Glycolipids and Fatty Acids of Two Dog Kidney Cell Lines

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Glycolipid and fatty acid compositions were studied in whole cells and plasma membranes from two dog kidney cell lines (Madin–Darby and SV40-transformed cells) grown in monolayer and suspension cultures. Glycolipids, which account for 5% or less of the total lipids in dog kidney cells, were substantially increased in plasma membranes relative to whole cells. Sialoglycolipids more complex than a Tay–Sachs-like ganglioside were not found in any whole-cell or plasma-membrane preparation of this study. Dog kidney cells transformed by SV40 virus contained primarily a less complex sialoglycolipid, haematoside. Neutral glycolipids comprised 26–43% of the total glycolipid content in Madin–Darby preparations, whereas in transformed cells and membranes neutral glycolipids constituted only 1–22% of the total glycolipid content. Ceramide trihexoside was found in Madin–Darby cultures, but not in transformed cultures. The values for short-chain fatty acids from neutral glycolipids and for saturated fatty acids were generally higher than the values for these fatty acids in calf serum.

Studies suggest that the plasma membrane is one of the prime regions affected when cells are transformed from a normal state to a malignant condition (Kalckar, 1965; Wallach, 1968; Pitot, 1974). Differences in the glycolipids of normal and malignant cells have been reported by several investigators (Inbar & Sachs, 1969; Nigam & Cantero, 1972; Robbins & Nicolson, 1975; Nicolson, 1976). Hakomori & Murakami (1968) reported that viral-transformed BHK cells have much smaller quantities of a complex glycolipid (haematoside) and increased quantities of a less-complex glycolipid (lactosylceramide). Polyoma-transformed BHK cells have limited activity to synthesize haematoside from sialic acid and lactosylceramide (Den *et al.*, 1971). Transformed cells grown to a high density also fail to synthesize complex types of glycolipids (Hakomori, 1970; Robbins & MacPherson, 1971). These observations suggest that malignant cells do not complete the carbohydrate portion of their glycolipids.

Although much of the work on glycolipids of cells and plasma membranes has been carried out on

rodent cell lines, it should be noted that rodents have an endogenous oncornavirus flora (Fenner *et al.*, 1974), which could significantly affect the results of studies utilizing these cell lines. On the other hand, dog cell lines offer advantages over rodent cell lines because dog cells have a limited viral flora with few, if any, oncornaviruses (Fenner *et al.*, 1974). Any differences in lipid patterns between normal and virally transformed dog whole cells and their plasma membranes should reflect the influence of the transforming virus.

This study presents the glycolipid composition of transformed and non-transformed dog kidney whole cells and their plasma membranes grown in monolayer and suspension cultures.

Materials and Methods

Cells

Two cell lines were used in this study, MDCK cells (Gaush *et al.*, 1966) and DKSV cells. Both cell lines were grown in monolayer and suspension cultures as described by Hougland *et al.* (1978). Plasma membranes were harvested from these cells by the fluorescein/mercuric acetate method of Warren *et al.* (1966).

Lipid extraction and fractionation

Total lipids were extracted from whole cells and plasma membranes by the five-step method of Weinstein *et al.* (1969).

Abbreviations used: BHK cells, baby-hamster kidney cells; MDCK cells, Madin–Darby dog kidney cells; DKSV cells, dog kidney cells transformed by SV40 virus.

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Sialoglycolipid isolation. Total lipid extracts were treated by the method of Weinstein & Marsh (1970) to separate sialoglycolipids from the chloroform/methanol phase containing ceramide hexosides, neutral lipids and phospholipids. The isolate sialoglycolipids were stored under N_2 in a Teflon-lined screw-capped tube at $-20^\circ C$ to prevent spontaneous hydrolysis of the lipid during storage.

Sialoglycolipid fractionation. Individual sialoglycolipids were separated on t.l.c. plates ($20\text{ cm} \times 200\text{ cm}$) coated with silica gel G (0.25 mm thick) as described by Ledeen *et al.* (1968). The sialoglycolipid bands were detected with I_2 vapour. A duplicate plate developed with the sample plate was sprayed with resorcinol hydrochloride (Svennerholm, 1957) to confirm that the bands contained sialoglycolipids. Individual sialoglycolipids from the sample plate were recovered by scraping each silica-gel band on to weighing paper and transferring the silica gel to a Pasteur pipette plugged with glass-wool. A solution of chloroform/methanol/water (20:20:1, by vol.) was used to elute the sialoglycolipids.

Neutral glycolipid solution. Ceramide hexosides were separated from the neutral lipids and phospholipids in the chloroform/methanol phase of the total lipid extracts by using a silicic acid column as described by Hougland *et al.* (1978).

Neutral glycolipid fractionation. Samples from a known volume of ceramide hexosides were separated on t.l.c. plates ($20\text{ cm} \times 20\text{ cm}$) coated with silica gel G (0.25 mm thick). The plates were activated (heated for 30 min at $100^\circ C$), spotted with the sample and developed in a tank equilibrated with chloroform/methanol/water (35:15:2, by vol.) (Suzuki & Chen, 1967). To detect ceramide hexoside spots, a t.l.c. plate that was developed along with the sample plate was sprayed with diphenylamine reagent (Krebs *et al.*, 1969). R_F values, determined for the ceramide hexoside spots on the sprayed plate, were used to locate the areas containing the ceramide hexoside lipids on unsprayed plates. The ceramide hexosides were recovered by using the procedure described above for individual sialoglycolipids.

Methanolysis and glycolipid determination

Individual sialoglycolipids and neutral glycolipids were hydrolysed in anhydrous methanolic 0.5M-HCl at $80^\circ C$ for 24 h (Sweeley & Walker, 1964). The hydrolysates were extracted with $3 \times 1\text{ ml}$ portions of hexane to remove the fatty acids, leaving carbohydrates in the lower methanolic phase.

Fatty acid determination. Fatty acids from the glycolipids were quantified as methyl esters by using g.l.c. as described by Hougland *et al.* (1978).

Hexose determination. A known quantity of arabinose was added to each glycolipid sample before methanolysis to serve as an internal standard.

Trimethylsilyl derivatives of the hexoses were prepared by the addition of 0.1 ml of a trimethylsilylation reagent composed of anhydrous pyridine/hexamethyldisilazane/trimethylchlorosilane/bis(trimethylsilyl)trifluoroacetamide (2:2:1:1, by vol.). After 15 min at $20^\circ C$, the contents of the tubes were dried under N_2 and dissolved in $30\text{ }\mu\text{l}$ of redistilled chloroform. Samples ($1\text{--}5\text{ }\mu\text{l}$) were analysed by g.l.c. in a Hewlett-Packard model 402 instrument. A glass column ($183\text{ cm} \times 0.6\text{ cm}$) packed with SE-30 on 80–100-mesh Gas-Chrom Q (5.5%, w/w) was operated at $160^\circ C$ with an He flow rate of 60 ml/min. The carbohydrates were quantified by using a Hewlett-Packard integrator, model 3370A.

Hexosamine determination. The lower methanolic phase containing the hexosamines was evaporated to dryness under N_2 , and the residue was dissolved in 0.5 ml of freshly prepared methanol/acetic anhydride (3:1, v/v) to which a few grains of silver acetate had been added. This mixture was incubated for about 30 min at $20^\circ C$. After the silver acetate was removed by filtration and the methanol was evaporated under N_2 , the residue was dried for about 30 min over KOH pellets in a vacuum desiccator to remove the acetic anhydride. The resulting residue was treated with the trimethylsilylation reagent described above for hexoses, and appropriate samples were injected into the g.l.c. instrument as before.

Sialic acid determination. Sialoglycolipids eluted from the silica gel were hydrolysed with methanolic HCl as described above. The methanolic phase was dried slowly under N_2 by the procedure of Yu & Ledeen (1970).

Trimethylsilyl derivatives of sialic acid were prepared as described for hexose derivatives. After incubation for 15 min at $20^\circ C$, the contents of the reaction were dried under N_2 and dissolved in $30\text{ }\mu\text{l}$ of redistilled chloroform. Samples ($3\text{ }\mu\text{l}$) were injected into a Hewlett-Packard g.l.c., model 402 instrument. A stainless-steel column ($214\text{ cm} \times 0.6\text{ cm}$) packed with SE-30 on 80–100-mesh Gas-Chrom Q (5.5%, w/w) was operated on a program with an He flow of 60 ml/min. The temperature program was isothermal at $190^\circ C$ for 10 min, followed by temperature increases of $7.5^\circ C/\text{min}$ to a maximum of $250^\circ C$. The total running time was approx. 25 min for all samples. The samples were quantified by using a Hewlett-Packard integrator, model 3370A.

Since the quantity of each glycolipid obtained in this study was extremely small and since each molecule of glycolipid contains only one molecule of glucose, the quantities of individual glycolipids were estimated on the basis of their glucose content. The molecular weight of each glycolipid fraction was calculated by assuming that sphingosine was the long-chain base, the amino sugars were present as the *N*-acylated derivatives, and the average fatty acid chain length was 18 carbon atoms. Thus the following

estimated molecular weights were obtained: ceramide monohexoside, 784; ceramide dihexoside, 964; ceramide trihexoside, 1144; haematoside, 1332; and Tay-Sachs-like ganglioside, 1528.

Results

Sialoglycolipids

Haematoside. Haematoside is present in all whole cells and plasma membranes of this study (Table 1). It accounts for about 50% or more of the total glycolipid content of MDCK plasma membranes and DKSV whole cells and plasma membranes. In addition, haematoside accounts for all of the sialoglycolipid in plasma membranes from suspension cultures of MDCK and DKSV and from monolayer cultures of the latter cells. From the $\mu\text{g}/\text{mg}$ dry wt. values (Table 1), it is evident that the amount of haematoside is elevated in all membrane preparations relative to the whole cells from which they are obtained.

Tay-Sachs glycolipid. A glycolipid that resembles the Tay-Sachs ganglioside is found in all MDCK preparations except in plasma membranes from suspension cultures. Almost 60% of the sialoglycolipid in whole DKSV cells from suspension culture consists of this amino sugar glycolipid. MDCK whole cells from monolayer and suspension cultures have 80 and 50% respectively of their sialoglycolipid as this compound. It also constitutes 20% of the sialoglycolipid in MDCK plasma membranes from monolayer culture.

Neutral glycolipids

Ceramide monohexoside. Ceramide monohexoside is present in all whole cells and plasma membranes

tested except in suspension-grown MDCK whole cells (Table 2). The amount ($\mu\text{g}/\text{mg}$ dry wt.) of this neutral glycolipid is greater in plasma membranes than in whole cells. Ceramide monohexoside is the predominant neutral glycolipid in DKSV cultures.

Ceramide dihexoside. All preparations except for plasma membranes from DKSV cultures contain small amounts of ceramide dihexoside (Table 2). In MDCK suspension cultures, ceramide dihexoside accounts for all the neutral glycolipid in whole cells and for 86% of the neutral glycolipid in plasma membranes.

Glycolipids constitute a small percentage (5% or less) of the total lipid content of these whole cells and plasma membranes (Table 3). All plasma membranes have substantially more glycolipid ($\mu\text{g}/\text{mg}$ dry wt.) than do whole cells. Sialoglycolipids account for almost 80–100% of the glycolipids in DKSV culture preparations, whereas MDCK culture preparations have 60–75% of their total glycolipids as sialoglycolipid. All MDCK culture preparations also have more of their total glycolipids as neutral glycolipids than do DKSV culture preparations. In general, neutral glycolipids contribute much less than sialoglycolipid to the total lipid content of these whole cells and plasma membranes.

Fatty acids of MDCK cells

The fatty acid profiles of calf serum, which is representative of the serum used in the culture media, and of glycolipids from MDCK whole cells and plasma membranes are presented in Tables 4 and 5 respectively. In monolayer cultures, the values for short-chain fatty acids (C_{12} – C_{14}) from neutral glycolipids are 2–6-fold higher in MDCK whole cells and plasma membranes than in calf serum. On the other

Table 1. Sialoglycolipids of dog kidney cells and plasma membranes

Haematoside contains: glucose, galactose, sialic acid (1:1:1, molar proportions); and Tay-Sachs glycolipid contains: glucose, galactose, galactosamine, sialic acid (1:1:1:1, molar proportions). See the Materials and Methods section for the determination of sialoglycolipids from whole cells and their plasma membranes in monolayer and suspension culture.

Culture	Haematoside			Tay-Sachs glycolipid		
	($\mu\text{g}/\text{mg}$ dry wt.)	(% of total glycolipid)	(% of total sialoglycolipid)	($\mu\text{g}/\text{mg}$ dry wt.)	(% of total glycolipid)	(% of total sialoglycolipid)
Monolayer						
MDCK cells	0.38	14	22	1.30	50	78
MDCK membranes	16.20	59	80	4.17	15	20
DKSV cells	1.34	86	100	0	0	0
DKSV membranes	1.84	78	100	0	0	0
Suspension						
MDCK cells	0.60	28	50	0.60	29	50
MDCK membranes	5.27	62	100	0	0	0
DKSV cells	1.32	41	42	1.86	58	58
DKSV membranes	11.89	99	100	0	0	0

Table 2. *Neutral glycolipids of dog kidney cells and plasma membranes*

Ceramide monohexoside contains glucose only; ceramide dihexoside contains glucose and galactose (1:1, molar ratio); ceramide trihexoside contains glucose and galactose (1:2, mol ratio). See the Materials and Methods section for determination of neutral glycolipids from whole cells and their membranes in monolayer and suspension culture.

Culture	Ceramide monohexoside			Ceramide dihexoside			Ceramide trihexoside		
	($\mu\text{g}/\text{mg}$ dry wt.)	(% of total glyco-lipid)	(% of total neutral glyco-lipid)	($\mu\text{g}/\text{mg}$ dry wt.)	(% of total glyco-lipid)	(% of total neutral glyco-lipid)	($\mu\text{g}/\text{mg}$ dry wt.)	(% of total glyco-lipid)	(% of total neutral glyco-lipid)
Monolayer									
MDCK cells	0.78	30	86	0.01	*	14	0.12	5	14
MDCK membranes	4.61	17	67	1.78	7	33	0.51	2	7
DKSV cells	0.19	12	85	0.04	2	15	0	0	0
DKSV membranes	0.53	22	100	0	0	0	0	0	0
Suspension									
MDCK cells	0	0	0	0.52	25	57	0.40	18	43
MDCK membranes	0.45	5	14	2.65	33	86	0	0	0
DKSV cells	0.02	1	50	0.02	1	50	0	0	0
DKSV membranes	0.16	1	100	0	0	0	0	0	0

*Trace (less than 0.5%).

Table 3. *Summary of glycolipids from dog kidney cells and plasma membranes*

Culture	Sialoglycolipid			Neutral glycolipid	
	($\mu\text{g}/\text{mg}$ dry wt.)	(% of total glycolipid)	(% of total lipid)	(% of total glycolipid)	(% of total lipid)
Monolayer					
MDCK cells	2.59	64	1	35	1
MDCK membranes	26.78	74	4	26	1
DKSV cells	1.57	86	*	14	*
DKSV membranes	2.39	78	1	22	1
Suspension					
MDCK cells	2.09	57	1	43	*
MDCK membranes	8.50	62	1	38	*
DKSV cells	3.23	99	1	1	*
DKSV membranes	12.04	99	5	1	*

* Trace (less than 0.5%).

hand, sialoglycolipids contain almost no short-chain fatty acids. The unsaturated fatty acid (C_{18}) values for all glycolipids are decreased substantially, except for that for neutral glycolipid of monolayer whole cells, which is similar to that of calf serum. Stearic acid (C_{18}) values from sialoglycolipids are 2–3-fold greater in whole cells and plasma membranes from monolayer cultures than in calf serum. The percentage of unsaturated fatty acids in glycolipids is less in MDCK whole cells and plasma membranes from monolayer-grown samples than in calf serum.

Short-chain fatty acid values from neutral glycolipids are elevated 2–6-fold in MDCK whole cells and plasma membranes of suspension cultures in

comparison with calf serum; however, essentially no short-chain fatty acids are found in their sialoglycolipids. Stearic acid from sialoglycolipids accounts for 40–55% of all fatty acids in suspension-grown whole cells and plasma membranes, whereas stearic acid values from neutral glycolipids are similar to that in calf serum. Only the unsaturated fatty acid (C_{18}) value from neutral glycolipids of suspension-grown whole cells is nearly equal to that in calf serum; values for the other preparations are less. The limited distribution of fatty acids in sialoglycolipids of MDCK plasma membranes from suspension cultures may have been due to the small sample available for analysis (results not shown).

Table 4. *Fatty acid composition of calf serum*

Fatty acid profiles were obtained by equating the total nmol of fatty acid recovered to 100% and determining the percentage of each fatty acid relative to this total. The percentages have been rounded to the nearest whole number.

Fatty acids		Percentage of total fatty acid
Lauric	C ₁₂	1
Myristic	C ₁₄	3
Pentadecanoic	C ₁₅	1
Palmitic	C ₁₆	21
Palmitoleic	C _{16:1}	6
Margaric	C ₁₇	1
Stearic	C ₁₈	15
Unsaturated	C ₁₈	46
Long-chain	≥C ₂₀	7
Unsaturated (%)		52
Saturated (%)		48

Fatty acids of DKSV cells

Fatty acid profiles of glycolipids from DKSV whole cells and their plasma membranes are presented in Table 6. The values for short-chain fatty acids (C₁₂–C₁₄) from sialoglycolipids are much lower in whole cells and plasma membranes grown in monolayers than in calf serum; however, short-chain fatty acid values from neutral glycolipids are elevated 4–6-fold. Palmitic acid values from sialoglycolipids of DKSV

whole cells in monolayer culture are elevated, whereas those values from neutral glycolipids are less than or similar to that value in calf serum. Stearic acid values from sialoglycolipids of whole cells and plasma membranes are increased 2.5–4-fold, whereas those values from neutral glycolipids are similar to the stearic acid value of calf serum. Unsaturated fatty acid (C₁₈) values, except from neutral glycolipids of DKSV whole cells, are 2–10-fold less in monolayer whole cells and plasma membranes than in calf serum. The limited distribution of fatty acids from sialoglycolipids of plasma membranes from suspension culture may also have been due to the small sample size available for analysis (results not shown). It is noteworthy that about 5% of the fatty acids from sialoglycolipids of plasma membranes are unsaturated, whereas 30–50% of the fatty acids from calf serum and from other glycolipids of whole cells and plasma membranes are unsaturated.

In suspension cultures of DKSV whole cells and plasma membranes, short-chain fatty acid values from sialoglycolipids are decreased compared with calf serum values, whereas those values from neutral glycolipids are elevated. The palmitoleic acid value from all glycolipids is greatly decreased in DKSV whole cells and plasma membranes from both monolayer and suspension cultures. Relative to calf serum, stearic acid values are increased in all glycolipids of DKSV culture preparations from suspension cultures, except in neutral glycolipids from the plasma membranes. All values for unsaturated fatty acid

Table 5. *Fatty acids from glycolipids of MDCK whole cells and plasma membranes*

Fatty acid profiles were obtained by equating the total nmol of fatty acid recovered to 100% and determining the percentage of each fatty acid relative to this total. The fatty acids are reported as percentages of total fatty acid recovered.

		Monolayer culture				Suspension culture			
		Whole cells		Plasma membranes		Whole cells		Plasma membranes	
Fatty acids		Sialo-glyco-lipid	Neutral glyco-lipid	Sialo-glyco-lipid	Neutral glyco-lipid	Sialo-glyco-lipid	Neutral glyco-lipid	Sialo-glyco-lipid	Neutral glyco-lipid
Lauric	C ₁₂	0	3	0	10	0	4	0	7
Tridecanoic	C ₁₃	0	*	0	0	0	0	0	1
Myristic	C ₁₄	1	6	*	13	*	4	0	17
Pentadecanoic	C ₁₅	*	1	*	1	1	2	0	2
Palmitic	C ₁₆	27	22	24	26	18	12	29	24
Palmitoleic	C _{16:1}	*	3	0	0	*	12	0	3
Margaric	C ₁₇	4	4	1	1	7	3	0	1
Stearic	C ₁₈	32	13	44	17	39	13	57	17
Unsaturated	C ₁₈	32	43	28	30	28	42	15	23
Nonadecanoic	C ₁₉	1	1	0	0	1	*	0	2
Long-chain	≥C ₂₀	4	5	2	2	6	9	0	3
Unsaturated (%)		33	45	28	29	28	53	15	26
Saturated (%)		67	55	71	71	72	47	85	74

* Trace (less than 0.5%).

Table 6. *Fatty acids from glycolipids of DKSv whole cells and plasma membranes*

Fatty acid profiles were obtained by equating the total nmol of fatty acid recovered to 100% and determining the percentage of each fatty acid relative to this total. The fatty acids are reported as a percentage of total fatty acid recovered.

		Monolayer culture				Suspension culture			
		Whole cells		Plasma membranes		Whole cells		Plasma membranes	
Fatty acids		Sialo-glyco-lipid	Neutral glyco-lipid	Sialo-glyco-lipid	Neutral glyco-lipid	Sialo-glyco-lipid	Neutral glyco-lipid	Sialo-glyco-lipid	Neutral glyco-lipid
Lauric	C ₁₂	0	6	0	8	0	4	0	9
Tridecanoic	C ₁₃	0	2	0	1	0	1	0	3
Myristic	C ₁₄	1	8	0	15	2	4	2	10
Pentadecanoic	C ₁₅	1	*	0	5	1	5	*	11
Palmitic	C ₁₆	31	14	40	21	29	24	23	22
Palmitoleic	C _{16:1}	1	2	0	3	0	*	3	1
Margaric	C ₁₇	1	1	0	2	1	3	1	1
Stearic	C ₁₈	37	19	56	16	45	22	33	13
Unsaturated	C ₁₈	27	42	5	25	27	27	35	24
Nonadecanoic	C ₁₉	0	1	0	1	*	1	0	1
Long-chain	≥C ₂₀	1	5	0	3	4	9	4	5
Unsaturated (%)		29	44	5	28	17	27	38	25
Saturated (%)		71	56	95	72	82	73	62	75

* Trace (less than 0.5%).

(C₁₈) as well as the percentage of unsaturated fatty acid for all glycolipids are less in DKSv whole cells and plasma membranes from suspension culture than in calf serum.

Discussion

Since the complete function of glycolipids in cells is not yet known, information is needed about changes in glycolipid profiles of the cell and its plasma membrane as the result of cell division, contact with other cells and transformation by viruses or chemicals. One group of membrane glycolipids, gangliosides (sialoglycolipids), is of special interest. Gangliosides and other glycolipids on the cell membrane are involved as receptor sites, receiving external signals from the environment (Nicolson, 1976) and binding cholera toxin (Van Heyningen *et al.*, 1971; Cuatrecasas, 1973*a,b,c,d*; Holmgren *et al.*, 1973) and thyrotropin (thyroid-stimulating hormone; Mullin *et al.*, 1976; Meldoleci *et al.*, 1977). Besançon & Ankel (1974) and Vengris *et al.* (1976) have indicated a possible interaction between gangliosides and interferon.

Most of the data used for comparing glycolipids have been developed from rodent cell lines. Neutral glycolipids and sialoglycolipids from virally (polyoma) and spontaneously transformed BHK cells contain smaller quantities of haematoside and increased quantities of lactosylceramide (Hakomori & Murakami, 1968; Hakomori, 1970). Unlike that in transformed BHK cells, the haematoside content

is not decreased in transformed dog kidney cells (Table 1), transformed 3T3 cells (Brady & Mora, 1970) or established fibroblast cell lines from inbred mouse strains (Mora *et al.*, 1969).

The complex gangliosides G_{D1a} ganglioside (disialoganglioside), G_{M1} ganglioside (monosialoganglioside) and G_{M2} (Tay-Sachs) ganglioside are virtually absent in both transformed and non-transformed BHK cells (Hakomori, 1970) and are decreased in transformed 3T3 cells (Brady & Mora, 1970). In this study, gangliosides similar to D_{D1a} and G_{M1} gangliosides were not found in the dog kidney cells. However, a ganglioside similar to G_{M2} ganglioside was detected in low concentration. The lack of complex gangliosides in DKSv cells and in transformed BHK cells, which are not agglutinated by soya-bean agglutinin, is noteworthy, because transformed fibroblasts from rat, mouse and human origin that contain complex gangliosides are agglutinable (Herschman, 1972). It is likely that transformed dog kidney cells would not be agglutinated by soya-bean agglutinin; however, agglutination studies were not included in the present investigation.

Although dog kidney cells differ from rodent cells, some glycolipid patterns, such as ceramide trihexoside (Table 2), are similar. Detectable amounts of ceramide trihexoside are not found in transformed BHK or DKSv cells, but are present in MDCK and non-transformed BHK cells. The present data for glycolipids of dog kidney whole cells and plasma membranes support the findings reported by Hakomori (1973), Gahmberg *et al.* (1974) and

Steiner *et al.* (1974), namely that glycolipids of transformed cells have less complex, hence simpler, glycolipids than do normal or non-transformed cells.

The distribution of fatty acids from glycolipids of dog kidney cells resembles that of neural tissue more than that of non-neural tissue. Glycolipids of dog kidney cells and their plasma membranes generally contain small quantities of long-chain fatty acids ($\geq C_{20}$) and large quantities of other fatty acids ($\leq C_{18}$), whereas glycolipids from dog intestine (McKibbin, 1969) and BP/C3H ascites-sarcoma cells (Gray, 1965) have a high content of behenic (C_{22}) and lignoceric (C_{24}) fatty acids respectively. Glycolipids from spleen (Philppart *et al.*, 1965) and dog intestine (McKibbin, 1969) have 60% or more of their fatty acids as long-chain fatty acids ($\geq C_{20}$). Stearic acid (C_{18}) accounts for 86–95% of the fatty acids from brain gangliosides (Trams *et al.*, 1962) and C_{14} – C_{18} fatty acids predominate in L cells and plasma membranes (Weinstein *et al.*, 1969).

The purpose of the high content of short-chain fatty acids (C_{12} – C_{14}) in neutral glycolipids from dog kidney cells is not known; however, Kritchevsky & Howard (1970) reported that cells *in vitro* derive a spectrum of fatty acids from the serum used in the growth medium. Short-chain fatty acids may aid in movement of the protein–lipid complex through the membrane lipid matrix if neutral glycolipids are tightly bound to protein.

Changes in the glycolipids of mammalian cells resulting from viral transformation generally have been studied by using monolayer-grown cells and, less frequently, their plasma membranes. Consequently the present data for the glycolipids of normal and virally transformed dog kidney cells and plasma membranes from monolayer and suspension cultures are unique.

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