Factors affecting the glycosphingolipid composition of murine tissues

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ABSTRACT The effects of genetic strain, sex, age, and pathological state on the distribution and concentration of glycosphingolipids in mouse kidneys and livers were studied. The concentrations of glycosphingolipids and phospholipids in the kidneys and livers of different strains were compared. The major glycosphingolipid in the kidneys of male and female BALB/c, C3H/He, C57/BL, A, and C57×A (F_1 hybrid) mice was a sulfatide, monoglycosyl-(3-sulfate) ceramide; monoglycosyl ceramide was the major component in livers. The kidneys of males of all strains contained significant amounts of diglycosyl ceramide, but those of females contained, at most, only traces. Glycosphingolipid concentration in the male kidneys of C57/BL and C57×A (F_1 hybrid) was much higher than in the female and was also much higher than in the male kidneys of C3H/He, BALB/c, and A strains. The kidneys of "old" (36 wk) male and female C3H/He mice contained much higher proportions of monoglycosyl ceramide than 10–12-wk-old adults. The distributions of glycosphingolipids in kidneys of female C3H/He mice with BP8 ascites tumors and male C57×A (F_1 hybrid) mice with EL4 ascites tumors differed from those in the normal mice. An unknown lipid, present in all glycosphingolipid extracts from kidney and liver, was tentatively identified as cholesterol sulfate.

SUPPLEMENTARY KEY WORDS genetic strain · sex · age · pathological state · ascites tumors · sulfatide · diglycosyl ceramide · cholesterol sulfate

PREVIOUS STUDIES (1) showed that the glycosphingolipid[1] composition in the kidneys of C3H mice with strain specific BP8 ascites (sarcoma) tumors was different from that in the kidneys of normal mice. It was also shown that the amounts of neutral glycosyl ceramides in mouse kidneys varied with strain, and that the structure of the major diglycosyl ceramide was not the same in all strains (2).

In a recent study of the distribution and concentration of glycosphingolipids in the kidneys and livers of several strains of mice, we found that the glycosphingolipid composition of kidneys from male and female mice differed. Because of this finding the results of some of our earlier work, in which we used mixed batches of mice of both sexes, were of doubtful value. Accordingly, some of the work was repeated and expanded, and the results are reported in this paper.

METHODS AND MATERIALS

Analytical Methods

Phosphorus was estimated either by the method of Gray and Macfarlane (3) or by that of Bartlett (4). GSL was estimated as sphingosine by the method of Lauter and Trams (5). When the amount of available GSL was limited, a more sensitive fluorescence procedure was used (6).

Total lipids were extracted from the tissues (7), and the total neutral glycosyl ceramides and sulfatides were isolated (8).

Identification of GSL by Thin-Layer Chromatography

A sample of the mixture of neutral glycosyl ceramides and sulfatides from each tissue was separated into individual components by two-dimensional chromatography on small (8.2 cm × 8.2 cm) thin-layer plates of Silica Gel H (9). The first solvent was chloroform–

[1] The nomenclature is that suggested by the IUPAC-IUB Commission on Biochemical Nomenclature (see 1968. *Biochim. Biophys. Acta.* **152:** 1). However, in this paper the term glycosphingolipid excludes those which contain sialic acid, i.e. gangliosides.

Abbreviations: GSL, glycosphingolipid(s); MGC, monoglycosyl ceramide; DGC, diglycosyl ceramide; TGC, triglycosyl ceramide; AG, aminoglycolipid (a tetraglycosyl ceramide containing *N*-acetylgalactosamine); MGSC, monoglycosyl(-sulfate) ceramide; DGSC, diglycosyl(-sulfate) ceramide (both classed as sulfatide).

methanol–water 65:25:4, (by vol). After development the plate was dried over P_2O_5 (in vacuo) for 20 min and then developed in the second direction with the tetrahydrofuran–methylal–methanol–4 N aqueous ammonia 10:5:5:1 (by vol). This solvent was removed by heating the plate at 140°C for 15 min. The lipids were made visible by spraying the plate with 50% H_2SO_4 and then by heating it at 160–180°C for 20 min. All compounds were identified with reference to standards of known structure (1, 2, 10).

Materials

Kidneys and liver (and occasionally, lungs and spleen) were removed from normal male and female adult mice (10–12 wk old) immediately after their death. The mice were of the following inbred strains: BALB/c, A, C3H/He, C57 × A (F_1 hybrid), and C57/BL. Tissues were also removed from some older (36 wk) C3H/He mice. All strains originated from stock at the MRC Laboratory Animals Centre, Carshalton.

The same tissues were also removed from male and female C3H/He mice carrying BP8 ascites (sarcoma) tumors and C57 × A (F_1 hybrid) carrying EL4 ascites (leukemia) tumors. The mice were killed 9–11 days after an intraperitoneal injection of approximately 8×10^5 cells. At that time they were alert and appeared in good physical condition, but they were rather plump.

Several batches of male and female mice of each strain were studied. A batch usually consisted of 4, 6, or 12 mice although one batch consisted of 125 male and 75 female C57 × A (F_1 hybrid) carrying EL4 tumors.

RESULTS

BALB/c Mice

The major component in the kidneys of both sexes was a sulfatide, MGSC (Figs. 1a and b). Small amounts of DGSC were also present. The identities of both sulfatides were confirmed by the fact that their chromatographic properties on thin-layer plates of Silica Gel H and on columns of DEAE-cellulose (11) were indistinguishable from those of the authentic compounds, galactosyl-(3-sulfate)-(1 → 1)-ceramide and galactosyl-(3-sulfate)-(1 → 4)-glucosyl-(1 → 1)-ceramide, respectively, and by the fact that they were hydrolyzed to MGC and DGC, respectively, by 0.05 N methanolic HCl at room temperature (22°) (12). DGC was present in kidneys from male mice but was not found in kidneys of the females. Both the male and female contained TGC, AG, and an unknown compound X (see below). The concentration of GSL/g of kidney was slightly higher in the male (0.23 μmole) than in the female (0.19 μmole) (Fig. 2).

The major GSL component in the liver from both sexes was MGC. There was a substantial amount of DGC, small amounts of TGC, AG, compound X, and traces of MGSC. The GSL concentration in the liver was much lower than in the kidney (Fig. 2).

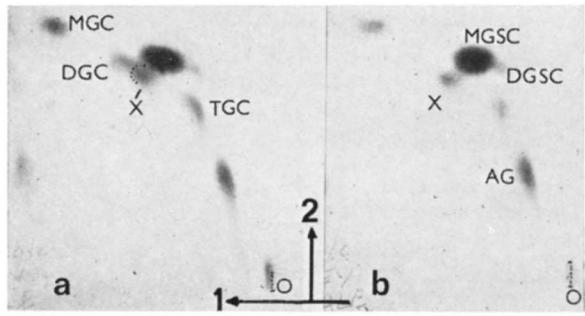

FIG. 1. The glycosphingolipid composition in kidneys of (*a*) male and (*b*) female adult BALB/c mice. *O*, origin; *1*, direction of first solvent; *2*, direction of second solvent. Note absence of DGC in *b*. Lipid samples were approximately (*a*) 80 μg and (*b*) 80 μg.

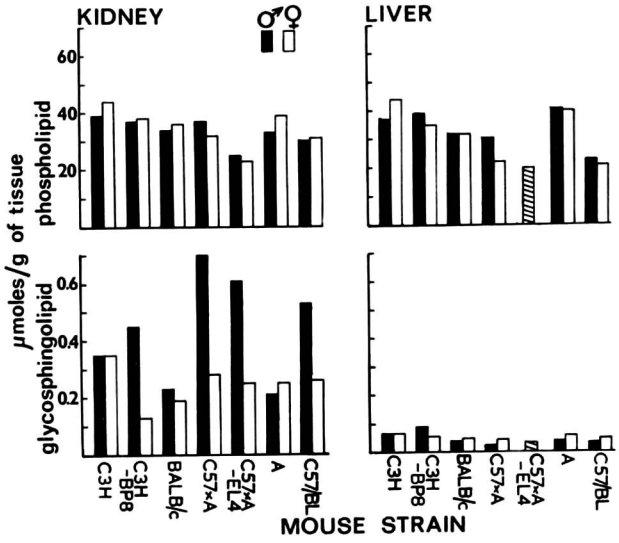

FIG. 2. Glycosphingolipid and phospholipid concentrations in male and female adult (10–12 wk) mouse kidneys and livers. BP8, an ascites (sarcoma) tumor carried by C3H/He mice; EL4, an ascites (leukemia) tumor carried by C57×A (F_1 hybrid) mice. The column with etched lines represents the value for a batch of mice comprised of both sexes. Batch deviation from given the following values of kidney glycosphingolipids—C3H/He: ♂ ±12%, ♀ ±6%; C3H/He/BP8: ♂ ±10%, ♀ ±6%; BALB/c: ♂ ±11%, ♀ ±15%; C57×A: ♂ ±9%, ♀ ±10%; A: ♂ ±15%, ♀ ±15%; C57/BL: ♂ ±10%, ♀ ±10%. Only one large batch of kidneys from C57×A/EL4 males and one of kidneys from C57×A/EL4 females were analyzed.

A Mice

The distributions of GSL in the kidneys were similar to those in BALB/c mice, MGSC being the major component in both sexes. There was DGC in the kidneys of male mice but only a trace was present in the kidneys of the females. The relative amounts of compound X in the total GSL of both sexes was slightly higher than in BALB/c kidneys.

The GSL distribution was the same in the livers from male and female mice and was similar to that in BALB/c liver, except that there was a larger amount of compound X, almost as much as of MGC.

C3H/He Mice

The GSL pattern in kidneys of male mice was similar to that in BALB/c males (Fig. 1a) except that TGC was the major component of the neutral glycosyl ceramides. In females (Fig. 3a), only a trace of DGC was present. The distribution of GSL in the kidneys of males carrying BP8 ascites (sarcoma) tumors was similar to that in normal males. In contrast to the normal female, however, only trace quantities of TGC were present in the kidney GSL of the females carrying tumors (Fig. 3b). The GSL concentration in the females with tumors was was also much lower than normal (Fig. 2).

The distributions of GSL in the livers of male and female mice were similar (Fig. 4) and were not altered by the presence of a BP8 tumor in either the male or the female. However, the GSL concentration in the livers of males with tumors was double that of females with tumors (Fig. 2).

The GSL in lungs and spleen of normal mice (mixed sexes) were quantitatively similar to those in lungs and spleen of mice with BP8 tumors. The kidney GSL of 36-wk-old males and females (batches of 12) were also examined (Fig. 5). Compared with normal 10–12-wk-old males and females, both sexes showed a relative increase in the amount of MGC and a decrease in the amount of MGSC (e.g. compare females, Figs. 3a and 5a).

C57 × A (F_1 Hybrid) Mice

DGC was not detected in the kidneys of female mice, but it was present in the kidneys of males (Figs. 6a and b). MGSC was the major component in both sexes. The GSL concentration was much higher in males (0.7 μmoles/g of tissue) than in females (0.28 μmoles/g of tissue).

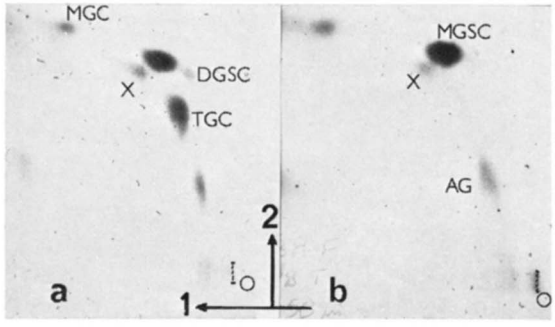

FIG. 3. The glycosphingolipid compositions in kidneys of (*a*) normal female adult C3H/He mice and (*b*) females carrying BP8 ascites tumor. Note only traces of TGC in *b*. Lipid samples were approximately (a) 80 μg and (b) 80 μg.

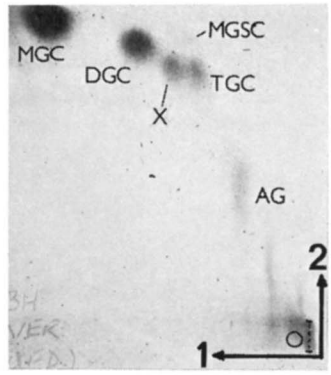

FIG. 4. The glycosphingolipid composition of the liver of the normal C3H/He mouse (10–12 wk old). What is shown is a sample (100 μg) of the combined GSL fractions from livers of both sexes, since the GSL distributions in male and female were similar.

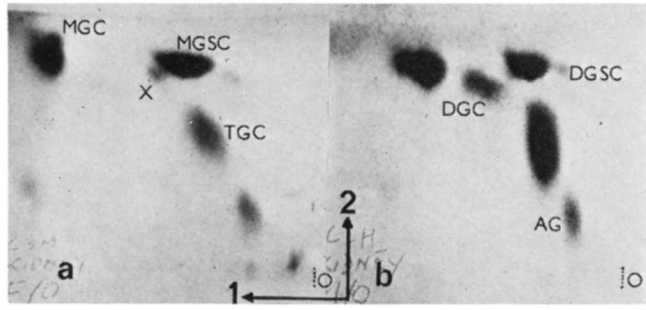

FIG. 5. Glycosphingolipid compositions in the kidneys of old (36 wk) female (*a*) and male (*b*) C3H/He mice. Lipid samples were approximately (*a*) 120 μg and (*b*) 160 μg.

The GSL pattern in the kidneys of females carrying EL4 ascites (leukemia) tumors was similar to the pattern in normal mice (Fig. 6a), but the kidneys of males with tumors (Fig. 6c) contained relatively much more DGC and TGC, and much less MGSC than those of normal males (Fig. 6b).

The liver GSL in both sexes, with or without ascites tumors, were similar to those in C3H livers. MGC was the major component, and TGC and AG were the minor components. There was nearly as much compound X as MGC, and there was more compound X than MGSC, which in turn was greater in amount than DGC.

C57/BL Mice

The patterns of distribution in kidneys of male and female mice were similar to those in C57×A (F_1 hybrid) except that the TGC and AG were present in approximately equal proportions. The major components, MGSC, accounted for 30 and 50% of the total GSL (6) in the kidneys of male and female mice, respectively. The GSL concentration in the kidneys of the males (0.53 μmoles/g of tissue) was double that in the female kidneys (0.26 μmoles/g of tissue).

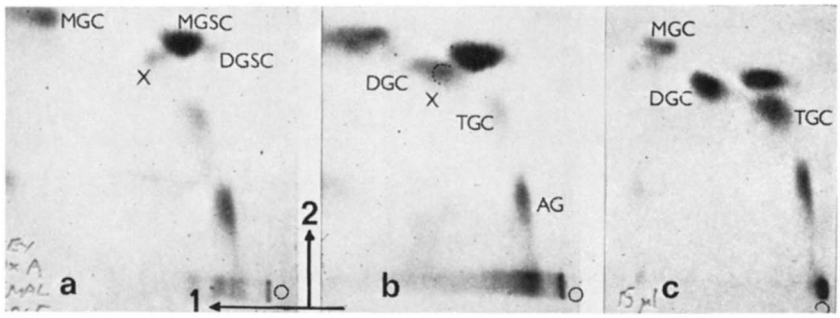

FIG. 6. Glycosphingolipid compositions in the kidneys of C57×A (F_1 hybrid) mice. *a*, female; *b*, male; *c*, male carrying EL4 ascites tumor. Comparing *c* with *b*, note increase in DGC and TGC relative to MGSC (sulfatide). Lipid samples were approximately (*a*) 80 μg, (*b*) 120 μg, (*c*) 120 μg.

The patterns of the liver GSL in both sexes were similar to those in the C57×A (F_1 hybrid).

Compound X

The compound was found in the kidneys and livers of normal mice of all strains so far examined. Like galactosyl-(3-sulfate)-(1 → 1)-ceramide, it was retained on DEAE-cellulose chloroform–methanol 2:1 (v/v) was used as eluant, but it was eluted by chloroform–methanol containing 0.5 N aqueous lithium chloride (11). It had a slightly higher mobility than galactosyl-(3-sulfate)-(1 → 1)-ceramide on thin-layer plates of Silica Gel H developed in chloroform–methanol–water 65:25:4 (by vol.). After hydrolysis with 0.5 N methanolic HCl at 80°C for 18 hr, a lipid component was obtained and was identified as cholesterol by thin-layer chromatography and gas–liquid chromatography (3% OV-1 on Gas-Chrom Q at 250°C). Authentic cholesterol was used as a reference compound. Inasmuch as infrared analysis of compound X suggested that a sulfate group was present (peaks at 1225 cm^{-1} and 830 cm^{-1}), cholesterol sulfate was synthesized by the method of Mumma (13) and was compared with compound X.

The behavior of cholesterol sulfate in relation to galactosyl-(3-sulfate)-(1 → 1)-ceramide (see above) was indistinguishable from that of compound X. Also the infrared spectrum of compound X and its chromatographic properties on silicic acid-impregnated paper developed in chloroform–methanol 19:1 (v/v) were both similar to those of the synthetic cholesterol sulfate (14, 15). It was thus concluded that compound X was cholesterol sulfate.

DISCUSSION

The biological functions of the neutral glycosyl ceramides and sulfatides are not known, but, since they have haptenic functions (16) and are probably exclusive to the plasma membranes of the mammalian cell (17), they may contribute either to the surface properties of the cell or to certain membrane functions, such as permeability. It is reasonable to suppose that a cell has a GSL composition which is best suited to its requirements for normal function and survival. But what factors are likely to control the GSL composition?

The two most obvious factors are tissue and species specificities. Each tissue has a pattern (e.g., Figs. 3a and 4) which, broadly speaking is the same in most mammalian species although concentrations may vary. For instance, kidney GSL is relatively rich in sulfatide (human [18], rat, rabbit, baboon, and hamster[2]), but its concentration varies with the species. In human kidneys (18) sulfatide is about 14% of the GSL, but in mouse kidneys it ranges from 30- 50% of the total GSL. Inbred strains of a species also have different GSL compositions in some tissues. For example, the diglycosyl moiety of the DGC in kidneys of mice differs with the strain (2). The results presented in this paper show that the concentration of GSL in the kidney is much higher in male C57/BL and C57 ×A (F_1 hybrid) than in male A, BALB/c, and C3H strains (Fig. 2). In contrast, the qualitative compositions[2] and concentrations of the phospholipids, the major tissue lipids, are similar in all strains (Fig. 2). The GSL pattern in the liver varies with mouse strain, mainly in the proportions of MGC and MGSC present. The concentration of GSL is only 10–20% of that in the kidneys (cf. phospholipids, Fig. 2).

An important factor which governs the GSL patterns in some tissues, and which up to the present time has usually been ignored in lipid studies, is the sex of the animal. In all mouse strains examined there was little or no DGC in the kidneys of female mice but always a substantial amount in the male (Figs. 1a, 5b, and 6b). Since DGC was absent from the kidneys of both male and female mice up to 2 wk old[3], the differences in adults may be determined by hormones which are known to have considerable influence on the function of

[2] Gray, G. M. Unpublished results.
[3] Hay, J. B. Unpublished results.

mouse kidneys, e.g., the excretion of protein by the male is controlled by testosterone (19). Testosterone also stimulates the activity of β-glucuronidase, which is twice as active in the male as in the female (20). The large difference in GSL concentrations between kidneys of males and females of the C57/BL and C57 × A (F_1 hybrid) strains may also be hormonally determined.

The DGC in the kidneys of C57/BL, BALB/c, and A strains is predominantly galactosyl-(1 → 4)-galactosyl-(1 → 1)-ceramide (2). Hauser and Hildebrand (21) were unable to demonstrate the synthesis of this DGC from monogalactosyl ceramide and UDP-galactose in C57/BL kidneys, but they did show that they could synthesize lactosyl ceramide from monoglucosyl ceramide. However, we have obtained a net synthesis of digalactosyl ceramide from monogalactosyl ceramide and UDP-galactose with homogenates of both C57/BL male and C57/BL female kidneys at rates two to three times faster than the synthesis of lactosyl ceramide from glucosyl ceramide.[4]

GSL metabolism may be considerably modified in certain pathological conditions. The hereditary diseases (collectively classed as sphingolipidoses) such as Gaucher's disease (22), Fabry's disease (23), and metachromatic leucodystrophy (24) are all characterized by an increased concentration of one or more of the GSL in certain tissues. The GSL in the kidneys of C3H/He (1) and C57×A (F_1 hybrid) mice are changed in the presence of a strain specific ascites tumor. The sex of the mouse and the type of tumor both seem to determine the change. Thus the presence of the BP8 (sarcoma) tumor affects the kidney GSL in the female C3H/He mouse (Figs. 3a and b) whereas the EL4 (leukemia) tumor in C57×A (F_1 hybrid) mouse affects it only in the male (Figs. 6b and c). The change in the GSL pattern is quite different in the two strains, and in the kidney of the female C3H/He, the total GSL is less than normal (Fig. 2). These results, though few, suggest that one sex is more susceptible than the other to a tumor, and that the connection between the tumor and a change in tissue GSL may be a disturbance of the mouse's hormone balance. A recent finding, which also supports the idea that GSL synthesis and metabolism are influenced by hormones, is that the GSL of BP8 cells grown in C3H/He males contains relatively more DGC than cells grown in C3H/He females.[5]

Age is another factor. Martensson (18) found that the concentration of GSL in human kidneys decreased with increasing age, but the proportions of the components remained about the same. Our studies so far have been qualitative only, but the GSL in the kidneys of old mice (36 wk) of both sexes showed a relative increase in MGC and a decrease in MGSC (Fig. 5) as compared with GSL from the kidneys of younger animals (10–12 weeks). This pattern might result from a decline in activity of some of the enzymes which synthesize these lipids.

The evidence from these studies, though limited to one mammalian species, indicates that the GSL pattern in a tissue is dependent on a number of factors, some of which are perhaps interrelated. It is generally accepted that there are characteristic patterns and concentrations of GSL for different tissues and species, but, under certain conditions, sex, strain, age, and disease can each cause conspicuous changes in the distribution and concentration of GSL. In contrast, apart from reports (25–27) that hormones are probably responsible for the differences in phosphatidylcholine synthesis and fatty acid distribution in the liver phospholipids of male and female rats, there is little evidence that the phospholipids are significantly affected by the strain, age, or the health of the animal.

It is not known why the metabolism of GSL is so sensitive to these factors, but on the assumption that the GSL are exclusively in the plasma membrane, the idea of a possible connection between changes in the GSL pattern and changes in the permeability of the membrane and the cell's response to certain internal or external stimuli, is an attractive one.

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References

1. Adams, E. P., and G. M. Gray. 1967. *Nature* (*London*). **216:** 277.
2. Adams, E. P., and G. M. Gray. 1968. *Chem. Phys. Lipids.* **2:** 147.
3. Gray, G. M., and M. G. Macfarlane. 1958. *Biochem. J.* **70:** 409.
4. Bartlett, G. R. 1959. *J. Biol. Chem.* **234:** 466.
5. Lauter, C. J., and E. G. Trams. 1962. *J. Lipid Res.* **3:** 136.
6. Coles, L., and G. M. Gray. 1970. *J. Lipid Res.* **11:** 164.
7. Gray, G. M., and M. G. MacFarlane. 1961. *Biochem. J.* **81:** 480.
8. Gray, G. M. 1967. *Biochim. Biophys. Acta.* **144:** 511.
9. Gray, G. M. 1967. *Biochim. Biophys. Acta.* **144:** 519.
10. Adams, E. P., and G. M. Gray. 1967. *Chem. Phys. Lipids.* **1:** 368.

[4] Coles, L. Unpublished results.
[5] Hay, J. B. Unpublished results.

11. Svennerholm, L., and H. Thorin. 1962. *J. Lipid Res.* **3:** 483.
12. Stoffyn, P., and A. Stoffyn. 1963. *Biochim. Biophys. Acta.* **70:** 107.
13. Mumma, R. O. 1966. *Lipids.* **1:** 221.
14. Moser, H. W., A. B. Moser, and J. C. Orr. 1966. *Biochim. Biophys. Acta.* **116:** 146.
15. Rice, L. I., E. H. Rice, L. Spolter, W. Marx, and J. S. O'Brien. 1968. *Arch. Biochem. Biophys.* **127:** 37.
16. Rapport, M. M., L. Graf, and H. Schneider. 1964. *Arch. Biochem. Biophys.* **105:** 431.
17. Dod, B. J., and G. M. Gray. 1968. *Biochem. J.* **110:** 50P.
18. Martensson, E. 1966. Glycolipids of human kidney. Ph.D. Thesis. University of Göteborg, Göteborg, Sweden.
19. Wicks, L. F. 1941. *Proc. Soc. Exp. Biol. Med.* **48:** 395.
20. Morrow, A. G., D. M. Carroll, and E. M. Greenspan. 1951. *J. Nat. Cancer Inst.* **11:** 663.
21. Hauser, G., and J. Hildebrand. 1969. *Fed. Proc.* **28:** 595.
22. Suomi, W. D., and B. W. Agranoff. 1965. *J. Lipid Res.* **6:** 211.
23. Sweeley, C. C., and B. Klinoski. 1965. *In* The Metabolic Basis of Inherited Disease. Stanbury J. B., J. B. Wyngaarden, and D. S. Frederickson, editors. McGraw-Hill, Inc., New York. 2nd edition.
24. O'Brien, J. S. 1964. *Biochem. Biophys. Res. Commun.* **15:** 484.
25. Lyman, R. L., A. Shannon, R. Ostwald, and P. Miljanich. 1964. *Can. J. Biochem.* **42:** 365.
26. Natori, Y. 1963. *J. Biol. Chem.* **238:** 2075.
27. Lyman, R. L., J. Tinoco, P. Bouchard, G. Sheehan, R. Ostwald, and P. Miljanich. 1967. *Biochim. Biophys. Acta.* **137:** 107.