from human

**Fig. 1.** Thin layer chromatographic separation of neutral glycosphingolipids from normal human skin fibroblasts in the solvent system, chloroform-methanol-water (11:4:0.6). A separation of human erythrocyte glycosphingolipids is included for comparison.

**Fig. 2.** Thin layer chromatographic separation of gangliosides from normal human skin fibroblasts in the solvent system, chloroform-methanol-2.5 N NH₄OH (60:10:9). Sample 1 is the gangliosides from 30 x 10⁶ cells, Sample 2, the gangliosides from 125 x 10⁶ cells; a gray matter ganglioside mixture is included for comparison.
Glycosphingolipid Structure—The carbohydrate moieties of normal fibroblast glycosphingolipids appeared identical to analogous compounds isolated from human blood and tissue. No precise information as to the nature of the anomeric linkages was obtained but since GL-3 accumulates in fibroblasts from patients with Fabry's disease (21), the terminal galactose residue must be α-(1 → 4), since GL-2a accumulates in fibroblasts from a patient with lactosylceramidosis (22) the galactose-glucose linkage is probably β-(1 → 4), and since GL-4 accumulates in GM₂ gangliosidosis type II fibroblasts (23) the linkage of the terminal galNAc is β-(1 → 3) to galactose. Mass spectrometric analysis of GL-4 (Table II) showed it to be identical to human erythrocyte globoside (20, 24) although the actual anomeric configuration of the monosaccharides cannot yet be determined by mass spectrometry.

**Fatty Acid Composition of Glycosphingolipids**—The distribution of glycosphingolipids in the chloroform-methanol-water (Folch) partition was investigated. Only trace amounts of GL-1a, GL-3, and GL-4 were found in the upper phase (Table III). The only neutral glycosphingolipid which constituted more than 5% of the total was GL-2a, which may have arisen from the degradation of GM₂ during the processing of the upper phase. The major sialoglycosphingolipid, GM₃, appeared to be partitioned almost equally between the two phases while GD₃ was exclusively in the upper phase. Reextraction of the cells with CHCl₃-CH₃OH, 1:1 did not increase the yield of sialoglycosphingolipids. Neutral and phospholipids of fetal calf and calf serum contained

![Figure 3](image_url)

**Figure 3.** Gas liquid chromatographic analysis of GL-1a, GL-2a, and GL-3 as trimethylsilyl derivatives of their methylglycosides. Separations were achieved on 3% OV-1 with temperature programming from 160° at 2° per min. Three peaks, representing the furanose, α- and β-pyranose forms, are obtained for galactose and two peaks (α- and β-pyranose) for glucose. Mannitol is the internal standard.

![Figure 4](image_url)

**Figure 4.** Gas liquid chromatographic analysis of GL-4, GM₃, and GD₃ as trimethylsilyl derivatives of their methylglycosides. Separations were achieved on 3% OV-1 with temperature programming from 160° at 2° per min. Two peaks (β- and α-pyranose) are obtained for galNAc and one major peak (α-) for NANA.

### Table I

**Glycosphingolipids in fibroblasts cultured from skin biopsies of normal patients**

The results are the mean of duplicate analyses on cells from four normal patients. Cells were grown in medium supplemented with 10% fetal calf serum and 10% calf serum as described in the text.

| Glycosphingolipid | 1 | 2 | 3 | 4 | Mean |
|-------------------|---|---|---|---|------|
| GL-1a             | 0.65 | 0.41 | 0.47 | 0.23 | 0.44 |
| GL-1b             | 0.65 | 0.08 | 0.63 | 0.01 | 0.08 |
| GL-2a             | 0.22 | 0.15 | 0.19 | 0.22 | 0.20 |
| GL-3              | 0.78 | 0.67 | 0.61 | 0.56 | 0.66 |
| GL-4              | 0.41 | 0.42 | 0.25 | 0.23 | 0.32 |
| GM₃               | 1.69 | 0.88 | 0.75 | 1.02 | 1.00 |
| GD₃               | 0.23 | 0.20 | 0.21 | 0.17 | 0.20 |
principally C16:0, C18:0, C18:1, C18:2, and C20:4 fatty acids. In contrast, the major fatty acids in fibroblast glycosphingolipids were C20:0 and C22:0, with C18:1 and C24:1 the only unsaturated fatty acids present in significant amounts (minor components of GL-3 and GL-4 as shown in Table IV). It seems unlikely, therefore, that serum fatty acids represent a direct source of fibroblast glycosphingolipid fatty acids. Both GL-2a and GM2 were resolved into two bands by the thin layer chromatography system. Fatty acid analysis showed that the faster moving band in each case contained more of the shorter chain length fatty acids, although the separation of C19:0 and C20:0 was not complete. The virtual absence of C19:0, C20:0, and C21:0 fatty acids accounts for the gap between the two bands.

Sphingosine Base Composition of Glycosphingolipids—The predominant sphingosine base (70%) was C18-sphingosine (sphingosine-4-ene) which is characteristic of human visceral glycosphingolipids (23). Only trace amounts of longer (C20 and C22) or shorter (C16 and C18) chain homologs were found, but the amount of C18-dihydrosphingosine (sphinganine) (30%) was higher than that found in most tissues (20, 25).

Origin of Fibroblast Glycosphingolipids—Since calf serum was used as a supplement to the medium, the glycosphingolipid composition of both fetal calf serum and calf serum was examined. In contrast to human serum (16) calf serum contained very low levels of glycosphingolipids; fetal calf serum contained virtually none (Table V). The total amount of serum (and therefore glycosphingolipid) used in the growth of 1 g dry weight of cells is actually quite large (Table V). However, analysis of the culture medium after exposure to normally metabolizing fibroblasts for 48 hours, showed very little change in the concentration of glycosphingolipids. Increasing the amount of serum supplement from 10% to 30% had little effect on the intracellular glycosphingolipid content and in fact produced a decrease when expressed on a pmole per g dry weight basis (Table VI). The sialic acid in human fibroblasts, as judged by gas liquid chromatography (18) following mild acid (0.01 x HCl) hydrolysis or methanolysis for 2 hours, was found to be exclusively of the N-acetyl type. One exception was a strain from a patient with

| Table II
| Comparison of mass spectra between fibroblast GL-4 and human erythrocyte GL-4 |

One mole of glycosphingolipid contains one mole of sphingosine (major fragment for C18-sphingosine, m/e 311), therefore, the intensities of the major ions are expressed relative to that of m/e 311 (19). Since human skin fibroblasts contain some dihydro-sphingosine (m/e 313), intensities are expressed relative to the total intensity of ions due to long chain bases, i.e. m/e 311 + 313.

| m/e | Human skin fibroblast GL-4 | Human red cell GL-4 |
|-----|-----------------------------|---------------------|
| 103 rel. | 2.50 | 2.42 |
| 175 rel. | 1.30 | 1.10 |
| 191 rel. | 0.88 | 0.90 |
| 204 rel. | 3.46 | 3.59 |
| 217 rel. | 3.93 | 3.54 |
| 243 rel. | 0.88 | 1.22 |
| 271 rel. | 0.88 | 1.33 |
| 315 rel. | 0.56 | 0.62 |
| 330 rel. | 1.82 | 1.86 |
| 420 rel. | 2.96 | 3.20 |

| Table III
| Glycosphingolipid composition of upper and lower Folch partition phases |

| Glycosphingolipid | Upper phase | Lower phase |
|-------------------|-------------|-------------|
| GL-1a             | <0.01       | 0.27        |
| GL-1b             | <0.01       | 0.01        |
| GL-2a             | 0.06        | 0.17        |
| GL-3              | <0.01       | 0.55        |
| GL-4              | <0.01       | 0.21        |
| GM1               | 0.40        | 0.62        |
| GM2               | 0.27        | <0.01       |

| Table IV
| Fatty acid composition of glycosphingolipids in human skin fibroblasts |

| Fatty acid | GL-1a | GL-3b | GL-4b | GMa | GMB |
|------------|-------|-------|-------|-----|-----|
| C16:0      | 18.6  | 12.1  | 21.3  | 9.1 | 10.4 | 4.4  | 16.7 |
| C18:0      | 3.0   | 2.9   | 6.2   | 2.2 | 3.7  | 1.7  | 3.6  |
| C18:1      | 11.3  | 9.2   | 22.8  | 6.8 | 8.6  | 6.4  | 12.8 |
| C18:2      | 5.3   | 2.5   | 3.5   | 2.0 | 2.9  | 2.5  | 3.5  |
| C18:3      | 3.4   | 1.2   | 2.4   | 0.5 | 1.5  | 1.5  | 1.7  |
| C22:0      | 17.0  | 27.6  | 15.5  | 23.2 | 26.6 | 29.8 | 22.1 |
| C22:1      | 4.3   | 7.3   | 2.3   | 7.9 | 7.0  | 8.2  | 5.3  |
| C24:0      | 16.8  | 35.9  | 10.9  | 47.0 | 38.1 | 44.7 | 26.3 |
| C24:1      | 1.8   | 0.6   | 0.9   | 1.1 | 0.5  | 0.3  | 1.3  |

# Ratio of fatty acid chain length to number of double bonds.
# Also contained C14:0, C15:0, C16:0, and C18:1 fatty acids (5.9%).
# U, faster moving (upper) band; L, slower moving (lower) band after thin layer chromatography in chloroform-methanol-water (10:40:6).
# Also contained C14:0, C15:0, C16:1, and C18:1 fatty acids (2.0%).
The medium used in these studies was modified Eagle's, prepared as described in the text.

| Glycosphingolipid | Calf serum (10%) | Fetal calf serum (10%) | Conditioned (used) medium* | Total amount of glycosphingolipids fed to cells during 1-week growth period |
|-------------------|-----------------|------------------------|---------------------------|-----------------------------------------------|
| GL-1a             | 0.033           | 0.008                  | 0.034                     | 1.24                                          |
| GL-2a             | 0.020           | 0.005                  | 0.023                     | 1.00                                          |
| GL-3              | 0.000           | 0.004                  | 0.015                     | 0.50                                          |
| GL-4              | 0.010           | 0.001                  | 0.015                     | 0.44                                          |
| Glb3              | 0.035           | 0.002                  | 0.034                     | 1.46                                          |

* This medium initially contained 10% calf serum and 10% fetal calf serum.

Mixture of N-glycolyl and N-acetyl neuraminic acids.

### Table VI

**Effect of serum concentration on glycosphingolipid content of normal human fibroblasts**

The cells were grown to confluency under all three conditions, the rate of growth being much slower in 10% FCS*. Apart from serum, the medium is as described in the text.

| Medium | GL-1a | GL-2a | GL-3 | GL-4 | GM3 |
|--------|-------|-------|------|------|-----|
| 10%    | 0.41  | 0.27  | 1.27 | 0.53 | 1.03|
| 10:10* | 0.40  | 0.15  | 0.78 | 0.25 | 0.79|
| 20:10* | 0.37  | 0.15  | 0.59 | 0.42 | 0.88|

* Modified Eagle’s medium containing 10% fetal calf serum.
* Modified Eagle’s medium containing 10% fetal calf serum and 10% calf serum.

Hurler’s disease where a mixture of N-acetyl- and N-glycosyl-neuraminylgalactosylglucosylceramide (Gq3) was found (26). Gas liquid chromatography analysis showed calf serum to contain significant amounts of N-glycolyl Gq3.

**Glycosphingolipids in Other Mammalian Cells in Culture**

The glycosphingolipid content appears specific for the cell type but independent of the medium. Thus Swiss and Balb mouse 3T3 fibroblasts, 1300 mouse neuroblastoma (NB41A and NB2a) and rat RGC-6 glial tumor (27) cell lines, grown in the same medium as human fibroblasts showed a completely different spectrum of glycosphingolipids (Table VII). The analyses of BHK 21/13 cells (where the major glycosphingolipid is GM3) are in agreement with a qualitative report (28) by Hakomori et al. and mouse 3T3 cells are in agreement with the report on their ganglioside composition by Kumar et al. (11). Glycosphingolipids GL-1a, GL-2a, and GM3 from BHK 21/13 cells showed a predominance of C16:0, C18:0, and C18:1 fatty acids in contrast to the presence of longer chain length fatty acids in human fibroblast glycosphingolipids. The similarity in fatty acid composition between individual glycosphingolipids in the BHK cells supported the concept of direct metabolic interconversion as previously found for human skin fibroblasts (Table IV).

The synthesis of glycosphingolipids was studied by using D-

![Fig. 5. Semilogarithmic plot to show the incorporation of [U-14C]glucose into the hexose moiety of human skin fibroblast glycosphingolipids. GL-1a, ○-○; GL-2a, ■-■; GL-3, □-□; GL-4, ■-■; GM3, △-△; GD3, ▲-▲.](http://www.jbc.org/)

[U-14C]glucose. The data in Fig. 5 show that maximum labeling of all six glycosphingolipids occurred between 24 hours and 48 hours. After this time (even if the medium was not changed) there was a rapid loss of 90% of the 14C label over the next 24 hours followed by a much more gradual loss of the final 10%, the half-life in this slow phase was 2 to 3 days. Although most of the label in the glycosphingolipid was found in the hexose moiety, significant labeling of the sphingosine and fatty acid moiety occurred. Both the rate and extent of loss of label roughly
paralled the loss of label from the hexose moiety. An example of this effect for GL-1a is given in Table VIII. Thus glycosphingolipids in cultured human skin fibroblasts are metabolized as whole units and do not appear to undergo exchange reactions such as acyltransferase. However, the reutilization of ceramide cannot be ruled out without further study.

### DISCUSSION

We have shown in this study that human skin fibroblasts synthesize and degrade the six glycosphingolipids (GL-1a, GL-2a, GL-3, GL-4, GM3, and GD3) associated with visceral organs such as liver and spleen. Such glycosphingolipids are believed to be associated with the plasma membrane of cells (28). Of the glycosphingolipids associated with nervous tissue, GL-1b was detected in trace amounts but sulfatide (GL-1bS) was undetectable. In fact, sulfatide has only been reported to occur in one cell type of cultured cell, neoplastic mast cells (30) and our studies on various mammalian fibroblast lines such as BHK, and mouse 3T3 (Swiss and Balb), did not reveal measurable sulfatide levels (Table VII). Since it would be desirable to study the gangliosides (GM1 and GM2) in cultured skin fibroblasts. Further, fatty acid analysis of the fibroblast glycosphingolipids revealed the high content of behenate (C57:0) and lignocerate (C56:0) characteristic of visceral organs. The similarity in fatty acid composition between the six glycosphingolipids was sufficient to support the concept of metabolic interconversion as indicated above. It is perhaps significant that the major monosialoglycosphingolipid of visceral tissue (GM3) contains predominantly long chain (hydrophobic) fatty acids, whereas that of brain (GM1) contains predominantly stearate (C18:0) (less hydrophobic).

Using [U-14C]glucose as precursor, it was possible to show that 14C is incorporated into the hexose, fatty acid, and sphingosine moieties of skin fibroblast glycosphingolipids. Maximum incorporation occurred between 24 and 48 hours and 90% of the label was lost between 48 and 72 hours. A semilogarithmic plot of specific activity versus time emphasized the biphasic nature of the decay curve, suggesting that there were two pools of glycosphingolipid. Previous studies on the turnover rates of membrane lipids (32) indicate a value of 2 to 3 days, comparable to the values found for glycosphingolipids (GL-3, T1/2 = 2.4; GL-4, T1/2 = 2.9) between Day 3 and Day 7.

The rapid metabolism of 14C-labeled glycosphingolipids in normal skin fibroblasts indicates that catabolic enzyme deficiencies should be readily detectable. The application of these techniques to the study of such inborn lysosomal hydrolase deficiencies is described in the subsequent paper (23).

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### REFERENCES

1. Harwood, S. L. (1979) Chem. Phys. Lipids 5, 90–115
2. Rapport, M. M. and Graf, L. (1969) Progr. Allergy 13, 273–331

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**Table VIII**

| Time after pulse (hrs) | GL-1a | GL-2a | GL-3 | GL-4 | GL-5a | GL-6a |
|------------------------|-------|-------|------|------|-------|-------|
| 6                      | 4,320 | 255   | 75   |      |       |       |
| 24                     | 960,000 | 22,000 | 26,400 |      |       |       |
| 35                     | 750,000 | 17,000 | 30,000 |      |       |       |
| 48                     | 425,000 | 10,650 | 17,000 |      |       |       |
| 72                     | 32,000  | 3,400  | 5,700  |      |       |       |
| 102                    | 11,200  | 2,740  | 2,300  |      |       |       |
| 168                    | 5,800   | 1,260  | 1,350  |      |       |       |
Glycosphingolipids in Cultured Human Skin Fibroblasts: I. CHARACTERIZATION AND METABOLISM IN NORMAL FIBROBLASTS
Glyn Dawson, Reuben Matalon and Albert Dorfman

J. Biol. Chem. 1972, 247:5944-5950.

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