Fiske, C. H., and Subbarow, Y. (1925), *J. Biol. Chem. 66*, 375.
Hori, S. H. (1966), *Histochemie 7*, 297.
Kuby, S. A., Noda, L., and Lardy, H. A. (1954), *J. Biol. Chem. 210*, 65.
Lorand, L., Schuel, H., Demovski, R. A., and Meisler, J. (1965), *in* Molecular Biology of Muscular Contraction, Ebashi, F., Ed., Tokyo, Igaku Shoin.
Morrison, J. F., and James, E. (1965), *Biochem. J. 97*, 37.
Nanninga, L. B., and Mommaerts, W. F. H. M. (1960), *Proc. Natl. Acad. Sci. U. S. 46*, 1155.
Noda, L., Kuby, S. A., and Lardy, H. A. (1954), *J. Biol. Chem. 210*, 83.
Noda, L., Nihei, T., and Morales, M. F. (1960), *J. Biol. Chem. 235*, 2830.
Ouellet, L., Laidler, K. J., and Morales, M. F. (1952), *Arch. Biochem. Biophys. 39*, 37.
Vasil'ev, V. P. (1957), *Zh. Fiz. Khim. 31*, 419.
Yagi, K., and Mase, R. (1962), *J. Biol. Chem. 237*, 397.

The Degradation of Tritiated Dihydrosphingosine in the Intact Rat*

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ABSTRACT: Tritiated dihydrosphingosine, prepared by the catalytic reduction of sphingosine with tritium gas in the presence of platinum on charcoal, was administered by intravenous injection to mature rats. The animals were sacrificed after periods of time which varied from 15 to 90 min and the total lipids of the livers were isolated. Between 18 and 26% of the injected radioactivity were present in the liver lipids and were shown to be about equally divided between the long-chain base groups and the fatty acid groups. The largest part of the tritium of the fatty acid fraction was present in palmitic acid. Both the amount and the intramolecular distribution of the radioactivity in the palmitic acid led to the conclusion that this compound was formed directly from the injected dihydrosphingosine by reactions which resulted in the metabolic removal of carbon atoms 1 and 2 from the lipid base molecule. Evidence was obtained that sphingosine and phytosphingosine are probably not intermediates in the enzymatic degradation of dihydrosphingosine.

Studies on the distribution of sphingosine and dihydrosphingosine in various animal tissues which were analyzed according to the method described by Schmidt *et al.* (1966) revealed that only a small percentage of the total lipid bases in rat liver and kidney could be accounted for as dihydrosphingosine. Since dihydrosphingosine is believed to be a precursor of sphingosine, we attempted to obtain data pertinent to the mechanism by which the characteristic proportions of sphingosine to dihydrosphingosine in tissues are maintained by injecting tritium-labeled dihydrosphingosine into rats and measuring the appearance of tritium in sphingosine. In the course of these experiments, it was noted that in addition to the tritium labeling of both dihydrosphingosine- and sphingosine-containing sphingolipids, a considerable amount of radioactivity was recovered in the fatty acids of rat liver following the injection of [^{3}H]-dihydrosphingosine.

The conversion of phytosphingosine into pentadecanoic acid has been reported by Barenholz and Gatt (1967) who injected rats with labeled phytosphingosine obtained by growing cultures of *Hansenula ciferri* in the presence of ^{3}H- or ^{14}C-labeled precursors. While the present study was in progress, Stoffel and his collaborators (1967a–c) reported observations concerning the *in vivo* metabolism of radioactively labeled lipid bases which were prepared by organic synthesis. Their data show that in contrast to phytosphingosine, both sphingosine and dihydrosphingosine are degraded to palmitic acid and ethanolamine. In the present communication, observations providing an independent confirmation of the conclusions of Stoffel *et al.* (Stoffel and Sticht, 1967a,b; Stoffel *et al.*, 1967) as well as additional information which eliminates sphingosine as a required intermediate in the biological degradation of dihydrosphingosine are described.

Experimental Section

Solvents. All solvents used in these experiments were

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ACS reagent grade. The petroleum ether (bp 30–60°) and pyridine which were employed in preparing samples for gas–liquid partition chromatography were purified by drying and distillation before use. Solvent mixtures given in the text are defined on a volume to volume basis.

trans-2-Hexadecenoic Acid. Tetradecanal and malonic acid (both obtained from Aldrich Chemical Co.) were condensed to yield *trans*-2-hexadecenoic acid as described by Shapiro *et al.* (1958a). After crystallization from hexane, the white crystalline compound melted at 48–48.5° (lit. (Shapiro *et al.*, 1958a) mp 48–49°). Gas–liquid partition chromatography of the methyl ester yielded a single symmetrical peak.

Dihydrosphingosine was prepared by hydrogenating sphingosine in the apparatus and according to the technique described by Brown and Brown (1966). This compound was found to be contaminated with small amounts of other ninhydrin-positive materials, but was suitable as a reference compound for thin-layer chromatography. A mixture of *erythro-* and *threo*-dihydrosphingosine was synthesized by the procedure of Shapiro *et al.* (1958b). The compound was shown to be pure by thin-layer chromatography, melting range, and gas–liquid partition chromatography of the trimethylsilyl derivative.

Tritiated Dihydrosphingosine. Sphingosine sulfate was prepared from purified beef brain sphingolipids by methanolysis as described by Carter *et al.* (1947). The free base was liberated with alkali and purified by crystallization from heptane. Sphingosine (100 mg) dissolved in 10 ml of absolute ethanol containing 50 mg of 10% platinum on charcoal was allowed to react for 30 min with 3.0 Ci of tritium gas. Reduction was then carried to completion with hydrogen gas. Labile tritium was removed with 5.0 ml of ethanol and 5.0 ml of chloroform. The solvents were removed by vacuum distillation.[1]

The product of this reaction was found to be contaminated with a number of radioactive substances which had a chromatographic mobility that differed from authentic dihydrosphingosine. For this reason, the [^{3}H]dihydrosphingosine was always purified before use in these experiments. The labeled dihydrosphingosine sample (3–10 mCi) was spotted on a thin-layer plate of silica gel G together with marker spots of dihydrosphingosine and the plate was developed with the solvent system of Sambasivarao and McCluer (1963). The zone on the plate corresponding to the reference samples was scraped out and eluted with chloroform–methanol (2:1). The eluate was brought to dryness *in vacuo* and dissolved in methanol. This solution was extracted three times with two volumes of petroleum ether. The methanol layer was then made to volume with chloroform–methanol (2:1) and aliquots of these solutions were used shortly after preparation for injection and determination of purity. The dihydrosphingosine content of these preparations was determined by the ninhydrin method of Schmidt *et al.* (1966). The specific activities of these preparations were found to be 4.01–4.06 $\times$ 10^8 dpm/μmole.

The purity of the [^{3}H]dihydrosphingosine was checked by converting samples into the trimethylsilyl derivative by the procedure of Carter and Gaver (1967) and by subsequent gas–liquid partition chromatography. The chromatography of one of these samples under the conditions described below yielded essentially only one peak which contained 90% of the recovered radioactivity. This peak corresponded in retention time to the trimethylsilyl derivative of synthetic dihydrosphingosine. In other assays, small quantities of the purified [^{3}H]dihydrosphingosine were mixed with the synthetic compound and converted into the trimethylsilyl derivative. A single peak was obtained which contained 90% or more of the recovered radioactivity.

Gas–Liquid Partition Chromatography. All analyses by gas–liquid partition chromatography were carried out with a Barber-Colman Model 10 apparatus equipped with a ^{90}Sr detector. The fatty acid methyl esters were separated on a 6 ft $\times$ 4 mm i.d. U-shaped glass column packed with 15% polybutanediol succinate on Chromosorb W (80–100 mesh). This column was usually operated at about 175°. The flash heater and detector temperatures were maintained 40–50° higher than the column temperature. Argon was passed through the column at a rate of 70–100 ml/min. The methyl esters were identified by comparison with standards obtained from Applied Science Laboratories and the areas of the peaks were determined with a planimeter.

The trimethylsilyl derivatives of the long-chain bases were separated on a 6 ft $\times$ 4 mm i.d. U-shaped glass column packed with 3.8% silicone rubber (UC W98) on Diatoport S (both obtained from Hewlett-Packard Co.). Excellent separations of these compounds were obtained with a column temperature of 170–200°. The detector and flash heater temperatures were about 40° higher than those of the column. A flow rate of about 50 ml/min of argon was maintained. The retention times were calculated using methyl stearate as a reference standard. Precise agreement with the values reported by Gaver and Sweeley (1965) was obtained.

The radioactive compounds were trapped for analysis by passing the effluent gas from the gas chromatograph into 15-cm tubes containing glass wool. The radioactive compounds were eluted from the glass wool into counting vials with portions of the scintillation fluid. Between 75 and 95% of the radioactivity in the methyl esters were recovered in this manner. The recoveries were generally lower in the case of the trimethylsilyl derivatives of the long-chain bases (presumably due to incomplete formation of the derivatives) but in some experiments up to 90% of the injected activity could be recovered. When methyl palmitate was collected for chemical degradation, the same procedure was used except that the glass wool was eluted with chloroform–methanol (2:1).

Determination of Radioactivity. A Nuclear-Chicago liquid scintillation spectrophotometer was used for all assays reported here. Samples were counted in 10 ml of a toluene solution containing 0.4% 2,5-diphenyl-

[1] These operations were performed by the New England Nuclear Corp.

TABLE I: Radioactivity Recovered in the Lipid Fractions of Rat Liver at Various Times after the Injection of [^{3}H]-Dihydrosphingosine.

Expt	Time (min)	[^{3}H]Dihydrosphingosine Administered		Radioactivity Recovered						dpm of Methyl Palmitate × 100/ dpm of Total Fatty Acid Esters
				Total Lipids		Long-Chain Bases		Fatty Acid Esters		
		μmole	dpm × 10^6	dpm × 10^6	%[a]	dpm × 10^6	%[a]	dpm × 10^6	%[a]	
I	15	0.114	46.0	11.8	25.6	7.45	16.2	4.18	9.10	80.6
I	60	0.114	46.0	10.6	23.0	5.23	11.4	4.48	9.75	73.8
II	30	0.0432	17.4	4.02	23.1	2.19	12.6	1.49	8.57	79.3
II	90	0.0432	17.4	3.63	20.9	1.89	10.9	1.66	9.55	69.5
III	30	0.0905	36.5	6.71	18.4	2.79	7.65	3.37	9.23	89.0[b]

[a] Calculated as a percentage of the administered dose. [b] Purified by thin-layer chromatography prior to gas–liquid partition chromatography.

oxazole and 0.005% 1,4-bis[2-(5-phenyloxazolyl)]benzene. An internal standard of [^{3}H]toluene was used to permit the conversion of the observed counts into disintegrations per minute.

The Oxidative Cleavage of Dihydrosphingosine. A mixture of 54 μg of purified [^{3}H]dihydrosphingosine and 1.4 mg of synthetic dihydrosphingosine was treated with the periodate–permangenate reagent of von Rudloff (1956) according to the micromodification of Greene *et al.* (1965). The isolated fatty acids were converted into their methyl esters and separated by gas–liquid partition chromatography. The methyl ester of palmitic acid which is the chief product of the reaction was isolated and degraded as described below.

Chemical Degradation of Methyl Palmitate. The distribution of tritium in palmitic acid was determined by the stepwise degradation of the methyl palmitate fractions isolated by gas–liquid partition chromatography. The labeled methyl palmitate was combined with the unlabeled compound and palmitic acid was liberated by saponification. Palmitic acid was sequentially degraded to pentadecanoic and tetradecanoic acid by the Anker (1952) modification of the method of Hunsdiecker and Hunsdiecker (1942). The technique of Anker (1952) was followed except for the modification that the alcohols produced by the decarboxylation and debromination of the fatty acid silver salts were oxidized as described by Brady *et al.* (1960). In most cases, the fatty acids were purified by crystallization from both methanol and petroleum ether before the determination of radioactivity. By observing the precautions recommended by Foster and Bloom (1963), the yields of the fatty acids were sufficiently high to permit accurate determinations of their specific activities. The extent of conversion into the next lower homolog was shown to be almost complete by gas–liquid partition chromatography of the products.

Preparation of Lipid Fractions. Total lipid extracts were prepared from the whole livers of injected animals as described by Folch *et al.* (1957). Aliquots of the total lipid extracts were taken to dryness under nitrogen and methanolyzed in sealed tubes with methanolic 2 N sulfuric acid. The methanolysates were extracted repeatedly with petroleum ether. The methanol–sulfuric acid layer was neutralized and brought to dryness *in vacuo*. The residue was partitioned between chloroform and acidified water. The chloroform phase was removed and the extraction was repeated several times with additional aliquots of chloroform. This procedure results in the separation of the methanolyzed lipid extract into two fractions: a petroleum ether fraction, containing the fatty acid methyl esters and nonsaponifiable lipids, and a chloroform extract which contains the liberated long-chain bases. These extracts will be referred to as the fatty acid ester fraction and the long-chain base fraction. This separation technique was adapted from a method for the determination of the long-chain bases which is described fully elsewhere (Schmidt *et al.*, 1966). The procedure has been carefully checked and gives excellent recoveries of the long-chain bases.

Results

In the experiments which are reported here, Wistar rats weighing between 180 and 300 g were fasted overnight and injected in the tail vein with 0.10 ml of a solution of purified [^{3}H]dihydrosphingosine in dimethyl sulfoxide. The animals were sacrificed after the indicated periods of time and the total lipid, the fatty acid ester, and the long-chain base fractions were prepared from the livers as described above. Preliminary experiments indicated that only small amounts of radioactivity were present in other organs. The radioactivity present in each of these fractions as well as that of the individual esters in the fatty acid ester fraction was determined. These data are summarized in Table I.

It is significant that in each of these samples 25% or

TABLE II: The Distribution of Tritium in the Total Fatty Acids of Rat Liver following Injection of [^{3}H]-Dihydrosphingosine.[a]

Fatty Acid	Rel Retention Time	% Total Mass	% Radioactivity
14:0	0.255	0.4	1.4
15:0	0.360	1.0	1.4
16:0	0.519	12.4	73.8[b]
16:1	0.567	5.3	
17:0	0.706	2.3	2.3
18:0	1.00	18.1	14.2
18:1	1.10	15.1	6.8[c]
18:2	1.31	17.5	
18:3	1.42	2.4	
20:4	2.94	21.5	

[a] These results are the average values obtained from the gas–liquid partition chromatography of the fatty acid ester fraction (Table I, expt I, 60-min sample). An average of 83% of the injected activity was recovered in this assay. Further details on the assay procedure are given in the text. [b] In this experiment, 16:0 and 16:1 were collected together; other assays showed over 90% of the activity in the 16:0 fraction. [c] Includes all activity from 18:1 to 20:4.

TABLE III: The Tritium Content of the Fatty Acids Determined by the Chemical Degradation of Methyl [^{3}H]Palmitate.[a]

	Source of Palmitic Acid			
	Nonenzymatic Degradation of [^{3}H]Dihydrosphingosine[b]		Metabolic Degradation of [^{3}H]Dihydrosphingosine[c]	
Fatty Acid	dpm/μmole	% ^{3}H	dpm/μmole	% ^{3}H
Palmitic	13,590	100	2,000	100
Pentadecanoic	10,950	80.6	1,582	79.2
Tetradecanoic	3,618	26.6	638	31.8

[a] The methyl palmitate isolated by gas–liquid partition chromatography from either the nonenzymatic or enzymatic breakdown products of [^{3}H]dihydrosphingosine was combined with unlabeled methyl palmitate and saponified. The liberated palmitic acid was sequentially degraded to tetradecanoic acid. Further details are given in the text. [b] Methyl [^{3}H]palmitate (*ca.* 5×10^5 dpm, <0.1 μg) was combined with 1.07 g of carrier methyl palmitate. [c] Methyl [^{3}H]palmitate (*ca.* 2.5×10^4 dpm, 4.5 mg) from fatty acid esters (expt III, Table I) was combined with 307 mg of carrier methyl palmitate.

more of the total radioactivity which was recovered from the liver could be accounted for as methyl palmitate. Similar results were obtained in other experiments (not reported in detail here) in which rats of different ages were used and the interval between injection and sacrifice was prolonged. The amount of radioactivity in the liver lipids declined after several hours, but palmitic acid was always the most highly labeled fatty acid.

Control experiments were conducted in which [^{3}H]-dihydrosphingosine was added to nonradioactive total lipid extracts from liver and carried through the methanolysis and extraction procedure. The fact that only small amounts of radioactivity could be found in the fatty acid ester fraction excludes the possibility that the observed breakdown of [^{3}H]dihydrosphingosine could have occurred during the work-up procedure.

The fatty acid ester fractions were subjected to analysis by gas–liquid partition chromatography and the results of a typical run are given in Table II. Although some variations in the percentages of the individual fatty acid esters were noted in the different samples, in each case, the bulk (70% or more) of the radioactivity could be recovered as methyl palmitate. The palmitoleate ester fraction was found to contain 10% or less of the tritium present in the palmitate fraction, undoubtedly due in part to the fact that there is a slight overlap between these two peaks. Samples of methyl *trans*-2-hexadecenoate were found to have a retention time which differed markedly from that of methyl palmitate and methyl palmitoleate, but was between that found for methyl heptadecanoate and methyl stearate. Only small amounts of radioactivity could be found in the peaks corresponding to methyl pentadecanoate and methyl heptadecanoate. This would appear to rule out the possibility that these compounds or *trans*-2-hexadecenoate (which would be collected with the C_{17} ester) are necessarily intermediates in dihydrosphingosine breakdown. The tritium which is found in the C_{18} fatty acids is possibly a result of the metabolic elongation of the carbon chain of labeled palmitate.

To provide evidence that the labeled palmitic acid found after the injection of [^{3}H]dihydrosphingosine is formed directly from the long-chain base, the intramolecular distribution of tritium in the palmitate molecule was determined. Methyl palmitate which was isolated from the gas–liquid partition chromatographic effluents of one of the fatty acid ester fractions (Table I, expt III) was combined with carrier methyl palmitate and saponified to yield the free acid. This palmitic acid sample was then sequentially degraded to pentadecanoic and tetradecanoic acids which were analyzed for radioactivity. A sample of purified [^{3}H]dihydrosphingosine from the same preparation was oxidatively cleaved to palmitic acid with periodate–permanganate as described in the preceding section. After conversion into the methyl ester and isolation by gas–liquid partition chromatography, the labeled ester was mixed with carrier methyl palmitate and degraded to its lower homologs by the same procedure used above. The results of these determinations are given in Table III.

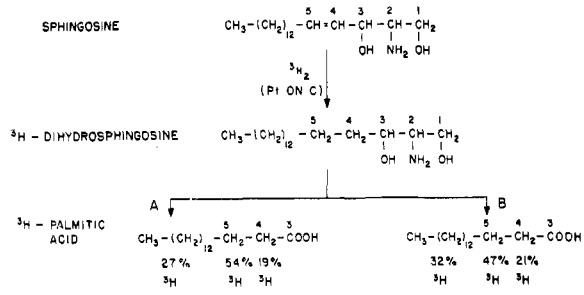

FIGURE 1: A scheme showing the intramolecular distribution of tritium in the palmitic acid derived from [^{3}H]dihydrosphingosine. (A) Produced by nonenzymatic cleavage with periodate–permanganate. (B) Produced metabolically. The isotope distribution was determined by the chemical degradation procedure described in the text.

The data in Table III show that the catalytic reduction of sphingosine with tritium gas did not result in an even distribution of the isotope between carbon atoms 4 and 5. A similar observation has recently been reported by Weiss and Stiller (1967) who reduced the *N*-carbobenzoxy derivative of sphingosine. The isotope distribution determined by these workers was somewhat different than that reported in the present study; this must be due to the fact that the compound reduced and the conditions of reduction were not the same.

A comparison of the labeling pattern in the metabolically derived palmitic acid with that of the palmitate produced by oxidative cleavage of [^{3}H]dihydrosphingosine reveals that they are similar. This similarity would not be retained if the labeled dihydrosphingosine were catabolized to small fragments and resynthesized to palmitic acid.

Discussion

The schematic diagram in Figure 1 shows the general outline and results of the experiments reported in this communication. It has been demonstrated that a large fraction of the injected dihydrosphingosine is converted to palmitic acid. The intramolecular distribution of tritium in this acid was found to be similar to that of the palmitic acid sample obtained by the nonenzymatic degradation of tritiated dihydrosphingosine from the same preparation. From these facts, it may be concluded that dihydrosphingosine is metabolically converted into palmitic acid by the removal of carbon atoms 1 and 2 from the long-chain base. Barenholz and Gatt (1967) reported very similar experiments with radioactive phytosphingosine. They found that phytosphingosine is cleaved between carbon atoms 3 and 4 to yield pentadecanoic acid as the principal product. This led them to suggest that the degradation of dihydrosphingosine may proceed as shown in eq 1.

dihydrosphingosine ⟶
sphingosine ⟶ phytosphingosine
↓
pentadecanoic acid (1)

The results described in the present communication show that dihydrosphingosine is not metabolized *via* phytosphingosine as suggested by Barenholz and Gatt (1967) and confirm the observations of Stoffel and his coworkers (Stoffel and Sticht, 1967a,b; Stoffel *et al.*, 1967). These investigators employed synthetically prepared sphingolipid bases and related derivatives to show that the products of both sphingosine and dihydrosphingosine degradation were palmitic acid and ethanolamine. They also demonstrated that 3-ketodihydrosphingosine is an intermediate in the synthesis of both sphingosine and dihydrosphingosine in agreement with the biosynthetic pathway proposed by Braun and Snell (1967). The degradation products obtained after the injection of radioactive 3-ketodihydrosphingosine led Stoffel *et al.* (1967) to suggest that this compound is also the first intermediate in the breakdown of dihydrosphingosine. The findings of Stoffel and his coworkers (Stoffel and Sticht, 1967a,b; Stoffel *et al.*, 1967), however, do not resolve the question as to whether or not the degradation of dihydrosphingosine to palmitic acid must be preceded by the conversion of the saturated lipid base into sphingosine or a related 4,5-unsaturated derivative. The results of our studies show that it is unlikely that sphingosine or its 3-keto derivative is a necessary intermediate in the degradation of dihydrosphingosine to palmitate, because the isotope distribution in the palmitate corresponds to that in the nonpolar portion of the dihydrosphingosine chain. The labeled dihydrosphingosine which was used in our study contained a part of the tritium in the 4,5 positions and another part in the remainder of the alkyl chain. Desaturation of this compound to sphingosine would have resulted in a large shift in the tritium distribution in that part of the lipid base molecule which is a precursor of palmitate. Conceivably, the degradation of sphingosine to palmitic acid might result in the intermediary formation of 2,3-hexadecenoic acid, although the mechanism of this degradation is as yet unexplored. With synthetic 2,3-hexadecenoic acid as a carrier, we did not detect any significant amount of radioactivity in the peak of this unsaturated fatty acid during gas–liquid partition chromatography of the liver fatty acids of rats that had been injected with tritium-labeled dihydrosphingosine.

Since appreciable percentages of the radioactivity of the injected dihydrosphingosine were found in the total sphingosine of the liver, and since free sphingosine is rapidly converted into palmitate according to Stoffel and Sticht (1967a,b), a shift in the isotope distribution in the palmitate is theoretically possible although the degradation of dihydrosphingosine to palmitate does not require that it is desaturated to sphingosine. Most likely, however, enzymatically formed sphingosine is immediately utilized in the tissues for the incorporation into ceramides. This assumption is suggested by the findings of E. L. Hogan and G. Schmidt (unpublished data) that no free lipid bases are detectable in normal tissues by sensitive chromatographic techniques. Thus, the rate of *in vivo* conversion into palmitate of metabolically formed sphingosine is most likely negligible in comparison to that of its injected precursor, dihydrosphingosine.

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References

Anker, H. S. (1952), *J. Biol. Chem. 194*, 177.
Barenholz, Y., and Gatt, S. (1967), *Biochem. Biophys. Res. Commun. 27*, 319.
Brady, R. O., Bradley, R. M., and Trams, E. G. (1960), *J. Biol. Chem. 235*, 3093.
Braun, P. E., and Snell, E. E. (1967), *Proc. Natl. Acad. Sci. U. S. 58*, 298.
Brown, C. A., and Brown, H. C. (1966), *J. Org. Chem. 31*, 3989.
Carter, H. E., and Gaver, R. C. (1967), *J. Lipid Res. 8*, 391.
Carter, H. E., Norris, W. P., Glick, F. J., Phillips, G. E., and Harris, R. (1947), *J. Biol. Chem. 170*, 269.
Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957), *J. Biol. Chem. 226*, 497.
Foster, D. W., and Bloom, B. (1963), *J. Biol. Chem. 238*, 888.
Gaver, R. C., and Sweeley, C. C. (1965), *J. Am. Oil Chemists' Soc. 42*, 294.
Greene, M. L., Kaneshiro, T., and Law, J. H. (1965), *Biochim. Biophys. Acta 98*, 582.
Hunsdiecker, H., and Hunsdiecker, C. (1942), *Ber. Deut. Chem. Ges. 75*, 291.
Sambasivarao, K., and McCluer, R. H. (1963), *J. Lipid Res. 4*, 106.
Schmidt, G., Hogan, E. L., Kjeta-Fyda, A., Tanaka, T., Joseph, J., Feldman, N. I., Collins, R. A., and Keenan, R. W. (1966), *in* Inborn Disorders of Sphingolipid Metabolism, Aronson, S. M., and Volk, B. W., Ed., New York, N. Y., Pergamon, p 325.
Shapiro, D., Segal, H., and Flowers, H. M. (1958a), *J. Am. Chem. Soc. 80*, 1194.
Shapiro, D., Segal, H., and Flowers, H. M. (1958b), *J. Am. Chem. Soc. 80*, 2170.
Stoffel, W., LeKim, D., and Sticht, G. (1967), *Z. Physiol. Chem. 348*, 1571.
Stoffel, W., and Sticht, G. (1967a), *Z. Physiol. Chem. 348*, 941.
Stoffel, W., and Sticht, G. (1967b), *Z. Physiol. Chem. 348*, 1345.
von Rudloff, E. (1956), *Can. J. Chem. 34*, 1413.
Weiss, B., and Stiller, R. L. (1967), *J. Biol. Chem. 242*, 2903.

CORRECTION

In the paper "Conformation of Cytochromes. III. Effect of Urea, Temperature, Extrinsic Ligands, and pH Variation on the Conformation of Horse Heart Ferricytochrome *c*," by Yash P. Myer, Volume 7, February 1968, page 765, the following correction should be made.

In column 2 on page 775, lines 34 and 36 should read "containing eight amino acid residues from 14 to 21)" and "11 to 21) in the aromatic absorption region do not," respectively.